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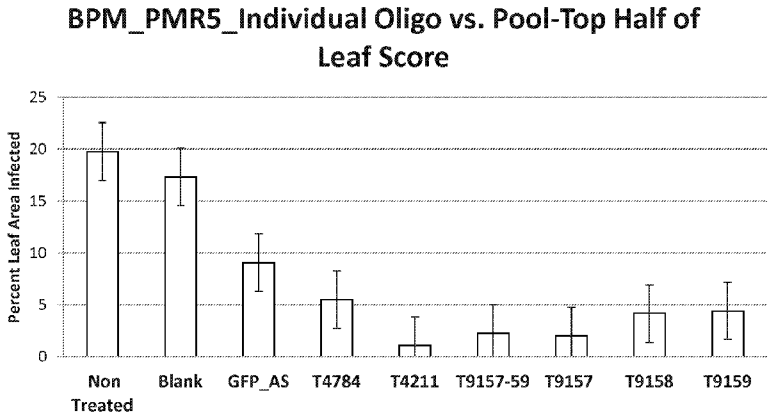
(54) Title: METHODS AND COMPOSITIONS FOR PLANT PEST CONTROL



(57) Abstract: Provided are methods and compositions to improve fungal disease resistance and/or nematode resistance in various crop plants. Also provided are combinations of compositions and methods to improve fungal disease resistance and/or nematode resistance in various crop plants. Powdery mildews are fungal diseases that affect a wide range of plants including cereals, grasses, vegetables, ornamentals, weeds, shrubs, fruit trees, broad-leaved shade and forest trees, that is caused by different species of fungi in the order Erysiphales.

METHODS AND COMPOSITIONS FOR PLANT PEST CONTROL

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This international patent application claims the benefit of U.S. Provisional patent Application No. 61/752,703, filed January 15, 2013, which is incorporated herein by reference in its entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] A sequence listing is provided herewith as a part of this International Patent application via the USPTO's EFS system in file named "40_71_59225_SEQ_LISTING" which is 70,527 bytes in size (measured in MS-Windows®), contains 128 sequences, was created on January 14, 2014, and is incorporated herein by reference in its entirety.

BACKGROUND

[0003] Powdery mildews are fungal diseases that affect a wide range of plants including cereals, grasses, vegetables, ornamentals, weeds, shrubs, fruit trees, broad-leaved shade and forest trees, that is caused by different species of fungi in the order *Erysiphales*. The disease is characterized by spots or patches of white to grayish, talcum-powder-like growth that produce tiny, pinhead-sized, spherical fruiting structures (the cleistothecia or overwintering bodies of the fungus), that are first white, later yellow-brown and finally black. The fungi that cause powdery mildews are host specific and cannot survive without the proper host plant. They produce mycelium (fungal threads) that grow only on the surface of the plant and feed by sending haustoria, or root-like structures, into the epidermal cells of the plant. The fungi overwinter on plant debris as cleistothecia or mycelia. In the spring, the cleistothecia produce spores that are moved to susceptible hosts by rain, wind or insects.

[0004] Powdery mildew disease is particularly prevalent in temperate and humid climates, where they frequently cause significant yield losses and quality reductions in various agricultural settings including greenhouse and field farming. This affects key cereals (e.g. barley and wheat), horticultural crops (e.g. grapevine, pea and tomato) and economically important ornamentals (e.g. roses). Limited access to natural sources of resistance to powdery mildews, rapid changes in pathogen virulence and the time consuming introgression of suitable resistance genes into elite varieties has led to the widespread use of fungicides to control the disease. This has not surprisingly led to the evolution and spread of fungicide

resistance, which is especially dramatic amongst the most economically important powdery mildews.

[0005] Downy mildew diseases are caused by oomycete microbes from the family *Peronosporaceae* that are parasites of plants. *Peronosporaceae* are obligate biotrophic plant pathogens and parasitize their host plants as an intercellular mycelium using haustoria to penetrate the host cells. The downy mildews reproduce asexually by forming sporangia on distinctive white sporangiophores usually formed on the lower surface of infected leaves. These constitute the "downy mildew" and the initial symptoms appear on leaves as light green to yellow spots. The sporangia are wind-dispersed to the surface of other leaves. Depending on the genus, the sporangia may germinate by forming zoospores or by germ-tube. In the latter case, the sporangia behave like fungal conidia and are often referred to as such. Sexual reproduction is via oospores.

[0006] Most *Peronosporaceae* are pathogens of herbaceous dicots. Some downy mildew genera have relatively restricted host ranges, e.g. *Basidiophora*, *Paraperonospora*, *Protobremia* and *Bremia* on *Asteraceae*; *Perofascia* and *Hyaloperonospora* almost exclusively on *Brassicaceae*; *Viennotia*, *Graminivora*, *Poakatesthia*, *Sclerospora* and *Peronosclerospora* on *Poaceae*, *Plasmoverna* on *Ranunculaceae*. However, the largest genera, *Peronospora* and *Plasmopara*, have very wide host ranges.

[0007] In commercial agriculture, downy mildews are a particular problem for growers of crucifers, grapes and vegetables that grow on vines. *Peronosporaceae* of economic importance include *Plasmopara viticola* which infect grapevines, *Peronospora tabacina* which causes blue mold on tobacco, *Bremia lactucae,* a parasite on lettuce, and *Plasmopara halstedii* on sunflower.

[0008] Rusts (*Pucciniales*, formerly *Uredinales*) are obligate biotrophic parasites of vascular plants. Rusts affect a variety of plants; leaves, stems, fruits and seeds and is most commonly seen as coloured powder, composed of tiny aeciospores which land on vegetation producing pustules, or uredia, that form on the lower surfaces. During late spring or early summer, yellow orange or brown, hairlike or ligulate structures called telia grow on the leaves or emerge from bark of woody hosts. These telia produce teliospores which will germinate into aerial basidiospores, spreading and causing further infection.

SUMMARY

[0009] The present embodiments provide for compositions comprising polynucleotide molecules and methods for treating a plant to alter or regulate gene or gene transcript

expression in the plant, for example, by providing RNA or DNA for inhibition of expression. Various aspects provide compositions comprising polynucleotide molecules and related methods for topically applying such compositions to plants to regulate endogenous PMR5 genes in a plant cell. The polynucleotides, compositions, and methods disclosed herein are useful in decreasing levels of PMR5 transcript and improving fungal disease and/or nematode resistance of a plant.

[0010] In one aspect, the polynucleotide molecules are provided in compositions that can permeate or be absorbed into living plant tissue to initiate localized, partially systemic, or systemic gene inhibition or regulation. In certain embodiments, the polynucleotide molecules ultimately provide to a plant, or allow the *in planta* production of, RNA that is capable of hybridizing under physiological conditions in a plant cell to RNA transcribed from a target endogenous gene or target transgene in the plant cell, thereby effecting regulation of the endogenous PMR5 target gene. In certain embodiments, regulation of the PMR5 target genes, such as by silencing or suppression of the target gene, leads to the upregulation of another gene that is itself affected or regulated by decreasing the PMR5 target gene's expression.

[0011] In some embodiments, the topical application of a composition comprising an exogenous polynucleotide and a transfer agent to a plant or plant part according to the methods described herein does not necessarily result in nor require the exogenous polynucleotide's integration into a chromosome of the plant. In some embodiments, the topical application of a composition comprising an exogenous polynucleotide and a transfer agent to a plant or plant part according to the methods described herein does not necessarily result in nor require transcription of the exogenous polynucleotide from DNA integrated into a chromosome of the plant. In certain embodiments, topical application of a composition comprising an exogenous polynucleotide and a transfer agent to a plant according to the methods described herein also does not necessarily require that the exogenous polynucleotide be physically bound to a particle, such as in biolistic mediated introduction of polynucleotides associated with a gold or tungsten particles into internal portions of a plant, plant part, or plant cell. An exogenous polynucleotide used in certain methods and compositions provided herein can optionally be associated with an operably linked promoter sequence in certain embodiments of the methods provided herein. However, in other embodiments, an exogenous polynucleotide used in certain methods and compositions provided herein is not associated with an operably linked promoter sequence. Also, in certain

embodiments, an exogenous polynucleotide used in certain methods and compositions provided herein is not operably linked to a viral vector.

[0012] In certain embodiments, methods for improving fungal disease resistance and/or nematode resistance in a plant comprising topically applying compositions comprising a polynucleotide and a transfer agent that suppress the target PMR5 gene are provided. In certain embodiments, methods for selectively suppressing the target PMR5 gene by topically applying the polynucleotide composition to a plant surface at one or more selected seed, vegetative, or reproductive stage(s) of plant growth are provided. Such methods can provide for gene suppression in a plant or plant part on an as needed or as desired basis. In certain embodiments, methods for selectively suppressing the target PMR5 gene by topically applying the polynucleotide composition to a plant surface at one or more pre-determined seed, vegetative, or reproductive stage(s) of plant growth are provided. Such methods can provide for PMR5 gene suppression in a plant or plant part that obviates any undesired or unnecessary effects of suppressing the genes expression at certain seed, vegetative, or reproductive stage(s) of plant development.

[0013] In certain embodiments, methods for selectively improving fungal disease resistance and/or nematode resistance in a plant by topically applying the polynucleotide composition to the plant surface at one or more selected seed, vegetative, or reproductive stage(s) are provided. Such methods can provide for improved fungal disease resistance and/or nematode disease resistance in a plant or plant part on an as needed or as desired basis. In certain embodiments, methods for selectively improving fungal disease and/or nematode resistance in a plant by topically applying the polynucleotide composition to the plant surface at one or more predetermined seed, vegetative, or reproductive stage(s) are provided. Such methods can provide for improving fungal disease and/or nematode resistance in a plant or plant part that obviates any undesired or unnecessary effects of suppressing PMR5 gene expression at certain seed, vegetative, or reproductive stage(s) of plant development.

[0014] Polynucleotides that can be used to suppress a PMR5 include, but are not limited to, any of: i) polynucleotides comprising at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a gene or a transcript of the gene(s) of Table 2 (SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11) or encoding a protein of Table 3 (SEQ ID NO: 41-48, or 49); ii) polynucleotides comprising at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a gene encoding a PMR5 or PMR5-like protein of Table 3 comprising a polynucleotide of SEQ ID NO:41-48, or 49; or, polynucleotides comprising at least 18 contiguous nucleotides that are essentially identical or

essentially complementary to a polynucleotides of SEQ ID NO:12-19, 21-37, 53-127 or 128. Methods and compositions that provide for the topical application of certain polynucleotides in the presence of transfer agents can be used to suppress PMR5 gene expression in an optimal manner. In certain embodiments, the compositions provided herein can be applied on an "as needed" basis upon scouting for the occurrence of fungal disease or nematodes. In certain embodiments, the compositions can be applied in a manner that obviates any deleterious effects on yield or other characteristics that can be associated with suppression of PMR5 gene expression in a crop plant. The applied polynucleotides are complementary to the PMR5 target host gene in plants and their topical application leads to suppression of the PMR5 gene's activity.

[0015] Provided herein are compositions and methods for controlling plant fungal diseases. Plant fungal diseases that can be controlled with the methods and compositions provided herein include, but are not limited to, obligate biotrophic powdery mildew, downy mildew and rust fungal infestations in plants. In certain embodiments, methods and compositions for reducing expression of one or more host plant PMR5 polynucleotide and/or protein molecules in one or more cells or tissues of the plant such that the plant is rendered less susceptible to fungal infections from the order *Erysiphales*, the family *Peronosporaceae* or the order *Pucciniales*, are provided. In certain embodiments, nucleotide and amino acid sequences of plant PMR5 genes which can be downregulated by methods and compositions provided herein to increase plant resistance to powdery mildew, downy mildew or rust infection are disclosed.

[0016] Also provided herein are methods and compositions that provide for reductions in expression of PMR5 target polynucleotide and protein molecules in at least the cells of a plant root and for improved resistance to nematodes. Nematodes that can be controlled by the methods and compositions provided herein include, but are not limited to, root knot nematodes (such as *Meloidogyne sp.*), cyst nematodes (such as *Globodera sp.* and *Heterodera* sp.), lesion nematodes (such as *Pratylenchus sp.*), and the like. In certain embodiments, PMR5 expression is reduced in plant root cells from which nematodes feed by providing topically to plant leaves, shoots, roots and/or seeds compositions comprising polynucleotides that comprise at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or to a transcript of the PMR5 gene; and a transfer agent.

[0017] Also provided are methods and compositions where topically induced reductions in PMR5 transcript or protein levels are used to achieve powdery mildew, downy mildew or rust

control while minimizing deleterious pleotropic effects in the host plant. Such methods and compositions provide for optimized levels of PMR5 gene inhibition and/or optimized timing of PMR5 gene inhibition.

[0018] Certain embodiments are directed to methods for producing a plant exhibiting an improvement in fungal disease resistance and/or nematode resistance comprising topically applying to a plant surface a composition that comprises:

a. at least one polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or to a transcript of the gene; and

b. a transfer agent, wherein the plant exhibits an improvement in fungal disease resistance and/or nematode resistance that results from suppression of the PMR5 gene. In certain embodiments, the polynucleotide molecule comprises sense ssDNA, sense ssRNA, dsRNA, dsDNA, a double stranded DNA/RNA hybrid, anti-sense ssDNA, or anti-sense ssRNA. In certain embodiments, the polynucleotide is selected from the group consisting of SEQ ID NO: 12-19, 21-37, 53-127, or 128, or wherein the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. In certain embodiments: (a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127 and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11; (b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4; (c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3, or 6, 53, 54, 55, or 56; (d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1; (e) the plant is a corn plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7; (f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and

the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10; (g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or, (h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9. In certain embodiments, the composition comprises any combination of two or more polynucleotide molecules. In certain embodiments, the polynucleotide is at least 18 to about 24, about 25 to about 50, about 51 to about 100, about 101 to about 300, about 301 to about 500, or at least about 500 or more residues in length. In certain embodiments, the composition further comprises a non-polynucleotide herbicidal molecule, a polynucleotide herbicidal molecule, a polynucleotide that suppresses an herbicide target gene, an insecticide, a fungicide, a nematocide, or a combination thereof. In certain embodiments, the composition further comprises a non-polynucleotide herbicidal molecule and the plant is resistant to the herbicidal molecule. In certain embodiments, the transfer agent comprises an organosilicone preparation. In certain embodiments, the polynucleotide is not operably linked to a viral vector. In certain embodiments, the polynucleotide is not integrated into the plant chromosome. Further embodiments are directed to: a plant made according to any of the above-described methods; progeny of plants that exhibit the improvements in fungal disease resistance and/or nematode resistance; seed of the plants, wherein seed from the plants exhibits the improvement in fungal disease resistance and/or nematode resistance; and a processed product of the plants, the progeny plants, or the seeds, wherein the processed products exhibit the improvement in fungal disease resistance and/or nematode resistance. In certain embodiments, the processed product of the plant or plant part exhibits an improved attribute relative to a processed product of an untreated control plant and the improved attribute results from the improved fungal disease resistance and/or nematode resistance. An improved attribute of a processed product can include, but is not limited to, decreased mycotoxin content, improved nutritional content, improved storage characteristics, improved flavor, improved consistency, and the like when compared to a processed product obtained from an untreated plant or plant part.

[0019] An additional embodiment is directed to a composition comprising a polynucleotide molecule that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of the gene, wherein the polynucleotide is not operably linked to a promoter; and, b) a transfer agent. In certain

embodiments, the polynucleotide is selected from the group consisting of SEQ ID NO: 12-19, 21-38, 53-127, and 128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11, or wherein the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. In certain embodiments:(a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11; (b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4; (c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3, or 6, 53, 54, 55, or 56; (d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1; (e) the plant is a corn plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7; (f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10; (g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or, (h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9. In certain embodiments, the polynucleotide is at least 18 to about 24, about 25 to about 50, about 51 to about 100, about 101 to about 300, about 301 to about 500, or at least about 500 or more residues in length. In certain embodiments, the composition further comprises a non-polynucleotide herbicidal molecule, a polynucleotide herbicidal molecule, a polynucleotide that suppresses an herbicide target gene, an insecticide,

a fungicide, a nematocide, or a combination thereof. In certain embodiments, the transfer agent is an organosilicone preparation. In certain embodiments, the polynucleotide is not physically bound to a biolistic particle.

[0020] Another embodiment is directed to a method of making a composition comprising the step of combining at least:(a) a polynucleotide molecule comprising at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of a plant, wherein the polynucleotide is not operably linked to a promoter or a viral vector; and, (b) a transfer agent. In certain embodiments, the polynucleotide is obtained by *in vivo* biosynthesis, *in vitro* enzymatic synthesis, or chemical synthesis. In certain embodiments, the method further comprises combining with the polynucleotide and the transfer agent at least one of a non-polynucleotide herbicidal molecule, a polynucleotide herbicidal molecule, an insecticide, a fungicide, and/or a nematocide. In certain embodiments, the transfer agent is an organosilicone preparation.

[0021] Yet another embodiment is directed to a method of identifying a polynucleotide for improving fungal disease resistance and/or nematode resistance in a plant comprising; (a) selecting a population of polynucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of a plant; (b) topically applying to a surface of at least one of the plants a composition comprising at least one polynucleotide from the population and an transfer agent to obtain a treated plant; and, (c) identifying a treated plant that exhibits suppression of the PMR5 gene or exhibits an improvement in fungal disease resistance or exhibits an improvement in nematode resistance, thereby identifying a polynucleotide that improves fungal disease resistance and/or nematode resistance in the plant. In certain embodiments, the selection of the population of polynucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of a plant can be effected by identifying polynucleotides that can suppress a PMR5 gene via Virus Induced Gene Silencing (VIGS). Those polynucleotides that can suppress a PMR5 gene via VIGS are disassociated from the VIGS vector, topically applied to a surface of a plant in a composition comprising at least one of those polynucleotides and a treated plant that exhibits suppression of the PMR5 gene or exhibits an improvement in fungal disease resistance or nematode resistance is identified, thus identifying a polynucleotide that improves fungal disease resistance and/or nematode resistance in the plant. In certain embodiments, the polynucleotide is selected from the group consisting of SEQ ID NO: 12-19, 21-38, 53-127, and 128, or wherein the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

or 11. In certain embodiments: (a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11; (b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4; (c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3, or 6, 53, 54, 55, or 56; (d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1; (e) the plant is a corn plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7; (f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10; (g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or, (h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9.

[0022] A further embodiment is directed to a plant comprising an exogenous polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of the gene, wherein the exogenous polynucleotide is not operably linked to a promoter or to a viral vector, is not integrated into the chromosomal DNA of the plant, and is not found in a non-transgenic plant; and, wherein the plant exhibits an improvement in fungal disease resistance and/or nematode resistance that results from suppression of the PMR5 gene. In certain embodiments, plant further comprises an organosilicone compound or a component thereof. In certain embodiments, the polynucleotide is selected from the group consisting of SEQ ID NO: 12-19, 21-38, 53-127,

and128, or comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. In certain embodiments (a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11; (b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4; (c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3 or 6; (d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1; (e) the plant is a corn plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7; (f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10; (g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or, (h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9.

[0023] An additional embodiment is directed to a plant part comprising an exogenous polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of the gene, wherein the exogenous polynucleotide is not operably linked to a promoter or to a viral vector and is not found in a non-transgenic plant; and, wherein the plant part exhibits an improvement in fungal disease resistance and/or nematode resistance that results from suppression of the PMR5 gene. In certain embodiments, the polynucleotide is selected from the group

consisting of SEQ ID NO: 12-19, 21-38, 53-127, and 128, or wherein the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. In certain embodiments: (a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11; (b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4; (c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3, or 6, 53, 54, 55, or 56; (d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1; (e) the plant is a corn plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7; (f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10; (g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or, (h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9. In certain embodiments, the plant part is a flower, meristem, ovule, stem, tuber, fruit, anther, pollen, leaf, root, or seed. In certain embodiments, the plant part is a seed. Also provided are processed plant products obtained from any of the aforementioned plant parts, wherein the processed plant products exhibit an improved attribute relative to a processed plant product of an untreated control plant and wherein the improved attribute results from the improved fungal disease resistance and/or nematode resistance. In certain embodiments, the processed product is a meal, a pulp, a feed, or a food product. Another embodiment is directed to a plant that exhibits an

improvement in fungal disease resistance and/or nematode resistance, wherein the plant was topically treated with a composition that comprises: (a) at least one polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or to a transcript of the gene; and (b) a transfer agent; and, wherein the plant exhibits an improvement in fungal disease resistance and/or nematode resistance that results from suppression of the PMR5 gene.

[0024] Also provided herein are transgenic plants, plant parts, plant cells, and processed plant products containing a transgene comprising a heterologous promoter that is operably linked to a polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of the PMR5 gene. Such transgenes can be integrated into the genome of the transgenic plant or provided in recombinant viral genomes that can be propagated in the plant. In certain embodiments, the transgene confers an improvement in fungal disease resistance and/or nematode resistance to the transgenic plants or plant parts that contain the transgene. In certain embodiments, the polynucleotide is selected from the group consisting of SEQ ID NO: 12-19, 21-38, 57-127, and 128, encodes an RNA comprising or consisting of SEQ ID NO: 53, 54, 55, 56, or their complements, encodes an RNA that is essentially identical or essentially complementary to SEQ ID NO: 53, 54, 55, 56, or comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. In certain embodiments: (a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11; (b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4; (c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3, 6, 53, 54, 55, or 56, or encodes an RNA comprising or consisting of SEQ ID NO: 53, 54, 55, or 56; (d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1; (e) the plant is a corn

plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7; (f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10; (g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or, (h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9. In certain embodiments, the transgenic plant part is a flower, meristem, ovule, stem, tuber, fruit, anther, pollen, leaf, root, or seed. Processed plant products containing the transgene include, but are not limited to, a meal a pulp, a feed, or a food product obtainable from the transgenic plant parts. In certain embodiments, the processed plant products exhibit an improved attribute relative to a processed plant product of an untreated control plant and wherein the improved attribute results from the improved fungal disease resistance and/or nematode resistance conferred by the transgene. In certain embodiments, the processed product is a meal, a pulp, a feed, or a food product. Also provided herein are methods for obtaining transgenic plants exhibiting an improvement in fungal disease resistance and/or nematode resistance comprising the steps of introducing any of the aforementioned transgenes into the genome of a plant and selecting for a transgenic plant wherein expression of an endogenous PMR5 gene is suppressed, thereby obtaining a plant exhibiting an improvement in fungal disease resistance and/or nematode resistance. Also provided herein are methods for improving fungal disease resistance and/or nematode resistance in plants that comprise growing transgenic plants comprising any of the aforementioned transgenes wherein expression of an endogenous PMR5 is suppressed in the presence of fungi and/or nematodes, wherein fungal disease resistance and/or nematode resistance of the transgenic plants is improved in comparison to a control plant that lack a transgene that suppresses an endogenous PMR5 gene in the control plant.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Figure 1 presents a bootstrapped phylogenetic tree of PMR5 proteins.

[0026] Figure 2 presents barley powdery mildew disease control measurements in barley plants treated with various liquids. Certain results were obtained with liquids that contained certain nucleic acids as indicated in the labels along the X-axis.

[0027] Figure 3 presents barley powdery mildew disease control measurements (percentage of the top half of the leaf area infected) in barley plants treated with various liquids. Certain results were obtained with liquids that contained certain individual oligonucleotides as indicated in the labels along the X-axis.

[0028] Figure 4 presents barley powdery mildew disease control measurements (percentage of the leaf area infected) in barley plants treated with various liquids. Certain results were obtained with liquids that contained certain nucleic acids as indicated in the labels along the X-axis.

[0029] Figure 5 presents Root Knot Nematode disease control measurements (number of RKN eggs/gram of root tissue) in soybean plants treated with various liquids. Certain results were obtained with liquids that contained certain nucleic acids as indicated in the labels along the X-axis.

DETAILED DESCRIPTION

I. Definitions

[0030] The following definitions and methods are provided to better define the present embodiments and to guide those of ordinary skill in the art in the practice of the embodiments disclosed in the present application. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

[0031] Where a term is provided in the singular, the inventors also contemplate aspects described by the plural of that term.

[0032] As used herein, the terms "DNA," "DNA molecule," and "DNA polynucleotide molecule" refer to a single-stranded DNA or double-stranded DNA molecule of genomic or synthetic origin, such as, a polymer of deoxyribonucleotide bases or a DNA polynucleotide molecule.

[0033] As used herein, the terms "DNA sequence," "DNA nucleotide sequence," and "DNA polynucleotide sequence" refer to the nucleotide sequence of a DNA molecule.

[0034] As used herein, the term "gene" refers to any portion of a nucleic acid that provides for expression of a transcript or encodes a transcript. A "gene" thus includes, but is not limited to, a promoter region, 5' untranslated regions, transcript encoding regions that can include intronic regions, and 3' untranslated regions.

[0035] As used herein, the terms "RNA," "RNA molecule," and "RNA polynucleotide molecule" refer to a single-stranded RNA or double-stranded RNA molecule of genomic or synthetic origin, such as, a polymer of ribonucleotide bases that comprise single or double stranded regions.

[0036] Unless otherwise stated, nucleotide sequences in the text of this specification are given, when read from left to right, in the 5' to 3' direction. The nomenclature used herein is that required by Title 37 of the United States Code of Federal Regulations § 1.822 and set forth in the tables in WIPO Standard ST.25 (1998), Appendix 2, Tables 1 and 3.

[0037] As used herein, a "plant surface" refers to any exterior portion of a plant. Plant surfaces thus include, but are not limited to, the surfaces of flowers, stems, tubers, fruit, anthers, pollen, leaves, roots, or seeds. A plant surface can be on a portion of a plant that is attached to other portions of a plant or on a portion of a plant that is detached from the plant.

[0038] As used herein, the phrase "polynucleotide is not operably linked to a promoter" refers to a polynucleotide that is not covalently linked to a polynucleotide promoter sequence that is specifically recognized by either a DNA dependent RNA polymerase II protein or by a viral RNA dependent RNA polymerase in such a manner that the polynucleotide will be transcribed by the DNA dependent RNA polymerase II protein or viral RNA dependent RNA polymerase. A polynucleotide that is not operably linked to a promoter can be transcribed by a plant RNA dependent RNA polymerase.

[0039] As used herein, any polynucleotide sequences of SEQ ID NO: 12-19, 21-37, or 38, though displayed in the sequence listing in the form of ssDNA, encompass all other polynucleotide forms such as dsDNA equivalents, ssDNA equivalents, ssRNA equivalents, ssRNA complements, dsRNA, and ssDNA complements.

[0040] As used herein, a first nucleic-acid sequence is "operably" connected or "linked" with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to an RNA and/or protein-coding sequence if the promoter provides for transcription or expression of the RNA or coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, are in the same reading frame.

[0041] As used herein, the phrase "organosilicone preparation" refers to a liquid comprising one or more organosilicone compounds, wherein the liquid or components contained therein, when combined with a polynucleotide in a composition that is topically applied to a target plant surface, enable the polynucleotide to enter a plant cell. Examples of organosilicone

preparations include, but are not limited to, preparations marketed under the trade names "Silwet®" or "BREAK-THRU®" and preparations provided in Table 1. In certain embodiments, an organosilicone preparation can enable a polynucleotide to enter a plant cell in a manner permitting a polynucleotide suppression of target gene expression in the plant cell.

[0042] As used herein, the phrase "provides for an improvement in fungal disease resistance and/or nematode resistance" refers to any measurable increase in a plants resistance to fungal- and/or nematode- induced damage. In certain embodiments, an improvement in fungal disease resistance and/or nematode resistance in a plant or plant part can be determined in a comparison to a control plant or plant part that has not been treated with a composition comprising a polynucleotide and a transfer agent. When used in this context, a control plant is a plant that has not undergone treatment with polynucleotide and a transfer agent. Such control plants would include, but are not limited to, untreated plants or mock treated plants.

[0043] As used herein, the phrase "provides for a reduction", when used in the context of a transcript or a protein in a plant or plant part, refers to any measurable decrease in the level of transcript or protein in a plant or plant part. In certain embodiments, a reduction of the level of a transcript or protein in a plant or plant part can be determined in a comparison to a control plant or plant part that has not been treated with a composition comprising a polynucleotide and a transfer agent. When used in this context, a control plant or plant part is a plant or plant part that has not undergone treatment with polynucleotide and a transfer agent. Such control plants or plant parts would include, but are not limited to, untreated or mock treated plants and plant parts.

[0044] As used herein, the phrase "wherein said plant does not comprise a transgene" refers to a plant that lacks either a DNA molecule comprising a promoter that is operably linked to a polynucleotide or a recombinant viral vector.

[0045] As used herein, the phrase "suppressing expression" or "suppression", when used in the context of a gene, refers any measurable decrease in the amount and/or activity of a product encoded by the gene. Thus, expression of a gene can be suppressed when there is a reduction in levels of a transcript from the gene, a reduction in levels of a protein encoded by the gene, a reduction in the activity of the transcript from the gene, a reduction in the activity of a protein encoded by the gene, any one of the preceding conditions, or any combination of the preceding conditions. In this context, the activity of a transcript includes, but is not limited to, its ability to be translated into a protein and/or to exert any RNA-mediated

biologic or biochemical effect. In this context, the activity of a protein includes, but is not limited to, its ability to exert any protein-mediated biologic or biochemical effect. In certain embodiments, a suppression of gene expression in a plant or plant part can be determined in a comparison of gene product levels or activities in a treated plant to a control plant or plant part that has not been treated with a composition comprising a polynucleotide and a transfer agent. When used in this context, a control plant or plant part is a plant or plant part that has not undergone treatment with polynucleotide and a transfer agent. Such control plants or plant parts would include, but are not limited to, untreated or mock treated plants and plant parts.

[0046] As used herein, the term "transcript" corresponds to any RNA that is produced from a gene by the process of transcription. A transcript of a gene can thus comprise a primary transcription product which can contain introns or can comprise a mature RNA that lacks introns.

[0047] As used herein, the term "liquid" refers to both homogeneous mixtures such as solutions and non-homogeneous mixtures such as suspensions, colloids, micelles, and emulsions.

II. Overview

[0048] Provided herein are certain methods and polynucleotide compositions that can be applied to living plant cells/tissues to suppress expression of target genes and that provide improved fungal disease resistance and/or nematode resistance to a crop plant. Also provided herein are plants and plant parts exhibiting fungal disease resistance and/or nematode resistance as well as processed products of such plants or plant parts. The compositions may be topically applied to the surface of a plant, such as to the surface of a leaf, and include a transfer agent. Aspects of the method can be applied to various crops, for example, including but not limited to: i) row crop plants including, but are not limited to, corn, barley, sorghum, soybean, cotton, canola, sugar beet, alfalfa, sugarcane, rice, and wheat; ii) vegetable plants including, but not limited to, tomato, potato, sweet pepper, hot pepper, melon, watermelon, cucumber, eggplant, cauliflower, broccoli, lettuce, spinach, onion, peas, carrots, sweet corn, Chinese cabbage, leek, fennel, pumpkin, squash or gourd, radish, Brussels sprouts, tomatillo, garden beans, dry beans, or okra; iii) culinary plants including, but not limited to, basil, parsley, coffee, or tea; iv) fruit plants including but not limited to apple, pear, cherry, peach, plum, apricot, banana, plantain, table grape, wine grape, citrus, avocado, mango, or berry; v) a tree grown for ornamental or commercial use, including, but not limited to, a fruit or nut

tree; or, vi) an ornamental plant (e. g., an ornamental flowering plant or shrub or turf grass). The methods and compositions provided herein can also be applied to plants produced by a cutting, cloning, or grafting process (i. e., a plant not grown from a seed) that include fruit trees and plants. Fruit trees produced by such processes include, but are not limited to, citrus and apple trees. Plants produced by such processes include, but are not limited to, avocados, tomatoes, eggplant, cucumber, melons, watermelons, and grapes as well as various ornamental plants.

[0049] Without being bound by theory, the compositions and methods of the present embodiments are believed to operate through one or more of the several natural cellular pathways involved in RNA-mediated gene suppression as generally described in Brodersen and Voinnet (2006), *Trends Genetics*, 22:268-280; Tomari and Zamore (2005) *Genes & Dev.*, 19:517-529; Vaucheret (2006) *Genes Dev.*, 20:759-771; Meins et al. (2005) *Annu. Rev. Cell Dev. Biol.*, 21:297-318; and Jones-Rhoades et al. (2006) *Annu. Rev. Plant Biol.*, 57:19-53. RNA-mediated gene suppression generally involves a double-stranded RNA (dsRNA) intermediate that is formed intra-molecularly within a single RNA molecule or inter-molecularly between two RNA molecules. This longer dsRNA intermediate is processed by a ribonuclease of the RNAase III family (Dicer or Dicer-like ribonuclease) to one or more shorter double-stranded RNAs, one strand of which is incorporated into the RNA-induced silencing complex ("RISC"). For example, the siRNA pathway involves the cleavage of a longer double-stranded RNA intermediate to small interfering RNAs ("siRNAs"). The size of siRNAs is believed to range from about 19 to about 25 base pairs, but the most common classes of siRNAs in plants include those containing 21 to 24 base pairs (See, Hamilton et al. (2002) *EMBO J.*, 21:4671-4679).

Polynucleotides

[0050] As used herein, "polynucleotide" refers to a DNA or RNA molecule containing multiple nucleotides and generally refers both to "oligonucleotides" (a polynucleotide molecule of 18-25 nucleotides in length) and longer polynucleotides of 26 or more nucleotides. Embodiments include compositions including oligonucleotides having a length of 18-25 nucleotides (18-mers, 19-mers, 20-mers, 21-mers, 22-mers, 23-mers, 24-mers, or 25-mers), or medium-length polynucleotides having a length of 26 or more nucleotides (polynucleotides of 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 110, about 120, about 130, about

140, about 150, about 160, about 170, about 180, about 190, about 200, about 210, about 220, about 230, about 240, about 250, about 260, about 270, about 280, about 290, or about 300 nucleotides), or long polynucleotides having a length greater than about 300 nucleotides (*e. g.*, polynucleotides of between about 300 to about 400 nucleotides, between about 400 to about 500 nucleotides, between about 500 to about 600 nucleotides, between about 600 to about 700 nucleotides, between about 700 to about 800 nucleotides, between about 800 to about 900 nucleotides, between about 900 to about 1000 nucleotides, between about 300 to about 500 nucleotides, between about 300 to about 600 nucleotides, between about 300 to about 700 nucleotides, between about 300 to about 800 nucleotides, between about 300 to about 900 nucleotides, or about 1000 nucleotides in length, or even greater than about 1000 nucleotides in length, for example up to the entire length of a target gene including coding or non-coding or both coding and non-coding portions of the target gene). Where a polynucleotide is double-stranded, its length can be similarly described in terms of base pairs.

[0051] Polynucleotide compositions used in the various embodiments include compositions including oligonucleotides, polynucleotides, or a mixture of both, including: RNA or DNA or RNA/DNA hybrids or chemically modified oligonucleotides or polynucleotides or a mixture thereof. In certain embodiments, the polynucleotide may be a combination of ribonucleotides and deoxyribonucleotides, for example, synthetic polynucleotides consisting mainly of ribonucleotides but with one or more terminal deoxyribonucleotides or synthetic polynucleotides consisting mainly of deoxyribonucleotides but with one or more terminal dideoxyribonucleotides. In certain embodiments, the polynucleotide includes non-canonical nucleotides such as inosine, thiouridine, or pseudouridine. In certain embodiments, the polynucleotide includes chemically modified nucleotides. Examples of chemically modified oligonucleotides or polynucleotides are well known in the art; see, for example, U.S. Patent Publication 2011/0171287, U.S. Patent Publication 2011/0171176, U.S. Patent Publication 2011/0152353, U.S. Patent Publication 2011/0152346, and U.S. Patent Publication 2011/0160082, which are herein incorporated by reference. Illustrative examples include, but are not limited to, the naturally occurring phosphodiester backbone of an oligonucleotide or polynucleotide which can be partially or completely modified with phosphorothioate, phosphorodithioate, or methylphosphonate internucleotide linkage modifications, modified nucleoside bases or modified sugars can be used in oligonucleotide or polynucleotide synthesis, and oligonucleotides or polynucleotides can be labeled with a fluorescent moiety (*e.g.*, fluorescein or rhodamine) or other label (*e.g.*, biotin).

[0052] Polynucleotides can be single- or double-stranded RNA, single- or double-stranded DNA, double-stranded DNA/RNA hybrids, and modified analogues thereof. In certain embodiments, the polynucleotides that provide single-stranded RNA in the plant cell may be: (a) a single-stranded RNA molecule (ssRNA), (b) a single-stranded RNA molecule that self-hybridizes to form a double-stranded RNA molecule, (c) a double-stranded RNA molecule (dsRNA), (d) a single-stranded DNA molecule (ssDNA), (e) a single-stranded DNA molecule that self-hybridizes to form a double-stranded DNA molecule, (f) a single-stranded DNA molecule including a modified Pol III gene that is transcribed to an RNA molecule, (g) a double-stranded DNA molecule (dsDNA), (h) a double-stranded DNA molecule including a modified Pol III gene that is transcribed to an RNA molecule, and (i) a double-stranded, hybridized RNA/DNA molecule, or combinations thereof. In certain embodiments, these polynucleotides can comprise both ribonucleic acid residues and deoxyribonucleic acid residues. In certain embodiments, these polynucleotides include chemically modified nucleotides or non-canonical nucleotides. In certain embodiments of the methods, the polynucleotides include double-stranded DNA formed by intramolecular hybridization, double-stranded DNA formed by intermolecular hybridization, double-stranded RNA formed by intramolecular hybridization, or double-stranded RNA formed by intermolecular hybridization. In certain embodiments where the polynucleotide is a dsRNA, the anti-sense strand will comprise at least 18 nucleotides that are essentially complementary to the target gene. In certain embodiments the polynucleotides include single-stranded DNA or single-stranded RNA that self-hybridizes to form a hairpin structure having an at least partially double-stranded structure including at least one segment that will hybridize to RNA transcribed from the gene targeted for suppression. Not intending to be bound by any mechanism, it is believed that such polynucleotides are or will produce single-stranded RNA with at least one segment that will hybridize to RNA transcribed from the gene targeted for suppression. In certain embodiments, the polynucleotides can be operably linked to a promoter – generally a promoter functional in a plant, for example, a pol II promoter, a pol III promoter, a pol IV promoter, or a pol V promoter.

[0053] The polynucleotide molecules are designed to modulate expression by inducing regulation or suppression of an endogenous gene in a plant and are designed to have a nucleotide sequence essentially identical or essentially complementary to the nucleotide sequence of an endogenous gene of a plant or to the sequence of RNA transcribed from an endogenous gene of a plant, which can be coding sequence or non-coding sequence. These effective polynucleotide molecules that modulate expression are referred to herein as "a

trigger, or triggers". By "essentially identical" or "essentially complementary" it is meant that the trigger polynucleotides (or at least one strand of a double-stranded polynucleotide) have sufficient identity or complementarity to the endogenous gene or to the RNA transcribed from the endogenous gene (e.g. the transcript) to suppress expression of the endogenous gene (e.g.to effect a reduction in levels or activity of the gene transcript and/or encoded protein). Polynucleotides of the methods and compositions provided herein need not have 100 percent identity to a complementarity to the endogenous gene or to the RNA transcribed from the endogenous gene (i.e. the transcript) to suppress expression of the endogenous gene (i.e. to effect a reduction in levels or activity of the gene transcript or encoded protein). Thus, in certain embodiments, the polynucleotide or a portion thereof is designed to be essentially identical to, or essentially complementary to, a sequence of at least 18 or 19 contiguous nucleotides in either the target gene or messenger RNA transcribed from the target gene (e.g. the transcript). In certain embodiments, an "essentially identical" polynucleotide has 100 percent sequence identity or at least about 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent sequence identity when compared to the sequence of 18 or more contiguous nucleotides in either the endogenous target gene or to an RNA transcribed from the target gene (e.g. the transcript). In certain embodiments, an "essentially complementary" polynucleotide has 100 percent sequence complementarity or at least about 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent sequence complementarity when compared to the sequence of 18 or more contiguous nucleotides in either the target gene or RNA transcribed from the target gene.

[0054] In certain embodiments, polynucleotides used in the methods and compositions provided herein can be essentially identical or essentially complementary to any of: i) conserved regions of PMR5 genes of both monocot and dicot plants; ii) conserved regions of PMR5 genes of monocot plants; or iii) conserved regions of PMR5 genes of dicot plants. Such polynucleotides that are essentially identical or essentially complementary to such conserved regions can be used to improve fungal disease resistance and/or nematode disease resistance by suppressing expression of PMR5 genes in any of: i) both dicot and monocot plants, including, but not limited to, corn, barley, wheat, sorghum, rice, cucumber, pea, *Medicago sp.*, soybean, pepper, tomato, and grape; ii) monocot plants, including, but not limited to, corn, barley, wheat, sorghum, and rice, and; or iii) dicot plants, including, but not limited to, cucumber, pea, *Medicago sp.*, soybean, pepper, tomato, and grape.

[0055] Polynucleotides containing mismatches to the target gene or transcript can thus be used in certain embodiments of the compositions and methods provided herein. In certain

embodiments, a polynucleotide can comprise at least 19 contiguous nucleotides that are essentially identical or essentially complementary to said gene or said transcript or comprises at least 19 contiguous nucleotides that are essentially identical or essentially complementary to the target gene or target gene transcript. In certain embodiments, a polynucleotide of 19 continuous nucleotides that is essentially identical or essentially complementary to the endogenous target gene or to RNA transcribed from the target gene (e.g. the transcript) can have 1 or 2 mismatches to the target gene or transcript. In certain embodiments, a polynucleotide of 20 or more nucleotides that contains a contiguous 19 nucleotide span of identity or complementarity to the endogenous target gene or to an RNA transcribed from the target gene can have 1 or 2 mismatches to the target gene or transcript. In certain embodiments, a polynucleotide of 21 continuous nucleotides that is essentially identical or essentially complementary to the endogenous target gene or to RNA transcribed from the target gene (e.g. the transcript) can have 1, 2, or 3 mismatches to the target gene or transcript. In certain embodiments, a polynucleotide of 22 or more nucleotides that contains a contiguous 21 nucleotide span of identity or complementarity to the endogenous target gene or to an RNA transcribed from the target gene can have 1, 2, or 3 mismatches to the target gene or transcript. In designing polynucleotides with mismatches to an endogenous target gene or to an RNA transcribed from the target gene, mismatches of certain types and at certain positions that are more likely to be tolerated can be used. In certain embodiments, mismatches formed between adenine and cytosine or guanosine and uracil residues are used as described by Du et al. Nucleic Acids Research, 2005, Vol. 33, No. 5 1671–1677. In certain embodiments, mismatches in 19 base pair overlap regions can be at the low tolerance positions 5, 7, 8 or 11 (from the 5' end of a 19 nucleotide target) with well tolerated nucleotide mismatch residues, at medium tolerance positions 3, 4, and 12-17, and/or at the high tolerance nucleotide positions at either end of the region of complementarity (i.e. positions 1, 2, 18, and 19) as described by Du et al. Nucleic Acids Research, 2005, Vol. 33, No. 5 1671–1677. It is further anticipated that tolerated mismatches can be empirically determined in assays where the polynucleotide is applied to the plants via the methods provided herein and the treated plants assayed for suppression of PMR5 expression or appearance of fungal disease resistance and/or nematode resistance.

[0056] In certain embodiments, polynucleotide molecules are designed to have 100 percent sequence identity with or complementarity to one allele or one family member of a given target gene coding or non-coding sequence of a PMR5 target gene. In other embodiments, the polynucleotide molecules are designed to have 100 percent sequence identity with or

complementarity to multiple alleles or family members of a given PMR5 target gene. In certain embodiments, the polynucleotide can thus comprise at least 18 contiguous nucleotides that are identical or complementary to SEQ ID NO: 1-19, 21-38, or 53-129. In certain embodiments, the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1-19, 21-38, or 53-128.

[0057] In certain embodiments, polynucleotide compositions and methods provided herein typically effect regulation or modulation (e. g., suppression) of gene expression during a period during the life of the treated plant of at least 1 week or longer and typically in systemic fashion. For instance, within days of treating a plant leaf with a polynucleotide composition as described herein, primary and transitive siRNAs can be detected in other leaves lateral to and above the treated leaf and in apical tissue. In certain embodiments, methods of systemically suppressing expression of a gene in a plant, the methods comprising treating said plant with a composition comprising at least one polynucleotide and a transfer agent, wherein said polynucleotide comprises at least 18 or at least 19 contiguous nucleotides that are essentially identical or essentially complementary to a gene or a transcript encoding a PMR5 gene of the plant are provided, whereby expression of the gene in said plant or progeny thereof is systemically suppressed in comparison to a control plant that has not been treated with the composition.

[0058] Compositions used to suppress a target gene can comprise one or more polynucleotides that are essentially identical or essentially complementary to multiple genes, or to multiple segments of one or more genes. In certain embodiments, compositions used to suppress a target gene can comprise one or more polynucleotides that are essentially identical or essentially complementary to multiple consecutive segments of a target gene, multiple non-consecutive segments of a target gene, multiple alleles of a target gene, or multiple target genes from one or more species.

[0059] In certain embodiments, the polynucleotide includes two or more copies of a nucleotide sequence (of 18 or more nucleotides) where the copies are arranged in tandem fashion. In another embodiment, the polynucleotide includes two or more copies of a nucleotide sequence (of 18 or more nucleotides) where the copies are arranged in inverted repeat fashion (forming an at least partially self-complementary strand). The polynucleotide can include both tandem and inverted-repeat copies. Whether arranged in tandem or inverted repeat fashion, each copy can be directly contiguous to the next, or pairs of copies can be separated by an optional spacer of one or more nucleotides. The optional spacer can be unrelated sequence (*i. e.*, not essentially identical to or essentially complementary to the

copies, nor essentially identical to, or essentially complementary to, a sequence of 18 or more contiguous nucleotides of the endogenous target gene or RNA transcribed from the endogenous target gene). Alternatively the optional spacer can include sequence that is complementary to a segment of the endogenous target gene adjacent to the segment that is targeted by the copies. In certain embodiments, the polynucleotide includes two copies of a nucleotide sequence of between about 20 to about 30 nucleotides, where the two copies are separated by a spacer no longer than the length of the nucleotide sequence.

Tiling

[0060] Polynucleotide trigger molecules can be identified by "tiling" gene targets in random length fragments, e.g. 200-300 polynucleotides in length, with partially overlapping regions, e.g. 25 or so nucleotide overlapping regions along the length of the target gene. Multiple gene target sequences can be aligned and polynucleotide sequence regions with homology in common are identified as potential trigger molecules for multiple targets. Multiple target sequences can be aligned and sequence regions with poor homology are identified as potential trigger molecules for selectively distinguishing targets. To selectively suppress a single gene, trigger sequences may be chosen from regions that are unique to the target gene either from the transcribed region or the non-coding regions, e.g., promoter regions, 3' untranslated regions, introns and the like.

[0061] Polynucleotides fragments are designed along the length of the full length coding and untranslated regions of a PMR5 gene or family member as contiguous overlapping fragments of 200-300 polynucleotides in length or fragment lengths representing a percentage of the target gene. These fragments are applied topically (as sense or anti-sense ssDNA or ssRNA, dsRNA, or dsDNA) to determine the relative effectiveness in providing the yield/quality phenotype. Fragments providing the desired activity may be further subdivided into 50-60 polynucleotide fragments which are evaluated for providing the yield/quality phenotype. The 50-60 base fragments with the desired activity may then be further subdivided into 19-30 base fragments which are evaluated for providing the yield/quality phenotype. Once relative effectiveness is determined, the fragments are utilized singly, or in combination in one or more pools to determine effective trigger composition or mixture of trigger polynucleotides for providing the yield/quality phenotype.

[0062] Coding and/or non-coding sequences of gene families in the crop of interest are aligned and 200-300 polynucleotide fragments from the least homologous regions amongst the aligned sequences are evaluated using topically applied polynucleotides (as sense or anti-

sense ssDNA or ssRNA, dsRNA, or dsDNA) to determine their relative effectiveness in providing the yield/quality phenotype. The effective segments are further subdivided into 50-60 polynucleotide fragments, prioritized by least homology, and reevaluated using topically applied polynucleotides. The effective 50-60 polynucleotide fragments are subdivided into 19-30 polynucleotide fragments, prioritized by least homology, and again evaluated for induction of the yield/quality phenotype. Once relative effectiveness is determined, the fragments are utilized singly, or again evaluated in combination with one or more other fragments to determine the trigger composition or mixture of trigger polynucleotides for providing the yield/quality phenotype.

[0063] Coding and/or non-coding sequences of gene families in the crop of interest are aligned and 200-300 polynucleotide fragments from the most homologous regions amongst the aligned sequences are evaluated using topically applied polynucleotides (as sense or anti-sense ssDNA or ssRNA, dsRNA, or dsDNA) to determine their relative effectiveness in inducing the yield/quality phenotype. The effective segments are subdivided into 50-60 polynucleotide fragments, prioritized by most homology, and reevaluated using topically applied polynucleotides. The effective 50-60 polynucleotide fragments are subdivided into 19-30 polynucleotide fragments, prioritized by most homology, and again evaluated for induction of the yield/quality phenotype. Once relative effectiveness is determined, the fragments may be utilized singly, or in combination with one or more other fragments to determine the trigger composition or mixture of trigger polynucleotides for providing the yield/quality phenotype.

[0064] Also, provided herein are methods for identifying a preferred polynucleotide for improving fungal disease and/or nematode resistance in a plant. Populations of candidate polynucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of the gene can be generated by a variety of approaches, including but not limited to, any of the tiling, least homology, or most homology approaches provided herein. Such populations of polynucleotides can also be generated or obtained from any of the polynucleotides or genes provided herewith in SEQ ID NO:1-19 or 21-38 or 53-128. Such populations of polynucleotides can also be generated or obtained from any genes that are orthologous to the genes provided herewith in SEQ ID NO:1-11. Such populations of polynucleotides can also be generated or obtained from any genes that encode proteins that are orthologous to a protein of Table 3 (SEQ ID NO:41-48, or 49). Any of the aforementioned populations of polynucleotides can also be selected by testing candidate polynucleotides for suppression of PMR5 via Viral Induced Gene Silencing (VIGS) methods.

In certain embodiments, selection of polynucleotides that can potentially provide PMR5 gene suppression when applied to plants with a transfer agent can be effected by Viral Induced Gene Silencing (VIGS) methods. In general, a candidate PMR5 suppression sequence is tested by insertion of that sequence into a cloned viral genome that can be introduced into a target plant or target plant cell to effect PMR5 suppression. Various methods and vectors used for suppression of gene targets by VIGS can be adapted for use in testing for suppression of PMR5 genes by use of appropriate candidate PMR5 suppression sequences disclosed herein. VIGS methods and vectors that can be used for performing VIGS in dicot plants include, but are not limited to, those disclosed in U.S. Patent Nos. 5,922,602, 6,635,805, 6,369,296, and 7,229,829, which are each incorporated herein by reference in their entireties with respect to their disclosure of VIGS vectors and methods. VIGS methods and vectors that can be used for performing VIGS in monocot plants include, but are not limited to, those disclosed in US Patent No. 6,800,748, which is incorporated herein by reference in its entirety with respect to its disclosure of VIGS vectors and methods. Candidate polynucleotide sequences can be tested for PMR5 suppression with VIGS vectors and methods based on cloned Hordeivirus (including, but not limited to, barley stripe mosaic virus ("BSMV"), poa semilatent virus ("PSLV"), lychnis ringspot virus ("LRSV"), and anthoxanthum latent blanching virus ("ALBV")), tobacco mosaic virus (TMV), Cucumber Green Mottle Mosaic virus watermelon strain (CGMMV-W); Brome Mosaic virus (BMV), Potyvirus (including, but not limited to, Rice Necrosis virus, and Potato Virus Y (PVY)), Rice tungro bacilliform virus (RTBV) and Geminivirus (including, but not limited to, Tomato Golden Mosaic Virus (ToGMV)) genomes. Useful ToGMV vectors that can be used are described by Revington et al. (Plant Cell. 1989 October; 1(10): 985–992). Useful methods for effecting VIGS via vacuum infiltration mediated agroinfection methods described in Yan et al. (Plant Cell Rep (2012) 31:1713–1722) can be adapted for testing candidate PMR5 suppression sequences in *Agrobacterium*-based VIGS vectors. Such polynucleotides can be topically applied to a surface of plants in a composition comprising at least one polynucleotide from said population and a transfer agent to obtain treated plants. Treated plants that exhibit suppression of the PMR5 gene and/or exhibit an improvement fungal disease and/or nematode resistance are identified, thus identifying a preferred polynucleotide that improves improving fungal disease and/or nematode resistance in a plant. Suppression of the gene can be determined by any assay for the levels and /or activity of a gene product (i.e. transcript or protein). Suitable assays for transcripts include, but are not limited to, semi-quantitative or quantitative reverse transcriptase PCR® (qRT-PCR) assays. Suitable assays

for proteins include, but are not limited to, semi-quantitative or quantitaive immunoassays, biochemical activity assays, or biological activity assays. In certain embodiments, the polynucleotides can be applied alone. In other embodiments, the polynucleotides can be applied in pools of multiple polynucleotides. When a pool of polynucleotides provides for suppression of the PMR5 gene and/or an improvement in fungal disease resistance and/or nematode disease resistance are identified, the pool can be de-replicated and retested as necessary or desired to identify one or more preferred polynucleotide(s) that improves fungal disease resistance and/or nematode disease resistance in a plant.

[0065] Methods of making polynucleotides are well known in the art. Such methods of making polynucleotides can include *in vivo* biosynthesis, *in vitro* enzymatic synthesis, or chemical synthesis. In certain embodiments, RNA molecules can be made by either *in vivo* or *in vitro* synthesis from DNA templates where a suitable promoter is operably linked to the polynucleotide and a suitable DNA –dependent RNA polymerase is provided. DNA – dependent RNA polymerases include, but are not limited to, E. coli or other bacterial RNA polymerases as well as the bacteriophage RNA polymerases such as the T7, T3, and SP6 RNA polymerases. Commercial preparation of oligonucleotides often provides two deoxyribonucleotides on the 3' end of the sense strand. Long polynucleotide molecules can be synthesized from commercially available kits, for example, kits from Applied Biosystems/Ambion (Austin, TX) have DNA ligated on the 5' end that encodes a bacteriophage T7 polymerase promoter that makes RNA strands that can be assembled into a dsRNA. Alternatively, dsRNA molecules can be produced from expression cassettes in bacterial cells that have regulated or deficient RNase III enzyme activity. Long polynucleotide molecules can also be assembled from multiple RNA or DNA fragments. In some embodiments design parameters such as Reynolds score (Reynolds et al. *Nature Biotechnology* 22, 326 - 330 (2004) and Tuschl rules (Pei and Tuschl, Nature Methods 3(9): 670-676, 2006) are known in the art and are used in selecting polynucleotide sequences effective in gene silencing. In some embodiments random design or empirical selection of polynucleotide sequences is used in selecting polynucleotide sequences effective in gene silencing. In some embodiments the sequence of a polynucleotide is screened against the genomic DNA of the intended plant to minimize unintentional silencing of other genes.

[0066] While there is no upper limit on the concentrations and dosages of polynucleotide molecules that can be useful in the methods and compositions provided herein, lower effective concentrations and dosages will generally be sought for efficiency. The concentrations can be adjusted in consideration of the volume of spray or treatment applied to

plant leaves or other plant part surfaces, such as flower petals, stems, tubers, fruit, anthers, pollen, leaves, roots, or seeds. In one embodiment, a useful treatment for herbaceous plants using 25-mer polynucleotide molecules is about 1 nanomole (nmol) of polynucleotide molecules per plant, for example, from about 0.05 to 1 nmol polynucleotides per plant. Other embodiments for herbaceous plants include useful ranges of about 0.05 to about 100 nmol, or about 0.1 to about 20 nmol, or about 1 nmol to about 10 nmol of polynucleotides per plant. In certain embodiments, about 40 to about 50 nmol of a ssDNA polynucleotide are applied. In certain embodiments, about 0.5 nmol to about 2 nmol of a dsRNA is applied. In certain embodiments, a composition containing about 0.5 to about 2.0 mg/mL, or about 0.14 mg/mL of dsRNA or ssDNA (21-mer) is applied. In certain embodiments, a composition of about 0.5 to about 1.5 mg/mL of a long dsRNA polynucleotide (i.e. about 50 to about 200 or more nucleotides) is applied. In certain embodiments, about 1 nmol to about 5 nmol of a dsRNA is applied to a plant. In certain embodiments, the polynucleotide composition as topically applied to the plant contains the at least one polynucleotide at a concentration of about 0.01 to about 10 milligrams per milliliter, or about 0.05 to about 2 milligrams per milliliter, or about 0.1 to about 2 milligrams per milliliter. In certain embodiments, a composition of about 0.5 to about 1.5 mg/mL of a long dsRNA polynucleotide (i.e. about 50 to about 200 or more nucleotides) is applied. Very large plants, trees, or vines may require correspondingly larger amounts of polynucleotides. When using long dsRNA molecules that can be processed into multiple oligonucleotides, lower concentrations can be used. To illustrate embodiments, the factor 1X, when applied to oligonucleotide molecules is arbitrarily used to denote a treatment of 0.8 nmol of polynucleotide molecule per plant; 10X, 8 nmol of polynucleotide molecule per plant; and 100X, 80 nmol of polynucleotide molecule per plant.

[0067] The polynucleotide compositions described herein are useful in compositions, such as liquids that comprise polynucleotide molecules, alone or in combination with other components either in the same liquid or in separately applied liquids that provide a transfer agent. As used herein, a transfer agent is an agent that, when combined with a polynucleotide in a composition that is topically applied to a target plant surface, enables the polynucleotide to enter a plant cell. In certain embodiments, a transfer agent is an agent that conditions the surface of plant tissue, e. g., seeds, leaves, stems, roots, flowers, or fruits, to permeation by the polynucleotide molecules into plant cells. The transfer of polynucleotides into plant cells can be facilitated by the prior or contemporaneous application of a polynucleotide-transferring agent to the plant tissue. In some embodiments the transferring agent is applied subsequent to the application of the polynucleotide composition. The

polynucleotide transfer agent enables a pathway for polynucleotides through cuticle wax barriers, stomata and/or cell wall or membrane barriers into plant cells. Suitable transfer agents to facilitate transfer of the polynucleotide into a plant cell include agents that increase permeability of the exterior of the plant or that increase permeability of plant cells to oligonucleotides or polynucleotides. Such agents to facilitate transfer of the composition into a plant cell include a chemical agent, or a physical agent, or combinations thereof. Chemical agents for conditioning or transfer include (a) surfactants, (b) an organic solvent or an aqueous solution or aqueous mixtures of organic solvents, (c) oxidizing agents, (d) acids, (e) bases, (f) oils, (g) enzymes, or combinations thereof. Embodiments of the method can optionally include an incubation step, a neutralization step (e.g., to neutralize an acid, base, or oxidizing agent, or to inactivate an enzyme), a rinsing step, or combinations thereof. Embodiments of agents or treatments for conditioning of a plant to permeation by polynucleotides include emulsions, reverse emulsions, liposomes, and other micellar-like compositions. Embodiments of agents or treatments for conditioning of a plant to permeation by polynucleotides include counter-ions or other molecules that are known to associate with nucleic acid molecules, *e. g.*, inorganic ammonium ions, alkyl ammonium ions, lithium ions, polyamines such as spermine, spermidine, or putrescine, and other cations. Organic solvents useful in conditioning a plant to permeation by polynucleotides include DMSO, DMF, pyridine, *N*-pyrrolidine, hexamethylphosphoramide, acetonitrile, dioxane, polypropylene glycol, other solvents miscible with water or that will dissolve phosphonucleotides in non-aqueous systems (such as is used in synthetic reactions). Naturally derived or synthetic oils with or without surfactants or emulsifiers can be used, *e. g.*, plant-sourced oils, crop oils (such as those listed in the 9th Compendium of Herbicide Adjuvants, publicly available on the worldwide web (internet) at herbicide.adjuvants.com can be used, *e. g.*, paraffinic oils, polyol fatty acid esters, or oils with short-chain molecules modified with amides or polyamines such as polyethyleneimine or *N*-pyrrolidine. Transfer agents include, but are not limited to, organosilicone preparations.

[0068] In certain embodiments, an organosilicone preparation that is commercially available as Silwet® L-77 surfactant having CAS Number 27306-78-1 and EPA Number: CAL.REG.NO. 5905-50073-AA, and currently available from Momentive Performance Materials, Albany, New York can be used to prepare a polynucleotide composition. In certain embodiments where a Silwet L-77 organosilicone preparation is used as a pre-spray treatment of plant leaves or other plant surfaces, freshly made concentrations in the range of about 0.015 to about 2 percent by weight (wt percent) (*e. g.*, about 0.01, 0.015, 0.02, 0.025,

0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.5 wt percent) are efficacious in preparing a leaf or other plant surface for transfer of polynucleotide molecules into plant cells from a topical application on the surface. In certain embodiments of the methods and compositions provided herein, a composition that comprises a polynucleotide molecule and an organosilicone preparation comprising Silwet L-77 in the range of about 0.015 to about 2 percent by weight (wt percent) (*e. g.*, about 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.5 wt percent) is used or provided. In certain embodiments of the methods and compositions provided herein, a composition that comprises a polynucleotide molecule and an organosilicone preparation comprising Silwet L-77 in the range of about 0.3 to about 1 percent by weight (wt percent) or about 0.5 to about 1% by weight (wt percent) is used or provided.

[0069] In certain embodiments, any of the commercially available organosilicone preparations provided in the following Table 1 can be used as transfer agents in a polynucleotide composition. In certain embodiments where an organosilicone preparation of Table 1 is used as a pre-spray treatment of plant leaves or other surfaces, freshly made concentrations in the range of about 0.015 to about 2 percent by weight (wt percent) (*e. g.*, about 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.5 wt percent) are efficacious in preparing a leaf or other plant surface for transfer of polynucleotide molecules into plant cells from a topical application on the surface. In certain embodiments of the methods and compositions provided herein, a composition that comprises a polynucleotide molecule and an organosilicone preparation of Table 1 in the range of about 0.015 to about 2 percent by weight (wt percent) (*e. g.*, about 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.5 wt percent) is used or provided.

[0070] Table 1. Examples of organosilicone preparations

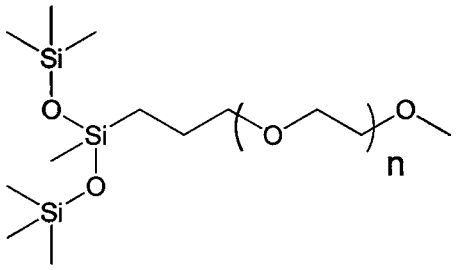
Name	CAS number	Manufacturer [1,2]
BREAK-THRU® S 321	na	Evonik Industries AG
BREAK-THRU ® S 200	67674-67-3	Evonik Industries AG
BREAK-THRU ® OE 441	68937-55-3	Evonik Industries AG
BREAK-THRU ® S 278	27306-78-1	Evonik Goldschmidt
BREAK-THRU ® S 243	na	Evonik Industries AG
Silwet® L-77	27306-78-1	Momentive Performance Materials
Silwet® HS 429	na	Momentive Performance Materials
Silwet® HS 312	na	Momentive Performance Materials
BREAK-THRU ® S 233	134180-76-0	Evonik Industries AG
Silwet® HS 508		Momentive Performance Materials
Silwet® HS 604		Momentive Performance Materials

[1] Evonik Industries AG, Essen, Germany

[2] Momentive Performance Materials, Albany, New York

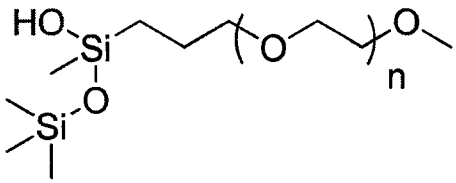
[0071] Organosilicone preparations used in the methods and compositions provided herein can comprise one or more effective organosilicone compounds. As used herein, the phrase "effective organosilicone compound" is used to describe any organosilicone compound that is found in an organosilicone preparation that enables a polynucleotide to enter a plant cell. In certain embodiments, an effective organosilicone compound can enable a polynucleotide to enter a plant cell in a manner permitting a polynucleotide mediated suppression of a target gene expression in the plant cell. In general, effective organosilicone compounds include, but are not limited to, compounds that can comprise: i) a trisiloxane head group that is covalently linked to, ii) an alkyl linker including, but not limited to, an n-propyl linker, that is covalently linked to, iii) a poly glycol chain, that is covalently linked to, iv) a terminal group. Trisiloxane head groups of such effective organosilicone compounds include, but are not limited to, heptamethyltrisiloxane. Alkyl linkers can include, but are not limited to, an n-propyl linker. Poly glycol chains include, but are not limited to, polyethylene glycol or polypropylene glycol. Poly glycol chains can comprise a mixture that provides an average chain length "n" of about "7.5". In certain embodiments, the average chain length "n" can vary from about 5 to about 14. Terminal groups can include, but are not limited to, alkyl groups such as a methyl group. Effective organosilicone compounds are believed to include,

but are not limited to, trisiloxane ethoxylate surfactants or polyalkylene oxide modified heptamethyl trisiloxane.



(Compound I: polyalkyleneoxide heptamethyltrisiloxane, average n=7.5).

One organosilicone compound believed to be ineffective comprises the formula:



[0072] In certain embodiments, an organosilicone preparation that comprises an organosilicone compound comprising a trisiloxane head group is used in the methods and compositions provided herein. In certain embodiments, an organosilicone preparation that comprises an organosilicone compound comprising a heptamethyltrisiloxane head group is used in the methods and compositions provided herein. In certain embodiments, an organosilicone composition that comprises Compound I is used in the methods and compositions provided herein. In certain embodiments, an organosilicone composition that comprises Compound I is used in the methods and compositions provided herein. In certain embodiments of the methods and compositions provided herein, a composition that comprises a polynucleotide molecule and one or more effective organosilicone compound in the range of about 0.015 to about 2 percent by weight (wt percent) (*e. g.*, about 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, 0.055, 0.06, 0.065, 0.07, 0.075, 0.08, 0.085, 0.09, 0.095, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.5 wt percent) is used or provided.

[0073] In certain embodiments, the polynucleotide compositions that comprise an organosilicone preparation can comprise a salt such as ammonium chloride, tetrabutylphosphonium bromide, and/or ammonium sulfate. Ammonium chloride, tetrabutylphosphonium bromide, and/or ammonium sulfate can be provided in the polynucleotide composition at a concentration of about 0.5% to about 5% (w/v). An ammonium chloride, tetrabutylphosphonium bromide, and/or ammonium sulfate concentration of about 1% to about 3%, or about 2% (w/v) can also be used in the polynucleotide compositions that comprise an organosilicone preparation. In certain embodiments, the polynucleotide compositions can comprise an ammonium salt at a concentration greater or equal to 300 millimolar. In certain embodiments, the polynucleotide compositions that comprise an organosilicone preparation can comprise ammonium sulfate at concentrations from about 80 to about 1200 mM or about 150 mM to about 600 mM.

[0074] In certain embodiments, the polynucleotide compositions can also comprise a phosphate salt. Phosphate salts used in the compositions include, but are not limited to, calcium, magnesium, potassium, or sodium phosphate salts. In certain embodiments, the polynucleotide compositions can comprise a phosphate salt at a concentration of at least about 5 millimolar, at least about 10 millimolar, or at least about 20 millimolar. In certain embodiments, the polynucleotide compositions will comprise a phosphate salt in a range of about 1mM to about 25mM or in a range of about 5mM to about 25mM. In certain embodiments, the polynucleotide compositions can comprise sodium phosphate at a concentration of at least about 5 millimolar, at least about 10 millimolar, or at least about 20 millimolar. In certain embodiments, the polynucleotide compositions can comprise sodium phosphate at a concentration of about 5 millimolar, about 10 millimolar, or about 20 millimolar. In certain embodiments, the polynucleotide compositions will comprise a sodium phosphate salt in a range of about 10mM to about 160mM or in a range of about 20mM to about 40mM. In certain embodiments, the polynucleotide compositions can comprise a sodium phosphate buffer at a pH of about 6.8.

[0075] In certain embodiments, other useful transfer agents or adjuvants to transfer agents that can be used in polynucleotide compositions provided herein include surfactants and/or effective molecules contained therein. Surfactants and/or effective molecules contained therein include, but are not limited to, sodium or lithium salts of fatty acids (such as tallow or tallowamines or phospholipids) and organosilicone surfactants. In certain embodiments, the polynucleotide compositions that comprise a transfer agent are formulated with counter-ions or other molecules that are known to associate with nucleic acid molecules. Illustrative

examples include, but are not limited to, tetraalkyl ammonium ions, trialkyl ammonium ions, sulfonium ions, lithium ions, and polyamines such as spermine, spermidine, or putrescine. In certain embodiments, the polynucleotide compositions are formulated with a non-polynucleotide herbicide. Non-polynucleotide herbicidal molecules include, but are not limited to, glyphosate, auxin-like benzoic acid herbicides including dicamba, chloramben and TBA, glufosinate, auxin-like herbicides including phenoxy carboxylic acid herbicide, pyridine carboxylic acid herbicide, quinoline carboxylic acid herbicide, pyrimidine carboxylic acid herbicide, and benazolin-ethyl herbicide, sulfonylureas, imidazolinones, bromoxynil, delapon, cyclohezanedione, protoporphyrionogen oxidase inhibitors, and 4-hydroxyphenyl-pyruvate-dioxygenase inhibiting herbicides.

[0076] In certain embodiments, the polynucleotides used in the compositions that are essentially identical or essentially complementary to the PMR5 target gene or transcript will comprise the predominant nucleic acid in the composition. Thus in certain embodiments, the polynucleotides that are essentially identical or essentially complementary to the target gene or transcript will comprise at least about 50%, 75%, 95%, 98%, or 100% of the nucleic acids provided in the composition by either mass or molar concentration. However, in certain embodiments, the polynucleotides that are essentially identical or essentially complementary to the target gene or transcript can comprise at least about 1% to about 50%, about 10% to about 50%, about 20% to about 50%, or about 30% to about 50% of the nucleic acids provided in the composition by either mass or molar concentration. Also provided are compositions where the polynucleotides that are essentially identical or essentially complementary to the target gene or transcript can comprise at least about 1% to 100%, about 10% to 100%, about 20% to about 100%, about 30% to about 50%, or about 50% to a 100% of the nucleic acids provided in the composition by either mass or molar concentration.

[0077] Polynucleotides comprising ssDNA, dsDNA, ssRNA, dsRNA, or RNA/DNA hybrids that are essentially identical or complementary to certain plant target genes or transcripts and that can be used in compositions containing transfer agents that include, but are not limited to, organosilicone preparations, to suppress those target genes when topically applied to plants are disclosed in co-assigned U.S. Patent Application No. 13/042,856 (US20110296556). Various polynucleotide herbicidal molecules, compositions comprising those polynucleotide herbicidal molecules and transfer agents that include, but are not limited to, organosilicone preparations, and methods whereby herbicidal effects are obtained by the topical application of such compositions to plants are also disclosed in co-assigned U.S. Patent Application No. 13/042,856, and those polynucleotide herbicidal molecules,

compositions, and methods are incorporated herein by reference in their entireties. Genes encoding proteins that can provide tolerance to an herbicide and/or that are targets of a herbicide are collectively referred to herein as "herbicide target genes". Herbicide target genes include, but are not limited to, a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a glyphosate oxidoreductase (GOX), a glyphosate decarboxylase, a glyphosate-N-acetyl transferase (GAT), a dicamba monooxygenase, a phosphinothricin acetyltransferase, a 2,2- dichloropropionic acid dehalogenase, an acetohydroxyacid synthase, an acetolactate synthase, a haloarylnitrilase, an acetyl-coenzyme A carboxylase (ACCase), a dihydropteroate synthase, a phytoene desaturase (PDS), a protoporphyrin IX oxygenase (PPO), a hydroxyphenylpyruvate dioxygenase (HPPD), a para-aminobenzoate synthase, a glutamine synthase, a cellulose synthase, a beta tubulin, and a serine hydroxymethyltransferase gene. The effects of applying certain compositions comprising polynucleotides that are essentially identical or complementary to certain herbicide target genes and transfer agents on plants containing the herbicide target genes was shown to be potentiated or enhanced by subsequent application of an herbicide that targets the same gene as the polynucleotide in co-assigned U.S. Patent Application No. 13/042,856 (US20110296556).. For example, compositions comprising polynucleotides targeting the EPSPS herbicide target gene were potentiated by glyphosate in experiments disclosed in co-assigned U.S. Patent Application No. 13/042,856 (US20110296556).

[0078] In certain embodiments of the compositions and methods disclosed herein, the composition comprising a polynucleotide and a transfer agent can thus further comprise a second polynucleotide comprising at least 19 contiguous nucleotides that are essentially identical or essentially complementary to a transcript to a protein that confers resistance to a herbicide. In certain embodiments, the second polynucleotide does not comprise a polynucleotide that is essentially identical or essentially complementary to a transcript encoding a protein of a target plant that confers resistance to said herbicidal molecule. Thus, in an non-limiting embodiment, the second polynucleotide could be essentially identical or essentially complementary to a transcript encoding a protein that confers resistance to a herbicide in a weed (such as an EPSPS encoding transcript) but would not be essentially identical or essentially complementary to a transcript encoding a protein that confers resistance to that same herbicide in a crop plant.

[0079] In certain embodiments, the polynucleotide compositions that comprise a transfer agent can comprise glycerin. Glycerin can be provided in the composition at a concentration of about 0.1% to about 1% (w/v or v/v). A glycerin concentration of about 0.4% to about

0.6%, or about 0.5% (w/v or v/v) can also be used in the polynucleotide compositions that comprise a transfer agent.

[0080] In certain embodiments, the polynucleotide compositions that comprise a transfer agent can further comprise organic solvents. Such organic solvents include, but are not limited to, DMSO, DMF, pyridine, N-pyrrolidine, hexamethylphosphoramide, acetonitrile, dioxane, polypropylene glycol, other solvents miscible with water or that will dissolve phosphonucleotides in non-aqueous systems (such as is used in synthetic reactions).

[0081] In certain embodiments, the polynucleotide compositions that comprise a transfer agent can further comprise naturally derived or synthetic oils with or without surfactants or emulsifiers. Such oils include, but are not limited to, plant-sourced oils, crop oils (such as those listed in the 9th Compendium of Herbicide Adjuvants, publicly available on line at www.herbicide.adjuvants.com), paraffinic oils, polyol fatty acid esters, or oils with short-chain molecules modified with amides or polyamines such as polyethyleneimine or N-pyrrolidine.

[0082] In some embodiments, methods include one or more applications of the composition comprising a polynucleotide and a transfer agent or one or more effective components contained therein. In certain embodiments of the methods, one or more applications of a transfer agent or one or more effective components contained therein can precede one or more applications of the composition comprising a polynucleotide and a transfer agent. In embodiments where a transfer agent and/or one or more effective molecules contained therein is used either by itself as a pre-treatment or as part of a composition that includes a polynucleotide, embodiments of the polynucleotide molecules are double-stranded RNA oligonucleotides, single-stranded RNA oligonucleotides, double-stranded RNA polynucleotides, single-stranded RNA polynucleotides, double-stranded DNA oligonucleotides, single-stranded DNA oligonucleotides, double-stranded DNA polynucleotides, single-stranded DNA polynucleotides, chemically modified RNA or DNA oligonucleotides or polynucleotides or mixtures thereof.

[0083] Compositions and methods as described herein are useful for modulating or suppressing the expression of an endogenous PMR5 target gene or transgenic PMR5 target gene in a plant cell or plant. In certain embodiments of the methods and compositions provided herein, expression of PMR5 target genes can be suppressed completely, partially and/or transiently to result in an improvement in in fungal disease resistance and/or nematode resistance. In various embodiments, a PMR5 target gene includes coding (protein-coding or translatable) sequence, non-coding (non-translatable) sequence, or both coding and non-

coding sequence. In some embodiments, compositions can include polynucleotides and oligonucleotides designed to target multiple PMR5 genes, or multiple segments of one or more PMR5 genes. The target gene can include multiple consecutive segments of a target PMR5 gene, multiple non-consecutive segments of a PMR5 target gene, multiple alleles of a target gene, or multiple PMR5 target genes from one or more species. PMR5 target genes include, but are not limited to, the endogenous PMR5 plant genes of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. PMR5 target genes include, but are not limited to, PMR5 plant genes that encode proteins that are orthologous to the proteins of SEQ ID NO:41-48, or 49. PMR5 target genes include, but are not limited to, PMR5 plant genes that encode the proteins of SEQ ID NO:41-48, or 49 or essentially homologous proteins having between about 1, 2, 3,4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, deletions, or insertions.

[0084] Target genes and plants containing those target genes can be obtained from: i) row crop plants including, but are not limited to, corn, soybean, cotton, canola, sugar beet, alfalfa, sugarcane, rice, and wheat; ii) vegetable plants including, but not limited to, tomato, potato, sweet pepper, hot pepper, melon, watermelon, cucumber, eggplant, cauliflower, broccoli, lettuce, spinach, onion, peas, carrots, sweet corn, Chinese cabbage, leek, fennel, pumpkin, squash or gourd, radish, Brussels sprouts, tomatillo, garden beans, dry beans, or okra; iii) culinary plants including, but not limited to, basil, parsley, coffee, or tea; iv) fruit plants including but not limited to apple, pear, cherry, peach, plum, apricot, banana, plantain, table grape, wine grape, citrus, avocado, mango, or berry; v) a tree grown for ornamental or commercial use, including, but not limited to, a fruit or nut tree; or, vi) an ornamental plant (e.g., an ornamental flowering plant or shrub or turf grass). The methods and compositions provided herein can also be applied to plants produced by a cutting, cloning, or grafting process (i.e., a plant not grown from a seed) include fruit trees and plants that include, but are not limited to, citrus, apples, avocados, tomatoes, eggplant, cucumber, melons, watermelons, and grapes as well as various ornamental plants. Such row crop, vegetable, culinary, fruit, tree, or ornamental plants exhibiting improvements in fungal disease resistance and/or nematode resistance that result from suppressing PMR5 gene expression are provided herein. Such row crop, vegetable, culinary, fruit, tree, or ornamental plant parts or processed plant products exhibiting improvements in fungal disease resistance and/or nematode resistance that result from suppressing PMR5 gene expression are also provided herein. Such plant parts can include, but are not limited to, flowers, stems, tubers, fruit, anthers, meristems, ovules, pollen, leaves, or seeds. Such processed plant products obtained from the plant parts can include, but are not limited to, a meal, a pulp, a feed, or a food product.

[0085] A method for modulating or suppressing expression of an PMR5 gene in a plant including (a) conditioning of a plant to permeation by polynucleotides and (b) treatment of the plant with the polynucleotide molecules, wherein the polynucleotide molecules include at least one segment of 18 or more contiguous nucleotides cloned from or otherwise identified from the PMR5 target gene in either anti-sense or sense orientation, whereby the polynucleotide molecules permeate the interior of the plant and induce modulation of the target gene is provided. The conditioning and polynucleotide application can be performed separately or in a single step. When the conditioning and polynucleotide application are performed in separate steps, the conditioning can precede or can follow the polynucleotide application within minutes, hours, or days. In some embodiments more than one conditioning step or more than one polynucleotide molecule application can be performed on the same plant. In embodiments of the method, the segment can be cloned or identified from (a) coding (protein-encoding), (b) non-coding (promoter and other gene related molecules), or (c) both coding and non-coding parts of the PMR5 target gene. Non-coding parts include DNA, such as promoter regions or the RNA transcribed by the DNA that provide RNA regulatory molecules, including but not limited to: introns, 5' or 3' untranslated regions, and microRNAs (miRNA), trans-acting siRNAs, natural anti-sense siRNAs, and other small RNAs with regulatory function or RNAs having structural or enzymatic function including but not limited to: ribozymes, ribosomal RNAs, t-RNAs, aptamers, and riboswitches. In certain embodiments where the polynucleotide used in the composition comprises a promoter sequence essentially identical to, or essentially complementary to, at least 18 contiguous nucleotides of the promoter of the endogenous target gene, the promoter sequence of the polynucleotide is not operably linked to another sequence that is transcribed from the promoter sequence.

[0086] Compositions comprising a polynucleotide and a transfer agent provided herein can be topically applied to a plant or plant part by any convenient method, e.g., spraying or coating with a powder, or with a liquid composition comprising any of an emulsion, suspension, or solution. Such topically applied sprays or coatings can be of either all or of any a portion of the surface of the plant or plant part. Similarly, compositions that comprise a transfer agent or other pre-treatment can in certain embodiments be applied to the plant or plant part by any convenient method, e.g., spraying or wiping a solution, emulsion, or suspension. Compositions comprising a polynucleotide and a transfer agent provided herein can be topically applied to plant parts that include, but are not limited to, flowers, stems, tubers, meristems, ovules, fruit, anthers, pollen, leaves, or seeds.

[0087] Application of compositions comprising a polynucleotide and a transfer agent to seeds is specifically provided herein. Seeds can be contacted with such compositions by spraying, misting, immersion, and the like.

[0088] In certain embodiments, application of compositions comprising a polynucleotide and a transfer agent to plants, plant parts, or seeds in particular can provide for an improvement in fungal disease resistance and/or nematode resistance in progeny plants, plant parts, or seeds derived from those treated plants, plant parts, or seeds. In certain embodiments, progeny plants, plant parts, or seeds derived from those treated plants, plant parts, or seeds will exhibit an improvement in fungal disease resistance and/or nematode resistance that result from suppressing expression of a PMR5 gene. In certain embodiments, the methods and compositions provided herein can provide for an improvement in fungal disease resistance and/or nematode resistance in progeny plants or seeds as a result of epigenetically inherited suppression of PMR5 expression. In certain embodiments, such progeny plants exhibit an improvement in fungal disease resistance and/or nematode resistance from epigenetically inherited suppression of PMR5 gene expression that is not caused by a transgene where the polynucleotide is operably linked to a promoter, a viral vector, or a copy of the polynucleotide that is integrated into a non-native location in the chromosomal DNA of the plant. Without seeking to be limited by theory, progeny plants or seeds derived from those treated plants, plant parts, or seeds can exhibit an improvement in an improvement in fungal disease resistance and/or nematode resistance through an epigenetic mechanism that provides for propagation of an epigenetic condition where suppression of PMR5 gene expression occurs in the progeny plants, plant parts, or plant seeds. In certain embodiments, progeny plants or seeds exhibiting an improvement in fungal disease resistance and/or nematode resistance as a result of epigenetically inherited suppression of PMR5 gene expression can also exhibit increased methylation, and in particular, increased methylation of cytosine residues, in the endogenous PMR5 gene of the plant. Plant parts, including seeds, of the progeny plants that exhibit an improvement in an improvement in fungal disease resistance and/or nematode resistance as a result of epigenetically inherited suppression of PMR5 gene expression, can also in certain embodiments exhibit increased methylation, and in particular, increased methylation of cytosine residues, in the endogenous PMR5 gene. In certain embodiments, DNA methylation levels in DNA encoding the endogenous PMR5 gene can be compared in plants that exhibit an improvement in fungal disease resistance and/or nematode resistance and control plants that do not exhibit an improvement in fungal disease resistance and/or nematode resistance to correlate the presence of the an improvement in fungal disease

resistance and/or nematode resistance to epigenetically inherited suppression of PMR5 gene expression and to identify plants that comprise the epigenetically inherited improvement in fungal disease resistance and/or nematode resistance.

[0089] Various methods of spraying compositions on plants or plant parts can be used to topically apply to a plant surface a composition comprising a polynucleotide that comprises a transfer agent. In the field, a composition can be applied with a boom that extends over the crops and delivers the composition to the surface of the plants or with a boomless sprayer that distributes a composition across a wide area. Agricultural sprayers adapted for directional, broadcast, or banded spraying can also be used in certain embodiments. Sprayers adapted for spraying particular parts of plants including, but not limited to, leaves, the undersides of leaves, flowers, stems, male reproductive organs such as tassels, meristems, pollen, ovules, and the like can also be used. Compositions can also be delivered aerially, such as by a crop dusting airplane. In certain embodiments, the spray can be delivered with a pressurized backpack sprayer calibrated to deliver the appropriate rate of the composition. In certain embodiments, such a backpack sprayer is a carbon dioxide pressurized sprayer with a 11015 flat fan or equivalent spray nozzle with a customized single nozzle assembly (to minimize waste) at a spray pressure of about 0.25 MPa and/or any single nozzle sprayer providing an effective spray swath of 60 cm above the canopy of 3 to 12 inch tall growing plants can be used. Plants in a greenhouse or growth chamber can be treated using a track sprayer or laboratory sprayer with a 11001XR or equivalent spray nozzle to deliver the sample solution at a determined rate. An non-limiting rate is about 140 L/ha at about 0.25 MPa pressure.

[0090] In certain embodiments, it is also contemplated that a plant part can be sprayed with the composition comprising a polynucleotide that comprises a transfer agent. Such plant parts can be sprayed either pre-or post-harvest to provide for an improvement in fungal disease resistance and/or nematode resistance in the plant part that results from suppression of PMR5 gene expression. Compositions can be topically applied to plant parts attached to a plant by a spray as previously described. Compositions can be topically applied to plant parts that are detached from a plant by a spray as previously described or by an alternative method. Alternative methods for applying compositions to detached parts include, but are not limited to, passing the plant parts through a spray by a conveyor belt or trough, or immersing the plant parts in the composition.

[0091] Compositions comprising polynucleotides and transfer agents can be applied to plants or plant parts at one or more developmental stages as desired and/or as needed. Application of compositions to pre-germination seeds and/or to post-germination seedlings is provided in

certain embodiments. Seeds can be treated with polynucleotide compositions provided herein by methods including, but not limited to, spraying, immersion, or any process that provides for coating, imbibition, and/or uptake of the polynucleotide composition by the seed. Seeds can be treated with polynucleotide compositions using seed batch treatment systems or continuous flow treatment systems. Seed coating systems are at least described in U.S. Patent Numbers 6,582,516, 5,891,246, 4,079,696, and 4,023,525. Seed treatment can also be effected in laboratory or commercial scale treatment equipment such as a tumbler, a mixer, or a pan granulator. A polynucleotide composition used to treat seeds can contain one or more other desirable components including, but not limited to liquid diluents, binders to serve as a matrix for the polynucleotide, fillers for protecting the seeds during stress conditions, and plasticizers to improve flexibility, adhesion and/or spreadability of the coating. In addition, for oily polynucleotide compositions containing little or no filler, drying agents such as calcium carbonate, kaolin or bentonite clay, perlite, diatomaceous earth or any other adsorbent material can be added. Use of such components in seed treatments is described in U.S. Patent No. 5,876,739. Additional ingredients can be incorporated into the polynucleotide compositions used in seed treatments. Such ingredients include but are not limited to: conventional sticking agents, dispersing agents such as methylcellulose (Methocel A15LV or Methocel A15C, for example, serve as combined dispersant/sticking agents for use in seed treatments), polyvinyl alcohol (e.g., Elvanol 51-05), lecithin (e.g., Yelkinol P), polymeric dispersants (e.g., polyvinylpyrrolidone/vinyl acetate PVPNA S-630), thickeners (e.g., clay thickeners such as Van Gel B to improve viscosity and reduce settling of particle suspensions), emulsion stabilizers, surfactants, antifreeze compounds (e.g., urea), dyes, colorants, and the like that can be combined with compositions comprising a polynucleotide and a transfer agent. Further ingredients used in compositions that can be applied to seeds can be found in McCutcheon's, vol. 1, "Emulsifiers and Detergents," MC Publishing Company, Glen Rock, N.J., U.S.A., 1996 and in McCutcheon's, vol. 2, "Functional Materials," MC Publishing Company, Glen Rock, N.J., U.S.A., 1996. Methods of applying compositions to seeds and pesticidal compositions that can be used to treat seeds are described in U.S. Patent Application publication 20080092256, which is incorporated herein by reference in its entirety.

[0092] Application of the compositions in early, mid-, and late vegetative stages of plant development is provided in certain embodiments. Application of the compositions in early, mid-and late reproductive stages is also provided in certain embodiments. Application of the compositions to plant parts at different stages of maturation is also provided.

[0093] The following examples are included to demonstrate examples of certain embodiments. Those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLES

Example 1. Polynucleotides related to the PMR5 target gene sequences.

[0094] The target PMR5 genes and/or transcripts are provided in Table 2 and the sequence listing. Such genes, and protein sequences encoded by the PMR5 genes (Table 3), can be used to identify orthologous PMR5 genes and transcripts from other plants not provided herewith. Such orthologous genes and their transcripts can then serve as targets of polynucleotides provided herein or as a source of polynucleotides that are specifically designed to target the orthologous genes or transcripts.

[0095] The target PMR5 polynucleotide molecule at least occurs in the genome of plants provided in Table 2. The PMR5 genes provided in Table 3, or their corresponding transcripts, can be used as targets of polynucleotide compositions comprising a polynucleotide that of at least 18 contiguous nucleotides that are essentially identical or essentially complementary to those genes or transcripts. The proteins and genes respectively provided in Tables 2 and 3, or sequences contained within those proteins or genes can also be used to obtain orthologous PMR5 genes from plants not listed in Tables 2 and 3. Such orthologous genes and their transcripts can then serve as targets of polynucleotides provided herein or as a source of polynucleotides that are specifically designed to target the orthologous genes or transcripts.

[0096] Table 2. Target PMR5 gene sequences

SEQ ID NO:1	*Lactuca sativa* (lettuce)	Contains PMR5-like gene coding region from lettuce
SEQ ID NO:2	*Solanum lycopersicum* (tomato)	Contains PMR5-like gene coding region from tomato
SEQ ID NO:3	*Cucumis sativus* (cucumber)	Contains PMR5-like gene coding region from cucumber
SEQ ID NO:4	*Hordeum vulgare* (barley)	Contains PMR5-like gene coding region from barley
SEQ ID NO:5	*Triticum aestivum* (wheat)	Contains PMR5-like gene coding region from wheat
SEQ ID NO:6	*Cucumis sp* (cucumber)	Contains PMR5-like gene coding region from cucumber

SEQ ID NO:7	*Zea mays* (corn)	Contains PMR5-like gene coding region from corn
SEQ ID NO:8	*Glycine max* (soybean)	Contains PMR5-like gene coding region from soybean
SEQ ID NO:9	*Oryza sativa* (rice)	Contains PMR5-like gene coding region from rice
SEQ ID NO:10	*Solanum lycopersicum* (tomato)	Contains PMR5-like promoter and 5' untranslated region from tomato PMR5-like gene
SEQ ID NO:11	*Glycine max* (soybean)	Contains PMR5-like promoter and 5' untranslated region from soybean PMR5-like gene

[0097] Table 3. Target gene encoded protein sequences

SEQ ID NO:	***Species***	**Description**
SEQ ID NO:41	*Lactuca sativa*	PMR5-like protein encoded by SEQ ID NO:41
SEQ ID NO:42	*Solanum lycopersicum*	PMR5-like protein encoded by SEQ ID NO:42
SEQ ID NO:43	*Cucumis sativus*	PMR5-like protein encoded by SEQ ID NO:43
SEQ ID NO:44	*Hordeum vulgare*	PMR5-like protein encoded by SEQ ID NO:44
SEQ ID NO:45	*Triticum aestivum*	PMR5-like protein encoded by SEQ ID NO:45
SEQ ID NO:46	*Cucumis sp*	PMR5-like protein encoded by SEQ ID NO:46
SEQ ID NO:47	*Zea mays*	PMR5-like protein encoded by SEQ ID NO:47
SEQ ID NO:48	*Glycine max*	PMR5-like protein encoded by SEQ ID NO:48
SEQ ID NO:49	*Oryza sativa*	PMR5-like protein encoded by SEQ ID NO:49

[0098] The sequence listing contains the target PMR5 DNA sequences from the indicated plant species of Table 2. For each gene having a DNA sequence provided in the sequence listing and listed in Table 2, polynucleotides such as single stranded or double stranded DNA or RNA fragments in sense and/or antisense orientation will be mixed with an organosilicone preparation. These compositions will be topically applied to plants to effect expression of the target genes in the specified plant to obtain the plants that exhibit disease resistance. In particular, plants that are resistant to powdery mildew, downy mildew, and/or rust infection and/or nematodes will be obtained through the application of such compositions.

Example 2. Polynucleotides that can be used to reduce PMR5 expression in various plants.

[0099] An set of polynucleotides that can be used to reduce expression of PMR5 genes in various plants is provided herewith as SEQ ID NOS: 12-19, 21-37, or 38. The SEQ ID NOS: 12-19, 21-37, or 38 describe ssDNA oligonucleotides and sense/antisense double stranded RNA targeted to the coding regions of PMR5 sequences from soybean and barley that are useful for downregulating PMR5 expression using methods described here. Other regions of

PMR5 genes can also be targeted to modify expression including the use of antisense DNA oligonucleotides against coding regions and/or targeting promoter regions using sense/antisense dsRNA, sense or antisense ssDNA as well as sense/antisense double stranded DNA. For example, a polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 can be used to downregulate expression of those PMR5 genes.

Example 3. Topical oligonucleotide application and powdery mildew testing methods

[00100] Barley seeds (Perry variety) are planted about ¼" into soil in 2 inch pots in the growth chamber and grown at 25°C with a 16hr light cycle in 50% humidity. Before polynucleotide application the plants are randomized. Application of polynucleotides (either ssDNA oligos and/or dsRNA) is performed by pipet application where 5μL of solution containing nucleotides is applied to both sides of the first leaf. The nucleotide solution applied consists of ~3-15 nmol of each ssDNA oligonucleotide or ~0.5-1 nmol dsRNA, 0.1-0.3% Silwet L-77, 5mM $NaPO_4$, and 1% AMS in Gibco ultra-pure water. Examples of polynucleotides include polynucleotides that comprise at least 18 contiguous nucleotides that are essentially identical or complementary to SEQ ID NO:4. Examples of polynucleotides also include polynucleotides of SEQ ID NO:21-37, and 38. Two days post treatment seedlings are infected with barley powdery mildew (*Blumeria graminis* f. sp. *hordei*). The growth chamber settings for the infection are as follows: 23°C, with a 12 hr light cycle in 70% humidity. At seven days post infection disease severity is scored for the percentage of leaf area covered with powdery mildew.

[00101] Data is analyzed using ANOVA Single Factor Analysis (α=0.1). The ½ LSD is calculated and custom error bars created for the bar graphs. Percent disease reduction is compared to formulation blank and nucleic acid control.

[00102] Cucumber seeds are planted in a 3-inch square pot and thinned to one plant per pot after emergence. When the first true leaf is fully expanded and the second leaf is opening, a polynucleotide solution such as ssDNA and/or dsRNA oligos directed to the promoter and/or targeting the coding region of a target gene of interest is applied to the first true leaf or the cotyledons. Examples of polynucleotides include polynucleotides that comprise at least 18 contiguous nucleotides that are essentially identical or complementary to SEQ ID NO:3 or 6. The nucleotide solution applied consists of 6-20nm of each ssDNA oligonucleotide or 0.5-4 nm dsRNA, 0.1 to 0.3 % L77 Silwet, 50mM $NaPO_4$ in a final volume of 40 microliters of water. Two days later the entire cucumber plant is inoculated with a shower of dry spores of

cucumber powdery mildew (*Sphaerotheca fuliginea*) shaken off diseased plants. Disease severity will be evaluated on the treated leaf and succeeding leaves 10 days later and at subsequent intervals.

[00103] Tomato seeds are planted in a 3-inch square pot and thinned to one plant per pot after emergence. Two weeks old tomato seedlings are treated with 6-20nm of each ssDNA oligonucleotide or 0.5- 4 nm dsRNA, 0.2 – 0.5% L77 Silwet, 50mM $NaPO_4$, 1% ammonium sulfate in a final volume of 30 microliters of water. Examples of polynucleotides include polynucleotides that comprise at least 18 contiguous nucleotides that are essentially identical or complementary to SEQ ID NO:2 or 10. Two to 4 days post spraying plants are inoculated with dry spores of tomato powdery mildew (*Oidium neolycopersici*) and 13 days post infection, disease development is scored for the percentage of leaf area covered with powdery mildew.

Example 4. Protection of Barley from powdery mildew by topical application of pooled ssDNA oligonucleotides.

[00104] Barley seeds are planted in 2 inch pots in the greenhouse essentially as described in Example 3. Six days later, barley seedlings were treated with the indicated oligos or a control formulation according to the method outlined in Table 4, where 4µl of the indicated solution is applied to both sides of a leaf. Treatment 1 -12 methods with the indicated ssDNA oligonucleotide(s) are described in Table 5. The description of the ssDNA oligonucleotides used is provided in Table 6. About 2 days post treatment, the seedlings were infected with spores of barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) and 7 days post infection, disease development was scored for the percentage of leaf area covered with powdery mildew. Results of this analysis are shown in Figures 1, 2 and corresponding Tables 7 and 8, respectively. ANOVA statistical calculations for the Table 7 are shown in Table 9 and the corresponding graph with LSD bar is shown in in Figure 1. ANOVA statistical calculations for the Table 8 are shown in Table 10 and the corresponding graph with LSD bar is shown in Figure 2.

[00105] Table 4. Formulation

Component	Final Concentration
PMR5 or Control Trigger Oligonucleotide	1.52nmol
NaPO4	5mM
AMS (Ammonium	1%

Sulfate)	
Silwet	0.25%

[00106] Table 5. Treatments

Trmnt	Trigger Type	Nucleotide ID#	Nucleotide Conc.	target gene	region	reps
1	X	Non Treated	X	X	X	5
2	X	Blank	X	X	X	5
3	ssDNA_AS	T4783-85[1]	.51nmol/oligo X 3 oligos	X	X	5
4	ssDNA_AS	GFP_AS[2] (SEQ ID NO:20)	1.52nmol/oligo	X	X	5
5	ssDNA_AS	DEGEN[3]	1.52nmol/oligo	X	X	5
6	ssDNA_AS	T4211 (SEQ ID NO: 39)	1.52nmol/oligo	MLO[4]	CDS	5
7	ssDNA_AS	T9154-56 (SEQ ID NO:21, 22, 23)	.51nmol/oligo X 3 oligos	PMR5	CDS	5
8	ssDNA_AS	T9157-59 (SEQ ID NO: 24, 25, 26)	.51nmol/oligo X 3 oligos	PMR5	CDS	5
9	ssDNA_AS	T9160-62 (SEQ ID NO: 27, 28, 29)	.51nmol/oligo X 3 oligos	PMR5	CDS	5
10	ssDNA_AS	T9163-65 (SEQ ID NO:30, 31, 32)	.51nmol/oligo X 3 oligos	PMR5	CDS	5
11	ssDNA_AS	T9166-68 (SEQ ID NO: 33, 34, 35)	.51nmol/oligo X 3 oligos	PMR5	CDS	5
12	ssDNA_AS	T9169-71 (SEQ ID NO: 33, 34, 35	.51nmol/oligo X 3 oligos	PMR5	CDS	5

[1] T4783-85 are randomly generated oligos that do not match cucumber, cotton, tomato, melon, lettuce, barley, soybean, maize genomes at >=20 bp with 100% identity (negative control).

[2] GFP_AS is an oligonucleotide (SEQ ID NO:20) directed against the *Aqueoria* Green Fluorescent Protein (negative control).

[3] DEGEN are a mixture of degenerate oligonucleotides (negative control).

[4] MLO is an positive control oligonucleotide (SEQ ID NO:39) that targets a barley (Mildew Resistance Locus O) gene(s).

[00107] Table 6. Oligonucleotides Used

Oligo name	Sequence	SEQ ID NO:	Length
T4782	ATGGGGGCTCCCGTTAATCCGAAGA	40	25
T4783	AGCGCCGGTAGCGAGCATACGTATG	50	25
T4784	ACGACTCTGCTTATTATACTCGGTC	51	25
T4784	GACATATTAGGGGCGACGTCTCCAA	52	25
T9154	GCGGAGCCGTCGACATCGCGGACCC	21	25
T9155	CACTTGTACCCGGTGTAGCCCTCCG	22	25
T9156	AGTTGAACTCCGCGTCGATGACCGG	23	25
T9157	GGAGTCCGGGCGGCCATAGAGCTGG	24	25
T9158	CGGCTTCCAGCGGTACCGGAGGTAG	25	25
T9159	GTCAAACCTGGGTAGCTCGCAGCTG	26	25
T9160	CTTCATCCGCGTCAAAAAGTCGGCG	27	25
T9161	TCCCCCACGAACATCACCGTCTTCC	28	25
T9162	CGACTCCCACTGGTTACGGCCCAGC	29	25
T9163	GCGCGGCGGCGTGCAGCAGGCAGAC	30	25
T9164	GGTCGGCGGAGACGAGCTGCGACGG	31	25
T9165	TCCCCTGCACCACGTCGATGTCCAC	32	25
T9166	GAGGTCCACCCAGTTGGCCCATGTG	33	25
T9167	TTATTGGGCCCAGCCAACGGGACCG	34	25
T9168	GCTCCTGGCCCATCGGCTGCGACGT	35	25
T9169	CCCTGCAGCACCGTTTTCGTCACCT	36	25
T9170	GAGCAAACGGACCGGGCTTTTCATC	37	25
T9171	CGCAGCGCCGACAGCGCCGTGATGT	38	25

[00108] Table 7. Results of Whole Leaf Assay

SUMMARY: Whole Leaf				
Groups	*Count*	*Sum*	*Average Percent Leaf Area Diseased*	*Variance*
Non Treated	10	255	25.5	213.6111
Blank	10	225	22.5	256.9444
T4783-85 (Random Oligonucleotide negative control)	10	108	10.8	106.8444
GFP_AS	10	170	17	173.3333
DEGEN	10	120	12	51.11111
T4211	10	85	8.5	39.16667
T9154-56	10	95	9.5	35.83333
T9157-59	10	58	5.8	15.73333
T9160-62	10	156	15.6	204.7111
T9163-65	10	122	12.2	223.7333
T9166-68	10	135	13.5	66.94444

T9169-71	10	105	10.5	63.61111

[00109] Table 8. Results of Top-half Leaf Analysis

SUMMARY: top half of leaf				
Groups	*Count*	*Sum*	*Average Percent Leaf Area Diseased*	*Variance*
Non Treated	10	225	22.5	390.2778
Blank	10	195	19.5	180.2778
T4783-85	10	56	5.6	60.26667
GFP_AS	10	181	18.1	219.4333
DEGEN	10	68	6.8	51.73333
T4211	10	19	1.9	8.1
T9154-56	10	31	3.1	14.98889
T9157-59	10	35	3.5	9.166667
T9160-62	10	77	7.7	48.23333
T9163-65	10	35	3.5	14.72222
T9166-68	10	55	5.5	55.83333
T9169-71	10	59	5.9	53.43333

[00110] Table 9. ANOVA Analysis of Table 7 Data (single factor)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3620.167	11	329.1060606	2.720676	0.003881	1.631129
Within Groups	13064.2	108	120.9648148			
Total	16684.37	119				
		Std of dif	48.38592593	6.955999		
		DF	1.6606			
		LSD	11.5511324			
		LSD/2	5.775566199			

[00111] Table 10. ANOVA Analysis of Table 8 Data (single factor)

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5593.667	11	508.5151515	5.515016	6.1133E-07	1.631129
Within Groups	9958.2	108	92.20555556			
Total	15551.87	119				

ANOVA						
		Std of dif	36.88222222	6.073074		
		DF	1.6606			
		LSD	10.08494592			
		LSD/2	5.04247296			

[00112] Figures 1 and 2 show that the percentage disease area was decreased numerically in plants treated with Silwet formulations containing certain barley PMR5 antisense DNA oligonucleotides relative to the Silwet formulation alone (Blank), the Silwet formulation combined with degenerate oligo mixture, or the Silwet formulation combined with a control GFP (Green Fluorescent Protein) oligonucleotide (SEQ ID NO:20).

[00113] Figure 2 shows that the percent leaf disease area was decreased by a statistically significant level in plants treated with Silwet formulations containing certain barley PMR5 antisense DNA oligonucleotides relative to the Silwet formulation alone (Blank) and the Silwet formulation combined with a control GFP (Green Fluorescent Protein) oligonucleotide (SEQ ID NO:20).

Example 5. Protection of Barley from powdery mildew by topical application of single PMR5 oligonucleotides

[00114] Barley seeds are planted in 2 inch pots in the greenhouse essentially as described in Example 3. Six days later, barley seedlings were treated with the indicated oligos or a control formulation according to the method outlined in Table 11, where 4µl of the indicated solution is applied to both sides of a leaf. Treatment 1 -9 methods with the indicated ssDNA oligonucleotide(s) are described in Table 12. The description of the ssDNA oligonucleotides used is provided in Table 13. About 2 days post oligonucleotide treatment, the seedlings were infected with spores of barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) and 7 days post infection, disease development was scored for the percentage of leaf area covered with powdery mildew. Results of this analysis are shown in Figures 3, 4 and corresponding Tables 14 and 15, respectively. ANOVA statistical calculations for the Table 14 results are shown in Table 16 and the corresponding graph with LSD bar is shown in in Figure 3. ANOVA statistical calculations for the Table 15 are shown in Table 17 and the corresponding graph with LSD bar is shown in in Figure 4.

[00115] Table 11. Formulations

Component	Final Concentration
PMR5 or Control Trigger Oligonucleotide	1.14nmol
$NaPO_4$	5mM
AMS (Ammonium Sulfate)	1%
Silwet	0.25%

[00116] Table 12. Treatments

Trtmnt	Nucleotide ID#	Nucelotide Conc.	target gene	region	reps
1	Non Treated	X	X	X	6
2	Blank	X	X	X	6
3	GFP_AS (SEQ ID NO:20)	1.14nmol/oligo	X	X	6
4	T4784 (SEQ ID NO:51)	1.14nmol/oligo	X	X	6
5	T4211 (SEQ ID NO: 39)	1.14nmol/oligo	MLO	CDS	6
6	T9157-59 oligonucleotide pool (SEQ ID NO:24, 25, 26)	.38nmol/oligo X 3 oligos	PMR5	CDS	6
7	T9157	1.14nmol/oligo	PMR5	CDS	6
8	T9158	1.14nmol/oligo	PMR5	CDS	6
9	T9159	1.14nmol/oligo	PMR5	CDS	6

[00117] Table 13. Oligonucleotides Used

Oligo name	Sequence	SEQ ID NO:	Length
T4784	ACGACTCTGCTTATTATACTCGGTC	51	25
T9157	GGAGTCCGGGCGGCCATAGAGCTGG	24	25
T9158	CGGCTTCCAGCGGTACCGGAGGTAG	25	25
T9159	GTCAAACCTGGGTAGCTCGCAGCTG	26	25

[00118] Table 14. Results of Whole Leaf Assay

Treatment	*Count*	*Sum*	*Average Percent Disease*	*Variance*
Non Treated	6	90	15	7.5
Blank	6	73	12.2	49.66667
GFP_AS	6	43.5	7.25	9.275
T4784	6	34.5	5.75	11.775
T4211	6	24	4	30

Treatment	*Count*	*Sum*	*Average Percent Disease*	*Variance*
T9157-59	6	42.5	7.08	35.14167
T9157	6	29	4.83	2.866667
T9158	6	38.5	6.42	5.941667
T9159	6	28	4.67	19.46667

[00119] Table 15. Results of Top-half Leaf Analysis

SUMMARY				
Groups	*Count*	*Sum*	*Average Percent Disease*	*Variance*
Non Treated	6	118.5	19.75	83.275
Blank	6	104	17.3	66.86667
GFP_AS	6	54.5	9.08	111.5417
T4784	6	33	5.5	15
T4211	6	6.5	1.08	0.941667
T9157-59	6	13.5	2.25	3.475
T9157	6	12	2	1.4
T9158	6	25	4.17	3.266667
T9159	6	26.5	4.42	8.241667

[00120] Table 16. ANOVA Analysis of Table 14 Data (single factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	659.2593	8	82.40741	4.321227	0.000641	1.810719
Within Groups	858.1667	45	19.07037			
Total	1517.426	53				
	Std of dif	6.35679	2.521268			
	DF	1.6808				
	LSD	4.237747				
	LSD/2	2.118873				

[00121] Table 17. ANOVA Analysis of Table 15 Data (single factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2234.759259	8	279.3449	8.551132	5.42746E-07	1.810719
Within Groups	1470.041667	45	32.66759			
Total	3704.800926	53				
		Std of dif	10.8892	3.299878		
		DF	1.6808			

Source of Variation	SS	df	MS	F	P-value	F crit
		LSD	5.546436			
		LSD/2	2.773218			

[00122] Figures 3 and 4 show that the percentage disease area was decreased numerically in plants treated with Silwet formulations containing certain barley PMR5 antisense DNA oligonucleotides relative to both the Silwet formulation alone (Blank), or the Silwet formulation combined with a control GFP (Green Fluorescent Protein) oligonucleotide (SEQ ID NO:20).

[00123] Figure 4 shows that the percentage disease area was decreased by a statistically significant level in plants treated with Silwet formulations containing certain barley PMR5 antisense DNA oligonucleotides relative to both the Silwet formulation alone (Blank) and the Silwet formulation combined with a control GFP (Green Fluorescent Protein) oligonucleotide (SEQ ID NO:20).

Example 6. Topical oligonucleotide application and nematode testing methods

Application of oligonucleotides to leaves for nematode control

[00124] Ten day old cucumber plants grown in sand are spotted with nucleotides, either ssDNA and/or dsRNA oligos directed to the promoter and/or targeting the coding region of a target gene of interest. The nucleotide solution applied consists of 6-20nm of each ssDNA oligonucleotide or 0.5-1 nm dsRNA, 0.1% L77 Silwet, 50mM NaPO4 in a final volume of 40uL water. Two cotyledon or leaves are spotted with 20uL of the nucleotide solution for a total of 40uL per plant. After 6-24 hours, 1000 vermiform eggs or 1000 J2 *Meloidogyne incognita* (RKN) are inoculated into each pot. Watering of the test plants is then restricted to only water as needed to prevent wilt for a period of 24 hours. After the 24 hour restricted watering, normal sub-irrigation watering is done for the duration of the test. Cucumber plants are harvested approximately 14 days after inoculation by washing sand off the roots. A root gall rating and visual phytotoxicity rating is assigned using the following scales: Gall rating scale (Gall: % root mass galled): 0 = 0-5%; 1 = 6-20%; 2 = 21-50%; and 3 = 51-100%. Visual phytotoxicity scale is also assigned (Vis. tox; visual reduction in root mass compared to the control): rs1 = mild stunting; rs2 = moderate stunting; rs3 = severe stunting.

[00125] Experiments in soybeans using soy cyst nematodes (SCN) are similar to the cucumber RKN assay except for the following changes. Soybean seeds are planted in 100% sand in two inch square plastic pots. The oligonucleotide solution is applied when the

soybeans show the first trifoliate beginning to emerge, about 10 to 12 days after planting. At least six hours after application of the oligonucleotide solution, the nematode soybean cyst nematode (SCN) inoculum (1000 vermiform eggs or 1000 J2s) is applied to the pots. Watering of the test plants is then restricted to only water as needed to prevent wilt for a period of 24 hours. After the 24 hour restricted watering, normal sub-irrigation watering is done for the duration of the test. Twenty eight days after inoculation the plants are harvested and cysts counted

[00126] Experiments in corn using lesion nematodes are similar to above except for the following changes. Corn plants growing in a sand:Turface mix 2:1 in 4 inch deep pots (Turface™ MVP, Profile Products, LLC., Buffalo Grove, IL) . Treatment with oligonucleotide solution is done when the plants are approximately 8-10 old. At least six hours after inoculation of the oligonucleotide solution, plants are inoculated with 2 gm of *P. scribneri* infested corn roots which are then removed from the pot after 7 days. Watering of the test plants is then restricted to only water as needed to prevent wilt for a period of 24 hours after inoculation. After the 24 hour restricted watering, normal sub-irrigation watering as needed is done for the duration of the test. 12-14 days post inoculation, plants are harvested and nematodes extracted for 6 days from the cut up roots in a mist tent.

Application of oligonucleotides to seeds for nematode control

[00127] Cucumber seeds are soaked approximately 5 - 72 hours in nucleotides, either ssDNA and/or dsRNA oligos directed to the promoter and/or targeting the coding region of a target of interest. Seeds can also be soaked in water for a few hours prior to soaking in oligonucleotide solution. Soaking solution consists of 20nm of each ssDNA nucleotide or 0.03-1nm dsRNA, .1% silwet L77, 50mM NaPO4 in a final volume 200uL in water. The radicals of the cucumber seeds emerge within 72 hours, after which the seeds are placed on germination paper until root length is approximately 2 inches. Seedlings are transplanted to sand vials for RKN inoculation 24 hours later. Ten mL dry sand is added to each vial and seedlings are planted by tilting the vial and laying the seedling in the correct orientation so that the cotyledons are just above the sand and then tilting back to cover the radicles with sand. 3.3 ml water is added to each vial and the vials placed in racks under fluorescent light banks. 500 vermiform eggs or 300 J2 RKN are inoculated in each tube in 50 uL of deionized or spring water. Harvest of the cucumber plants is performed 10 to 12 days after inoculation by washing sand off the roots. A root gall rating and visual phytotoxicity rating is assigned using the following scales: Gall rating scale (Gall: % root mass galled): 0 = 0-5%; 1 = 6-20%;

2 = 21-50%; and 3 = 51-100%. The average of the triplicate gall rating is then calculated: green = 0.00-0.33 (no galls); yellow = 0.67-1.33 (mild galling); orange = 1.67-2.33 (moderate galling); red = 2.67-3.00 (severe galling). Visual phytotoxicity scale is also assigned (Vis. tox; visual reduction in root mass compared to the control): rs1 = mild stunting; rs2 = moderate stunting; rs3 = severe stunting.

[00128] Experiments in soybeans using soy cyst nematodes (SCN) are similar to RKN assays except for the following changes. After 5 - 72 hours of soaking soybean seeds are planted in 100% sand in two inch square plastic pots. Seeds can also be soaked in water for a few hours prior to soaking in oligonucleotide solution. Seven days after planting the soybean seed, the nematode soybean cyst nematode (SCN) inoculum (1000 vermiform eggs or 1000 J2s) are applied to the pot. Watering of the test plants is then restricted to only water as needed to prevent wilt for a period of 24 hours. After the 24 hour restricted watering, normal sub-irrigation watering is done for the duration of the test. Twenty eight days after inoculation the test is harvested and cysts counted.

[00129] Experiments in corn using lesion nematodes are similar to above except for the following changes. After 5 - 72 hours of soaking corn seeds are planted in a sand:Turface mix 2:1 in 4 inch deep pots (Turface™ MVP, Profile Products, LLC., Buffalo Grove, IL). Seeds can also be soaked in water for a few hours prior to soaking in oligonucleotide solution. Inoculum of 2gm of roots *P. scribneri* infested corn roots are applied at seeding and removed from the pot after 7 days. Watering of the test plants is then restricted to only water as needed to prevent wilt for a period of 24 hours after inoculation. After the 24 hour restricted watering, normal sub-irrigation watering as needed is done for the duration of the test. 12-14 days post inoculation, plants are harvested and nematodes extracted for 6 days from the cut up roots in a mist tent.

[00130] RKN and SCN J2s are prepared from hatchbowls using the following solutions: RKN solution: 1L aerated tap water, 1ml of 50mg/ml kanamycin, 0.5ml of 20mg/ml imazalil sulfate; SCN solution: 1L aerated tap water, 1ml of 50mg/ml kanamycin, 0.5ml of 20mg/ml imazalil sulfate, 1430mg zinc sulfate.

[00131] Hatchbowls are autoclaved 6 oz bowls, lined with screen mesh and paper filter. Approximately 20ml of appropriate hatch solution is poured into each bowl. Eggs are then place in the bowls and covered with foil. The bowls are then placed in a 25 °C incubator overnight. The next day the hatched J2's are extracted, additional solution added as needed and replaced in the incubator. Each bowl is used for 2 weeks and then disposed.

Example 7. Protection of Soybean from Root Knot Nematodes (RKN)

[00132] Soybean variety W2 at the unifoliate stage were contacted with the indicated control and test solutions (Table 18) and inoculated with 2500 *Meloidogyne incognita* eggs 24 hrs later. The indicated amounts of oligomers were provided in 5mM $NaPO_4$, 1% Ammonium Sulfate, and 0.25% Silwet™ (wt percent). Approximately 50 µl of solution containing the ssDNA oligonucleotides of Table 19, in pools of 4 ssDNAs/pool, was applied to each plant and 4 plants were subjected to each treatment. Approximately 2500RKN (*M. incognita*) were inoculated into the soil about 1 day post treatment. Root weights and egg counts were recorded approximately 25 days post infection (Table 20). RKN infection was analyzed by comparing the number of eggs produced per gram of root tissue for each group as shown in Table 21 and Figure 5. ANOVA analysis of the Table 21 and Figure 5 egg/gram root data is provided in Table 22.

[00133] Table 18. Treatments

Trtmnt #	Description:	Oligos Final Conc.
1	PMR5 T6706-09 (SEQ ID NO:12, 13, 14, 15)	0.4 nmol/ul (each) 1.6 nmol/ul (total)
2	PMR5 T6714-17 (SEQ ID NO:16, 17, 18, 19)	0.4 nmol/ul (each) 1.6 nmol/ul (total)
3	GFP asDNA (SEQ ID NO:20)	1.6 nmol/ul (total)
4	blank	0 nmol/ul

[00134] Table 19. Oligonucleotides used

Oligo	Sequence	Location in Soybean PMR5 Gene (SEQ ID NO:8)	SEQ ID NO
T6706	TGTATCTGAGGTAATCAGAATCAGG	259..283	12
T6707	GAGGTCACAGTTGAGGGGTCTCCAT	285..309	13
T6708	AGAAACTCCACCCCATTGAACCTAG	311..335	14
T6709	CATCACAGTTTTGCCCTTCATTTGC	339..363	15
T6714	TGAAGAGAGCCTTGATGATCCCACC	638..662	16
T6715	CCTCCTAATTCCATATAATCCCACC	665..689	17
T6716	AGTTTCACCATAGCAGTTCTTTGTA	864..888	18
T6717	GCTGTGCCAGTGCTAGTAATTGGAG	890..914	19

[00135] Table 20. Summary of Root Weight Data

Treatment	Root Wt grams REP1	Root Wt grams REP2	Root Wt grams REP3	Root Wt grams REP4	AVG Root Wt grams	stdev
1 (PMR5 T6706-09)	13.3	13.8	11.7	15.3	13.30	1.81
2 (PMR5 T6714-17)	13.7	11.1	8.7	12.2	11.10	1.79
3 (GFP asDNA)	13	14.2	9.3	14.1	14.20	2.80
4 (blank)	13	13	11.5	11.1	13.00	1.00

[00136] Table 21. Eggs per Gram of Root

Treatment #	Oligos used	Count	Sum	Average Eggs/ gm Root	Variance
1	PMR5 T6706-09 (SEQ ID NO:12, 13, 14, 15)	4	756.1	189.025	13398.76
2	PMR5 T6714-17 (SEQ ID NO:16, 17, 18, 19)	4	226.5	56.625	697.9825
3	GFP asDNA (SEQ ID NO:20)	4	651	162.75	2425.937
4	none	4	691.3	172.825	6078.223

[00137] Table 22. ANOVA Analysis of Egg/gram Root Data

	Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
	Between Groups	43349.04	3	14449.68	2.557363	0.104065	3.490295
	Within Groups	67802.71	12	5650.226			
	Total	111151.7	15				
			df=1.782				
			std of diff=53.15				
			lsd=94.72				
			1/2lsd=47.4				

[00138] Example 8. Resistance of Cucumber seedlings to Root Knot Nematodes Cucumber seeds (Straight Eight, Burpee Seeds, Warminster, PA, USA) at 4 seeds/well were soaked in a 24 well plate (flat bottom). Soak solution of 200uL contained Ultrapure™ Gibco water, 20mM $NaPO_4$ buffer and nucleotides (either ssDNA (highest conc- 80nmol) and/or dsRNA (highest conc- 0.15nmol) oligos) for approximately 72 hours on a rockerat room temperature (~25°C). Surfactant was omitted in this assay. Controls included a negative control trigger molecule (eg. GFP or degenerate oligos that do not target endogenous cucumber sequences) and buffer alone. The radicals of the cucumber seeds emerged within 72 hours and the seeds were then placed on germination pouches (cyg™ Seed Germination Pouches, Mega International, Saint Paul, Minnesota; on the internet at mega-international.com/tech.htm) with 20mL of tap water for three days at 25°C with a 12 hr light cycle. Roots were approximately 2 inches in length and observations about effect of treatment on radicals could be assessed at this time. Seedlings were transplanted to sand vials after three days. The "QuickSand" assay was performed by adding 10 mL dry sand to each glass flat bottom vial, planting seedlings by tilting the vial, laying the seedling in an orientation so that the cotyledons are just above the sand and then tilting back to cover the radicals with sand. About 3.5 ml water was added to each vial and the vials placed in racks under fluorescent light banks at room temperature. After three days, 250 *Meloidogyne incognita* J2 were inoculated in each tube in 50 to 200 uL of aerated tap water. Harvest of the cucumber plants was performed 10 to 11 days after inoculation by washing sand off the roots. A root gall rating and visual phytotoxicity rating was assigned using the following scales: Gall rating scale (Gall: % root mass galled): 0 = 0-5%; 1 = 6-20%; 2 = 21-50%; and 3 = 51-100%. Visual phytotoxicity scale was also assigned (Vis. tox; visual reduction in root mass compared to the control): rs1 = mild stunting; rs2 = moderate stunting; rs3 = severe stunting. The following calculations are then done to determine efficacy: average of at least three replicates, standard deviation of four replicates, % reduction compared to control, single factor ANOVA test, ½ LSD value for data.

[00139] Pools of the dsRNAs T6860 (SEQ ID NO:53), T6861 (SEQ ID NO:54), T6862(SEQ ID NO:55) , and T6863 (SEQ ID NO:56) were tested for RKN control in the aforementioned cucumber seedling assay at 0.06nmol/each dsRNA and 0.15nmol/each dsRNA (Table 23).

[00140] Table 23. Control of RKN by PMR5 dsRNA pools

Treatment	Rep Score							AVG	stdev	% Reduction
PMR5 Pool T6860-63 coding (0.06nmol total)	30	35	35	25	35			32.00	4.47	30.0
PMR5 T6860-63 coding (0.15nmol total)	25	40	20					28.33	10.41	38.0
GFP control (0.15nmol total))	40	60	47.5		35	45	47.5	45.83	8.47	-0.3
No dsRNA	47.5	42.5	55	42.5	40	47.5	45	45.71	4.94	0.0

[00141] The Anova: Single factor analysis is provided in Tables 24, 25, and 26.

[00142] Table 24. Anova: Summary.

Groups	*Treatment*	*Count*	*Sum*	*Average*	*Variance*
Row 1	PMR5 T6860-63 coding (0.06nmol total)	2	36.47214	18.23607	378.8916
Row 2	PMR5 T6860-63 coding (0.15nmol total)	2	38.74166	19.37083	160.6529
Row 3	GFP control (0.15nmol total))	2	54.29895	27.14948	698.1731
Row 4	No dsRNA	2	50.6544	25.3272	831.2664

[00143] Table 25 ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	115.1635	3	38.38783	0.074216	0.970689	6.591382
Within Groups	2068.984	4	517.246			
Total	2184.148	7				

[00144] Table 26. ANOVA analysis

std of diff	8.04088	
df	*2.132*	Table
LSD	17.14316	
1/2 LSD	8.571578	

[00145] Individual dsRNA molecules (T6860 (SEQ ID NO:53), T6861 (SEQ ID NO:54), T6862 (SEQ ID NO:55), and T6863 (SEQ ID NO:56) were also tested for RKN control in the aforementioned cucumber seedling assay at 0.06nmol/each dsRNA and 0.15nmol/each dsRNA (Table 27).

[00146] Table 27. Individual dsRNA treatment results.

Treatment	**SEQ ID NO**	**Rep Score**								**AVG**	**stdev**	**% Reduction**
PMR5 T6860 (0.06nmol)	53					27.5	40	22.5	35	31.25	7.77	31.6
PMR5 T6861 (0.06nmol)	54					27.5	20	30	27.5	26.25	4.33	42.6
PMR5 T6862 (0.06nmol)	55					42.5	25	35	30	33.13	7.47	27.5
PMR5 T6863 (0.06nmol)	56					40	30	42.5	35	36.88	5.54	19.3
PMR5 T6860 (0.15nmol)	53					37.5	27.5	45	20	32.50	10.99	28.9
PMR5 T6861 (0.15nmol)	54					40	35	32.5	32.5	35.00	3.54	23.4

PMR5 T6862 (0.15nmol)	55					30	30	40	45	36.25	7.50	20.7
PMR5 T6863 (0.15nmol)	56					20	25	25	32.5	25.63	5.15	43.9
GFP control (0.15nmol)		40	60	47.5		35	45	47.5		45.83	8.47	-0.3
No dsRNA		47.5	42.5	55	42.5	40	47.5	45		45.71	4.94	0.0

Example 9. Control of *Phytophthora* Root Rot in Soybean

[00147] DNA oligos directed to either promoter (prm) or coding regions (CDS) of the PMR5 gene were tested for their ability to control *Phytophthora* root rot (PRR) on soybean. Disease development was good as the non-inoculated control roots were 3 fold larger than inoculated only roots that were not treated with oligos. In this test, all plants were fertilized with nitrogen by sub-irrigation one time before inoculation and were not chlorotic. In treatment 14, a small amount of fertilizer was also added directly to the pot before inoculation.

[00148] Root rate loss was calculated by the following:

non-inoculated root weight –treatment root weight=root weight loss.

[00149] Pool T6702-T6705 had statistically less root loss than the formulation blank, indicating that one or more of the oligos in that pool conferred control of *Phytophtora* Root Rot in soybean.

[00150] Table 28. Percentage reduction in root weight loss

					%reduction in root wt loss	
			root	root wt		
Trt#	Oligo Pool	SEQ ID NO:	wt	loss	FB	FC
1	as T6686- T6689 prm	57-60	2.3	5.1	-7.4	-17.4
2	as T6690- T6693 prm	61-64	3.2	4.1	12.2	4.1
3	as T6694- T6697 prm	65-68	3.0	4.3	8.5	0.0
4	as T6698- T6701 prm	69-72	2.3	5.0	-6.0	-15.9
5	as T6702-T6705 CDS	73-76	3.6	3.7	**20.7**	13.4
6	as T6706- T6709 CDS	77-80	2.5	4.8	-1.6	-11.0

7	as T6710- T6713 CDS	81-84	2.7	4.6	1.6	-7.6
8	as T6714- T6717 CDS	85-88	3.0	4.3	8.0	-0.6
9	as T6718- T6721 CDS	89-92	2.5	4.9	-3.2	-12.8
10	SEQ ID NO 20		3.0	4.3	8.0	0.0
11	formulation blank		2.6	4.7	0.0	-9.9
12	inoculation only		2.3	5.0		
13	not inoculated		7.3	0.0		

Legend: prm=promoter ; CDS=coding sequence

[00151] Table 29. Percentage root weight increase

			ROOT Weight		
				% root wt increase	
Trt#		SEQ ID NO:	average	FB	FC
1	as T6686-89 prm	57-60	2.3	-13.5	-25.0
2	as T6690-93 prm	61-64	3.2	22.1	5.8
3	as T6694-97 prm	65-68	3.0	15.4	0.0
4	as T6698-701 prm	69-72	2.3	-10.9	-22.8
5	as T6702-05 CDS	73-76	3.6	**37.5**	**19.2**
6	as T6706-09 CDS	77-80	2.5	-2.9	-15.8
7	as T6710-13 CDS	81-84	2.7	2.9	-10.8
8	as T6714-17 CDS	85-88	3.0	14.4	-0.8
9	as T6718-21 CDS	89-92	2.5	-5.8	-18.3
10	SEQ ID NO 20		3.0	14.4	-0.8
11	formulation blank		2.6	-1.0	-14.2
12	inoculation only		2.3	3.2	
13	not inoculated		7.3	3X larger than trt 12 or trt 14	
14	fertilized and inoculated		1.3	5.5	

Example 10. Use of VIGS to select polynucleotides that suppress expression of PMR5 genes

[00152] To select candidate polynucleotides that can potentially suppress endogenous PMR5 genes, polynucleotide sequences are introduced into a Tomato Golden Mosaic Virus (ToGMV) vector and tested for their ability to provide Virus-Induced Gene Silencing of the endogenous PMR5 gene in plants. Polynucleotide sequences that exhibit VIGS-mediated suppression of PMR5 are subsequently screened for their ability to suppress expression of PMR5 when applied to a plant with a transfer agent.

[00153] A modification of the sprout vacuum-infiltration-mediated agroinoculation method for virus-induced gene silencing protocol described in Yan et al. Plant Cell Rep (2012) 31:1713–1722 can be used. Surface sterilized tomato seeds are first germinated on ¼ Murashige-Skoog media plus Cefotaxime. After about 3 days, *Agrobacterium* component A containing ToGMoV:PMR5 Suppression Sequence and the ToGMoV component B are each separately inoculated into 10 mL Luria Broth with appropriate concentrations of spectinomycin, gentamycin, and chloramphenicol and shaken at 24°C for about 1-2 days to prepare an *Agrobacterium* inoculum containing the ToGMoV vector components. The A genome component is known to encode viral functions necessary for viral DNA replication, while the B genome component specifies functions necessary for spread of the virus through the infected plant (Revington, et al. Plant Cell. 1989 October; 1(10): 985–992). After about one to two days of growth, the *Agrobacterium* are pelleted by centrifugation and resuspended to a final OD600 of 0.05 in Infiltration Buffer (10mM MES, 10mM MgCl, 100uM Acetosyringone). The *Agrobacterium* A component and B component are mixed for use at a 1:1 ratio and an Infiltration buffer only control (Mock) is also prepared. The A and B component mixture and the mock Infiltration buffer control are then allowed to incubate at room temperature (~25°C) for 3-4 hours. About 3 mls of each sample (i.e. ToGMV vector with a given test PMR5 suppression sequence) is transferred into a small microtiter dish. Typically, 1 microtiter plate (6-24 wells) is used for each test ToGMV vector with a given test PMR5 suppression sequence and 1 microtiter plate is used for the mock control (Infiltration buffer only). About 3-5 sprouts are added to each well, a vacuum is pulled for 10 seconds and then stopped. Pulling and stopping of the vacuum is then repeated 2 more times. Vacuum infiltrated sprouts are planted in soil, taking care not to cross contaminate samples. This can be accomplished by changing gloves and using new tweezers. Planted sprouts are covered with humidity dome and left at room temperature (~25°C) overnight to recover. After a day, potted sprouts are transferred to a growth chamber. Phenotypes associated with PMR5 suppression (i.e. fungal and /or nematode resistance) can be observed by challenging potted plants that were agroinfected with a ToGMV vector containing a sequence that provides for suppression of the endogenous PMR5 gene but is not observed in plants subjected to the mock control (Infiltration buffer only) or a ToGMV vector containing a sequence that does not provide for suppression of the endogenous PMR5 gene.

What is claimed is:

1. A method for producing a plant exhibiting an improvement in fungal and/or nematode disease resistance comprising topically applying to a plant surface a composition that comprises:

a. at least one polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or to a transcript of said gene; and

b. a transfer agent, wherein said plant exhibits an improvement in fungal and/or nematode disease resistance that results from suppression of said PMR5 gene.

2. The method of claim 1, wherein said polynucleotide molecule comprises sense ssDNA, sense ssRNA, dsRNA, dsDNA, a double stranded DNA/RNA hybrid, anti-sense ssDNA, or anti-sense ssRNA.

3. The method of claim 1, wherein said polynucleotide is selected from the group consisting of SEQ ID NO: 12-19, 21-38, 53-127, and 128, or wherein said polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11.

4. The method of claim 3, wherein:

(a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11;

(b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4;

(c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3, 6, 53, 54, 55, or 56;
(d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1;
(e) the plant is a corn plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7;
(f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10;
(g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or,
(h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9.

5. The method of claim 1, wherein said composition comprises any combination of two or more polynucleotide molecules.

6. The method of claim 1, wherein said polynucleotide is at least 18 to about 24, about 25 to about 50, about 51 to about 100, about 101 to about 300, about 301 to about 500, or at least about 500 or more residues in length.

7. The method of claim 1, wherein said composition further comprises a non-polynucleotide herbicidal molecule, a polynucleotide herbicidal molecule, a polynucleotide that suppresses an herbicide target gene, an insecticide, a fungicide, a nematocide, or a combination thereof.

8. The method of claim 1, wherein said composition further comprises a non-polynucleotide herbicidal molecule and said plant is resistant to said herbicidal molecule.

9. The method of any one of claims 1-8, wherein said transfer agent comprises an organosilicone preparation.

10. The method of any one of claims 1-8, wherein said polynucleotide is not operably linked to a viral vector.

11. The method of any one of claims 1-8, wherein said polynucleotide is not integrated into the plant chromosome.

12. A plant obtained by the method of any one of claims 1-11.

13. The plant obtained by the method of claim 12, wherein a progeny plant or a plant part derived therefrom exhibits an improvement in fungal and/or nematode disease resistance.

14. A progeny plant of said plant of claim 12, wherein said progeny plant exhibits said improvement in fungal and/or nematode disease resistance.

15. A seed of said plant of claim 12, wherein said seed exhibits said improvement in fungal and/or nematode disease resistance.

16. A processed product of said plant of claim 12, wherein said processed product exhibits an improved attribute relative to a processed product of an untreated control plant and wherein said improved attribute results from said fungal and/or nematode disease resistance.

17. A processed product of said progeny plant of claim 14, wherein said processed product exhibits an improved attribute relative to a processed product of an untreated control plant and wherein said improved attribute results from said fungal and/or nematode disease resistance.

18. A processed product of said seed of claim 15, wherein said processed product exhibits an improved attribute relative to a processed product of an untreated control plant and wherein said improved attribute results from said fungal and/or nematode disease resistance.

19. A composition comprising a polynucleotide molecule that comprises at least 18

contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of said gene, wherein said polynucleotide is not operably linked to a promoter; and, b) a transfer agent.

20. The composition of claim 19, wherein said polynucleotide is selected from the group consisting of SEQ ID NO: 12-19, 21-38, 53-127, and 128, or wherein said polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11.

21. The composition of claim 19, wherein:
(a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11;
(b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4;
(c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3, or 6, 53, 54, 55, or 56;
(d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1;
(e) the plant is a corn plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7;
(f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10;
(g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or,

(h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9.

22. The composition of claim 19, wherein said polynucleotide is at least 18 to about 24, about 25 to about 50, about 51 to about 100, about 101 to about 300, about 301 to about 500, or at least about 500 or more residues in length.

23. The composition of claim 19, wherein said composition further comprises a non-polynucleotide herbicidal molecule, a polynucleotide herbicidal molecule, a polynucleotide that suppresses an herbicide target gene, an insecticide, a fungicide, a nematocide, or a combination thereof.

24. The composition of claim 19, wherein said transfer agent is an organosilicone preparation.

25. The composition of any one of claims 19-24, wherein said polynucleotide is not physically bound to a biolistic particle.

26. A method of making a composition comprising the step of combining at least:
a) a polynucleotide molecule comprising at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of a plant, wherein said polynucleotide is not operably linked to a promoter or a viral vector; and,
b) a transfer agent.

27. The method of claim 26, wherein said polynucleotide is obtained by *in vivo* biosynthesis, *in vitro* enzymatic synthesis, or chemical synthesis.

28. The method of claim 26, wherein said method further comprises combining with said polynucleotide and said transfer agent at least one of a non-polynucleotide herbicidal molecule, a polynucleotide herbicidal molecule, an insecticide, a fungicide, and/or a nematocide.

29. The method of any one of claims 26-28, wherein said transfer agent is an organosilicone preparation.

30. A method of identifying a polynucleotide for improving fungal and/or nematode disease resistance in a plant comprising;

a) selecting a population of polynucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of a plant;

b) topically applying to a surface of at least one of said plants a composition comprising at least one polynucleotide from said population and an transfer agent to obtain a treated plant; and,

c) identifying a treated plant that exhibits suppression of the PMR5 gene or exhibits an improvement in fungal and/or nematode disease resistance or exhibits an improvement in nematode resistance, thereby identifying a polynucleotide that improves fungal and/or nematode disease resistance in said plant.

31. The method of claim 30, wherein said polynucleotide is selected from the group consisting of wherein said polynucleotide is selected from the group consisting of SEQ ID NO: 12-19, 21-38, 53-127, and 128, or wherein said polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11.

32. The method of claim 30, wherein:

(a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11;

(b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4;

(c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3, or 6, 53, 54, 55, or 56;
(d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1;
(e) the plant is a corn plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7;
(f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10;
(g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or,
(h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9.

33. A plant comprising an exogenous polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of said gene, wherein said exogenous polynucleotide is not operably linked to a promoter or to a viral vector, is not integrated into the chromosomal DNA of the plant, and is not found in a non-transgenic plant; and, wherein said plant exhibits an improvement in fungal and/or nematode disease resistance that results from suppression of the PMR5 gene.

34. The plant of claim 33, wherein said plant further comprises an organosilicone compound or a component thereof.

35. The plant of claim 33 or claim 34, wherein said polynucleotide is selected from the group consisting of SEQ ID NO: 12-19, 21-38, 53-127, and 128, or wherein said polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11.

36. The plant of claim 33 or claim 34, wherein:

(a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11;

(b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4;

(c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3, or 6, 53, 54, 55, or 56;

(d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1;

(e) the plant is a corn plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7;

(f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10;

(g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or,

(h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9.

37. A plant part comprising an exogenous polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of said gene, wherein said exogenous polynucleotide is not operably linked to a promoter or to a viral vector and is not found in a non-transgenic plant; and, wherein said

plant part exhibits an improvement in fungal and/or nematode disease resistance that results from suppression of the PMR5 gene.

38. The plant part of claim 37, wherein said plant part further comprises an organosilicone compound or a metabolite thereof.

39. The plant part of claim 37 or claim 38, wherein said polynucleotide is selected from the group consisting of SEQ ID NO: 12-19, 21-38, 53-127, and 128, or wherein said polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO: 1, 2, 3, 4, 5, 6,7, 8, 9, 10, or 11.

40. The plant part of claim 37 or claim 38, wherein:

(a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11;

(b) the plant is a barley plant, the gene or the transcript is a barley PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4;

(c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:3, or 6, 53, 54, 55, or 56;

(d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1;

(e) the plant is a corn plant, the gene or the transcript is a corn PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7;

(f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or 10;

(g) the plant is a wheat plant, the gene or the transcript is a wheat PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or,
(h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9.

41. The plant part of any one of claims 37-40, wherein said plant part is a flower, meristem, ovule, stem, tuber, fruit, anther, pollen, leaf, root, or seed.

42. The plant part of claim 41, wherein said plant part is a seed.

43. A processed plant product obtained from the plant part of any one of claims 29-33 exhibiting an improved attribute relative to a processed plant product of an untreated control plant and wherein said improved attribute results from said improved disease tolerance.

44. The processed plant product of claim 43, wherein said product is a meal, a pulp, a feed, or a food product.

45. A plant that exhibits an improvement in fungal and/or nematode disease resistance, wherein said plant was topically treated with a composition that comprises:

a. at least one polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or to a transcript of said gene; and,

b. a transfer agent; and,

wherein said plant exhibits an improvement in fungal and/or nematode disease resistance that results from suppression of the PMR5 gene.

46. The plant of claim 45, wherein said transfer agent is an organosilicone preparation.

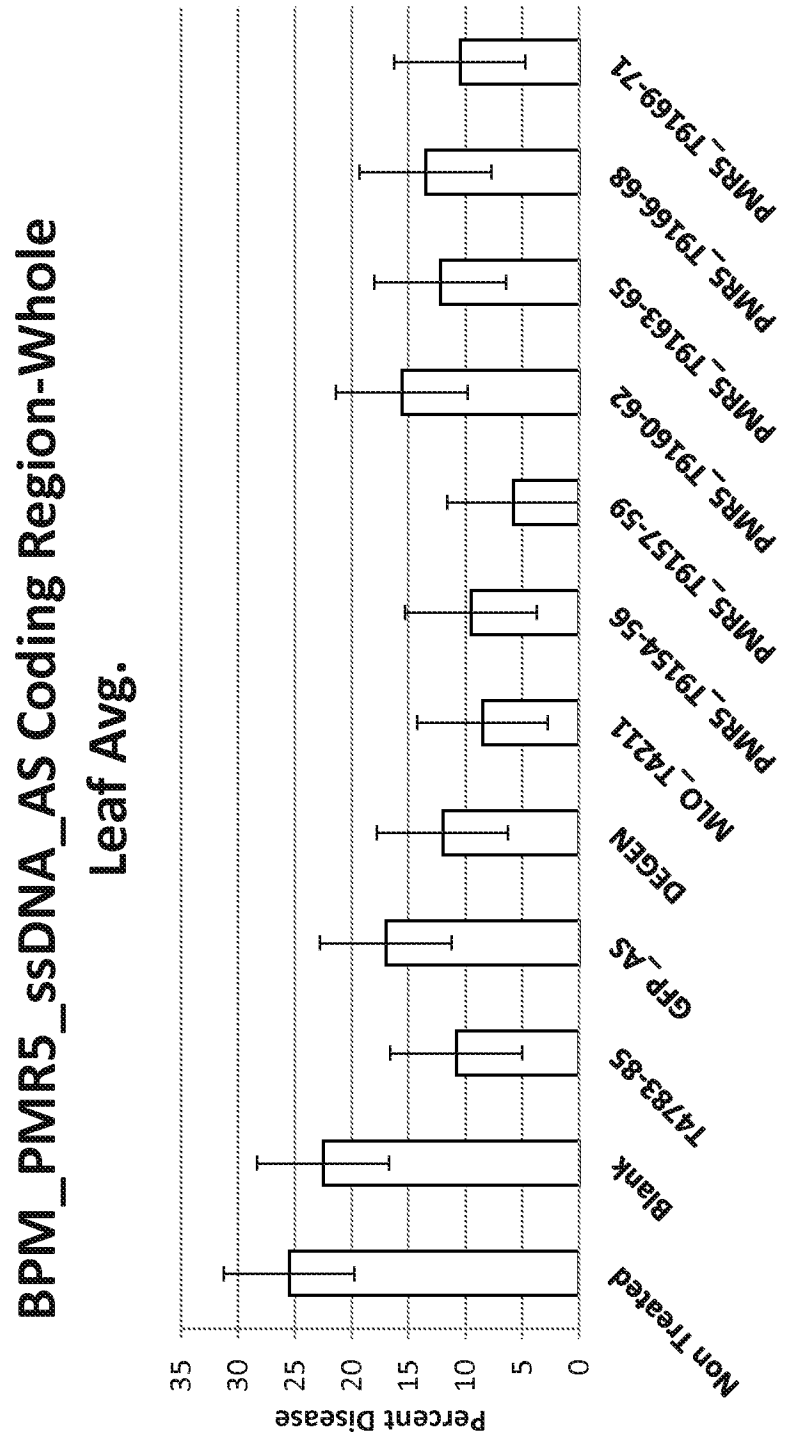

BPM_PMR5_ssDNA_AS Coding Region-Whole Leaf Avg.
Percent Disease
0
5
10
15
20
25
30
35
Non Treated
Blank
T4783-85
GFP_AS
DEGEN
MLO_T4211
PMR5_T9154-56
PMR5_T9157-59
PMR5_T9160-62
PMR5_T9163-65
PMR5_T9166-68
PMR5_T9169-71

FIG. 1

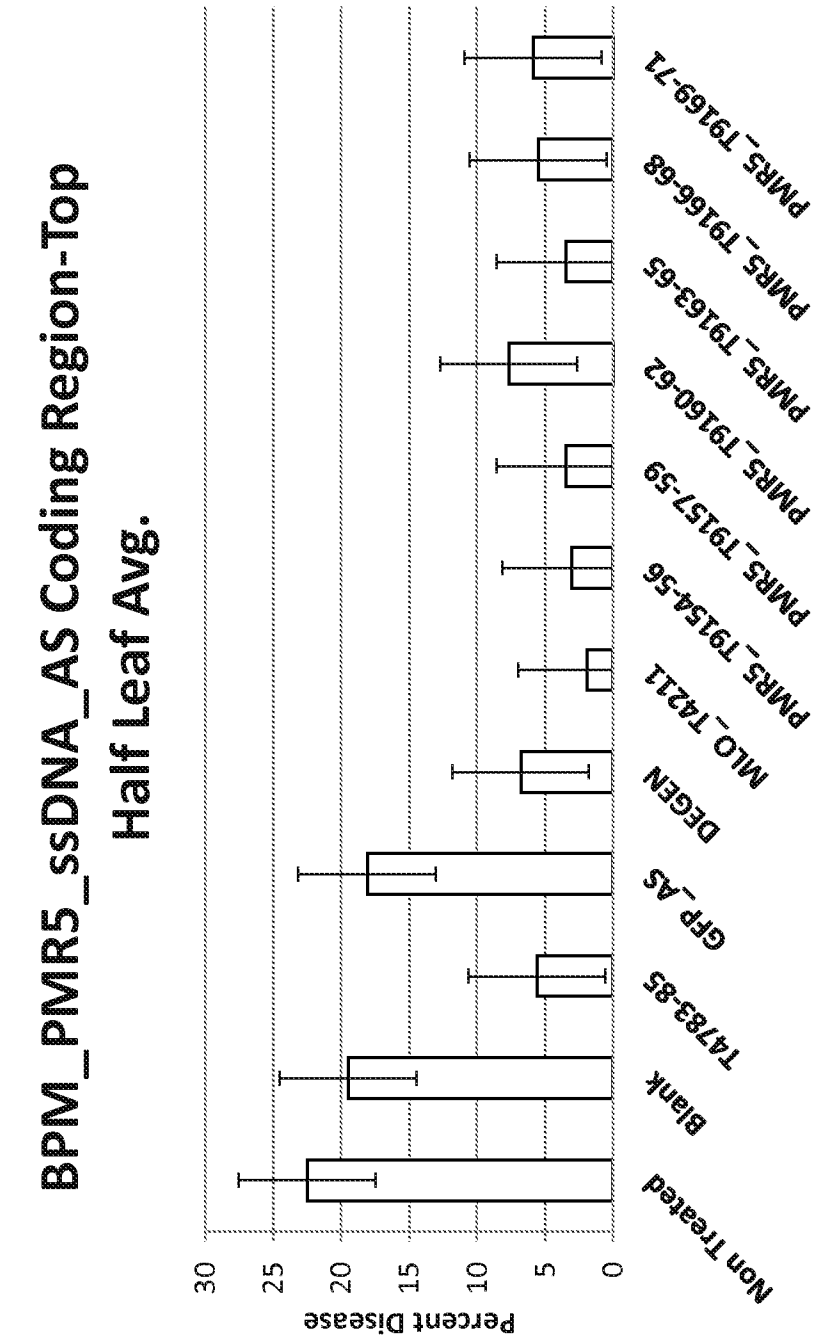

BPM_PMR5_ssDNA_AS Coding Region-Top
Half Leaf Avg.
Percent Disease
0
5
10
15
20
25
30
Non Treated
Blank
T4783-85
GFP_AS
DEGEN
MLO_T421.1
PMR5_T9154-56
PMR5_T9157-59
PMR5_T9160-62
PMR5_T9163-65
PMR5_T9166-68
PMR5_T9169-71

FIG. 2

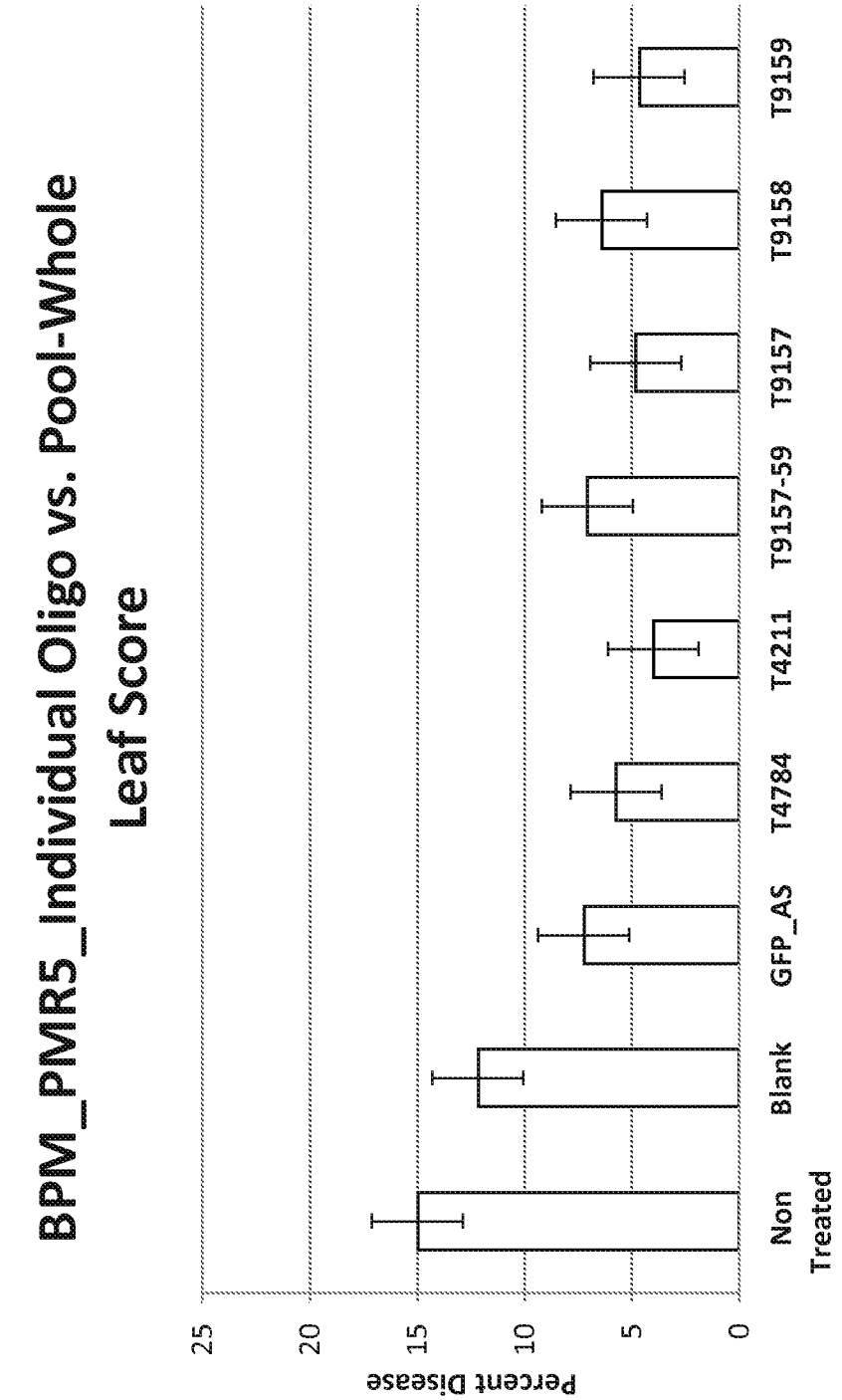

BPM_PMR5_Individual Oligo vs. Pool-Whole
Leaf Score
Percent Disease
25
20
15
10
5
0
Non Treated
Blank
GFP_AS
T4784
T4211
T9157-59
T9157
T9158
T9159

FIG. 3

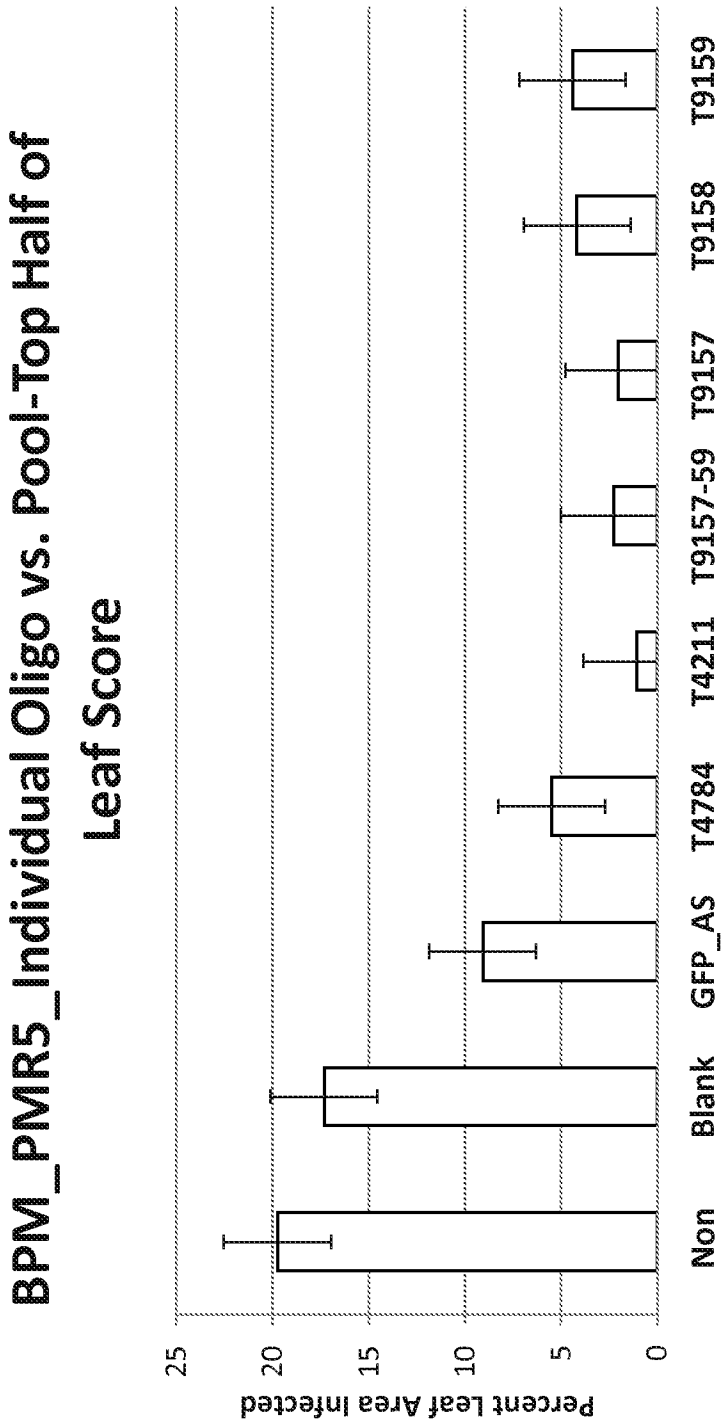

BPM_PMR5_Individual Oligo vs. Pool-Top Half of Leaf Score
Percent Leaf Area Infected
0
5
10
15
20
25
Non Treated
Blank
GFP_AS
T4784
T4211
T9157-59
T9157
T9158
T9159

FIG. 4

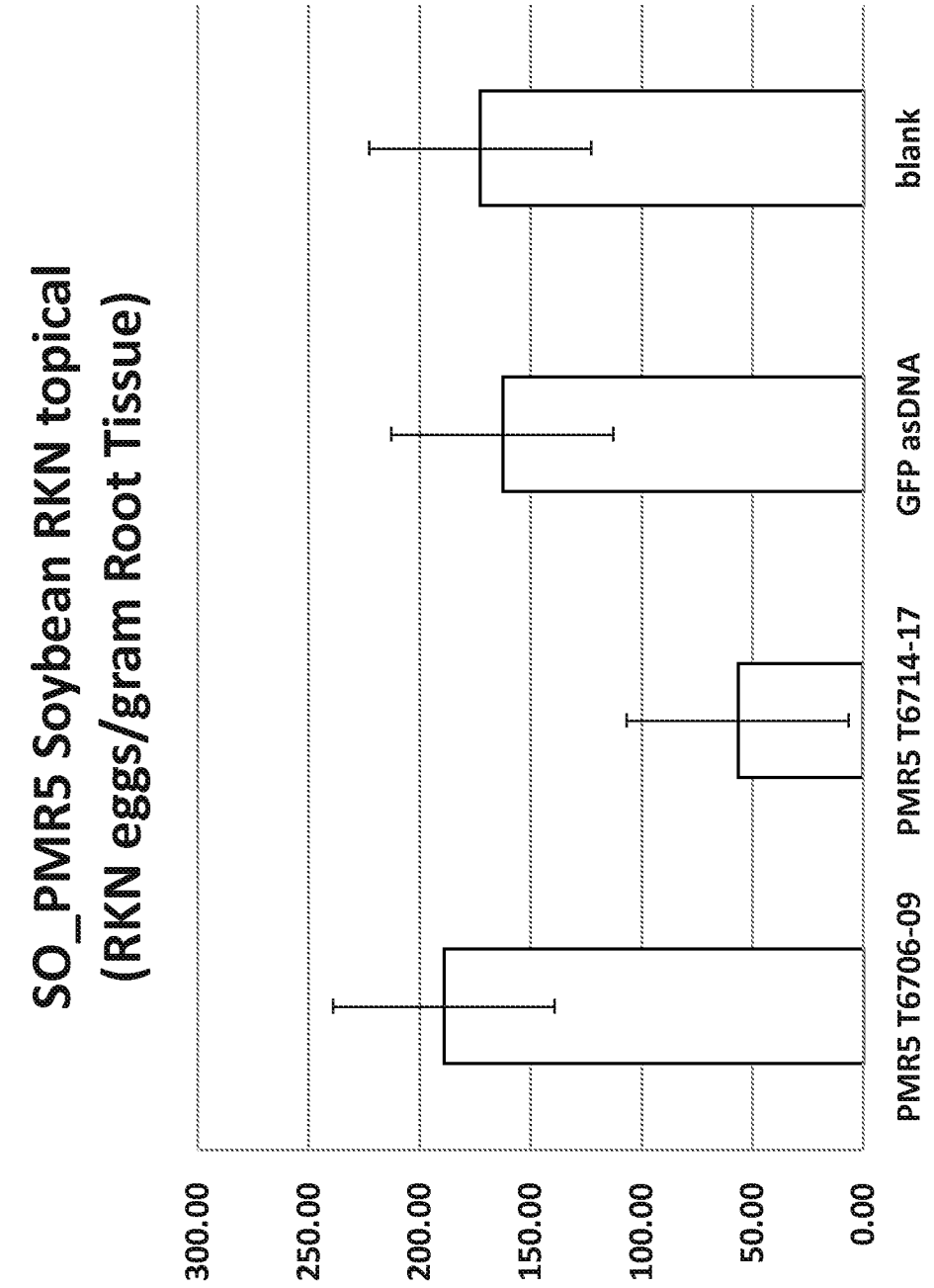

SO_PMR5 Soybean RKN topical
(RKN eggs/gram Root Tissue)
300.00
250.00
200.00
150.00
100.00
50.00
0.00
PMR5 T6706-09
PMR5 T6714-17
GFP asDNA
blank

FIG. 5

INTERNATIONAL SEARCH REPORT

PCT/US2014/011594 28.05.2014

International application No.
PCT/US2014/011594

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 63/00 (2014.01)
USPC - 435/468
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)
IPC(8) - A01N 61/00, 63/00, 65/00; C12N 15/00, 15/113, 15/29, 15/82; C12P 19/34 (2014.01)
USPC - 435/410, 419, 430, 468; 800/278, 285

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12N 15/8201, 15/8218, 15/8279, 15/8282, 15/8285 (2014.02)

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

PatBase, Google Patents, Google, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/0296556 A1 (SAMMONS et al) 01 December 2011 (01.12.2012) entire document	1, 2, 6, 9-11, 19, 22, 24-30, 33, 34, 37, 38, 45, 46
Y	YUAN et al. 'A High Throughput Barley Stripe Mosaic Virus Vector for Virus Induced Gene Silencing in Monocots and Dicots. PlosOne 6(10): 1-16 (e26468). 21 October 2011. entire document	1, 2, 6, 9-11, 19, 22, 24-30, 33, 34, 37, 38, 45, 46
A	US 6,864,404 B1 (VOGEL et al) 08 March 2005 (08.03.2005) entire document	1-4, 6, 9-11, 19-22, 24-40, 45, 46
A	WO 2012/142509 A1 (PAULY et al) 18 October 2012 (18.10.2012) entire document	1-4, 6, 9-11, 19-22, 24-40, 45, 46
P, X	US 2013/0318657 A1 (AVNIEL et al) 28 November 2013 (28.11.2013) entire document	1-4, 6, 9-11, 19-22, 24-40, 45, 46

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 May 2014	28 MAY 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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

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

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

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

3. Additional comments:

SEQ ID NO: 12 was searched.

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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.: 12-18, 41-44
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheets

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.:
1-4, 6, 9-11, 19-22, 24-40, 45, and 46, limited to SEQ ID NO: 12.

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.

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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 examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-11, 19-40, 45, and 46 are drawn to a method for producing a plant exhibiting an improvement in fungal and/or nematode disease resistance comprising topically applying to a plant surface a composition that comprises: a. at least one polynucleotide that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or to a transcript of said gene; and b. a transfer agent, wherein said plant exhibits an improvement in fungal and/or nematode disease resistance that results from suppression of said PMR5 gene; a composition and method of making a composition comprising the same; a method of identifying a polynucleotide for improving fungal and/or nematode disease resistance in a plant comprising the same; and a plant and plant part comprising the same.

The first invention of Group I+ is restricted to a method for producing a plant exhibiting an improvement in fungal and/or nematode disease resistance comprising topically applying to a plant surface a composition that consists of a. one polynucleotide selected to be SEQ ID NO: 12; and b. a transfer agent, wherein said plant exhibits an improvement in fungal and/or nematode disease resistance that results from suppression of said PMR5 gene; a composition and method of making a composition comprising the same; a method of identifying a polynucleotide for improving fungal and/or nematode disease resistance in a plant comprising the same; and a plant and plant part comprising the same. It is believed that claims 1-4, 6, 9-11, 19-22, 24-40, 45, and 46 read on this first named invention and thus these claims will be searched without fee to the extent that they read on a polynucleotide selected to be SEQ ID NO: 12.

Applicant is invited to elect additional methods and compositions with polynucleotides with specified SEQ ID NO for each polynucleotide to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be a method for producing a plant exhibiting an improvement in fungal and/or nematode disease resistance comprising topically applying to a plant surface a composition that consists of a. one polynucleotide selected to be SEQ ID NO: 13; and b. a transfer agent, wherein said plant exhibits an improvement in fungal and/or nematode disease resistance that results from suppression of said PMR5 gene; a composition and method of making a composition comprising the same; a method of identifying a polynucleotide for improving fungal and/or nematode disease resistance in a plant comprising the same; and a plant and plant part comprising the same. Additional methods and compositions will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

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

The Groups I+ formulas do not share a significant structural element, requiring the selection of alternatives for the plants, plant-specific PMR5 genes, and sequences where "(a) the plant is a soybean plant, the gene or the transcript is a soybean PMR5 gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:12-19, 57-127, and SEQ ID NO:128, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:8 or 11; (b) the plant is a barley plant, the gene or the transcript is a barley PMRS gene or transcript, and the polynucleotide molecule is selected from the group consisting of SEQ ID NO:21-37, and SEQ ID NO:38, or the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:4; (c) the plant is a cucumber plant, the gene or the transcript is a cucumber PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are
essentially identical or essentially complementary to SEQ ID NO:3, 6, 53, 54, 55, or 56; (d) the plant is a lettuce plant, the gene or the transcript is a lettuce PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:1; (e) the plant is a com plant, the gene or the transcript is a com PMRS gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:7; (f) the plant is a tomato plant, the gene or the transcript is a tomato PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:2 or; (g) the plant is a wheat plant, the gene or the transcript is a wheat PMRS gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:5; or, (h) the plant is a rice plant, the gene or the transcript is a rice PMR5 gene or transcript, and the polynucleotide comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to SEQ ID NO:9."

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The Groups I+ share the technical features of a method and composition for producing a plant exhibiting an improvement in fungal and/or nematode disease resistance comprising topically applying to a plant surface a composition that comprises: a. at least one polynucleotide molecule that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a PMR5 gene or to a transcript of said gene and b. a transfer agent, wherein said plant exhibits an improvement in fungal and/or nematode disease resistance that results from suppression of said PMR5 gene; wherein said polynucleotide is not operably linked to a promoter or a viral vector; step of combining; a method of identifying a polynucleotide for improving fungal and/or nematode disease resistance in a plant comprising; a) selecting a population of polynucleotides that are essentially identical or essentially complementary to a PMR5 gene or transcript of a plant; b) topically applying to a surface of at least one of said plants a composition comprising at least one polynucleotide from said population and an transfer agent to obtain a treated plant; and, c) identifying a treated plant that exhibits suppression of the PMRS gene or exhibits an improvement in fungal and/or nematode disease resistance or exhibits an improvement in nematode resistance, thereby identifying a polynucleotide that improves fungal and/or nematode disease resistance in said plant; a plant and a plant part comprising an exogenous polynucleotide, wherein said exogenous polynucleotide is not operably linked to a promoter or to a viral vector is not integrated into the chromosomal DNA of the plant, and is not found in a non-transgenic plant; a plant that exhibits an improvement in fungal and/or nematode disease resistance, wherein said plant was topically treated with a composition. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2011/0296556 A1 to Sammons et al., discloses a method (methods, Abstract) and composition (compositions, Para. [0004]) for producing a plant exhibiting an improvement in fungal and/or nematode disease resistance (disclosed herein are useful for regulating endogenous genes of a plant pest or pathogen, Para. [0004]; plant can be...produced, Para. [0071]; plant gene involved in responding to a pest or pathogen, thus providing control of plant pests or diseases, Para. [0074]; enhancing transfer of the polynucleotide into the interior of the plant, enhancing efficacy of the polynucleotides, Para. [0177]; challenge with an insect or nematode pest or with a viral, fungal, or bacterial pathogen, Para. [0072]) comprising topically applying to a plant surface a composition (topically applying onto a living plant a topically applied composition including polynucleotide molecules, Para. [0007]) that comprises: a. at least one polynucleotide molecule that comprises at least 18 contiguous nucleotides that are essentially identical or essentially complementary to a gene or to a transcript of said gene (As used herein, "polynucleotide" refers to a nucleic acid molecule containing multiple nucleotides...having a length of 18-25 nucleotides, Para. [0050]; The polynucleotides are designed to induce systemic regulation or suppression of an endogenous gene in a plant and are designed to have a sequence essentially identical or essentially complementary to the sequence, Para. [0054]); and b. a transfer agent (compositions are formulated with a...transferring agent, Para. [0053]; agent...includes...organosilicone, Para. [0068]), wherein said plant exhibits an improvement in fungal and/or nematode disease resistance that results from suppression of said gene (suppression of the target gene, Para. [0004]; plant gene involved in responding to a pest or pathogen, thus providing control of plant pests or diseases, Para. [0074]; enhancing transfer of the polynucleotide into the interior of the plant, enhancing efficacy of the polynucleotides, Para. [0177]); wherein said polynucleotide is not operably linked to a promoter or a viral vector (single-stranded polynucleotides functional in this invention have sequence complementarity that need not be 100% but is at least sufficient to permit hybridization to RNA transcribed from the target gene, Para. [0055]); step of combining (combinations thereof, Para. [0061]); a method of making a composition (methods of making polynucleotides, Para. [0058]; polynucleotide compositions of this invention, Para. [0059]), a method of identifying a polynucleotide (investigating reverse genetics by regulating or modulating an endogenous target gene in a plant...identify traits that can be imparted by topical application of polynucleotides, Para. [0072]; sequence of a polynucleotide is screened against the genomic DNA of the intended plant to minimize unintentional silencing of other genes, Para. [0058]) for improving fungal and/or nematode disease resistance in a plant (disclosed herein are useful for regulating endogenous genes of a plant pest or pathogen, Para. [0004]; plant can be...produced, Para. [0071]; plant gene involved in responding to a pest or pathogen, thus providing control of plant pests or diseases, Para. [0074]; enhancing transfer of the polynucleotide into the interior of the plant, enhancing efficacy of the polynucleotides, Para. [0177]; challenge with an insect or nematode pest or with a viral, fungal, or bacterial pathogen, Para. [0072]) comprising; a) selecting a population of polynucleotides that are essentially identical or essentially complementary to a gene or transcript of a plant (selecting polynucleotide sequences effective in gene silencing, Para. [0058]; as used herein, "polynucleotide" refers to a nucleic acid molecule containing multiple nucleotides...having a length of 18-25 nucleotides, Para. [0050]; The polynucleotides are designed to induce systemic regulation or suppression of an endogenous gene in a plant and are designed to have a sequence essentially identical or essentially complementary to the sequence, Para. [0054]); b) topically applying to a surface of at least one of said plants a composition comprising at least one polynucleotide from said population and an transfer agent to obtain a treated plant (topically applying onto a living plant a topically applied composition including polynucleotide molecules, Para. [0007]; compositions are formulated with a...transferring agent, Para. [0053]; agent... includes...organosilicone, Para. [0068]; plants were treated with an aqueous solution, Para. [0059]; treated plants were observed...The dsRNA polynucleotides combinations showing the greatest efficacy in this assay included the four "short" dsRNA molecules described in Example 1, Para. [0157]); and, c) identifying a treated plant that exhibits suppression of the gene or exhibits an improvement in fungal and/or nematode disease resistance or exhibits an improvement in nematode resistance (sequence of a polynucleotide is screened against the genomic DNA of the intended plant to minimize unintentional silencing of other genes, Para. [0058]; suppression of the target gene, Para. [0004]; plant gene involved in responding to a pest or pathogen, thus providing control of plant pests or diseases, Para. [0074]; enhancing transfer of the polynucleotide into the interior of the plant, enhancing efficacy of the polynucleotides, Para. [0177]; challenge with an insect or nematode pest or with a viral, fungal, or bacterial pathogen, Para. [0072]), thereby identifying a polynucleotide investigating reverse genetics by regulating or modulating an endogenous target gene in a plant...identify traits that can be imparted by topical application of polynucleotides, Para. [0072]; sequence of a polynucleotide is screened against the genomic DNA of the intended plant to minimize unintentional silencing of other genes, Para. [0058]) that improves fungal and/or nematode disease resistance in said plant (disclosed herein are useful for regulating endogenous genes of a plant pest or pathogen, Para. [0004]; plant can be...produced, Para. [0071]; plant gene involved in responding to a pest or pathogen, thus providing control of plant pests or diseases, Para. [0074]; enhancing transfer of the polynucleotide into the interior of the plant, enhancing efficacy of the polynucleotides, Para. [0177]; challenge with an insect or nematode pest or with a viral, fungal, or bacterial pathogen, Para. [0072]); a plant and a plant part comprising an exogenous polynucleotide (a plant with exogenous DNA or RNA for suppressing an endogenous gene, Para. [0008]), wherein said exogenous polynucleotide is not operably linked to a promoter or to a viral vector (single-stranded polynucleotides functional in this invention have sequence complementarity that need not be 100% but is at least sufficient to permit hybridization to RNA transcribed from the target gene, Para. [0055]), is not integrated into the chromosomal DNA of the plant, and is not found in a non-transgenic plant (the exogenous DNA is not integrated into a chromosome of the plant, the exogenous RNA is not transcribed from DNA integrated into a chromosome of the plant, Para. [0008]); a plant that exhibits an improvement in fungal and/or nematode disease resistance (disclosed herein are useful for regulating endogenous genes of a plant pest or pathogen, Para. [0004]; plant can be... produced, Para. [0071]; plant gene involved in responding to a pest or pathogen, thus providing control of plant pests or diseases, Para. [0074]; enhancing transfer of the polynucleotide into the interior of the plant, enhancing efficacy of the polynucleotides, Para. [0177]; challenge with an insect or nematode pest or with a viral, fungal, or bacterial pathogen, Para. [0072]), wherein said plant was topically treated with a composition (topically applying onto a living plant a topically applied composition including polynucleotide molecules, Para. [0007]).

"A High Throughput Barley Stripe Mosaic Virus Vector for Virus Induced Gene Silencing in Monocots and Dicots" entitled to Yuan et al., discloses a gene which is the PMR5 gene (PMR5 gene, Abstract; The potential effects of TaPMR5 silencing on powdery mildew Infections were assessed, Pg. 8, right-hand column, third paragraph).

It would have been obvious to one of ordinary skill in the art at the time of invention to modify the method, composition and plant of Sammons to include the PMR5 gene as taught by Yuan. The motivation would have been because down regulation of PMR5 in susceptible plants has a pronounced interference with the early stages of powdery mildew infection, which reduces pathogenesis (Yuan, Pg. 13 , left-hand column, first paragraph).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.