

**[0010]** Biotrophic fungi possess various strategies to access host nutrients. Some utilize extracellular growth; some use intercellular growth; other grow largely intercellularly, but with specialized hyphae (haustoria) that grow into plant cell apoplasts. Finally, some may grow intracellularly, during at least part of their lifecycle. In each of these cases, host responses to fungal infection are suppressed (Mendgen and Hahn, 2002).

**[0011]** It has previously been impractical to provide dsRNA molecules for control of fungal plant pathogens. Therefore, there has existed a need for improved methods of modulating gene expression by repressing, delaying or otherwise reducing gene expression within a particular fungal pathogen for the purpose of controlling pathogen infestation or to introduce novel phenotypic traits.

#### SUMMARY OF THE INVENTION

**[0012]** In one aspect, the invention provides a method of inhibiting expression of a target gene in a phytopathogenic microorganism. In certain embodiments, the method comprises modulating or inhibiting expression of one or more target genes in a phytopathogen that causes cessation of infection, growth, development, and/or reproduction, and eventually results in the death of the organism. The method comprises introduction of partial or fully, stabilized double-stranded RNA (dsRNA), including its modified forms such as small interfering RNA (siRNA) sequences, to the target phytopathogen, wherein the dsRNA inhibits expression of at least one or more target genes of the phytopathogen and wherein the inhibition exerts a deleterious effect upon the pathogen. The methods and associated compositions may be used for limiting or eliminating infection of a plant or plant cell by a phytopathogen, such as a fungus, in or on any host tissue or environment in which a pathogen is present by providing one or more compositions comprising the dsRNA molecules described herein in the host of the pathogen. The method will find particular benefit for protecting plants from fungal attack. In one embodiment, the pathogen is defined as a biotroph. In other embodiments, the pathogen is a necrotroph or a hemibiotroph. In a preferred embodiment, the pathogen is a fungus. The pathogen in particular may be a rust fungus, and may be the causal agent of Asian Soy Rust (e.g. *Phakopsora pachyrizi*).

**[0013]** In another aspect, the present invention provides exemplary nucleic acid compositions that are homologous to at least a portion of one or more native nucleic acid sequences in a target plant pathogenic microorganism. Specific examples of such nucleic acids provided by the invention are given in the attached sequence listing as SEQ ID NO:3-15; SEQ ID NO:18-23; SEQ ID NO:29; and SEQ ID NO:33-35.

**[0014]** In another aspect, the invention provides a method for designing and producing a nucleic acid molecule that is taken up in vitro or in planta by a plant pathogenic fungus in a form effective to allow for sequence specific suppression of fungal gene expression by an RNAi-mediated mechanism. In one embodiment, such a nucleic acid molecule is partially double stranded and is resistant to degradation by ribonuclease. In another embodiment, the nucleic acid is a siRNA. In another embodiment, the nucleic acid suppresses expression of a gene necessary for fungal growth. In yet another embodiment, the nucleic acid suppresses expression of a gene necessary for infection of host tissue by a fungus. In another embodiment, the nucleic acid suppresses expression of a gene necessary for fungal reproduction. In yet another embodi-

ment, the nucleic acid suppresses expression of a gene necessary for uptake of nutrients by a fungal cell.

**[0015]** In another embodiment, the invention provides a method for modulating expression of a target gene in a fungal cell, the method comprising: (a) transforming a plant cell with a vector comprising a nucleic acid sequence encoding a dsRNA operatively linked to a promoter and a transcription termination sequence; (b) culturing the transformed plant cell under conditions sufficient to allow for development of a plant cell culture comprising a plurality of transformed plant cells; (c) selecting for transformed plant cells that have integrated the vector into their genomes; (d) screening the transformed plant cells for expression of the dsRNA encoded by the vector; (e) selecting a plant cell that expresses the dsRNA; (f) optionally regenerating a plant from the plant cell that expresses the dsRNA; whereby expression of the gene in the plant is sufficient to modulate the expression of a target gene in a fungal cell that contacts the transformed plant or plant cell. Modulation of gene expression may include partial or complete suppression of such expression.

**[0016]** In yet another aspect, the invention provides a method for suppressing a gene expressed in a plant pathogen, such as a fungus or oomycete, that comprises the step of providing in the tissue of the host of the pathogen a gene suppressive amount of at least one dsRNA molecule transcribed from a nucleotide sequence as described herein, at least one segment of which is complementary to an mRNA sequence within the cells of the pathogen. The method may further comprise observing the death or growth inhibition, of the pathogen, and the degree of host symptomatology. A dsRNA molecule, including its modified form such as an siRNA molecule, taken up by a pathogenic microorganism in accordance with the invention may be at least from about 80, 95, 96, 97, 98, 99, or about 100% identical to a segment of a RNA molecule transcribed from a nucleotide sequence selected from the group consisting of SEQ ID NO:3-15; SEQ ID NO:18-23; SEQ ID NO:29; and SEQ ID NO:33-35. In specific embodiments, such a sequence may be defined as having the aforementioned identity to (a) two or more segments of a single gene, (b) one or more segments of different genes, or (c) two or more segments of two or more genes.

**[0017]** In another embodiment, the invention provides a nucleic acid that suppresses expression of a host plant gene that is necessary for establishment or maintenance of a fungal infection, or development of plant disease symptoms.

**[0018]** Accordingly, in another aspect of the present invention, a set of isolated and purified nucleotide sequences as set forth in SEQ ID NO:3-15; SEQ ID NO:18-23; SEQ ID NO:29; and SEQ ID NO:33-35 is provided. The present invention provides a stabilized dsRNA molecule for the expression of one or more miRNAs for inhibition of expression of a target gene in a phytopathogenic microorganism, expressed from these sequences and fragments thereof. A stabilized dsRNA, including a miRNA or siRNA molecule can comprise at least two coding sequences that are arranged in a sense and an antisense orientation relative to at least one promoter, wherein the nucleotide sequence that comprises a sense strand and an antisense strand are linked or connected by a spacer sequence of at least from about five to about one thousand nucleotides, wherein the sense strand and the antisense strand may be a different length, and wherein each of the two coding sequences shares at least 80% sequence identity, at least 90%, at least 95%, at least 98%, or 100% sequence identity, to any one or more nucleotide sequence(s)