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Genetic and molecular characterization of a blue light photoreceptor MGWC-1 in *Magnaporth oryzae*

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ABSTRACT

Three key factors involved in successful plant disease development include the presence of a susceptible host, a virulent pathogen, and a disease-conducive environment. Our understanding of how environmental factors influence disease-conducive or disease-suppressive conditions, and how a pathogen advantageously capitalizes on them, is quite limited. Utilizing the model pathosystem *Magnaporthe oryzae–Oryza sativa*, we found a significant light-dependent disease suppression. Our genetic data suggest that the blue-light receptor MGWC-1 in *M. oryzae* is involved in light-dependent disease suppression during the dark-phase (disease-conducive light condition) immediately after pathogen–host contact. Sensing "darkness" is accomplished by MGWC-1, a blue-light receptor in *M. oryzae*. To explore the potential molecular mechanisms of light-dependent disease suppression we performed a genome-wide microarray experiment and identified several groups of gene families that are differentially regulated during the light-to-dark transition. Our genetic and molecular data provide insights into how a fungal pathogen utilizes ambient light signals for successful disease development.

1. Introduction

An optimal environment, in addition to a susceptible host and a virulent pathogen, is important for successful plant disease development (Agrios, 2005). Light conditions are a major environmental factor for many biological activities. Perceiving ambient light and preparing for coming environmental changes through a circadian clock are important capacities for the survival of an organism (DeCoursey et al., 2000; Green et al., 2002; Ouyang et al., 1998). However, little is known concerning how specific environmental conditions, such as light, influence plant–microbe interactions at the molecular level.

One of the most characterized fungal photoreceptor is WHITE COLLAR-1 (WC-1) in *Neurospora crassa* (Corrochano, 2007). In *N. crassa*, most of known light-induced phenotypes are regulated by blue light, and all blue light-induced phenotypes are absent in either *white collar-1* (*wc-1*) or *white collar-2* (*wc-2*) mutants (Ballario and Macino, 1997). The *wc-1* and *wc-2* genes have been cloned (Ballario et al., 1996; Linden and Macino, 1997) and their products have been shown to interact with each other (Ballario et al., 1998; Cheng et al., 2001a; Denault et al., 2001). Based on these findings and sequence information showing that WC-1 and WC-2 are putative transcription factors, *wc-1* and *wc-2* have been proposed to mediate light-induced gene expression (Ballario et al., 1998, 1996; Lee et al., 2000; Linden and Macino, 1997; Talora et al., 1999). Both WC-1 and WC-2 are regulated post-translationally through phosphorylation (Schwerdtfeger and Linden, 2000, 2001; Talora et al., 1999). While light-induced phosphorylation of WC-1 is transient (Heintzen et al., 2001), phosphorylation of WC-2 is stable under constant light (LL). WC-1, working with WC-2 and cofactor FAD, has been confirmed to be a blue light photoreceptor (Froehlich et al., 2002; He et al., 2002).

A closely related plant pathogenic fungus *Magnaporthe oryzae*, the causal agent of rice blast disease, contains a homolog of WC-1, *M. oryzae* WC-1 (MGWC-1). Rice blast disease is one of the most devastating threats to the world's food supply. More than half of the world's population depends on rice as a stable food resource, and a conservative estimate suggests that *M. oryzae* is responsible for the loss of enough rice to feed 60 million people every year

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(Zeigler et al., 1994). However, the economic impact of *Magnaporthe* is not limited to rice production. Certain species of *Magnaporthe* infect other economically important grasses including barley, wheat, pearl millet and turf-grass, the latter being a concern for the golf industry (Landschoot and Hoyland, 1992).

M. oryzae is an excellent fungal model system for studying plant–microbe interactions and pathogenicity. Our previous genetic study suggested that *mgwc-1* in *M. oryzae* is an ortholog of *wc-1* in *N. crassa*, and demonstrated that MGWC-1 is responsible for light-dependent asexual development and spore discharge in *M. oryzae* (Lee et al., 2006). In addition to WC-1, additional molecules that play roles in fungal development have been characterized. For example, it has been shown that asexual development in *Aspergillus nidulans* is regulated by a protein complex sensing red and blue light (Purschwitz et al., 2008). Ambient light conditions also control secondary metabolites in *A. nidulans*, e.g. penicillin (Calvo, 2008). The *velvet* gene (*veA*), which physically and functionally interacts with photoreceptors, is also part of a protein complex that controls secondary metabolism in *A. nidulans* (Bayram et al., 2008). Thus, *veA* appears to be a molecular link between ambient light conditions and development/secondary metabolism in *A. nidulans* (Bayram et al., 2010; Calvo, 2008). Several review articles document recent research progress in fungal photoreceptors (Avalos and Estrada, 2010; Chen et al., 2010; Corrochano and Garre, 2010; Idnurm et al., 2010).

In our previous report, we demonstrated that the photoreceptor MGWC-1 plays an important role in asexual development of *M. oryzae* (Lee et al., 2006). In this report, we focused on genetic and molecular characterization of *mgwc-1* and the biological role of MGWC-1. Since both plants and pathogens use light as an important environmental signal, we hypothesized that ambient light conditions also play a role in pathogenic plant–microbe interactions. To test this, we relied on genetic and genomic approaches. Using genetic tools, we explored whether ambient light conditions influence pathogenicity of *M. oryzae*, and if the light receptor MGWC-1 participates in this regulation. Our data identify a disease-conducive and a disease-suppressive light condition, and that MGWC-1 is responsible for light-dependent disease suppression. Using microarray analysis, we also identified candidate genes that may play roles in MGWC-1-dependent disease suppression.

2. Materials and methods

2.1. Fungal strains and growth conditions

M. oryzae strain Guy11 is a parental strain of the *mgwc-1* knockout strain KOM1–02 (Lee et al., 2006). Wild type and mutants were grown on oatmeal agar plates for spore production. Cultures were stored on filter papers at −80 °C for long-term storage and mutants were cultured in a Hygromycin B-containing medium. Pathogenicity tests were performed with the spores produced from the fresh culture of the long-term stock. Conidia were collected by scraping the surface of 10 days old oatmeal agar plates with sterile distilled water followed by filtering through one layer of Miracloth (Calbiochem, Darmstadt, Germany), and used for RNA isolation or for further experiments. For expression analysis, a total of 1×10^6 conidia were inoculated and grown in 200 ml of complete media, CM (Talbot et al., 1993) contained in 500 ml Erlenmeyer flasks for 3 days at 25 °C shaking incubator. For stress experiments, mycelia were filtered through sterile filter paper, washed three times with sterile distilled water, and then, inoculated for 30 min under experimental stress conditions (below). Media for the nutritional stresses have been prepared as previously reported (Talbot et al., 1993). Hydrogen peroxide and sodium chloride were amended into CM for oxidative stress and osmotic stress to final concentrations of 2% and 4%, respectively. For heat shock treatment, mycelia were inoculated into CM broth preheated to 42 °C for 30 min. Similarly, mycelia were incubated in the CM at 4 °C for cold shock. To obtain appressorial RNA, conidial suspension was dropped on hydrophobic surface of GelBond film (FMC BioProducts, Rockland, ME) in a moistened box, and let the conidia germinate and form appressoria at 25 °C. Germlings with appressoria were harvested by scraping the surface of GelBond film with rubber scraper at 4 h (for initiation of appressoria formation) and 24 h (for mature appressoria) after incubation. Samples were frozen immediately after harvesting with liquid nitrogen, and stored at −80 °C until the total RNA was extracted from them.

2.2. Rice plants and pathogenicity test

Rice seeds (cultivars M201 and Lemont, kindly provided by Dr. Karen Moldehauer, Rice Research and Extension Center, Stuttgart, AR, United States) were germinated on moist filter papers in Petri dishes for 3 days in a 30 °C incubator. Seedlings were transplanted to 127-cm pots containing Cornell potting mix. Plants were grown in a greenhouse at 28–22 °C with a light and dark cycle of 16 and 8 h, respectively, for 2 weeks, until they were at the four-leaf stage. The lamps in the greenhouse were 400 Watt High Pressure Sodium luminaires manufactured by PL Lighting Systems (Beamsville, ON Canada). They provide about 70–75 micromoles/m^2/s of PAR light at bench level. Plants were transported to growth chambers and grown for another week prior to experimentation to allow for acclimatization to the growth chamber conditions (similar to greenhouse, but with humidity controlled at 90%). About 50 ml of 1% urea solution was applied to each pot and watered, if necessary. Pathogenicity of wild type and *mgwc-1* knock out strains were tested by spray inoculation using different cultivars. The spore suspension, 2×10^5 spores/ml in concentration, collected from 10-day old cultures grown on V8 media (Lee et al., 2006), was used to spray inoculate the plants using an artist's air brush (Paasche H No. 1, Paasche Airbrush Company, Chicago, IL) connected to a pump to maintain air pressure at 20 psi. Six-pots containing five different rice cultivars can be sprayed with around 5–10 ml spore suspension. Inoculated seedlings were incubated in a dew chamber at 25 °C for 24 h before being transferred to a Percival growth chamber set at cycling conditions, 28 °C 16 h light: 22 °C 8 h darkness. Four individuals performed a blind test for disease rating after 8 days of inoculation. We performed two different disease rating method, average disease rating and maximum disease rating. We found that both ratings agree each other and decide to report only the maximum disease rating; for measuring disease severity, we scored the most common and largest lesion on a leaf based on the scale of 0–9 (0 being complete resistance and 9 being most severe disease), Standard Evaluation System for Rice (http://www.knowledgebank.irri.org/ses/SES.htm). We repeated the pathogenicity test four times with the same results. Severity of disease was measured using a 0–9 scale of pathogenicity disease index (Fig. 5). Two-way ANOVA analysis followed by the Tukey's honestly significant difference test was performed using MATLAB. There were significant differences in light conditions and in genotypes of pathogen (In both cultivars, light condition $P < 0.001$; genotype $P < 0.01$).

2.3. Fungal transformation

Protoplasts generation and transformation were performed following established protocols (Kim et al., 2005). Protoplasts were generated from young mycelia of the KOM1 strain grown in CM with 10 mg/ml Lysing Enzyme (Sigma, St. Louis) in 20% sucrose solution, for osmotic stability. Protoplasts were harvested by filtration through four layers of Miracloth (Calbiochem, Darmstadt,