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(54) Title: COMPOSITIONS AND METHODS FOR MODIFYING MATURITY IN RICE PLANTS

(57) Abstract: Methods and compositions to modulate maturity of a rice plant are disclosed. The disclosure further discloses compositions, polynucleotide constructs, transformed host cells, plants and seeds exhibiting altered stature characteristics or produce plants that exhibit altered maturity parameters.



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COMPOSITIONS AND METHODS FOR MODIFYING MATURITY IN RICE PLANTS

FIELD

5 This disclosure relates to compositions and methods of modifying maturity in rice plants.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

10 The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file named "7438_ST25.txt" created on August 17, 2018 and having a size of 123.4 kilobytes and is filed concurrently with the specification. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND

15 Recent advances in plant genetic engineering have opened new doors to engineer plants to have improved characteristics or traits, such as maturity, stature, height and other architecture. Early or late maturity, depending on the need, is a desirable trait in crop breeding for a variety of crops of commercial interest. Maturity adaptations increase harvest index, favorably partition carbon and nutrients between grain and non-grain biomass, enhance fertilizer use, water use efficiency and play a role in increasing planting density.

SUMMARY

25 Provided herein is a method of modifying maturity, the method comprising introducing one or more nucleotide modifications through a targeted DNA break at a genomic locus of a plant, wherein the genomic locus comprises a polynucleotide involved in flowering time regulation (FTR) encoding a response regulator receiver domain containing protein, a CCT motif containing protein, BHLH transcription factor, TCP family transcription factor, NAC domain-containing protein, tubulin/FtsZ domain containing protein, hsp20/alpha crystallin protein, core histone H2A/H2B/H3/H4 putative protein, AAA-type ATPase family protein, universal stress protein domain containing protein, PHD finger family protein, or a methyl-binding domain protein, and wherein the plant maturity is modified compared to a control plant not comprising the one or more introduced genetic modifications. In certain embodiments, the FTR polynucleotide encodes a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of

35

SEQ ID NOs: 14-26. In certain embodiments, the targeted DNA modification targets more than one distinct genomic loci that is involved in FTR of the plant.

In certain embodiments, the targeted DNA modification is selected from the group consisting of insertion, deletion, single nucleotide polymorphism (SNP), and a polynucleotide modification, such that the expression of the FTR polypeptide is reduced. In certain embodiments, the targeted DNA modification results in one or more of the following: reduced expression of the FTR polynucleotide; reduced transcriptional activity of the protein encoded by the FTR polynucleotide; generation of one or more alternative spliced transcripts of the FTR polynucleotide; deletion of one or more DNA binding domains; frameshift mutation in one or more exons of the FTR polynucleotide; deletion of a substantial portion of the FTR polynucleotide or deletion of the full-length open reading frame of the FTR polynucleotide; repression of an enhancer motif present within a regulatory region encoding the FTR polynucleotide; modification of one or more nucleotides or deletion of a regulatory element operably linked to the expression of the FTR polynucleotide, wherein the regulatory element is present within a promoter, intron, 3'UTR, terminator or a combination thereof. In certain embodiments, the targeted DNA modification targets the genomic locus of the FTR polynucleotide such that the one or more nucleotide modifications are present within (a) the same coding region; (b) non-coding region; (c) regulatory sequence; (d) untranslated region, or (e) any combination of (a)-(d) of an endogenous polynucleotide encoding a polypeptide that is involved in maturity.

In certain embodiments, the targeted DNA modification is introduced by a RNA-guided endonuclease, a site-specific deaminase, or a site-specific endonuclease. In certain embodiments, the targeted DNA modification is through a genome modification technique selected from the group consisting of polynucleotide-guided endonuclease, CRISPR-Cas endonucleases, base editing deaminases, zinc finger nuclease, a transcription activator-like effector nuclease (TALEN), engineered site-specific meganucleases, or Argonaute. In certain embodiments, the targeted DNA modification is induced by using a guide RNA that corresponds to a target sequence comprising a polynucleotide that encodes a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-26.

In certain embodiments, the plant exhibits early maturity when the targeted DNA modification results in reduced expression or activity of the protein encoded by the FTR polynucleotide. In an embodiment the plant exhibits first flowering about 5 to 15 days earlier than a control plant.

In certain embodiments, the plant exhibits delayed maturity when the targeted DNA modification results in reduced expression or activity of the protein encoded by the FTR

polynucleotide. In an embodiment, maturity of the plant is delayed by about 5% to about 50% compared to a control plant as measured by the number of days to first or 50% flowering or panicle emergence or panicle initiation.

In certain embodiments, the modification in maturity is obtained in the absence of a substantial reduction in grain yield measure per plant or as a population of plants per unit area. In certain embodiments, the stature of the plant is not significantly altered compared to the control rice plant as measured by a reduction in plant height. In certain embodiments, the targeted DNA modification of the plant does not substantially alter root architecture of the plant or does not significantly increase root lodging, compared to a control plant not comprising the modification(s).

In certain embodiments, the plant is a rice plant. In certain embodiments, the rice plant is a female in bred line. In certain embodiments, the rice plant is a hybrid. In certain embodiments, the rice plant is *Oryza sativa* var. indica.

Also provided herein is a rice plant exhibiting early maturity comprising a modified genomic locus involved in flowering time regulation (FTR), wherein the genomic locus comprises one or more introduced mutations compared to a control plant and wherein the FTR genomic locus encodes a polypeptide that is at least 90% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 14-21. In certain embodiments, the rice plant exhibits early maturity in a range of about 5 to 15 days as measured by first flowering timing. In certain embodiments, the rice plant is a female in bred line. In certain embodiments, the rice plant is a hybrid. In certain embodiments, the rice plant is *Oryza sativa* var. indica. In certain embodiments, the modification in maturity is obtained in the absence of a substantial reduction in grain yield measure per plant or as a population of plants per unit area. In certain embodiments, the stature of the plant is not significantly altered compared to the control rice plant as measured by a reduction in plant height. In certain embodiments, the targeted DNA modification of the plant does not substantially alter root architecture of the plant or does not significantly increase root lodging, compared to a control plant not comprising the modification(s).

Provided herein is a rice plant exhibiting delayed maturity comprising a modified genomic locus involved in flowering time regulation (FTR), wherein the genomic locus comprises one or more introduced mutations compared to a control plant and wherein the FTR genomic locus encodes a polypeptide that is at least 90% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 22-26. In certain embodiments, the maturity of the rice plant is delayed by about 5% to about 50% compared to the control rice plant as measured by the number of days to first or 50% flowering or panicle emergence or panicle initiation. In certain embodiments, the rice plant is a female in

bred line. In certain embodiments, the rice plant is a hybrid. In certain embodiments, the rice plant is *Oryza sativa* var. indica. In certain embodiments, the modification in maturity is obtained in the absence of a substantial reduction in grain yield measure per plant or as a population of plants per unit area. In certain embodiments, the stature of the plant is not significantly altered compared to the control rice plant as measured by a reduction in plant height. In certain embodiments, the targeted DNA modification of the plant does not substantially alter root architecture of the plant or does not significantly increase root lodging, compared to a control plant not comprising the modification(s).

Also provided herein is a recombinant DNA construct comprising a polynucleotide sequence encoding an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 14-26, operably linked to at least one heterologous nucleic acid sequence.

Additionally, provided herein is a plant cell comprising a recombinant DNA construct comprising a polynucleotide sequence encoding an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 14-26, operably linked to at least one heterologous nucleic acid sequence.

Provided herein is a guide RNA sequence that targets a genomic locus of a plant cell, wherein the genomic locus comprises a polynucleotide that encodes a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-26. In certain embodiments, the guide RNA is present in a recombinant DNA construct.

Also provide are plant cells comprising a guide RNA sequence that targets a genomic loci of a plant cell, wherein the genomic loci comprises a polynucleotide that encodes a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-26, and plant cells comprising a recombinant DNA construct comprising a guide RNA sequence that targets a genomic loci of a plant cell, wherein the genomic loci comprises a polynucleotide that encodes a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-26.

Further provided herein is a plant, or seed produced therefrom, having stably incorporated into its genome a recombinant DNA construct comprising a guide RNA sequence that targets a genomic loci of the plant cell, wherein the genomic loci comprises a polynucleotide that encodes a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-26. In an embodiment, the plant is a monocot. In an embodiment, the plant is a rice plant. In certain embodiments, the plant further comprises a heterologous nucleic acid sequence

selected from the group consisting of: a reporter gene, a selection marker, a disease resistance gene, a herbicide resistance gene, an insect resistance gene; a gene involved in carbohydrate metabolism, a gene involved in fatty acid metabolism, a gene involved in amino acid metabolism, a gene involved in plant development, a gene involved in plant growth regulation, a gene involved in yield improvement, a gene involved in drought resistance, a gene involved in increasing nutrient utilization efficiency, a gene involved in cold resistance, a gene involved in heat resistance and a gene involved in salt resistance in plants.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

The disclosure can be more fully understood from the following detailed description and the accompanying Sequence Listing that form a part of this application, which are incorporated herein by reference.

The sequence descriptions summarize the Sequence Listing attached hereto, which is hereby incorporated by reference. The Sequence Listing contains one letter codes for nucleotide sequence characters and the single and three letter codes for amino acids as defined in the IUPAC-IUB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219(2):345-373 (1984).

Table 1: Sequence Listing Description

SEQ ID NOS	Description
SEQ ID NO: 1	DP1492 LOC_Os03g14669.2 Core histone H2A/H2B/H3/H4, putative, expressed; Nucleotide Sequence
SEQ ID NO: 2	DP1564 LOC_Os07g49460.1 Response regulator receiver domain containing protein, expressed; Nucleotide Sequence
SEQ ID NO: 3	DP2300-LOC_Os07g15770.1 CCT motif family protein, expressed; Nucleotide Sequence
SEQ ID NO: 4	DP0830 LOC_Os01g68700.1 BHLH transcription factor, putative, expressed; Nucleotide Sequence
SEQ ID NO: 5	DP1315- LOC_Os02g54140.1 hsp20/alpha crystallin family protein, putative, expressed; Nucleotide Sequence
SEQ ID NO: 6	DP1722 -LOC_Os07g38730.1 -tubulin/FtsZ domain containing protein, putative, expressed; Nucleotide Sequence
SEQ ID NO: 7	DP1856-LOC_Os03g60080.1-NAC domain-containing protein 67, putative, expressed; Nucleotide Sequence
SEQ ID NO: 8	DP1249 -LOC_Os04g44440.1-TCP family transcription factor, putative, expressed; Nucleotide Sequence
SEQ ID NO: 9	DP1103- LOC_Os11g43970- AAA-type ATPase family protein, putative, expressed; Nucleotide Sequence
SEQ ID NO: 10	DP0896- LOC_Os07g49290.1- PHD finger family protein, putative, expressed; Nucleotide Sequence

SEQ ID NO: 11	DP0885 LOC_Os02g02970.1- expressed protein; Nucleotide Sequence
SEQ ID NO: 12	DP0998 LOC_Os07g36600.1 universal stress protein domain containing protein, putative, expressed; Nucleotide Sequence
SEQ ID NO: 13	DP0995 LOC_Os05g33550 methyl-binding domain protein MBD; Nucleotide Sequence
SEQ ID NO: 14	DP1492 LOC_Os03g14669.2 Core histone H2A/H2B/H3/H4, putative, expressed; Amino Acid Sequence
SEQ ID NO: 15	DP1564 LOC_Os07g49460.1 Response regulator receiver domain containing protein, expressed; Amino Acid Sequence
SEQ ID NO: 16	DP2300-LOC_Os07g15770.1 CCT motif family protein, expressed; Amino Acid Sequence
SEQ ID NO: 17	DP0830 LOC_Os01g68700.1 BHLH transcription factor, putative, expressed; Amino Acid Sequence
SEQ ID NO: 18	DP1315- LOC_Os02g54140.1 hsp20/alpha crystallin family protein, putative, expressed; Amino Acid Sequence
SEQ ID NO: 19	DP1722 -LOC_Os07g38730.1 -tubulin/FtsZ domain containing protein, putative, expressed; Amino Acid Sequence
SEQ ID NO: 20	DP1856-LOC_Os03g60080.1-NAC domain-containing protein 67, putative, expressed; Amino Acid Sequence
SEQ ID NO: 21	DP1249 -LOC_Os04g44440.1-TCP family transcription factor, putative, expressed; Amino Acid Sequence
SEQ ID NO: 22	DP1103- LOC_Os11g43970- AAA-type ATPase family protein, putative, expressed; Amino Acid Sequence
SEQ ID NO: 23	DP0896- LOC_Os07g49290.1- PHD finger family protein, putative, expressed; Amino Acid Sequence
SEQ ID NO: 24	DP0885 LOC_Os02g02970.1- expressed protein; Amino Acid Sequence
SEQ ID NO: 25	DP0998 LOC_Os07g36600.1 universal stress protein domain containing protein, putative, expressed; Amino Acid Sequence
SEQ ID NO: 26	DP0995 LOC_Os05g33550 methyl-binding domain protein MBD; Amino Acid Sequence
SEQ ID NO: 27	DP1492_CR1
SEQ ID NO: 28	DP1492_CR2
SEQ ID NO: 29	DP1564_CR1
SEQ ID NO: 30	DP1564_CR2
SEQ ID NO: 31	DP2300_CR1
SEQ ID NO: 32	DP2300_CR2
SEQ ID NO: 33	DP0830_CR1
SEQ ID NO: 34	DP0830_CR2
SEQ ID NO: 35	DP1315_CR1
SEQ ID NO: 36	DP1315_CR2
SEQ ID NO: 37	DP1722_CR1
SEQ ID NO: 38	DP1722_CR2

SEQ ID NO: 39	DP1856_CR1
SEQ ID NO: 40	DP1856_CR3
SEQ ID NO: 41	DP1249_CR1
SEQ ID NO: 42	DP1249_CR2
SEQ ID NO: 43	DP1103_CR1
SEQ ID NO: 44	DP1103_CR2
SEQ ID NO: 45	DP0896_CR1
SEQ ID NO: 46	DP0896_CR2
SEQ ID NO: 47	DP0885_CR1
SEQ ID NO: 48	DP0885_CR2
SEQ ID NO: 49	DP0998_CR1
SEQ ID NO: 50	DP0998_CR2
SEQ ID NO: 51	DP0995_CR1
SEQ ID NO: 52	DP0995_CR2
SEQ ID NO: 53	DP1103_CR3
SEQ ID NO: 54	DP1103_CR4
SEQ ID NO: 55	DP0896_CR3
SEQ ID NO: 56	DP0896_CR4
SEQ ID NO: 57	DP0885_CR3
SEQ ID NO: 58	DP0885_CR4
SEQ ID NO: 59	DP0998_CR3
SEQ ID NO: 60	DP0998_CR4
SEQ ID NO: 61	DP0995_CR3
SEQ ID NO: 62	DP0995_CR4

DETAILED DESCRIPTION

The disclosure of all patents, patent applications, and publications cited herein are incorporated by reference in their entirety.

5 As used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to “a plant” includes a plurality of such plants, reference to “a cell” includes one or more cells and equivalents thereof known to those skilled in the art, and so forth.

10 As used herein, the transitional phrase “consisting essentially of” generally refers to a composition, method that includes materials, steps, features, components, or elements, in addition to those literally disclosed, provided that these additional materials, steps, features, components, or elements do not materially affect the basic and novel characteristic(s) of the claimed subject matter.

Provided herein are methods for modifying the maturity of a plant, such as a rice plant, comprising, consisting essentially of, or consisting of introducing one or more nucleotide modifications, through targeted DNA modification at a genomic locus of the plant.

As used herein, “modifying maturity,” “maturity modulation,” or the like, refers to any

5 detectable change in the time for a modified plant to reach a stage of development, as compared to a control plant (e.g., a wild-type plant that does not comprise the DNA modification). For example, maturity can be measured as the number of days to first

flowering, panicle emergence, or panicle initiation, or alternatively, maturity can be measured as the number of days to 50% flowering, panicle emergence, or panicle initiation.

10 The stage of development at which maturity is measured is not particularly limited.

However, in certain embodiments maturity is measured based on the number of days to first flowering, panicle emergence, or panicle initiation.

In the stages of rice plant maturity, panicle initiation (PI) (generally referred to as stage 4) is marked by the emergence of the panicle. Often, young panicles that emerge of

15 the last node, is a cone-shaped organ visible if the stem is dissected. The cone may become visible about 10 days after it is formed, depending on the maturity duration of the rice variety. During the development of the reproductive phase, the number of grains in the panicle is usually determined. For example, in short-duration varieties, maximum tillering, inter-node elongation, and panicle initiation are generally exhibited simultaneously or within

20 a short window. Depending on the duration of the varieties, such as for example, medium to long-duration, these stages occur in the order mentioned above. Timing of panicle initiation in rice may also be influenced by many factors, such as temperature and photoperiod to which certain varieties have adapted. Panicle initiation generally marks the beginning of the reproductive phase in rice plant and therefore is a measure of maturity when evaluating rice
25 plants.

Panicle development generally referred to as stage 5 in rice development, is characterized by the swelling of the bottom of the panicle leaf due to the panicle growing upwards inside the stem. After panicle initiation (stage 4), the panicle grows towards the top of the stem, causing a swelling in the stem called elongation. The organs of the flower
30 develop, and the panicle grows until it reaches its final size before appearing from the flag leaf, generally referred to as heading.

Heading and flowering, generally referred to as stage 6, is characterized by the emergence of the panicle from the bottom of the panicle/flag leaf, which may take about two to three weeks to emerge from the stem fully. Three days after heading, flowering occurs,
35 and the process continues until the panicle has completely appeared. Flowering generally means that the flower opens, and pollination takes place.

Milky stage, generally referred to as stage 7 occurs after fertilization and is characterized by swollen ovary, and the caryopsis develops until it reaches its mature size. The grain (caryopsis) is first aqueous and then reaches a milky consistency, observable when the grain is squeezed. The panicles are green and erect until this stage of rice reproductive development.

Dough stage, generally referred to as stage 8 is often characterized by softening of the grain and reaching a hard paste consistency after flowering. The erect panicle begins to droop along with the change in color of the grain that is characteristic of the variety e.g., yellow, red, black.

Maturity, generally referred to as stage 9 is often characterized by ripe grain and when the grain has reached its final size and maximum weight, characterized by the droopy appearance. Grains become hard and develop their characteristic colors. This stage is reached when about 85 to 90% of the panicle grains are ripe.

A "genomic locus of a plant" as used herein, generally refers to the location on a chromosome of the plant where a gene, such as a polynucleotide involved in flowering time regulation (FTR), is found. As used herein, "gene" includes a nucleic acid fragment that expresses a functional molecule such as, but not limited to, a specific protein coding sequence and regulatory elements, such as those preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence.

A "regulatory element" generally refers to a transcriptional regulatory element involved in regulating the transcription of a nucleic acid molecule such as a gene or a target gene. The regulatory element is a nucleic acid and may include a promoter, an enhancer, an intron, a 5'-untranslated region (5'-UTR, also known as a leader sequence), or a 3'-UTR or a combination thereof. A regulatory element may act in "cis" or "trans", and generally it acts in "cis", i.e. it activates expression of genes located on the same nucleic acid molecule, e.g. a chromosome, where the regulatory element is located.

An "enhancer" element is any nucleic acid molecule that increases transcription of a nucleic acid molecule when functionally linked to a promoter regardless of its relative position.

A "repressor" (also sometimes called herein silencer) is defined as any nucleic acid molecule which inhibits the transcription when functionally linked to a promoter regardless of relative position.

A "promoter" generally refers to a nucleic acid fragment capable of controlling transcription of another nucleic acid fragment. A promoter generally includes a core promoter (also known as minimal promoter) sequence that includes a minimal regulatory region to initiate transcription, that is a transcription start site. Generally, a core promoter

includes a TATA box and a GC rich region associated with a CAAT box or a CCAAT box. These elements act to bind RNA polymerase II to the promoter and assist the polymerase in locating the RNA initiation site. Some promoters may not have a TATA box or CAAT box or a CCAAT box, but instead may contain an initiator element for the transcription initiation
5 site. A core promoter is a minimal sequence required to direct transcription initiation and generally may not include enhancers or other UTRs.

The term "cis-element" generally refers to transcriptional regulatory element that affects or modulates expression of an operably linked transcribable polynucleotide, where the transcribable polynucleotide is present in the same DNA sequence. A cis-element may
10 function to bind transcription factors, which are trans-acting polypeptides that regulate transcription.

An "intron" is an intervening sequence in a gene that is transcribed into RNA but is then excised in the process of generating the mature mRNA. The term is also used for the excised RNA sequences. An "exon" is a portion of the sequence of a gene that is
15 transcribed and is found in the mature messenger RNA derived from the gene but is not necessarily a part of the sequence that encodes the final gene product.

The 5' untranslated region (5'UTR) (also known as a translational leader sequence or leader RNA) is the region of an mRNA that is directly upstream from the initiation codon. This region is involved in the regulation of translation of a transcript by differing mechanisms
20 in viruses, prokaryotes and eukaryotes.

The "3' non-coding sequences" refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic
25 acid tracts to the 3' end of the mRNA precursor.

"RNA transcript" generally refers to a product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When an RNA transcript is a perfect complimentary copy of a DNA sequence, it is referred to as a primary transcript or it may be a RNA sequence derived from posttranscriptional processing of a primary transcript and is
30 referred to as a mature RNA. "Messenger RNA" ("mRNA") generally refers to RNA that is without introns and that can be translated into protein by the cell. "cDNA" generally refers to a DNA that is complementary to and synthesized from an mRNA template using the enzyme reverse transcriptase. The cDNA can be single-stranded or converted into the double-stranded by using the Klenow fragment of DNA polymerase I. "Sense" RNA
35 generally refers to RNA transcript that includes mRNA and so can be translated into protein within a cell or in vitro. "Antisense RNA" generally refers to a RNA transcript that is

complementary to all or part of a target primary transcript or mRNA and that blocks expression or transcripts accumulation of a target gene. The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e. at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" generally refers to antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

"Targeted DNA modification" can be used synonymously with targeted DNA mutation and refers to the introduction of a site specification modification that alters or changes the nucleotide sequence at a specific genomic locus of the plant (e.g., rice).

In certain embodiments, the targeted DNA modification occurs at a genomic locus that comprises a polynucleotide involved in flowering time regulation (FTR).

In certain embodiments the polynucleotide involved in FTR is a polynucleotide that encodes a response regulator receiver domain containing protein, a CCT motif containing protein, BHLH transcription factor, TCP family transcription factor, NAC domain-containing protein, tubulin/FtsZ domain containing protein, hsp20/alpha crystallin protein, core histone H2A/H2B/H3/H4 putative protein, AAA-type ATPase family protein, universal stress protein domain containing protein, PHD finger family protein, and/or a methyl-binding domain protein.

The polynucleotide sequences, encoded proteins, and the corresponding genomic loci of the polynucleotides involved in FTR are known in the art or can be readily identified using routine methods in the art. In certain embodiments, the polynucleotide involved in FTR encodes a polypeptide comprising an amino acid sequence that is at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 14-26.

Sequence alignments and percent identity calculations may be determined using a variety of comparison methods designed to detect similar or identical sequences including, but not limited to, the Megalign® program of the LASERGENE® bioinformatics computing suite (DNASTAR® Inc., Madison, WI). Unless stated otherwise, multiple alignment of the sequences provided herein were performed using the Clustal V method of alignment (Higgins and Sharp (1989) *CAB/OS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments and calculation of percent identity of protein sequences using the Clustal V method are KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids these parameters are KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. After alignment of the sequences, using the Clustal V program, it is possible to obtain "percent identity" and "divergence" values by viewing the "sequence distances" table

on the same program; unless stated otherwise, percent identities and divergences provided and claimed herein were calculated in this manner.

Alternatively, the Clustal W method of alignment may be used. The Clustal W method of alignment (described by Higgins and Sharp, *CABIOS*. 5:151-153 (1989); Higgins, D. G. et al., *Comput. Appl. Biosci.* 8:189-191 (1992)) can be found in the MegAlign™ v6.1 program of the LASERGENE® bioinformatics computing suite (DNASTAR® Inc., Madison, Wis.). Default parameters for multiple alignment correspond to GAP PENALTY=10, GAP LENGTH PENALTY=0.2, Delay Divergent Sequences=30%, DNA Transition Weight=0.5, Protein Weight Matrix=Gonnet Series, DNA Weight Matrix=IUB. For pairwise alignments the default parameters are Alignment=Slow-Accurate, Gap Penalty=10.0, Gap Length=0.10, Protein Weight Matrix=Gonnet 250 and DNA Weight Matrix=IUB. After alignment of the sequences using the Clustal W program, it is possible to obtain “percent identity” and “divergence” values by viewing the “sequence distances” table in the same program.

In one embodiment the % sequence identity is determined over the entire length of the molecule (nucleotide or amino acid).

The targeted DNA modification described herein may be any modification known in the art such as, for example, insertion, deletion, single nucleotide polymorphism (SNP), and or a polynucleotide modification. Additionally, the targeted DNA modification in the genomic locus may be located anywhere in the genomic locus, such as, for example, a coding region of the encoded polypeptide (e.g., exon), a non-coding region (e.g., intron), a regulatory element, or untranslated region.

The type and location of the targeted DNA modification of the FTP polynucleotide is not particularly limited so long as the targeted DNA modification results in reduced expression or activity of the protein encoded by the FTR polynucleotide. In certain embodiments the targeted DNA modification is a deletion of one or more nucleotides, preferably contiguous, of the genomic locus.

As used herein “reduced,” “reduction,” or the like refers to any detectable decrease in an experimental group (e.g., rice plant with a targeted DNA modification described herein) as compared to a control group (e.g., wild-type rice plant that does not comprise the targeted DNA modification).

Accordingly, reduced expression of a protein comprises any detectable decrease in the total level of the protein in a sample and can be determined using routine methods in the art such as, for example, Western blotting and ELISA.

In certain embodiments, a reduction in the expression or activity of the protein encoded by the FTR polynucleotide is due to a targeted DNA modification at a genomic locus of a plant that results in one or more of the following: (a) reduced expression of the

FTR polynucleotide; (b) reduced transcriptional activity of the protein encoded by the FTR polynucleotide; (c) generation of one or more alternatively spliced transcripts of the FTR polynucleotide; (d) deletion of one or more DNA binding domains of the encoded FTR polypeptide; (e) frameshift mutation in one or more exons of the FTR polynucleotide; (f) deletion of a substantial portion of the FTR polynucleotide or deletion of the full open reading frame of the FTR polynucleotide; (g) repression of an enhance motif present within a regulatory region encoding the FTR polynucleotide; or (h) modification of one or more nucleotides or deletion of a regulatory element operably linked to the expression of the FTR polynucleotide wherein the regulatory element is present within a promoter, intron, 3'UTR, terminator or a combination thereof.

In certain embodiments, the targeted DNA modification at a genomic locus involved in FTR results in plants (e.g., rice) that exhibit early maturity compared to control plants. For example, plants exhibiting early flowering compared to control plants. In certain embodiments, the modified plant's first flowing occurs in a range of about 5 to 15 days (e.g., 5 to 14, 5 to 13, 5 to 12, 5 to 11, 5 to 10, 5 to 9, 5 to 8, 5 to 7, 5 to 6) earlier than a control plant. In certain embodiments, the targeted DNA modification that results in early maturity occurs at a genomic locus comprising a polynucleotide involved in FTR encoding a response regulator receiver domain containing protein, a CCT motif containing protein, BHLH transcription factor, TCP family transcription factor, NAC domain-containing protein, tubulin/FtsZ domain containing protein, hsp20/alpha crystallin protein, and/or core histone H2A/H2B/H3/H4 putative protein. In certain embodiments, the DNA modification that results in early maturity is a deletion at the genomic locus of the polynucleotide involved in FTR.

In other embodiments, the targeted DNA modification results in plants (e.g., rice) that exhibit delayed maturity compared to control plants. For example, plants exhibiting a delayed first flowering time compared to control plant. In certain embodiments, the maturity of the plant is reduced by about 5%-50% compared to a control plant. In other words, the modified plant takes about 5%-50% longer (e.g., numbers of days) to reach a particular stage of development, as compared to a control plant. In certain embodiments, the targeted DNA modification that results in delayed maturity occurs at a genomic locus comprising a polynucleotide involved in FTR encoding AAA-type ATPase family protein, universal stress protein domain containing protein, PHD finger family protein, or a methyl-binding domain protein. In certain embodiments, the DNA modification that results in delayed maturity is a deletion at the genomic locus of the polynucleotide involved in FTR.

In certain embodiments, the genomic locus has more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) targeted DNA modification. For example, the translated region and a regulatory element of a genomic locus may each comprise a targeted DNA modification.

In certain embodiments, the plant may have targeted DNA modifications at more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) genomic loci that are involved in flowering time regulation of the plant (e.g., rice).

In certain embodiments of the methods described herein, the DNA modification(s) that modifies the maturity of the plant does not adversely affect other agronomic traits. For example in certain embodiments, the targeted DNA modification at a genomic locus comprising a polynucleotide involved in FTR does not: (a) significantly reduce grain yield, as measured by per rice plant or as a population of rice plants per unit area, (b) significantly reduce stature, as measured by a reduction in plant height, and/or (c) does not significantly alter root architecture, and/or root lodging compared to a control plant that does not comprise the modification.

The targeted DNA modification of the genomic locus may be done using any genome modification technique known in the art. In certain embodiments the targeted DNA modification is through a genome modification technique selected from the group consisting of a polynucleotide-guided endonuclease, CRISPR-Cas endonucleases, base editing deaminases, zinc finger nuclease, a transcription activator-like effector nuclease (TALEN), engineered site-specific meganuclease, or Argonaute.

In some embodiments, the genome modification may be facilitated through the induction of a double-stranded break (DSB) or single-strand break, in a defined position in the genome near the desired alteration. DSBs can be induced using any DSB-inducing agent available, including, but not limited to, TALENs, meganucleases, zinc finger nucleases, Cas9-gRNA systems (based on bacterial CRISPR-Cas systems), guided cpf1 endonuclease systems, and the like. In some embodiments, the introduction of a DSB can be combined with the introduction of a polynucleotide modification template.

A polynucleotide modification template can be introduced into a cell by any method known in the art, such as, but not limited to, transient introduction methods, transfection, electroporation, microinjection, particle mediated delivery, topical application, whiskers mediated delivery, delivery via cell-penetrating peptides, or mesoporous silica nanoparticle (MSN)-mediated direct delivery.

The polynucleotide modification template can be introduced into a cell as a single stranded polynucleotide molecule, a double stranded polynucleotide molecule, or as part of a circular DNA (vector DNA). The polynucleotide modification template can also be tethered to the guide RNA and/or the Cas endonuclease. Tethered DNAs can allow for co-localizing target and template DNA, useful in genome editing and targeted genome regulation, and can also be useful in targeting post-mitotic cells where function of endogenous HR machinery is expected to be highly diminished (Mali et al. 2013 *Nature Methods* Vol. 10:

957-963.) The polynucleotide modification template may be present transiently in the cell or it can be introduced via a viral replicon.

A “modified nucleotide” or “edited nucleotide” refers to a nucleotide sequence of interest that comprises at least one alteration when compared to its non-modified nucleotide sequence. Such “alterations” include, for example: (i) replacement of at least one
5 nucleotide, (ii) a deletion of at least one nucleotide, (iii) an insertion of at least one nucleotide, or (iv) any combination of (i) – (iii).

The term “polynucleotide modification template” includes a polynucleotide that comprises at least one nucleotide modification when compared to the nucleotide sequence
10 to be edited. A nucleotide modification can be at least one nucleotide substitution, addition or deletion. Optionally, the polynucleotide modification template can further comprise homologous nucleotide sequences flanking the at least one nucleotide modification, wherein the flanking homologous nucleotide sequences provide sufficient homology to the desired nucleotide sequence to be edited.

The process for editing a genomic sequence combining DSB and modification
15 templates generally comprises: providing to a host cell, a DSB-inducing agent, or a nucleic acid encoding a DSB-inducing agent, that recognizes a target sequence in the chromosomal sequence and is able to induce a DSB in the genomic sequence, and at least one polynucleotide modification template comprising at least one nucleotide alteration when
20 compared to the nucleotide sequence to be edited. The polynucleotide modification template can further comprise nucleotide sequences flanking the at least one nucleotide alteration, in which the flanking sequences are substantially homologous to the chromosomal region flanking the DSB.

The endonuclease can be provided to a cell by any method known in the art, for
25 example, but not limited to, transient introduction methods, transfection, microinjection, and/or topical application or indirectly via recombination constructs. The endonuclease can be provided as a protein or as a guided polynucleotide complex directly to a cell or indirectly via recombination constructs. The endonuclease can be introduced into a cell transiently or can be incorporated into the genome of the host cell using any method known in the art. In
30 the case of a CRISPR-Cas system, uptake of the endonuclease and/or the guided polynucleotide into the cell can be facilitated with a Cell Penetrating Peptide (CPP) as described in WO2016073433 published May 12, 2016.

As used herein, a “genomic region” is a segment of a chromosome in the genome of a cell that is present on either side of the target site or, alternatively, also comprises a
35 portion of the target site. The genomic region can comprise at least 5-10, 5-15, 5-20, 5-25, 5-30, 5-35, 5-40, 5-45, 5- 50, 5-55, 5-60, 5-65, 5- 70, 5-75, 5-80, 5-85, 5-90, 5-95, 5-100, 5-

200, 5-300, 5-400, 5-500, 5-600, 5-700, 5-800, 5-900, 5-1000, 5-1100, 5-1200, 5-1300, 5-1400, 5-1500, 5-1600, 5-1700, 5-1800, 5-1900, 5-2000, 5-2100, 5-2200, 5-2300, 5-2400, 5-2500, 5-2600, 5-2700, 5-2800, 5-2900, 5-3000, 5-3100 or more bases such that the genomic region has sufficient homology to undergo homologous recombination with the corresponding region of homology.

TAL effector nucleases (TALEN) are a class of sequence-specific nucleases that can be used to make double-strand breaks at specific target sequences in the genome of a plant or other organism. (Miller et al. (2011) *Nature Biotechnology* 29:143–148).

Endonucleases are enzymes that cleave the phosphodiester bond within a polynucleotide chain. Endonucleases include restriction endonucleases, which cleave DNA at specific sites without damaging the bases, and meganucleases, also known as homing endonucleases (HEases), which like restriction endonucleases, bind and cut at a specific recognition site, however the recognition sites for meganucleases are typically longer, about 18 bp or more (patent application PCT/US12/30061, filed on March 22, 2012).

Meganucleases have been classified into four families based on conserved sequence motifs, the families are the LAGLIDADG, GIY-YIG, H-N-H, and His-Cys box families. These motifs participate in the coordination of metal ions and hydrolysis of phosphodiester bonds. HEases are notable for their long recognition sites, and for tolerating some sequence polymorphisms in their DNA substrates. The naming convention for meganuclease is similar to the convention for other restriction endonuclease. Meganucleases are also characterized by prefix F-, I-, or PI- for enzymes encoded by free-standing ORFs, introns, and inteins, respectively. One step in the recombination process involves polynucleotide cleavage at or near the recognition site. The cleaving activity can be used to produce a double-strand break. For reviews of site-specific recombinases and their recognition sites, see, Sauer (1994) *Curr Op Biotechnol* 5:521-7; and Sadowski (1993) *FASEB* 7:760-7. In some examples the recombinase is from the Integrase or Resolvase families.

Zinc finger nucleases (ZFNs) are engineered double-strand break inducing agents comprised of a zinc finger DNA binding domain and a double-strand-break-inducing agent domain. Recognition site specificity is conferred by the zinc finger domain, which typically comprising two, three, or four zinc fingers, for example having a C2H2 structure, however other zinc finger structures are known and have been engineered. Zinc finger domains are amenable for designing polypeptides which specifically bind a selected polynucleotide recognition sequence. ZFNs include an engineered DNA-binding zinc finger domain linked to a non-specific endonuclease domain, for example nuclease domain from a Type IIs endonuclease such as FokI. Additional functionalities can be fused to the zinc-finger binding domain, including transcriptional activator domains, transcription repressor

domains, and methylases. In some examples, dimerization of nuclease domain is required for cleavage activity. Each zinc finger recognizes three consecutive base pairs in the target DNA. For example, a 3 finger domain recognized a sequence of 9 contiguous nucleotides, with a dimerization requirement of the nuclease, two sets of zinc finger triplets are used to bind an 18 nucleotide recognition sequence.

Genome editing using DSB-inducing agents, such as Cas9-gRNA complexes, has been described, for example in U.S. Patent Application US 2015-0082478 A1, published on March 19, 2015, WO2015/026886 A1, published on February 26, 2015, WO2016007347, published on January 14, 2016, and WO201625131, published on February 18, 2016, all of which are incorporated by reference herein.

The term "Cas gene" herein refers to a gene that is generally coupled, associated or close to, or in the vicinity of flanking CRISPR loci in bacterial systems. The terms "Cas gene", "CRISPR-associated (Cas) gene" are used interchangeably herein. The term "Cas endonuclease" herein refers to a protein encoded by a Cas gene. A Cas endonuclease herein, when in complex with a suitable polynucleotide component, is capable of recognizing, binding to, and optionally nicking or cleaving all or part of a specific DNA target sequence. A Cas endonuclease described herein comprises one or more nuclease domains. Cas endonucleases of the disclosure includes those having a HNH or HNH-like nuclease domain and / or a RuvC or RuvC-like nuclease domain. A Cas endonuclease of the disclosure includes a Cas9 protein, a Cpf1 protein, a C2c1 protein, a C2c2 protein, a C2c3 protein, Cas3, Cas 5, Cas7, Cas8, Cas10, or complexes of these.

As used herein, the terms "guide polynucleotide/Cas endonuclease complex", "guide polynucleotide/Cas endonuclease system", " guide polynucleotide/Cas complex", "guide polynucleotide/Cas system", "guided Cas system" are used interchangeably herein and refer to at least one guide polynucleotide and at least one Cas endonuclease that are capable of forming a complex, wherein said guide polynucleotide/Cas endonuclease complex can direct the Cas endonuclease to a DNA target site, enabling the Cas endonuclease to recognize, bind to, and optionally nick or cleave (introduce a single or double strand break) the DNA target site. A guide polynucleotide/Cas endonuclease complex herein can comprise Cas protein(s) and suitable polynucleotide component(s) of any of the four known CRISPR systems (Horvath and Barrangou, 2010, *Science* 327:167-170) such as a type I, II, or III CRISPR system. A Cas endonuclease unwinds the DNA duplex at the target sequence and optionally cleaves at least one DNA strand, as mediated by recognition of the target sequence by a polynucleotide (such as, but not limited to, a crRNA or guide RNA) that is in complex with the Cas protein. Such recognition and cutting of a target sequence by a Cas endonuclease typically occurs if the correct protospacer-

adjacent motif (PAM) is located at or adjacent to the 3' end of the DNA target sequence. Alternatively, a Cas protein herein may lack DNA cleavage or nicking activity, but can still specifically bind to a DNA target sequence when complexed with a suitable RNA component. (See also U.S. Patent Application US 2015-0082478 A1, published on March 19, 2015 and US 2015-0059010 A1, published on February 26, 2015, both are hereby incorporated in its entirety by reference).

A guide polynucleotide/Cas endonuclease complex can cleave one or both strands of a DNA target sequence. A guide polynucleotide/Cas endonuclease complex that can cleave both strands of a DNA target sequence typically comprise a Cas protein that has all of its endonuclease domains in a functional state (e.g., wild type endonuclease domains or variants thereof retaining some or all activity in each endonuclease domain). Non-limiting examples of Cas9 nickases suitable for use herein are disclosed in U.S. Patent Appl. Publ. No. 2014/0189896, which is incorporated herein by reference.

Other Cas endonuclease systems have been described in PCT patent applications PCT/US16/32073, filed May 12, 2016 and PCT/US16/32028 filed May 12, 2016, both applications incorporated herein by reference.

"Cas9" (formerly referred to as Cas5, Csn1, or Csx12) herein refers to a Cas endonuclease of a type II CRISPR system that forms a complex with a crNucleotide and a tracrNucleotide, or with a single guide polynucleotide, for specifically recognizing and cleaving all or part of a DNA target sequence. Cas9 protein comprises a RuvC nuclease domain and an HNH (H-N-H) nuclease domain, each of which can cleave a single DNA strand at a target sequence (the concerted action of both domains leads to DNA double-strand cleavage, whereas activity of one domain leads to a nick). In general, the RuvC domain comprises subdomains I, II and III, where domain I is located near the N-terminus of Cas9 and subdomains II and III are located in the middle of the protein, flanking the HNH domain (Hsu et al, Cell 157:1262-1278). A type II CRISPR system includes a DNA cleavage system utilizing a Cas9 endonuclease in complex with at least one polynucleotide component. For example, a Cas9 can be in complex with a CRISPR RNA (crRNA) and a trans-activating CRISPR RNA (tracrRNA). In another example, a Cas9 can be in complex with a single guide RNA.

Any guided endonuclease can be used in the methods disclosed herein. Such endonucleases include, but are not limited to Cas9 and Cpf1 endonucleases. Many endonucleases have been described to date that can recognize specific PAM sequences (see for example –Jinek et al. (2012) Science 337 p 816-821, PCT patent applications PCT/US16/32073, filed May 12, 2016 and PCT/US16/32028 filed May 12, 2016 and Zetsche B et al. 2015. Cell 163, 1013) and cleave the target DNA at a specific position. It is

understood that based on the methods and embodiments described herein utilizing a guided Cas system one can now tailor these methods such that they can utilize any guided endonuclease system.

The guide polynucleotide can also be a single molecule (also referred to as single guide polynucleotide) comprising a crNucleotide sequence linked to a tracrNucleotide sequence. The single guide polynucleotide comprises a first nucleotide sequence domain (referred to as Variable Targeting domain or VT domain) that can hybridize to a nucleotide sequence in a target DNA and a Cas endonuclease recognition domain (CER domain), that interacts with a Cas endonuclease polypeptide. By "domain" it is meant a contiguous stretch of nucleotides that can be RNA, DNA, and/or RNA-DNA-combination sequence. The VT domain and /or the CER domain of a single guide polynucleotide can comprise a RNA sequence, a DNA sequence, or a RNA-DNA-combination sequence. The single guide polynucleotide being comprised of sequences from the crNucleotide and the tracrNucleotide may be referred to as "single guide RNA" (when composed of a contiguous stretch of RNA nucleotides) or "single guide DNA" (when composed of a contiguous stretch of DNA nucleotides) or "single guide RNA-DNA" (when composed of a combination of RNA and DNA nucleotides). The single guide polynucleotide can form a complex with a Cas endonuclease, wherein said guide polynucleotide/Cas endonuclease complex (also referred to as a guide polynucleotide/Cas endonuclease system) can direct the Cas endonuclease to a genomic target site, enabling the Cas endonuclease to recognize, bind to, and optionally nick or cleave (introduce a single or double strand break) the target site. (See also U.S. Patent Application US 2015-0082478 A1, published on March 19, 2015 and US 2015-0059010 A1, published on February 26, 2015, both are hereby incorporated in its entirety by reference.)

The term "variable targeting domain" or "VT domain" is used interchangeably herein and includes a nucleotide sequence that can hybridize (is complementary) to one strand (nucleotide sequence) of a double strand DNA target site. In some embodiments, the variable targeting domain comprises a contiguous stretch of 12 to 30 nucleotides. The variable targeting domain can be composed of a DNA sequence, a RNA sequence, a modified DNA sequence, a modified RNA sequence, or any combination thereof.

The terms "single guide RNA" and "sgRNA" are used interchangeably herein and relate to a synthetic fusion of two RNA molecules, a crRNA (CRISPR RNA) comprising a variable targeting domain (linked to a tracr mate sequence that hybridizes to a tracrRNA), fused to a tracrRNA (trans-activating CRISPR RNA). The single guide RNA can comprise a crRNA or crRNA fragment and a tracrRNA or tracrRNA fragment of the type II CRISPR/Cas system that can form a complex with a type II Cas endonuclease, wherein said guide

RNA/Cas endonuclease complex can direct the Cas endonuclease to a DNA target site, enabling the Cas endonuclease to recognize, bind to, and optionally nick or cleave (introduce a single or double strand break) the DNA target site.

The terms “guide RNA/Cas endonuclease complex”, “guide RNA/Cas endonuclease system”, “guide RNA/Cas complex”, “guide RNA/Cas system”, “gRNA/Cas complex”, “gRNA/Cas system”, “RNA-guided endonuclease”, “RGEN” are used interchangeably herein and refer to at least one RNA component and at least one Cas endonuclease that are capable of forming a complex, wherein said guide RNA/Cas endonuclease complex can direct the Cas endonuclease to a DNA target site, enabling the Cas endonuclease to recognize, bind to, and optionally nick or cleave (introduce a single or double strand break) the DNA target site. A guide RNA/Cas endonuclease complex herein can comprise Cas protein(s) and suitable RNA component(s) of any of the four known CRISPR systems (Horvath and Barrangou, 2010, Science 327:167-170) such as a type I, II, or III CRISPR system. A guide RNA/Cas endonuclease complex can comprise a Type II Cas9 endonuclease and at least one RNA component (e.g., a crRNA and tracrRNA, or a gRNA). (See also U.S. Patent Application US 2015-0082478 A1, published on March 19, 2015 and US 2015-0059010 A1, published on February 26, 2015, both are hereby incorporated in its entirety by reference).

The guide polynucleotide of the methods and compositions described herein may be any polynucleotide sequence that targets the genomic loci of a plant cell comprising a polynucleotide that encodes an amino acid sequence that is at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to a sequence selected from the group consisting of SEQ ID NOs: 14-26. In certain embodiments, the guide polynucleotide is a guide RNA. The guide polynucleotide may also be present in a recombinant DNA construct.

The guide polynucleotide can be introduced into a cell transiently, as single stranded polynucleotide or a double stranded polynucleotide, using any method known in the art such as, but not limited to, particle bombardment, Agrobacterium transformation or topical applications. The guide polynucleotide can also be introduced indirectly into a cell by introducing a recombinant DNA molecule (via methods such as, but not limited to, particle bombardment or Agrobacterium transformation) comprising a heterologous nucleic acid fragment encoding a guide polynucleotide, operably linked to a specific promoter that is capable of transcribing the guide RNA in said cell. The specific promoter can be, but is not limited to, a RNA polymerase III promoter, which allow for transcription of RNA with precisely defined, unmodified, 5'- and 3'-ends (DiCarlo et al., Nucleic Acids Res. 41: 4336-

4343; Ma et al., Mol. Ther. Nucleic Acids 3:e161) as described in WO2016025131, published on February 18, 2016, incorporated herein in its entirety by reference.

The terms “target site”, “target sequence”, “target site sequence”, “target DNA”, “target locus”, “genomic target site”, “genomic target sequence”, “genomic target locus” and “protospacer”, are used interchangeably herein and refer to a polynucleotide sequence such as, but not limited to, a nucleotide sequence on a chromosome, episome, or any other DNA molecule in the genome (including chromosomal, chloroplastic, mitochondrial DNA, plasmid DNA) of a cell, at which a guide polynucleotide/Cas endonuclease complex can recognize, bind to, and optionally nick or cleave. The target site can be an endogenous site in the genome of a cell, or alternatively, the target site can be heterologous to the cell and thereby not be naturally occurring in the genome of the cell, or the target site can be found in a heterologous genomic location compared to where it occurs in nature. As used herein, terms “endogenous target sequence” and “native target sequence” are used interchangeably herein to refer to a target sequence that is endogenous or native to the genome of a cell and is at the endogenous or native position of that target sequence in the genome of the cell. Cells include, but are not limited to, human, non-human, animal, bacterial, fungal, insect, yeast, non-conventional yeast, and plant cells as well as plants and seeds produced by the methods described herein. An “artificial target site” or “artificial target sequence” are used interchangeably herein and refer to a target sequence that has been introduced into the genome of a cell. Such an artificial target sequence can be identical in sequence to an endogenous or native target sequence in the genome of a cell but be located in a different position (i.e., a non-endogenous or non-native position) in the genome of a cell.

An “altered target site”, “altered target sequence”, “modified target site”, “modified target sequence” are used interchangeably herein and refer to a target sequence as disclosed herein that comprises at least one alteration when compared to non-altered target sequence. Such “alterations” include, for example: (i) replacement of at least one nucleotide, (ii) a deletion of at least one nucleotide, (iii) an insertion of at least one nucleotide, or (iv) any combination of (i) – (iii).

Methods for “modifying a target site” and “altering a target site” are used interchangeably herein and refer to methods for producing an altered target site.

The length of the target DNA sequence (target site) can vary, and includes, for example, target sites that are at least 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more nucleotides in length. It is further possible that the target site can be palindromic, that is, the sequence on one strand reads the same in the opposite direction on the complementary strand. The nick/cleavage site can be within the target sequence or

the nick/cleavage site could be outside of the target sequence. In another variation, the cleavage could occur at nucleotide positions immediately opposite each other to produce a blunt end cut or, in other Cases, the incisions could be staggered to produce single-stranded overhangs, also called “sticky ends”, which can be either 5' overhangs, or 3' overhangs. Active variants of genomic target sites can also be used. Such active variants can comprise at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the given target site, wherein the active variants retain biological activity and hence are capable of being recognized and cleaved by an Cas endonuclease. Assays to measure the single or double-strand break of a target site by an endonuclease are known in the art and generally measure the overall activity and specificity of the agent on DNA substrates containing recognition sites.

A “protospacer adjacent motif” (PAM) herein refers to a short nucleotide sequence adjacent to a target sequence (protospacer) that is recognized (targeted) by a guide polynucleotide/Cas endonuclease system described herein. The Cas endonuclease may not successfully recognize a target DNA sequence if the target DNA sequence is not followed by a PAM sequence. The sequence and length of a PAM herein can differ depending on the Cas protein or Cas protein complex used. The PAM sequence can be of any length but is typically 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotides long.

The terms “targeting”, “gene targeting” and “DNA targeting” are used interchangeably herein. DNA targeting herein may be the specific introduction of a knock-out, edit, or knock-in at a particular DNA sequence, such as in a chromosome or plasmid of a cell. In general, DNA targeting can be performed herein by cleaving one or both strands at a specific DNA sequence in a cell with an endonuclease associated with a suitable polynucleotide component. Such DNA cleavage, if a double-strand break (DSB), can prompt NHEJ or HDR processes which can lead to modifications at the target site.

A targeting method herein can be performed in such a way that two or more DNA target sites are targeted in the method, for example. Such a method can optionally be characterized as a multiplex method. Two, three, four, five, six, seven, eight, nine, ten, or more target sites can be targeted at the same time in certain embodiments. A multiplex method is typically performed by a targeting method herein in which multiple different RNA components are provided, each designed to guide an guidepolynucleotide/Cas endonuclease complex to a unique DNA target site.

The terms “knock-out”, “gene knock-out” and “genetic knock-out” are used interchangeably herein. A knock-out represents a DNA sequence of a cell that has been rendered partially or completely inoperative by targeting with a Cas protein; such a DNA

sequence prior to knock-out could have encoded an amino acid sequence, or could have had a regulatory function (e.g., promoter), for example. A knock-out may be produced by an indel (insertion or deletion of nucleotide bases in a target DNA sequence through NHEJ), or by specific removal of sequence that reduces or completely destroys the function of
5 sequence at or near the targeting site.

The guide polynucleotide/Cas endonuclease system can be used in combination with a co-delivered polynucleotide modification template to allow for editing (modification) of a genomic nucleotide sequence of interest. (See also U.S. Patent Application US 2015-0082478 A1, published on March 19, 2015 and WO2015/026886 A1, published on February
10 26, 2015, both are hereby incorporated in its entirety by reference.)

The terms “knock-in”, “gene knock-in”, “gene insertion” and “genetic knock-in” are used interchangeably herein. A knock-in represents the replacement or insertion of a DNA sequence at a specific DNA sequence in cell by targeting with a Cas protein (by HR, wherein a suitable donor DNA polynucleotide is also used). Examples of knock-ins are a
15 specific insertion of a heterologous amino acid coding sequence in a coding region of a gene, or a specific insertion of a transcriptional regulatory element in a genetic locus.

Various methods and compositions can be employed to obtain a cell or organism having a polynucleotide of interest inserted in a target site for a Cas endonuclease. Such methods can employ homologous recombination to provide integration of the polynucleotide
20 of interest at the target site. In one method provided, a polynucleotide of interest is provided to the organism cell in a donor DNA construct. As used herein, “donor DNA” is a DNA construct that comprises a polynucleotide of interest to be inserted into the target site of a Cas endonuclease. The donor DNA construct further comprises a first and a second region of homology that flank the polynucleotide of interest. The first and second regions of
25 homology of the donor DNA share homology to a first and a second genomic region, respectively, present in or flanking the target site of the cell or organism genome. By “homology” is meant DNA sequences that are similar. For example, a “region of homology to a genomic region” that is found on the donor DNA is a region of DNA that has a similar sequence to a given “genomic region” in the cell or organism genome. A region of
30 homology can be of any length that is sufficient to promote homologous recombination at the cleaved target site. For example, the region of homology can comprise at least 5-10, 5-15, 5-20, 5-25, 5-30, 5-35, 5-40, 5-45, 5-50, 5-55, 5-60, 5-65, 5-70, 5-75, 5-80, 5-85, 5-90, 5-95, 5-100, 5-200, 5-300, 5-400, 5-500, 5-600, 5-700, 5-800, 5-900, 5-1000, 5-1100, 5-1200, 5-1300, 5-1400, 5-1500, 5-1600, 5-1700, 5-1800, 5-1900, 5-2000, 5-2100, 5-2200, 5-2300, 5-2400, 5-2500, 5-2600, 5-2700, 5-2800, 5-2900, 5-3000, 5-3100 or more bases in
35 length such that the region of homology has sufficient homology to undergo homologous

recombination with the corresponding genomic region. "Sufficient homology" indicates that two polynucleotide sequences have sufficient structural similarity to act as substrates for a homologous recombination reaction. The structural similarity includes overall length of each polynucleotide fragment, as well as the sequence similarity of the polynucleotides.

- 5 Sequence similarity can be described by the percent sequence identity over the whole length of the sequences, and/or by conserved regions comprising localized similarities such as contiguous nucleotides having 100% sequence identity, and percent sequence identity over a portion of the length of the sequences.

- 10 The amount of sequence identity shared by a target and a donor polynucleotide can vary and includes total lengths and/or regions having unit integral values in the ranges of about 1-20 bp, 20-50 bp, 50-100 bp, 75-150 bp, 100-250 bp, 150-300 bp, 200-400 bp, 250-500 bp, 300-600 bp, 350-750 bp, 400-800 bp, 450-900 bp, 500-1000 bp, 600-1250 bp, 700-1500 bp, 800-1750 bp, 900-2000 bp, 1-2.5 kb, 1.5-3 kb, 2-4 kb, 2.5-5 kb, 3-6 kb, 3.5-7 kb, 4-8 kb, 5-10 kb, or up to and including the total length of the target site. These ranges
- 15 include every integer within the range, for example, the range of 1-20 bp includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 bps. The amount of homology can also be described by percent sequence identity over the full aligned length of the two polynucleotides which includes percent sequence identity of about at least 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%,
- 20 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%. Sufficient homology includes any combination of polynucleotide length, global percent sequence identity, and optionally conserved regions of contiguous nucleotides or local percent sequence identity, for example sufficient homology can be described as a region of 75-150 bp having at least 80% sequence identity to a region of the target locus.
- 25 Sufficient homology can also be described by the predicted ability of two polynucleotides to specifically hybridize under high stringency conditions, see, for example, Sambrook et al., (1989) *Molecular Cloning: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, NY); *Current Protocols in Molecular Biology*, Ausubel et al., Eds (1994) *Current Protocols*, (Greene Publishing Associates, Inc. and John Wiley & Sons, Inc.); and, Tijssen (1993)
- 30 *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes*, (Elsevier, New York).

- The structural similarity between a given genomic region and the corresponding region of homology found on the donor DNA can be any degree of sequence identity that allows for homologous recombination to occur. For example, the amount of homology or
- 35 sequence identity shared by the "region of homology" of the donor DNA and the "genomic region" of the organism genome can be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%,

81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, such that the sequences undergo homologous recombination

The region of homology on the donor DNA can have homology to any sequence flanking the target site. While in some embodiments the regions of homology share significant sequence homology to the genomic sequence immediately flanking the target site, it is recognized that the regions of homology can be designed to have sufficient homology to regions that may be further 5' or 3' to the target site. In still other embodiments, the regions of homology can also have homology with a fragment of the target site along with downstream genomic regions. In one embodiment, the first region of homology further comprises a first fragment of the target site and the second region of homology comprises a second fragment of the target site, wherein the first and second fragments are dissimilar.

As used herein, "homologous recombination" includes the exchange of DNA fragments between two DNA molecules at the sites of homology.

Further uses for guide RNA/Cas endonuclease systems have been described (See U.S. Patent Application US 2015-0082478 A1, published on March 19, 2015, WO2015/026886 A1, published on February 26, 2015, US 2015-0059010 A1, published on February 26, 2015, US application 62/023246, filed on July 07, 2014, and US application 62/036,652, filed on August 13, 2014, all of which are incorporated by reference herein) and include but are not limited to modifying or replacing nucleotide sequences of interest (such as a regulatory elements), insertion of polynucleotides of interest, gene knock-out, gene-knock in, modification of splicing sites and/or introducing alternate splicing sites, modifications of nucleotide sequences encoding a protein of interest, amino acid and/or protein fusions, and gene silencing by expressing an inverted repeat into a gene of interest.

Methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens*, and obtaining transgenic plants have been published, among others, for cotton (U.S. Patent No. 5,004,863, U.S. Patent No. 5,159,135); soybean (U.S. Patent No. 5,569,834, U.S. Patent No. 5,416,011); Brassica (U.S. Patent No. 5,463,174); peanut (Cheng et al., Plant Cell Rep. 15:653-657 (1996), McKently et al., Plant Cell Rep. 14:699-703 (1995)); papaya (Ling et al., Bio/technology 9:752-758 (1991)); and pea (Grant et al., Plant Cell Rep. 15:254-258 (1995)). For a review of other commonly used methods of plant transformation see Newell, C.A., Mol. Biotechnol. 16:53-65 (2000). One of these methods of transformation uses *Agrobacterium rhizogenes* (Tepfler, M. and Casse-Delbart, F., Microbiol. Sci. 4:24-28 (1987)). Transformation of soybeans using direct delivery of DNA has been published using PEG fusion (PCT Publication No. WO 92/17598), electroporation

(Chowrira et al., Mol. Biotechnol. 3:17-23 (1995); Christou et al., Proc. Natl. Acad. Sci. U.S.A. 84:3962-3966 (1987)), microinjection, or particle bombardment (McCabe et al., Biotechnology 6:923-926 (1988); Christou et al., Plant Physiol. 87:671-674 (1988)).

There are a variety of methods for the regeneration of plants from plant tissues. The particular method of regeneration will depend on the starting plant tissue and the particular plant species to be regenerated. The regeneration, development and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, Eds.; In Methods for Plant Molecular Biology; Academic Press, Inc.: San Diego, CA, 1988). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development or through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is used to pollinate regenerated plants. A transgenic plant of the present disclosure containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

Recombinant DNA constructs comprising one or more of the polynucleotide sequences set forth in SEQ ID NOs: 14-26 are provided herein.

Also provided are plants, plant cells, and/or seeds introduced with a polynucleotide described herein. In certain embodiments the plant, plant cell, or seed comprises a recombinant DNA construct comprising one or more of the polynucleotide sequences set forth in SEQ ID NOs: 14-26. In certain embodiments, the plant, plant cell, or seed comprises a recombinant DNA construct comprising one or more guide polynucleotides that target the genomic loci of a plant cell comprising a polynucleotide that encodes an amino acid sequence that is at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to a sequence selected from the group consisting of SEQ ID NOs: 14-26.

The polynucleotide of the plant, plant cell, or seed can be stably introduced or can be transiently expressed by the plant, plant cell, or seed. In certain embodiments, the polynucleotide is stably introduced into the plant, plant cell, or seed.

The terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", "nucleic acid fragment", and "isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may

be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. Nucleotides (usually found in their 5'-monophosphate form) are referred to by a single letter designation as follows: "A" for adenylate or deoxyadenylate (for RNA or DNA, respectively), "C" for cytidylate or deoxycytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deoxythymidylate, "R" for purines (A or G), "Y" for pyrimidines (C or T), "K" for G or T, "H" for A or C or T, "I" for inosine, and "N" for any nucleotide.

The term "recombinant DNA construct" or "recombinant expression construct" is used interchangeably and generally refers to a discrete polynucleotide into which a nucleic acid sequence or fragment can be moved. Preferably, it is a plasmid vector or a fragment thereof comprising the promoters of the present disclosure. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., EMBO J. 4:2411-2418 (1985); De Almeida et al., Mol. Gen. Genetics 218:78-86 (1989)), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by PCR and Southern analysis of DNA, RT-PCR and Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

The terms "plasmid", "vector" and "cassette" refer to an extra chromosomal element often carrying genes that are not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA fragments. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear or circular, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

The promoters for use in the vector may be derived in their entirety from a native gene or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental

conditions. Core promoters are often modified to produce artificial, chimeric, or hybrid promoters, and can further be used in combination with other regulatory elements, such as cis-elements, 5'UTRs, enhancers, or introns, that are either heterologous to an active core promoter or combined with its own partial or complete regulatory elements. In certain
5 embodiments the promoter of the recombinant DNA construct may be a tissue-specific promoter, developmentally regulated promoter, or a constitutive promoter.

"Tissue-specific promoter" and "tissue-preferred promoter" are used interchangeably to refer to a promoter that is expressed predominantly but not necessarily exclusively in one tissue or organ, but that may also be expressed in one specific cell. "Developmentally
10 regulated promoter" generally refers to a promoter whose activity is determined by developmental events. "Constitutive promoter" generally refers to promoters active in all or most tissues or cell types of a plant at all or most developing stages. As with other promoters classified as "constitutive" (e.g. ubiquitin), some variation in absolute levels of expression can exist among different tissues or stages. The term "constitutive promoter" or
15 "tissue-independent" are used interchangeably herein.

In certain embodiments the promoter of the recombinant DNA construct is heterologous to the expressed nucleotide sequence. A "heterologous nucleotide sequence" generally refers to a sequence that is not naturally occurring with the sequence of the disclosure. While this nucleotide sequence is heterologous to the sequence, it may be
20 homologous, or native, or heterologous, or foreign, to the plant host. However, it is recognized that the instant sequences may be used with their native coding sequences to increase or decrease expression resulting in a change in phenotype in the transformed seed. The terms "heterologous nucleotide sequence", "heterologous sequence", "heterologous nucleic acid fragment", and "heterologous nucleic acid sequence" are used
25 interchangeably herein.

The isolated promoter sequence comprised in the recombinant DNA construct of the present disclosure can be modified to provide a range of constitutive expression levels of the heterologous nucleotide sequence. Thus, less than the entire promoter regions may be utilized and the ability to drive expression of the coding sequence retained. However, it is
30 recognized that expression levels of the mRNA may be decreased with deletions of portions of the promoter sequences. Likewise, the tissue-independent, constitutive nature of expression may be changed.

Modifications of the isolated promoter sequences of the present disclosure can provide for a range of constitutive expression of the heterologous nucleotide sequence.
35 Thus, they may be modified to be weak constitutive promoters or strong constitutive promoters. Generally, by "weak promoter" is intended a promoter that drives expression of

a coding sequence at a low level. By “low level” is intended levels about 1/10,000 transcripts to about 1/100,000 transcripts to about 1/500,000 transcripts. Conversely, a strong promoter drives expression of a coding sequence at high level, or at about 1/10 transcripts to about 1/100 transcripts to about 1/1,000 transcripts. Similarly, a “moderate constitutive” promoter is somewhat weaker than a strong constitutive promoter like the maize ubiquitin promoter.

The term “operably linked” or “functionally linked” generally refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The terms “initiate transcription”, “initiate expression”, “drive transcription”, and “drive expression” are used interchangeably herein and all refer to the primary function of a promoter. As detailed throughout this disclosure, a promoter is a non-coding genomic DNA sequence, usually upstream (5') to the relevant coding sequence, and its primary function is to act as a binding site for RNA polymerase and initiate transcription by the RNA polymerase. Additionally, there is “expression” of RNA, including functional RNA, or the expression of polypeptide for operably linked encoding nucleotide sequences, as the transcribed RNA ultimately is translated into the corresponding polypeptide.

The term “expression”, as used herein, generally refers to the production of a functional end-product e.g., an mRNA or a protein (precursor or mature).

The term “expression cassette” as used herein, generally refers to a discrete nucleic acid fragment into which a nucleic acid sequence or fragment can be cloned or synthesized through molecular biology techniques.

“Transformation” as used herein generally refers to both stable transformation and transient transformation. “Stable transformation” generally refers to the introduction of a nucleic acid fragment into a genome of a host organism resulting in genetically stable inheritance. Once stably transformed, the nucleic acid fragment is stably integrated in the genome of the host organism and any subsequent generation. Host organisms containing the transformed nucleic acid fragments are referred to as “transgenic” organisms. “Transient transformation” generally refers to the introduction of a nucleic acid fragment into the nucleus, or DNA-containing organelle, of a host organism resulting in gene expression without genetically stable inheritance.

The term “introduced” means providing a nucleic acid (e.g., expression construct) or protein into a cell. Introduced includes reference to the incorporation of a nucleic acid into a

eukaryotic or prokaryotic cell where the nucleic acid may be incorporated into the genome of the cell, and includes reference to the transient provision of a nucleic acid or protein to the cell. Introduced includes reference to stable or transient transformation methods, as well as sexually crossing. Thus, "introduced" in the context of inserting a nucleic acid fragment (e.g., a recombinant DNA construct/expression construct) into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid fragment into a eukaryotic or prokaryotic cell where the nucleic acid fragment may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

The heterologous polynucleotide can be stably integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant DNA construct. The alterations of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods, by genome editing procedures that do not result in an insertion of a foreign polynucleotide, or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation are also methods of modifying a host genome.

"Transient expression" generally refers to the temporary expression of often reporter genes such as β -glucuronidase (GUS), fluorescent protein genes ZS-GREEN1, ZS-YELLOW1 N1, AM-CYAN1, DS-RED in selected certain cell types of the host organism in which the transgenic gene is introduced temporally by a transformation method. The transformed materials of the host organism are subsequently discarded after the transient gene expression assay.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J. et al., In *Molecular Cloning: A Laboratory Manual*; 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 1989 (hereinafter "Sambrook et al., 1989") or Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K., Eds.; In *Current Protocols in Molecular Biology*; John Wiley and Sons: New York, 1990 (hereinafter "Ausubel et al., 1990").

The plant, plant cells, and seeds of the compositions and methods described herein are not particularly limited and may be any plant species including, but not limited to, monocots and dicots. The terms "monocot" and "monocotyledonous plant" are used interchangeably herein. A monocot of the current disclosure includes rice. The terms

“dicot” and “dicotyledonous plant” are used interchangeably herein. A dicot of the current disclosure includes the following families: Brassicaceae, Leguminosae, and Solanaceae.

In certain embodiments the plant is a rice plant of the genus *Oryza*. In certain embodiments, the rice plant is *Oryza sativa*, optionally of the variety *indica*, or *Oryza glaberrima*. In certain embodiments, the rice plant is an inbred rice line (e.g., female inbred line), while in other embodiments the rice plant is a hybrid rice plant.

“Plant” includes reference to whole plants, plant organs, plant tissues, seeds and plant cells and progeny of same. Plant cells include, without limitation, cells from seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores.

The plant, plant cell, and seed, of the compositions and methods described herein may further comprise a heterologous nucleic acid sequence that confers advantageous properties, such as improved agronomics, to the plant, plant cell, and/or seed. The heterologous nucleic acid sequences are known to those of ordinary skill in the art, and can be routinely incorporated in the plant, plant cell, and/or seeds described herein using routine methods in the art, such as those described herein.

In certain embodiments the heterologous nucleic acid sequence is selected from the group consisting of a reporter gene, a selection marker, a disease resistance gene, a herbicide resistance gene, an insect resistance gene, a gene involved in carbohydrate metabolism, a gene involved in fatty acid metabolism, a gene involved in amino acid metabolism, a gene involved in plant development, a gene involved in plant growth regulation, a gene involved in yield improvement, a gene involved in drought resistance, a gene involved in increasing nutrient utilization efficiency, a gene involved in cold resistance, a gene involved in heat resistance and a gene involved in salt resistance in plants.

In certain embodiments, the present disclosure contemplates the transformation of a recipient cell with more than one advantageous gene. Two or more genes can be supplied in a single transformation event using either distinct gene-encoding vectors, or a single vector incorporating two or more gene coding sequences. Any two or more genes of any description, such as those conferring herbicide, insect, disease (viral, bacterial, fungal, and nematode), or drought resistance, oil quantity and quality, or those increasing yield or nutritional quality may be employed as desired.

EXAMPLES

The present disclosure is further defined in the following Examples. From the above discussion and these Examples, one skilled in the art can ascertain the essential

characteristics of this disclosure, and without departing from the spirit and scope thereof, can make various changes and modifications of the disclosure to adapt it to various usages and conditions. Thus, various modifications of the disclosure in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing
5 description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

EXAMPLE 1

Rice Gene Targets for Maturity Modulation

This example demonstrates the identification of rice gene targets for maturity modification.

Optimal flowering time is a critical factor that determines crop yield and hybrid seed production. Maturity can be generally described as time from seedling to harvest, change in
15 flowering time, either early or late influence the maturity of the plants. The modulation of flowering time to growing conditions is not possible without a better understanding of genes involved in flowering time and their regulation.

To identify genes involved in rice FTR, rice activation tag populations were screened in field conditions and analyzed for the flowering phenotype. Lines showing either late or
20 early flowering compared to control were identified, and genes present both upstream and downstream to the T-DNA insertion site in the genomic DNA were cloned and overexpressed under a constitutive promoter. The genes which gave an early or late flowering phenotype upon overexpression were selected to reverse the phenotype through a CRISPR-Cas mediated SDN1 genome editing approach.

EXAMPLE 2

Targeted DNA Modification of Genomic Loci Involved in Flowering Time Regulation

Targeted DNA modification of the genomic loci of the genes identified Example 1 was performed to determine the effect on maturity.

CRISPR/Cas9 assisted targeted genome editing offers to knock-out any gene
30 sequence and generate knock-outs through small deletions, internal small fragment deletions within the target gene or full-length gene deletion. Through CRISPR-Cas genome editing, new variations of the alleles are introduced directly in the elite target germplasm with minimal genetic drag associated with conventional breeding material. The target genes
35 include DP1492, DP1249, DP1856, DP1722, DP1315, DP0830, DP2300, DP1564, DP1103,

DP0998, DP0885, DP0896 and DP0995. Tables 1-5 below provide the guide polynucleotides and targeting strategies to knock-down expression of the target genes.

Table 1: CRISPR/Cas Targeted Sequences of Polynucleotides Involved in FTR

Target Sequence Designation	Target Sequence (5'-3')	Target Sequence SEQ ID NO
DP1492_CR1	AGCAGCGCCGGATGTGGACCAGGG	SEQ ID NO: 27
DP1492_CR2	AACCAGCAGCTACCCTACGCCGG	SEQ ID NO: 28
DP1564_CR1	AACGACGCCGTGCCTGGGGCTGGG	SEQ ID NO: 29
DP1564_CR2	CCGGATGGCGGACCAACCTCCGGT (target cut site is on the complimentary strand)	SEQ ID NO: 30
DP2300_CR1	ATGAGGAGTCGCCAAATTATCAGG	SEQ ID NO: 31
DP2300_CR2	CCGGCGGGCTCACGTTTCGACGTCT (target cut site is on the complimentary strand)	SEQ ID NO: 32
DP0830_CR1	CCAAGGGGGCTTCCGACGGCAAT (target cut site is on the complimentary strand)	SEQ ID NO: 33
DP0830_CR2	AGGAGAATTCCGGCAAGGGG	SEQ ID NO: 34
DP1315_CR1	AGCCCAAGTTGGTAGCCATGACGG	SEQ ID NO: 35
DP1315_CR2	CCAAGTCCGACATCCAGGTGCGT (target cut site is on the complimentary strand)	SEQ ID NO: 36
DP1722_CR1	ATCGGCCAGGCCGGGATCCAGG	SEQ ID NO: 37
DP1722_CR2	ACCCTGAGCAGCTCATCTCTGGG	SEQ ID NO: 38
DP1856_CR1	ATCTCTACAAGTTCGACCCGTGG	SEQ ID NO: 39
DP1856_CR3	ACTCGCACACCCACTCGTGGGG	SEQ ID NO: 40
DP1249_CR1	ACCTTCCAGGTCTACCGGCCCATGG	SEQ ID NO: 41
DP1249_CR2	AGCTCACCCGCGAGCTGGGG	SEQ ID NO: 42
DP1103_CR1	CCTATCAGTGTTCTCAGTTTTCT (target cut site is on the complimentary strand)	SEQ ID NO: 43
DP1103_CR2	CCAGAGTCGCAGAAAGATGCT (target cut site is on the complimentary strand)	SEQ ID NO: 44
DP0896_CR1	CCTTGCGCCCAGGCTCCGCTACGT (target cut site is on the complimentary strand)	SEQ ID NO: 45
DP0896_CR2	AGCTCATCTACACCACGAAGCGAGG	SEQ ID NO: 46

DP0885_CR1	ATGGTGGACATCGTGGCTTCGAGCGG	SEQ ID NO: 47
DP0885_CR2	CCTTCGGAAATTCGCATAGCGAT (target cut site is on the complimentary strand)	SEQ ID NO: 48
DP0998_CR1	CCGGAGTCCCCGGGGGTGTTCT (target cut site is on the complimentary strand)	SEQ ID NO: 49
DP0998_CR2	ACCACTGTGTGTGCCCCGTGGTGG	SEQ ID NO: 50
DP0995_CR1	CCTGACTGCTCGTGTGAAGACCCT (target cut site is on the complimentary strand)	SEQ ID NO: 51
DP0995_CR2	ACTGTTTCTCACAACTCCGTGTGG	SEQ ID NO: 52
DP1103_CR3	ATTCACACGTTACAAACGCACGGG	SEQ ID NO: 53
DP1103_CR4	ACCTTTGTGGTGCTTCCTTGTCTAGG	SEQ ID NO: 54
DP0896_CR3	ATCGGTTACGAATCAATCCTCTCGG	SEQ ID NO: 55
DP0896_CR4	GTGCGCTCTGTTCTGGATTCTGG	SEQ ID NO: 56
DP0885_CR3	AGAAGAAGGCGGATTCCTTTTTGG	SEQ ID NO: 57
DP0885_CR4	AAGAGGCCGGGAAACCTAGACCAGG	SEQ ID NO: 58
DP0998_CR3	ACTGCCCCACATGGTCCGCATTTGG	SEQ ID NO: 59
DP0998_CR4	TGTTTCTCATGCATGAAGGATAAGG	SEQ ID NO: 60
DP0995_CR3	GGCCCAGCAACGCGGCCCGAGG	SEQ ID NO: 61
DP0995_CR4	ATGGTTTTGAATCGATTGACCTGG	SEQ ID NO: 62

Table 2: Description of the Edits Observed in the Targeted FTR Genes

Gene	Target Sequence Designation	Target Sequence SEQ ID NO	Relative Start Position (bp)	Relative End Position (bp)	Edit
DP1492 full-length genomic sequence (SEQ ID NO: 1)	DP1492_CR1	SEQ ID NO: 27	2076 (based on SEQ ID NO: 1 position)	2099 (based on SEQ ID NO: 1 position)	Internal deletions
	DP1492_CR2	SEQ ID NO: 28	1462	1484	Internal deletions
DP1564 full-length genomic sequence (SEQ ID NO: 2)	DP1564_CR1	SEQ ID NO: 29	792 (based on SEQ ID NO: 2 position)	815 (based on SEQ ID NO: 2 position)	Internal deletions
	DP1564_CR2	SEQ ID NO: 30	834	857	Internal deletions

DP2300 full-length genomic sequence (SEQ ID NO: 3)	DP2300_CR1	SEQ ID NO: 31	14 (based on SEQ ID NO: 3 position)	37 (based on SEQ ID NO: 3 position)	Internal deletions
	DP2300_CR2	SEQ ID NO: 32	697	720	Internal deletions
DP0830 full-length genomic sequence (SEQ ID NO: 4)	DP0830_CR1	SEQ ID NO: 33	1136 (based on SEQ ID NO: 4 position)	1158 (based on SEQ ID NO: 4 position)	Internal deletions
	DP0830_CR2	SEQ ID NO: 34	1363	1382	Internal deletions
DP1315 full-length genomic sequence (SEQ ID NO: 5)	DP1315_CR1	SEQ ID NO: 35	40 (based on SEQ ID NO: 5 position)	63 (based on SEQ ID NO: 5 position)	Internal deletions
	DP1315_CR2	SEQ ID NO: 36	283	305	Internal deletions
DP1722 full-length genomic sequence (SEQ ID NO: 6)	DP1722_CR1	SEQ ID NO: 37	155 (based on SEQ ID NO: 6 position)	176 (based on SEQ ID NO: 6 position)	Internal deletions
	DP1722_CR2	SEQ ID NO: 38	1303	1325	Internal deletions
DP1856 full-length genomic sequence (SEQ ID NO: 7)	DP1856_CR1	SEQ ID NO: 39	238 (based on SEQ ID NO: 7 position)	260 (based on SEQ ID NO: 7 position)	Internal deletions
	DP1856_CR3	SEQ ID NO: 40	796	817	Internal deletions
DP1249 full-length genomic sequence (SEQ ID NO: 8)	DP1249_CR1	SEQ ID NO: 41	73 (based on SEQ ID NO: 8 position)	97 (based on SEQ ID NO: 8 position)	Internal deletions
	DP1249_CR2	SEQ ID NO: 42	293	312	Internal deletions
DP1103 full-length genomic sequence (SEQ ID NO: 9)	DP1103_CR1	SEQ ID NO: 43	2030 (based on SEQ ID NO: 9 position)	2053 (based on SEQ ID NO: 9 position)	Internal deletions
	DP1103_CR2	SEQ ID NO: 44	4020	4040	Internal deletions
DP0896 full-length genomic sequence (SEQ ID NO: 10)	DP0896_CR1	SEQ ID NO: 45	201 (based on SEQ ID NO: 10 position)	224 (based on SEQ ID NO: 10 position)	Internal deletions
	DP0896_CR2	SEQ ID NO: 46	896	920	Internal deletions
DP0885 full-length genomic sequence	DP0885_CR1	SEQ ID NO: 47	381 (based on SEQ ID NO: 11 position)	406 (based on SEQ ID NO: 11 position)	Internal deletions

(SEQ ID NO: 11)	DP0885_CR2	SEQ ID NO: 48	1048	1070	Internal deletions
DP0998 full-length genomic sequence (SEQ ID NO: 12)	DP0998_CR1	SEQ ID NO: 49	322 (based on SEQ ID NO: 12 position)	343 (based on SEQ ID NO: 12 position)	Internal deletions
	DP0998_CR2	SEQ ID NO: 50	857	880	Internal deletions
DP0995 full-length genomic sequence (SEQ ID NO: 13)	DP0995_CR1	SEQ ID NO: 51	1594 (based on SEQ ID NO: 13 position)	1617 (based on SEQ ID NO: 13 position)	Internal deletions
	DP0995_CR2	SEQ ID NO: 52	1873	1896	Internal deletions

Table 3: Description of Edits Expected in Variants Generated by Single Guide Constructs

Strategy	Construct	Description of the edits
Single guide	DP1249-CR1	Guide designed to create small deletions in exon; observed 1-2 base pair deletions at the target cut site
	DP1249-CR2	Guide designed to create small deletions in exon;
	DP1315_CR1	Guide designed to create small deletions in exon 1; observed 1-6 base pair deletion at the target cut site.
	DP1722_CR1	Guide designed to create small deletion in exon 1; observed single nucleotide deletions at the target cut site.
	DP1722_CR2	Guide designed to create small deletion in exon 2; observed 1-3 base pair deletions at the target cut site.
	DP1856-CR1	Guide designed to create small deletion in exon 1; observed 1-3 base pair deletions at the target cut site.
	DP1856-CR3	Guide designed to create small deletion in exon 2; observed 2-6 base pair deletions at the target cut site.
	DP0830_CR1	Guide designed to create small deletions in exon 1; observed 1-4 base pair deletions at the target cut site.
	DP1492_CR1	Guide designed to create small deletions in exon; observed 2-6 base pair deletions at the target cut site
	DP1492_CR2	Guide designed to create small deletions in exon

	DP2300_CR1	Guide designed to create small deletion in exon 1; observed 3-4 base pair deletions at the target cut site.
	DP2300_CR2	Guide designed to create small deletion in exon 2
	DP1564_CR1	NA
	DP1564_CR2	NA

Table 4: Description of Edits Expected in Variants Generated by Dual Guide Constructs

Strategy	Construct	Description of the edits
Dual guides	DP1249 CR1+ CR2	Designed two guides in exon to delete an internal fragment of ~215 bp in ordered to knock-out the target gene, DP1249.
	DP1315 CR1+ CR2	Designed two guides in exon 1 to delete an internal fragment of ~231 bp in ordered to knock-out the target gene, DP1315.
	DP1492 CR1+ CR2	Designed two guides in exon to delete an internal fragment of ~615 bp in ordered to knock-out the target gene, DP1492.
	DP1564 CR1 + CR2	Designed two guides in exon 1 to delete an internal fragment of ~30 bp to knock-out the target gene, DP1564.
	DP1722 CR1 + CR2	Designed two guides one in exon 1 and another one in exon 2 to delete an internal fragment of ~1149 to knock-out the target gene, DP1722.
	DP1856 CR1+ CR3	Designed two guides one in exon 1 and another one in exon 2 to delete an internal fragment of ~557 bp to knock-out the target gene, DP1856.
	DP2300 CR1 + CR2	Designed two guides one in exon 1 and another one in exon 2 to delete an internal fragment of ~671 bp to knock-out the target gene, DP2300.
	DP0830 CR1+CR2	Designed two guides one in exon 1 and another one in exon 2 to delete an internal fragment of ~322 bp to knock-out the target gene, DP0830.

Table 5: Description of Edits Expected in Variants Generated by Dual Guide Constructs

Strategy	Construct	Description of the edits
Dual guides	DP1103 CR1+ CR2	Designed two guides one in Exon 4 and one in exon 10 to delete an internal fragment of 1990 bp to knock out the target gene, DP1103.
	DP0896 CR1+ CR2	Designed two guides in Exon 1 to delete ~708bp fragment to knock out the target gene, DP0896.
	DP0885 CR1+ CR2	Designed two guides one in Exon 1 and another one in exon 2 to delete ~827bp fragment in order to knock-out the target gene, DP0885.
	DP0998 CR1+ CR2	Designed two guides to delete an internal fragment of ~550 bp to knock-out the target gene, DP0998.
	DP0995 CR1+ CR2	Designed two guides in Exon 2 to delete ~279bp fragment to knock out the target gene, DP0995
	DP1103 CR3+ CR4	Designed two guides one in upstream to the promoter region and another one in terminator region to delete 13584 bp fragment
	DP0896 CR3+ CR4	Designed two guides one in promoter and another one in terminator region to delete 3512 bp fragment
	DP0885 CR3+ CR4	Designed two guides one in promoter and another one in terminator region to delete 3516 bp fragment
	DP0998 CR3+ CR4	Designed two guides one in upstream to the promoter and another one in downstream to the terminator region to delete 7152 bp fragment
	DP0995 CR3+ CR4	Designed two guides one in promoter region and another one in downstream to the terminator region to delete 2992 bp fragment

EXAMPLE 3

Phenotypic Analysis of Rice Plants with Maturity Modulation

Rice transformation

The method used to generate genome edited variants through gene gun mediated particle bombardment is described below:

1) Seed sterilization and Callus induction: Seeds from rice inbred lines; IRV95, SDIA18G9C and SRPA17M5C were sterilized in 75% ethanol for 2-3 minutes and washed thoroughly with water and incubated in 4% sodium hypochlorite for 10 minutes. The seeds were then washed 5 times with water and dried completely at room temperature. The dried seeds were inoculated on callus inducing media and the plates were incubated at 28 °C in light for 5-7 days. After that the proliferating calli obtained from rice seeds were placed on osmotic media for 4 hours before being bombarded with DNA:gold particles.

2) Particle bombardment:

a. Preparation of gold micro carriers: Sufficient amount of gold particles (amount of gold particles depends on the number of bombardments) were weighed and placed in 2.0 ml eppendorf tubes. One ml of 100% ethanol was added to the tube and sonicated for 30 sec before centrifuging for 1 min. The pellet containing the gold particles was resuspended in 1 ml of 100% ethanol, vortexed for 30 seconds and centrifuged again. This step was repeated twice before resuspending the pellet in 1 ml of sterile water. Fifty µl of gold particle suspension was aliquoted into eppendorf tubes and stored at 4 °C.

b. DNA and gold particle preparation: Five µg of DNA, 50 µl of 2.5 mM CaCl₂ and 20 µl of 0.1 M spermidine were added to 50 µl of gold particle suspension; vortexed for 1-2 minutes and allowed the mixture to settle down for 5 minutes. The tubes were centrifuged for 2 minutes before discarding the supernatant. The pellet was resuspended in 40 µl of 100% ethanol and mixed gently by vortexing and 5 µl of sample was quickly dispensed onto macrocarrier disks and dried completely.

c. Particle bombardment using Bio-Rad gene gun (PDS 1000): Macro carrier disk carrying DNA:gold particle prep were loaded onto macro carrier disk holder and stopping screen was placed on top of the disk. Manufacturer's instructions were followed to deliver DNA:gold particles onto tissue samples which were placed on osmotic medium. After bombardment, the tissue samples were kept on the same osmotic medium for 24 h at 32 °C in dark.

3) Selection and regeneration of transformed variants: After 24 hours post bombardment, the samples were sub-cultured on to resting media and kept in dark at 28 °C for 5 days. The cultures were then transferred to selection media containing Hygromycin as

selectable agent. After 3-4 selection cycles, proliferating, hygromycin resistant and Zs-Yellow positive callus variants were sub-cultured onto regeneration media and then to rooting and hardening media to obtain stable lines. Each independent line was transferred to an individual pot in greenhouse and samples were collected to perform molecular and phenotypic analysis.

Phenotypic analysis

Flowering generally means that the flower opens, and pollination takes place. Days to 50% flowering (DFF) refers to number of days when 50% of the panicles reach the flowering state. The results of the phenotypic analysis of the T0 or T1 population are provided in Tables 6-8.

Table 6: Flowering Data Collected from T0 Variants

Gene	Target Sequence Designation	Target Sequence SEQ ID NO	Days to 50% flowering (DFF)
DP1492 full-length genomic sequence (SEQ ID NO: 1)	DP1492_CR1	SEQ ID NO: 27	66-86
	DP1492_CR2	SEQ ID NO: 28	NA
DP1564 full-length genomic sequence (SEQ ID NO: 2)	DP1564_CR1	SEQ ID NO: 29	NA
	DP1564_CR2	SEQ ID NO: 30	NA
DP2300 full-length genomic sequence (SEQ ID NO: 3)	DP2300_CR1	SEQ ID NO: 31	NA
	DP2300_CR2	SEQ ID NO: 32	NA
DP0830 full-length genomic sequence (SEQ ID NO: 4)	DP0830_CR1	SEQ ID NO: 33	54-66
	DP0830_CR2	SEQ ID NO: 34	NA
DP1315 full-length genomic sequence (SEQ ID NO: 5)	DP1315_CR1	SEQ ID NO: 35	51-68
	DP1315_CR2	SEQ ID NO: 36	NA
DP1722 full-length genomic sequence (SEQ ID NO: 6)	DP1722_CR1	SEQ ID NO: 37	58-69
	DP1722_CR2	SEQ ID NO: 38	59-76
DP1856 full-length genomic sequence (SEQ ID NO: 7)	DP1856_CR1	SEQ ID NO: 39	56-67
	DP1856_CR3	SEQ ID NO: 40	57-70

DP1249 full-length genomic sequence (SEQ ID NO: 8)	DP1249_CR1	SEQ ID NO: 41	58-68
	DP1249_CR2	SEQ ID NO: 42	NA
DP1103 full-length genomic sequence (SEQ ID NO: 9)	DP1103_CR1	SEQ ID NO: 43	In the process of generating variants
	DP1103_CR2	SEQ ID NO: 44	
DP0896 full-length genomic sequence (SEQ ID NO: 10)	DP0896_CR1	SEQ ID NO: 45	Generating variants
	DP0896_CR2	SEQ ID NO: 46	
DP0885 full-length genomic sequence (SEQ ID NO: 11)	DP0885_CR1	SEQ ID NO: 47	Generating variants
	DP0885_CR2	SEQ ID NO: 48	
DP0998 full-length genomic sequence (SEQ ID NO: 12)	DP0998_CR1	SEQ ID NO: 49	Generating variants
	DP0998_CR2	SEQ ID NO: 50	
DP0995 full-length genomic sequence (SEQ ID NO: 13)	DP0995_CR1	SEQ ID NO: 51	Generating variants
	DP0995_CR2	SEQ ID NO: 52	

Table 7: Flowering Data Collected from T0 Variants

Approach	Target sequences	Target SEQ ID NO	Days to 50% flowering
Dual Guide	DP1492 CR1+ CR2	SEQ ID NOs: 27 + 28	60-74
	DP1564 CR1 + CR2	SEQ ID NOs: 29 + 30	57-80
	DP2300 CR1 + CR2	SEQ ID NOs: 31 + 32	65-78
	DP1315 CR1+ CR2	SEQ ID NOs: 35 + 36	67-68
	DP1722 CR1 + CR2	SEQ ID NOs: 37 + 38	72-89
	DP1856 CR1+ CR3	SEQ ID NOs: 39 + 40	74-84
	DP1249 CR1+ CR2	SEQ ID NOs: 41 + 42	63-72
	DP0830 CR1+CR2	SEQ ID NOs 33 + 34	79-90

Table 8: Flowering Data Collected from T0 Variants

Gene	Target sequence designation	Target sequence ID No		No of mutants	Days to 50%flowering	
					Range	Average
DP0998	DP0998 CR3+CR4	Sequence ID Nos 59 + 60	Mutants	7	106-121	113
			NGS-WT	8	70-112	86
DP0896	DP0896 CR3+CR4	Sequence ID Nos 55+ 56	Mutants	8	88-108	102
			NGS-WT	5	69-100	88
DP0885	DP0885 CR3+CR4	Sequence ID Nos 57 + 58	Mutants	3	77-100	85
			NGS-WT	7	79-87	83
DP1103	DP1103 CR3 +CR4	Sequence ID Nos 53+ 54	In the process of generating variants			
DP0995	DP0995 CR3+CR4	Sequence ID Nos 61+ 62	In the process of generating variants			
IRV95		Seed derived wild-type		3	84-94	88

The results of these studies show that plant maturity can be shortened by modulating flowering time phenotype of plants through modulation of putative Core Histone H2A/H2B/H3/H4 gene (DP1492); response regulator receiver domain containing protein (DP1564); CCT motif family protein (DP2300); Putative BHLH transcription factor (DP0830); putative TCP family transcription factor (DP1249); putative NAC domain-containing protein 67 (DP1856); tubulin/FtsZ domain containing protein gene (DP1722) or putative hsp20/alpha crystallin family protein gene (DP1315). Phenotype upon knock-out of genes listed resulted in early flowering and shortened maturity with no known pleiotropic effects as compared to wild-type control rice plants. The guide RNA/Cas9 endonuclease system is used to target and induce a double strand break at a Cas9 endonuclease target site located within the coding region of the genes listed. Plants comprising small deletions or internal fragment deletions within the coding region of the genes were selected and evaluated for the shortened maturity phenotype.

Conversely, plant maturity can be delayed by modulating flowering time phenotype of plants through modulation of Universal stress protein domain containing protein (DP0998); putative AAA-type ATPase family protein gene (DP1103); putative PHD finger family protein gene (DP0896); methyl-binding domain protein gene (DP0995) or 'expressed protein' gene (DP0885) gene. Phenotype upon knock-out of genes listed will result in delayed flowering and eventually delayed maturity with no known pleiotropic effects as

compared to wild-type control rice plants. The guide RNA/Cas9 endonuclease system is used to target and induce a double strand break at a Cas9 endonuclease target site located within the coding region of the genes listed. Plants comprising deletion of few nucleotides or a small internal fragment within the coding region of the genes will be selected and evaluated for the late maturity phenotype.

EXAMPLE 4

Transgenic Plants Overexpressing Genes Involved in Flowering Time Regulation

Transgenic plants were generated by transforming constructs carrying the indicated gene (SEQ ID NOs 9, 10, 11, 12, 13) cloned under maize ubiquitin promoter and PINII terminator. Events generated were evaluated in greenhouse (T0) and net house (T1) conditions. Days to 50% flowering data was collected from all the T0 events generated and compared with the data collected from controls. T1 lines derived from T0 events which were transformed with either DP1103, DP0998 or DP0995 gene constructs were evaluated and collected both days to 50% flowering and days to maturity. Data collected from T1 lines, Table 9, overexpressing DP0995 gene under maize ubiquitin promoter showed 10-12 day earliness in flowering and maturity compared to seed derived wild-type plants.

Table 9: Flowering Data Collected from T1 Transgenic Rice Events

Gene	Sequence ID No	T0 event ID	No of T1 lines	Days to 50% flowering	Average	Days to Maturity
DP0995	SEQ ID NO: 13	321798813	10	74-79	76	106
		321798820	10	76-82	78	108
		321798822	10	79-84	81	111
		321798824	10	78-82	79	109
		321798826	10	77-83	81	121
DP0998	SEQ ID NO: 12	318816819	10	87-92	90	120
		318816825	10	89-92	91	121
		318816829	10	86-95	92	122
DP1103	SEQ ID NO: 9	320601056	10	92	92	122
		320601058	10	91-93	92	122
		320601072	10	87-94	93	123
		320601094	10	92-96	94	124

IRV95		Seed derived WT	30	89-95	92	122
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The results of these studies show that plant maturity can be delayed by modulating flowering time phenotype of plants through modulation of universal stress protein domain containing protein, putative, expressed (DP0998) and PHD finger family protein, putative, expressed (DP0896). The guide RNA/Cas9 endonuclease system is used to target and induce a double strand break at a Cas9 endonuclease target site located outside the coding region to delete the full-length gene. Plants comprising full-length gene deletion would be selected and evaluated for the late maturity phenotype.

10

CLAIMS

What is claimed is:

1. A method of modifying maturity of a rice plant, the method comprising introducing one
 5 or more nucleotide modifications through a targeted DNA modification at a genomic locus of the rice plant, wherein
 - (a) the genomic locus comprises a polynucleotide involved in flowering time regulation (FTR) encoding a response regulator receiver domain containing protein, a CCT motif containing protein, BHLH transcription factor, TCP
 10 family transcription factor, NAC domain-containing protein, tubulin/FtsZ domain containing protein, hsp20/alpha crystallin protein, core histone H2A/H2B/H3/H4 putative protein, AAA-type ATPase family protein, universal stress protein domain containing protein, PHD finger family protein, or a methyl-binding domain protein, and
 - (b) wherein the maturity of the rice plant is modulated compared to a control rice
 15 plant not comprising the one or more introduced DNA modifications.
2. The method of claim 1, wherein the rice plant exhibits early maturity when the targeted DNA modification results in reduced expression or activity of protein encoded by the FTR polynucleotide.
- 20 3. The method of claim 1, wherein the maturity is modified in the absence of a substantial reduction in grain yield measured per rice plant or as a population of rice plants per unit area.
4. The method of claim 1, wherein the one or more nucleotide modifications target more than one distinct genomic loci that are involved in the flowering time regulation of the rice
 25 plant.
5. The method of claim 1, wherein the maturity of the rice plant is delayed by about 5% to about 50% compared to the control rice plant as measured by the number of days to first or 50% flowering or panicle emergence or panicle initiation.
6. The method of claim 1, wherein the rice plant is a female inbred line.
- 30 7. The method of claim 1, wherein the rice plant is a hybrid.
8. The method of claim 1, wherein the stature of the rice plant is not significantly altered compared to the control rice plant as measured by a reduction in plant height.
9. The method of claim 1, wherein the targeted DNA modification of the rice plant does not substantially alter root architecture of the plant or does not significantly increase
 35 root lodging, compared to a control plant not comprising the modifications.

10. The method of claim 1, wherein the rice plant exhibits first flowering about 5 to 15 days earlier than the control plant.
11. The method of claim 1, wherein the rice plant is *Oryza sativa* var. indica.
12. The method of claim 1, wherein the FTR polynucleotide encodes a polypeptide
5 comprising an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-26.
13. The method of claim 1, wherein the FTR polynucleotide encodes a polypeptide comprising an amino acid sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-26.
- 10 14. The method of claim 1, wherein the targeted DNA modification is selected from the group consisting of insertion, deletion, single nucleotide polymorphism (SNP), and a polynucleotide modification, such that the expression of the FTR polynucleotide is reduced or affected.
- 15 15. The method of claim 1, wherein the targeted DNA modification targets the genomic locus of the FTR polynucleotide such that the one or more nucleotide modifications are present within (a) the same coding region; (b) non-coding region; (c) regulatory sequence; or (d) untranslated region, of an endogenous polynucleotide encoding a polypeptide that is involved in maturity.
- 20 16. The method of claim 1, wherein the targeted DNA modification is introduced by a RNA-guided endonuclease.
17. The method of claim 1, wherein the targeted DNA modification is introduced by a site-specific deaminase.
18. The method of claim 1, wherein the targeted DNA modification is introduced by a site-specific endonuclease.
- 25 19. The method of claim 1, wherein the FTR polynucleotide genomic locus comprises a DNA modification in a polynucleotide that encodes a polypeptide comprising an amino acid sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-26, such that the edit results in one or more of the following:
 - 30 (a) reduced expression of the FTR polynucleotide;
 - (b) reduced transcriptional activity of the protein encoded by the FTR polynucleotide;
 - (c) generation of one or more alternative spliced transcripts of the FTR polynucleotide;
 - 35 (d) deletion of one or more DNA binding domains
 - (e) frameshift mutation in one or more exons of the FTR polynucleotide;

- (f) deletion of a substantial portion of the FTR polynucleotide or deletion of the full-length open reading frame of the FTR polynucleotide;
 - (g) repression of an enhancer motif present within a regulatory region encoding the FTR polynucleotide;
 - 5 (h) modification of one or more nucleotides or deletion of a regulatory element operably linked to the expression of the FTR polynucleotide, wherein the regulatory element is present within a promoter, intron, 3'UTR, terminator or a combination thereof.
20. The method of claim 1, wherein the targeted DNA modification is induced by using a
- 10 guide RNA that corresponds to a target sequence comprising a polynucleotide that encodes a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-26.
21. The method of claim 1, wherein the targeted DNA modification is through a genome
- 15 modification technique selected from the group consisting polynucleotide-guided endonuclease, CRISPR-Cas endonucleases, base editing deaminases, zinc finger nuclease, a transcription activator-like effector nuclease (TALEN), engineered site-specific meganucleases, or Argonaute.
22. A rice plant exhibiting early maturity comprising a modified genomic locus involved in
- 20 flowering time regulation (FTR), wherein the genomic locus comprises one or more introduced mutations compared to a control plant and wherein the FTR genomic locus encodes a polypeptide that is at least 90% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 14-21.
23. A rice plant exhibiting delayed maturity comprising a modified genomic locus involved
- 25 in flowering time regulation (FTR), wherein the genomic locus comprises one or more introduced mutations compared to a control plant and wherein the FTR genomic locus encodes a polypeptide that is at least 90% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 22-26.
24. The rice plant of claim 22 or 23, wherein the rice plant is a female inbred line.
25. The rice plant of claim 22 or 23, wherein the rice plant is a hybrid.
- 30 26. The rice plant of claim 22 or 23, wherein the stature of the rice plant is not significantly altered compared to the control rice plant as measured by a reduction in plant height.
27. The rice plant of claim 22 or 23, wherein the maturity modulation of the rice plant does not substantially alter root architecture of the plant or does not significantly increase root lodging, compared to a control plant not comprising the modifications.
- 35 28. The rice plant of claim 22, wherein the rice plant exhibits early maturity in a range of about 5 to 15 days as measured by first flowering timing.

29. The rice plant of claim 23, wherein the maturity of the rice plant is delayed by about 5% to about 50% compared to the control rice plant as measured by the number of days to first or 50% flowering or panicle emergence or panicle initiation.
30. The rice plant of claim 22 or 23, wherein the rice plant is *Oryza sativa* var. indica.
- 5 31. The rice plant of claim 22, wherein the FTR polynucleotide encodes a polypeptide comprising an amino acid sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-21.
32. The rice plant of claim 23, wherein the FTR polynucleotide encodes a polypeptide comprising an amino acid sequence that is at least 95% identical to a sequence
10 selected from the group consisting of SEQ ID NOS: 22-26.
33. A recombinant DNA construct comprising a polynucleotide sequence encoding an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NOS: 14-26, operably linked to at least one heterologous nucleic acid sequence.
- 15 34. A plant cell comprising the recombinant construct of claim 33.
35. A guide RNA sequence that targets a genomic locus of a plant cell, wherein the genomic locus comprises a polynucleotide that encodes a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID NOS: 14-26.
- 20 36. A recombinant DNA construct that expresses the guide RNA of claim 35.
37. A plant cell comprising the guide RNA of claim 35.
38. A plant cell comprising the recombinant DNA construct of claim 36.
39. A plant having stably incorporated into its genome the recombinant DNA construct of claim 36.
- 25 40. The plant of claim 39 wherein said plant is a monocot plant.
41. The plant of claim 39, wherein the plant is rice.
42. A seed produced by the plant of claim 39.
43. The plant of claim 39, further comprising a heterologous nucleic acid sequence selected from the group consisting of: a reporter gene, a selection marker, a disease
30 resistance gene, a herbicide resistance gene, an insect resistance gene; a gene involved in carbohydrate metabolism, a gene involved in fatty acid metabolism, a gene involved in amino acid metabolism, a gene involved in plant development, a gene involved in plant growth regulation, a gene involved in yield improvement, a gene involved in drought resistance, a gene involved in increasing nutrient utilization
35 efficiency, a gene involved in cold resistance, a gene involved in heat resistance and a gene involved in salt resistance in plants.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/46608

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/82, C12N 15/05 (2019.01)

CPC - Y02A 40/146, C12N 15/8261, C12N 15/8243, C12N 15/827, C12N 2310/20

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0126047 A1 (AN et al.) 14 May 14 2009 (14.05.2009) Entire document, especially abstract, para [0056] [0024] [0025] [0026] [0029]	1-2, 4-11, 14-15 ----- 3, 12-13, 16-21
Y	LEE et al. Complex Regulatory Networks of Flowering Time in Rice. J Rice Res, 26 April 2015, vol 3, no 3, article 141, pp 1-9, pg 1 col 1, para 2, pg 4, col 2, para 2	3
Y	US 2016/0032309 A1 (JAPAN TOBACCO INC.) 04 February 2016 (04.02.2016) abstract, para [0037], [0038], [0045], [0150], SEQ ID NO: 5	12-13, 19-20
Y	US 2017/0321210 A1 (NATIONAL UNIVERSITY CORPORATION KOBE UNIVERSITY) 09 November 2017 (09.11.2017) para [0001], [0003], [0018], [0020],	16-18, 21

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

09 December 2019

Date of mailing of the international search report

27 DEC 2019

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/46608

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

Group I: Claims 1-21, directed to a method of modifying maturity of a rice plant, comprising introducing one or more nucleotide modifications at a genomic locus of the rice plant.

Group II: Claims 22-43, directed to an isolated rice plant comprising a modified (mutated) genomic locus involved in flowering time regulation (FTR) modified to exhibit a change in maturity (early or delayed maturity); a vector for said modification; and a guide RNA sequence that targets a genomic locus of a plant cell.

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-21

Remark on Protest

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

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US 19/46608

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

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

Special Technical Features

Group I requires a method of genetic modification of the genome of a plant, not required by group II.

Group II requires an isolated plant composition, the plant modified to exhibit a change in maturity, and guide RNA that targets a sequence of a genomic locus of a plant cell not required by groups I.

Common Technical Features

The common technical feature shared by Groups I and II that would otherwise unify the groups, is a plant polynucleotide that encodes a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 14-26. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by US 2016/0032309 A1 to Japan Tobacco Inc (hereinafter 'Japan'). Japan teaches SEQ ID NO: 5, a 742 amino acid polypeptide having 98.9% identity to the applicant's 742 amino acid FTR polynucleotide of SEQ ID NO: 15 (abstract "An object of the present invention is to provide nucleic acids capable of imparting high-yielding ability to plants. Another object of the present invention is to use such nucleic acids to produce transgenic plants at increased yield, as well as to provide methods for increasing the yield of plants.", para [0107] "The PRR7 protein derived from Nipponbare consists of the 742 amino acids represented by SEQ ID NO: 5 in the Sequence Listing", para [0111] "The PRR7 protein as referred to herein is one that comprises a PR domain and a CCT motif. It is known that PRR proteins are associated with the circadian clock of plants and ubiquitous in plants.").

The common technical feature shared by Groups I and II that would otherwise unify the groups, is a rice plant exhibiting early maturity comprising a modified genomic locus involved in flowering time regulation (FTR), wherein the genomic locus comprises one or more introduced mutations compared to a control plant. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by US 2009/0126047 A1 to An et al. (hereinafter 'An'). An discloses a method of modifying maturity of a rice plant, the method comprising introducing one or more nucleotide modifications through a targeted DNA modification at a genomic locus of the rice plant, wherein (a) the genomic locus comprises a polynucleotide involved in flowering time regulation (FTR), and (b) wherein the maturity of the rice plant is modulated compared to a control rice plant not comprising the one or more introduced DNA modifications (abstract; para [0056]: "The flowering-time regulator may be ... OsCOL4 ..., which is overexpressed or suppressed through the above method."; para [0025]: "In the present invention, the OsCOL4 activated mutant line (by induction of overexpression, etc.) exhibits delayed flowering-time phenotype with a delay of 15 or more days compared with wild-type (see FIG. 14), whereas the OsCOL4 suppressed mutant line exhibits early flowering-time phenotype which is early by approximately 10 days compared with wild-type.").

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

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