

## IN PLANTA RNAI CONTROL OF FUNGI

**[0001]** This application claims the priority of U.S. Provisional Patent Application 60/765,112, filed Feb. 3, 2006, the disclosure of which is incorporated herein by reference in its entirety.

### BACKGROUND OF THE INVENTION

**[0002]** 1. Field of the Invention

**[0003]** The present invention relates generally to genetic control of plant disease. More specifically, the present invention relates to recombinant DNA technologies for post-transcriptionally repressing or inhibiting expression of target coding sequences in the cell of a fungal plant pathogen or host to provide a protective effect.

**[0004]** 2. Description of Related Art

**[0005]** Plants are subject to multiple potential disease causing agents in the environment. Plant pathogens include various fungi, bacteria, viruses, nematodes, and algae, among others. A multitude of means have been utilized for attempting to control infection and disease by these pathogens. Compositions and agents for controlling infestations by pests such as bacteria, fungi, nematodes and viruses have been provided in the form of antibiotic compositions, antifungal compositions, nematocides, and antiviral compositions. Biological and cultural control methods have been attempted in numerous instances. Chemical compositions have typically been applied to surfaces on which pathogens are present or administered to pathogenic microorganisms in the form of pellets, powders, tablets, pastes, or capsules and the like, but the level of specificity of these compositions toward target organisms has often been less than desirable. Thus, there is a great need in the art for improvement of these methods and particularly for methods that would benefit the environment relative to the prior techniques.

**[0006]** Commercial crops and agroecosystems are often the targets of attack by pathogens. Substantial progress has been made in the last few decades towards developing more efficient methods and compositions for controlling plant pathogenic microorganisms, including chemical agents that have often been very effective in eradicating infectious agents. However, there are several disadvantages to using chemical agents. Chemical agents are not selective. Applications of chemical pesticides intended to control pathogens that are harmful to various crops and other plants exert their effects on non-target organisms as well, often effectively disrupting populations of beneficial microorganisms as well, for a period of time following application of the agent. Chemical agents may persist in the environment and often are slow to be metabolized, if at all. They may accumulate in the food chain, and particularly in the higher predator species. Repeated application of these chemical pesticidal agents may lead to the development of pathogen populations resistant to the agents. Accumulation of these chemical agents in species higher up the evolutionary ladder can also often occur. These agents may act as mutagens and/or carcinogens to cause irreversible and deleterious genetic modifications. Thus there has been a particularly long felt need for environmentally friendly methods for controlling or eradicating pathogen infestation on or in plants, i.e., methods that are selective, environmentally inert, non-persistent, and biodegradable, and that fit well into disease management schemes.

**[0007]** Antisense methods and compositions have been reported in the art and are believed to exert their effects through the synthesis of a single-stranded RNA molecule that in theory hybridizes *in vivo* to a substantially complementary sense strand RNA molecule. Antisense technology has been difficult to employ in many systems for three principal reasons. First, the antisense sequence expressed in the transformed cell is unstable. Second, the instability of the antisense sequence expressed in the transformed cell concomitantly creates difficulty in delivery of the sequence to a host, cell type, or biological system remote from the transgenic cell. Third, the difficulties encountered with instability and delivery of the antisense sequence create difficulties in attempting to provide a dose within the recombinant cell expressing the anti sense sequence that can effectively modulate the level of expression of the target sense nucleotide sequence.

**[0008]** Double stranded RNA mediated inhibition of specific genes in various organisms has been previously demonstrated. dsRNA mediated approaches to genetic control have been tested in the fruit fly *Drosophila melanogaster* (Kennerdell Cell 95:1017-1026). Kennerdell et. al. describe a method for delivery of dsRNA involving generating transgenic insects that express double stranded RNA molecules or injecting dsRNA solutions into the insect body or within the egg sac prior to or during embryonic development. Research investigators have previously demonstrated that double stranded RNA mediated gene suppression can be achieved in nematodes either by feeding or by soaking the nematodes in solutions containing double stranded or small interfering RNA molecules and by injection of the dsRNA molecules. Rajagopal et. al. (2002) described failed attempts to suppress an endogenous gene in larvae of the insect pest *Spodoptera litura* by feeding or by soaking neonate larvae in solutions containing dsRNA specific for the target gene, but was successful in suppression after larvae were injected with dsRNA into the hemolymph of 5<sup>th</sup> instar larvae using a microapplicator. Similarly, U.S. Patent App. Pub. No. 2003/0150017 prophetically describes a preferred locus for inhibition of the lepidopteran larvae *Helicoverpa armigera* using dsRNA delivered to the larvae by ingestion of a plant transformed to produce the dsRNA. Development of plant diseases, for instance viral diseases, are also reported to have been suppressed by RNAi approaches in plant cells (e.g. Lindbo & Dougherty, 2005).

**[0009]** To date, no published information exists on RNAi-mediated gene suppression in fungi where the double-stranded (dsRNA) or small interfering (siRNA) molecules are taken up from artificial growth media (*in vitro*) or from plant tissue (*in planta*). The literature contains examples of RNAi-mediated gene suppression via transformation of DNA constructs into fungal cells either treated by cell wall alterations or electroporation; in other words the typical DNA transformation protocols used in fungi for the past 20 years (Chicas, Cogoni, and Macino; Cottrell and Doering; Mouyna et al.; Raponi and Arndt; Reese and Doering; Kadotani). Suppression of fungal infection of barley by interfering with expression of a plant gene via RNAi-mediated gene suppression has also been reported (Schultheiss et al.). The lack of RNAi-mediated gene suppression via fungal uptake of dsRNA molecules might have been due to degradation of the RNA outside of the cell or an inherent inability of fungal cells to take up dsRNA from the environment.