VIROID-DERIVED POLYNUCLEOTIDES FOR MODIFICATION OF PLANTS

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy, created on July 19, 2021, is named 51484-002WO2_Sequence_Listing_7_19_21_ST25 and is 528,405 bytes in size.

FIELD OF THE INVENTION

Provided herein are plant-modifying polynucleotides for use in a variety of agricultural and commercial applications.

BACKGROUND

Plant viroids are circular, single-stranded RNAs capable of invading plants. There is need in the art for plant-modifying polynucleotides (e.g., polynucleotides derived from viroids) for use in a variety of agricultural and commercial applications.

SUMMARY

In one example, disclosed herein is a method of delivering an effector to a eukaryote, comprising providing to the eukaryote a composition comprising a recombinant polynucleotide comprising: (i) a single-stranded RNA (ssRNA) viroid sequence and (ii) a heterologous RNA sequence comprising or encoding an effector, whereby the effector comprised by or encoded by the heterologous RNA sequence is delivered to the eukaryote.

In one aspect, the present disclosure refers to a method of delivering an effector to a plant, comprising providing to the plant a composition comprising a recombinant polynucleotide comprising: (i) a single-stranded RNA (ssRNA) plant viroid sequence and (ii) a heterologous RNA sequence that is not a viroid sequence comprising or encoding an effector, whereby the effector comprised by or encoded by the heterologous RNA sequence is delivered to the plant.

In some examples, the eukaryote is a plant, a fungus, or an animal.

In some examples, the composition is provided to a plant, plant tissue, or plant cell, or a processed product thereof, wherein the eukaryote consumes or contacts the plant, plant tissue, or plant cell, or processed product thereof, whereby the effector is delivered to the eukaryote.

In some examples, (a) the ssRNA viroid sequence is a viroid genome or a derivative thereof or (b) the ssRNA viroid sequence is a viroid genome fragment or a derivative thereof.

In some examples, the ssRNA viroid sequence is a sequence of a viroid from the family Pospiviroidae or Avsunviroidae. In some examples, the viroid is potato spindle tuber viroid (PSTVd) or eggplant latent viroid (ELVd).

In some examples, the ssRNA viroid sequence has at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs:51-54, SEQ ID NOs:65-66, SEQ ID NO:68, SEQ ID NO:75, SEQ ID NOs:77-79, SEQ ID NOs:84-96, SEQ ID NOs:98-107, SEQ ID NOs:123-124, SEQ ID NOs:126-132, SEQ ID NO:134, SEQ ID NOs:136-143, SEQ ID NOs:145-150, SEQ ID NOs:153-154, SEQ ID NO:159, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:196, SEQ ID NO:242, SEQ ID NO:268, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:289, SEQ ID NO:451, SEQ ID NOs:458-459, and SEQ ID NO:467.

In some examples, the ssRNA viroid sequence has at least 90% sequence identity to SEQ ID NO:51 or SEQ ID NO:50.

In some examples, the ssRNA viroid sequence does not contain a pathogenicity domain.
In some examples, the RNA sequence comprising or encoding the effector is not a viroid sequence and (a) has a biological effect on a plant or (b) has a biological effect on an animal or fungus that consumes or contacts the plant.
In some examples, the effector comprises or is encoded by an ssRNA sequence.
In some examples, the effector is an siRNA.
In some examples, the heterologous RNA sequence comprises coding RNA, non-coding RNA, or both coding and non-coding RNA.
In some examples, the effector comprises coding RNA, non-coding RNA, or both coding and non-coding RNA.
In some examples, the effector comprises non-coding RNA comprising at least one regulatory RNA or at least one interfering RNA that regulates a target gene or its transcript in a target cell.
In some examples, the target cell is selected from the group consisting of a plant cell, an animal cell, and a fungal cell.
In some examples, the effector modifies a trait, phenotype, or genotype in the target cell. In some examples, modifying comprises reducing expression of the target gene. In some examples, modifying comprises increasing expression of the target gene. In some examples, modifying comprises (a) editing the target gene or (b) regulating the target gene.
In some examples, the ssRNA viroid sequence effects one or more results selected from the group consisting of entry into a tissue or cell of the eukaryote; transmission through a tissue or cell or subcellular component of the eukaryote; replication in a tissue or cell of the eukaryote; targeting to a tissue or cell of the eukaryote; and binding to a factor in a tissue or cell of the eukaryote.
In some examples, the recombinant polynucleotide lacks free ends and/or is circular.
In some examples, the composition is topically delivered to a plant. In some examples, the topical delivery is spraying, leaf rubbing, soaking, coating, injecting, seed coating, or delivery through root uptake.
In another example, disclosed herein is a composition comprising a recombinant polynucleotide comprising: (a) a single-stranded RNA (ssRNA) viroid sequence that is a viroid genome or a derivative thereof or a viroid genome fragment or a derivative thereof, and (b) a heterologous RNA sequence that is not a viroid sequence and comprises or encodes an effector.
In one aspect, the present disclosure refers to a composition comprising a recombinant polynucleotide comprising: (a) a single-stranded RNA (ssRNA) plant viroid sequence that is a plant viroid genome or a derivative thereof or a plant viroid genome fragment or a derivative thereof, and (b) a heterologous RNA sequence that is not a viroid sequence and comprises or encodes an effector.
In some examples, the viroid genome is (a) a genome of a viroid from the family Pospiviroidae or Avsunviroidae, or (b) a genome of potato spindle tuber viroid (PSTVd) or eggplant latent viroid (ELVd).
In some examples, the ssRNA viroid sequence has at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs:51-54, SEQ ID NOs:65-66, SEQ ID NO:68, SEQ ID NO:75, SEQ ID NOs:77-79, SEQ ID NOs:84-96, SEQ ID NOs:98-107, SEQ ID NOs:123-124, SEQ ID NOs:126-132, SEQ ID NO:134, SEQ ID NOs:136-143, SEQ ID NOs:145-150, SEQ ID NOs:153-154, SEQ ID NO:159, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:196, SEQ ID NO:242, SEQ ID NO:268, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:289, SEQ ID NO:451, SEQ ID NOs:458-459, and SEQ ID NO:467.
In some examples, the ssRNA viroid sequence has at least 90% sequence identity to SEQ ID NO:51 or SEQ ID NO:50.

In some examples, the effector comprises non-coding RNA comprising at least one regulatory RNA or at least one interfering RNA or at least one guide RNA that regulates or modifies a target gene or its transcript in a target cell, wherein the target cell is a plant cell, an animal cell, or a fungal cell.
In some examples, the effector (a) modifies expression of a target gene in a eukaryotic cell; or (b) has a biological effect on a plant or on an animal or fungus that consumes or contacts the plant.
In some examples, the composition is (a) formulated for delivery to a plant or to the environment in which the plant grows; or (b) formulated for delivery to an animal or fungus.
In another example, disclosed herein is a composition comprising a recombinant polynucleotide comprising: (i) a single-stranded RNA (ssRNA) viroid sequence and (ii) a heterologous RNA sequence comprising or encoding an effector, the composition being formulated for topical delivery to a plant.
In some examples, the ssRNA viroid sequence is a viroid genome or a derivative thereof.
In some examples, the ssRNA viroid sequence is a viroid genome fragment or a derivative thereof.
In some examples, the recombinant polynucleotide encodes at least two ssRNA viroid sequences.
In some examples, the topical delivery is spraying, leaf rubbing, soaking, coating, injecting, seed coating, or delivery through root uptake.
In some examples, the composition further comprises an additional formulation component.
In some examples, the composition does not comprise an additional formulation component.

In some examples, the ssRNA viroid sequence comprises a sequence of at least 40 ribonucleotides which is at least 80% identical to a sequence, or fragment thereof, listed in Table 1. In some examples, the ssRNA viroid sequence has at least 90% identity to a sequence of Table 1. In some examples, the ssRNA viroid sequence has at least 95% identity to a sequence of Table 1. In some examples, the ssRNA viroid sequence has at least 98% identity to a sequence of Table 1. In some examples, the ssRNA viroid sequence has at least 99% identity to a sequence of Table 1.

In some examples, the sequence of Table 1 is SEQ ID NO: 50.

In some examples, the sequence of Table 1 is SEQ ID NO: 51.

In some examples, the viroid is from the family Pospiviroidae or Avsunviroidae.

In some examples, the viroid is eggplant latent viroid (ELVd), potato spindle tuber viroid (PSTVd), hop stunt viroid, coconut cadang-cadang viroid, apple scar skin viroid, Coleus blumei viroid 1, avocado sunblotch viroid, peach latent mosaic viroid, chrysanthemum chlorotic mottle viroid, or Dendrobium viroid.

In some examples, the viroid is ELVd. In some examples, the viroid is PSTVd.

In some examples, the ssRNA viroid sequence comprises a sequence that is at least 80% identical to a sequence listed in Table 2 or Table 3. In some examples, the ssRNA viroid sequence has at least 90% identity to a sequence of Table 2 or Table 3. In some examples, the ssRNA viroid sequence has at least 95% identity to a sequence of Table 2 or Table 3. In some examples, the ssRNA viroid sequence has at least 98% identity to a sequence of Table 2 or Table 3. In some examples, the ssRNA viroid sequence has at least 99% identity to a sequence of Table 2 or Table 3.

In some examples, each of the at least two ssRNA viroid sequences are at least 80% identical to a sequence listed in Table 2 or Table 3.

In some examples, the recombinant polynucleotide encodes a sequence that is at least 80% identical to SEQ ID NO: 884 and encodes a sequence that is at least 80% identical to SEQ ID NO: 885.

In some examples, the recombinant polynucleotide encodes a sequence that is at least 80% identical to SEQ ID NO: 886 and encodes a sequence that is at least 80% identical to SEQ ID NO: 887.
In some examples, the recombinant polynucleotide encodes a sequence that is at least 80% identical to SEQ ID NO: 888 and encodes a sequence that is at least 80% identical to SEQ ID NO: 889.
In some examples, the recombinant polynucleotide encodes a sequence that is at least 80% identical to SEQ ID NO: 890 and encodes a sequence that is at least 80% identical to SEQ ID NO: 891.
In some examples, the recombinant polynucleotide encodes a sequence that is at least 80% identical to SEQ ID NO: 892 and encodes a sequence that is at least 80% identical to SEQ ID NO: 893.
In some examples, the recombinant polynucleotide comprises 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 ssRNA viroid sequences that are at least 80% identical to a sequence listed in Table 2 or Table 3.
In some examples, the ssRNA viroid sequence comprises, in secondary structure, one or more of a replication motif, a transmission motif, a targeting motif, or a binding motif.
In some examples, the ssRNA viroid sequence does not contain a pathogenicity domain.

In some examples, the ssRNA viroid sequence comprises an internal loop, a stem-loop, a bulge loop, or a pseudoknot.

In some examples, the ssRNA viroid sequence comprises a replication domain, a transmission domain, a targeting domain, or a binding domain. In some examples, the transmission domain is a tissue transmission domain, a cell-cell transmission domain, or a subcellular transition domain. In some examples, the targeting domain is a tissue targeting domain, a cell targeting domain, or a subcellular targeting domain. In some examples, the targeting domain binds to a host cell. In some examples, the targeting domain is a nuclear targeting sequence or a nuclear exclusion sequence. In some examples, the binding domain binds a molecular target in the plant. In some examples, the binding domain binds DICER.

In some examples, the RNA sequence comprising or encoding the effector is not a viroid sequence and has a biological effect on a plant.

In some examples, the effector comprises or is encoded by an ssRNA sequence.

In some examples, the effector comprises a coding sequence. In some examples, the coding sequence encodes a protein or a polypeptide.

In some examples, the effector is a regulatory RNA. In some examples, the regulatory RNA is a lncRNA, circRNA, tRF, tRNA, rRNA, snRNA, snoRNA, or piRNA. In some examples, the effector is an interfering RNA. In some examples, the effector is a dsRNA or a hpRNA. In some examples, the effector is a microRNA (miRNA) or a pre-miRNA. In some examples, the effector is a phasiRNA. In some examples, the effector is a hcsiRNA. In some examples, the effector is a natsiRNA. In some examples, the effector is a guide RNA.

In some examples, the effector binds a target host cell factor. In some examples, the target host cell factor is a nucleic acid, a protein, a DNA, or an RNA.

In some examples, the recombinant polynucleotide further comprises an internal ribosome entry site (IRES), a 5' homology arm, a 3' homology arm, a polyadenylation sequence, a group I permuted intron-exon (PIE) sequence, an RNA cleavage site, a ribozyme, a DICER-binding sequence, an mRNA fragment comprising an intron, an exon, a combination of one or more introns and exons, an untranslated region (UTR), an enhancer region, a Kozak sequence, a start codon, or a linker. In some examples, the

ribozyme is a hammerhead ribozyme, a riboswitch, or a twister/tornado. In some examples, the DICER-binding sequence flanks the effector.

In some examples, the recombinant polynucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 additional heterologous sequence elements.

In some examples, the recombinant polynucleotide lacks free ends. In some examples, the recombinant polynucleotide is circular.

In some examples, the recombinant polynucleotide comprises at least one free end. In some examples, the recombinant polynucleotide is concatemeric. In some examples, the recombinant polynucleotide is linear.

In another example, disclosed herein is a cell comprising a composition of any of the above examples.

In some examples, the cell is a plant cell. In some examples, the plant cell is a monocot cell or a dicot cell. In some examples, the plant cell is a protoplast.

In some examples, the cell has been transiently transformed with the recombinant polynucleotide.

In some examples, the cell has been stably transformed with the recombinant polynucleotide.

In another example, disclosed herein is a composition according to any of the above examples, further comprising a plant cell.

In another example, disclosed herein is a liposome comprising a composition according to any of the above examples.

In another example, disclosed herein is a vesicle comprising a composition according to any of the above examples.

In another example, disclosed herein is a formulation comprising a composition according to any of the above examples.

In some examples, the formulation is a liquid, a gel, or a powder.

In some examples, the formulation is configured to be sprayed on plants, to be rubbed on leaves, to be coated on seeds, or to be delivered to roots.

In another example, disclosed herein is a method of delivering an effector to a plant, a plant tissue, or a plant cell, comprising providing to a plant, plant tissue, or plant cell a composition according to any one of the above examples, whereby the effector comprised by or encoded by the heterologous RNA sequence is delivered to the plant, plant tissue, or plant cell.

In some examples, the plant is a monocot or a dicot.

In some examples, the plant cell is a protoplast.

In some examples, providing the composition to the plant, plant tissue, or plant cell comprises delivering the composition to a leaf, root, stem, flower, seed, xylem, phloem, apoplast, symplast, meristem, fruit, embryo, microspore, pollen, pollen tube, ovary, ovule, or explant for transformation of the plant.

In some examples, the fruit is a pre-harvest fruit. In some examples, the fruit is a post-harvest fruit.

In another example, disclosed herein is a method of modifying a trait, phenotype, or genotype in a plant cell, comprising providing to the plant cell a composition according to any of the above examples.

In some examples, modifying comprises expressing in the plant a heterologous protein encoded by the RNA sequence comprising or encoding an effector.

In some examples, modifying comprises reducing expression of a target gene of the plant.
In some examples, modifying comprises increasing expression of a target gene of the plant.
In some examples, modifying comprises editing a target gene of the plant.
In some examples, modifying comprises regulating a target gene in the plant.
In some examples, the ssRNA viroid sequence effects one or more results selected from the group consisting of entry into a tissue or cell of the plant; transmission through a tissue or cell or subcellular component of the plant; replication in a tissue or cell of the plant; targeting to a tissue or cell of the plant; and binding to a factor in a tissue or cell of the plant.
In another example, disclosed herein is a composition comprising a recombinant polynucleotide comprising: (i) a single-stranded RNA (ssRNA) viroid sequence and (ii) a heterologous RNA sequence comprising or encoding an effector, wherein the ssRNA viroid sequence does not include a chloroplast localization sequence.

Other features and advantages of the disclosure will be apparent from the following Detailed Description and the Claims.

DEFINITIONS

As used herein, the term “internal ribosome entry site” or “IRES” refers to a sequence (e.g., an RNA sequence) capable of recruiting a ribosome and translation machinery to initiate translation from an RNA sequence. An IRES element is generally between 100-800 nucleotides. In embodiments, the efficiency or effectiveness of an IRES in the composition and methods described herein is tested, e.g., by introducing the IRES into a circular RNA expression vector and assaying for levels of expression of a downstream cistronic protein such as firefly luciferase using enzymatic reactions, or fluorescent readouts using reporters such as green fluorescent protein (GFP). An appropriate IRES can be obtained from plant and plant viral IRES sequences such as encephalomyocarditis virus IRES (ECMV), maize hsp101 IRES 5’UTR, crucifer infecting tobamovirus crTMV CR-CP 148 IRES, tobacco etch virus (TEV) IRES 5’UTR and hibiscus chlorotic ringspot virus (HCRSV) IRES. In addition, in embodiments, an IRES sequence is derived from non-plant eukaryotic virus sequences that include but are not limited to: acute bee paralysis virus (ABPV), classical swine fever virus (CSFV), coxsackievirus B3 virus (CVB3), encephalomyocarditis virus (ECMV), enterovirus 71 (E71), hepatitis A virus (HAV), human rhinovirus (HRV2), human rhinovirus (HRV2), human lymphotropic virus (HTLV) and polyoma virus (PV). Examples of IRES sequence useful in the compositions and methods described herein are shown in Table 4.
As used herein, the term “untreated” refers to an organism (e.g., a eukaryote, e.g., a plant, a fungus, or an animal) that has not been contacted with or delivered a recombinant polynucleotide (e.g., viroid-derived vector) described herein, including a separate organism that has not been delivered the recombinant polynucleotide, the same organism undergoing treatment assessed at a time point prior to delivery of the recombinant polynucleotide, or the same organism undergoing treatment assessed at an untreated part of the organism (that is, at an area of the organism not contacted with the recombinant polynucleotide).
As used herein, the term “effective amount,” “effective concentration,” or “concentration effective to” refers to an amount of a recombinant polynucleotide (e.g., viroid-derived vector) or a composition thereof,