Supervision

MOLECULAR GENETIC STUDIES ON SOME FUNGAL DISEASES IN RICE

By

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ABSTRACT

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Rice is the most important food crop in the developing world and the staple food of more than half of the world's population. Rice farming engages one million families in Egyptian population. Rice blast disease that caused by *Magnaporthe grisea* may affect rice production by 5% yield losses in normal infected seasons and varies from 30 to 50% yield losses in epidemic seasons. The aim of work is to isolate and identify some differentially expressed mRNA related to the *Magnaporthe grisea* infection in rice. Microarray was used to measure different levels in gene expression in resistance rice variety (G177) according to artificial infection with fungus spores The results indicated that there are 181 differentially expressed genes, where 96 genes were down regulated and 85 were up regulated with minimum fold change of two and p-value cut of 0.05. These up and down regulated genes affected a number of defense pathways that play major roles in *M.grisea* infection in rice. Some prominent impacts on gene regulation include up regulation of phospholipase A2 gene which enhances phospholipid A2 (PLA2) mechanism activation. On the other hand, few examples of down regulated genes include fructokinase 2, Leucine Rich Repeat family protein. It is evident that many of these genes are involved in critical pathways associated with resistance and/or susceptibility to blast disease. Consequently, this could shed some light on the mechanism of action of these genes on blast resistance in rice.

Key words: Rice, Magnaporthe grisea, Microarray.

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Abbreviation	Full Name
ABA	Absicic acid
AFP	Antifungal protein
ALDH	Aldehyde dehydrogenase
BAS	biotrophy-associated secreted
Bzip	basic region/leucine zipper
Camp	cyclic AMP
ESTs	expressed sequence tags
ET	ethylene
ETI	effector-triggered immunity
G171	Rice variety giza 171
G177	Rice variety giza 177
HEX1	hexagonal peroxisome
JA	jasmonic acid
LRR	leucine-rich repeat
MAPK	mitogen activated protein kinase
NO	Nitric oxide
PAL	phenylalanine ammonialyase
PAL2	Phospholipases A2
PAMP	pathogen associated molecular patterns
PCR	Polymerase chain reaction

PDF	protein defensin
PI	proteinase inhibitor
PRRs	pattern recognition receptors
PTI	PAMP-triggered immunity
QTLs	Quantitative trait loci
RLK	receptor-like kinase
ROS	reactive oxygen species
RT-PCR	Real time polymerase chain reaction
SA	salicylic acid
SAGE	Serial Analysis of Gene Expression
SSRs	simple sequence repeats
STS	sequence tagged site
TAG	Triacylglycerol
TFs	transcription factors
TRR	Tetratricopeptide
ABA	Absicic acid
AFP	Antifungal protein
ALDH	Aldehyde dehydrogenase
BAS	biotrophy-associated secreted
Bzip	basic region/leucine zipper
Camp	cyclic AMP

LIST OF ABBREVIATIONS

ESTs	expressed sequence tags
ET	ethylene
ETI	effector-triggered immunity
G171	Rice variety giza 171
G177	Rice variety giza 177
HEX1	hexagonal peroxisome
JA	jasmonic acid
LRR	leucine-rich repeat
MAPK	mitogen activated protein kinase
NO	Nitric oxide
PAL	phenylalanine ammonialyase
PAL2	Phospholipases A2
PAMP	pathogen associated molecular patterns

I. INTRODUCTION

Rice (*Oryza sativa L.*) is an essential crop, providing a carbohydrate source for more than one-third of the world's population and it is widely cultivated in arable land worldwide **(Jiang *et al.,* 2011)**. It is the first food crop whose genome has been completely sequenced, more than once and for both Indica and Japonica subspecies, reflecting its importance as major source of food world-wide **(Raghuvanshi *et al*., 2010)**. It has emerged as an ideal model species for the study of crop genomics due to its commercial value, small genome size (430 Mb), diploid origin and close relationship with other important cereal crops **(Ram *et al*., 2012).**

Rice productivity is very sensitive to a variety of biotic and abiotic factors. Both of them may cause losses in plant growth and yield. To cope with these stresses, plants have evolved several mechanisms to tolerate abiotic stresses and resist biotic stresses. Plants have several physical and chemical barriers to protect themselves from pathogen attack. They induce a set of defense mechanisms that allow them to restrict pathogen growth. Among all the biotic stresses of rice, blast disease alone causes yield loss of up to 50 %. The fungus grows in all rice-growing areas across the world and attacks almost all the aerial parts of rice plant typically, leaves and panicles **(Mohan *et al*., 2012).**

Comparative studies in the grasses laid the foundation for comparative genomics. Comprehensive data sets are in place for the major crop plants like rice, wheat, maize, barley, sorghum, and oats which provide evidence for the presence of genetic colinearity between genomes **(Raghuvanshi *et al*., 2010)**. Therefore, comparative sequence analysis provides valuable information about the functional sequences encoded by a genome, mainly by exploring the conserved DNA sequences that code for proteins or RNAs or regulatory elements. Recently, the progress in functional genomics data has shifted from basic

sequence analysis to detailed studies of functional attributes such as gene expression or protein-protein interactions **(Movahedi *et al*., 2011)**.

The analysis of gene expression, or more correctly transcript abundance, is widely carried out in a variety of laboratories in various disciplines. Northern blotting, quantitative RT-PCR (QRT-PCR) and microarray approaches are commonly used to assess transcript abundance **(Narsai *et al*., 2010).** The development and improvement of high-density microarrays have permitted a fast expansion of the gene expression analysis in the past 20 years **(Pariset *et al*., 2009).**

Gene expression profiling is accelerating the progress toward a comprehensive understanding of the genetic mechanisms that control responses to environmental stress. Microarray analysis has been developed to obtain overall gene expression profiles in various plants. Microarray profiling and the recently introduced TAG-based sequencing approaches are proven technologies for estimating gene expression **(Mizuno *et al*., 2010)**. Microarray technology has significantly increased its application-base and popularity. Initially developed to measure expression levels of given transcripts, it provides a snap-shot of the dynamic cellular transcriptomes which have been extracted from an isolated tissue-type. A common application of this technology is the comparison of the same tissue-type at the same stage of development between an experimental treatment, or diseased tissue compared to a wild-type control **(Crismani *et al*., 2011).**

This study aims to identify inducible genes in rice due to early infection of *Magnaporthe grisea* and predict the different defense mechanisms to resist this infection using microarray technology coupled with bioinformatics analysis.

II. REVIEW OF LITERATURE

1. Rice

Rice *(Oryza sativa L.)* is one of the largest single use of land for producing food, covering nine percent of the earth's arable land. It is the predominant staple food for 17 countries in both Asia and the Pacific besides, nine countries in Africa including Egypt **(Laxuman *et al*., 2011).** About 20% of the total calorie consumption worldwide comes from rice as the principle source of nourishment **(Nandan *et al*., 2010).**It is also considered the major source of calories for more than half of the total global population **(Pandey *et al*., 2012).** It forms the staple diet for more than 2.7 billion people **(Rangare *et al*., 2012).**

Reflecting its importance as a global food security and the necessity of increasing its production, United Nations General Assembly decided to celebrate the year 2004 as "International Year of Rice" with the theme of "Rice is Life**"(FAO, 2004).** The International Rice Research Institute at Philippines, estimated that in order to feed the growing global population, rice production must increase by another one-third by the year 2020 **(Devi and Sharme, 2010).**

Unfortunately like most plants, rice production is affected by a variety of factors that limit its production. These factors could be biotic (viruses, bacteria, fungi, insects and nematode), and/or abiotic (drought, salinity, submergence and soil toxicity). Among all biotic stresses, rice blast disease is the most destructive disease of rice world-wide **(Rauyaree *et al*., 2001).**

2. Blast disease

Rice blast disease is caused by a heterothallic ascomycetes fungus called *Magnaporthe grisea,* which grows in all rice-growing areas across the world and attacks almost all the aerial parts of rice plant typically, leaves and panicles **(Mohan *et al*., 2012).** It has been reported that it can

also attack the roots using specific pathway **(Sesma and Osbourn, 2004).** Around 50% of production may be lost in a field moderately affected by infection. Each year the fungus destroys rice enough to feed an estimated 60 million people **(Devi and Sharma, 2010).**

Brondani *et al.* (2000) constructed an AG microsatellite-enriched genomic DNA library for *Magnaporthe grisea* (anamorph *Pyricularia grisea*), the causal agent of rice blast. They isolated and sequenced seventy two DNA clones containing microsatellite repeats in order to develop a series of new PCR-based molecular markers to be used in genetic studies of the fungus. They selected twenty four clones to design primer pairs for the PCR amplification of microsatellite alleles. Single spore cultures of *M. grisea* isolated from rice and wheat in Brazil, Colombia and China were genotyped by three microsatellite loci. They noticed that isolates from southern Brazil were predominantly monomorphic at the tested SSRs loci, indicating a low level of genetic variability in these samples. However, seven alleles were observed at the MGM-1 locus in isolates from central Brazil, and at least nine alleles were detected at the same locus in a sample of Colombian isolates. They concluded that polymorphism analysis at SSRs loci is a simple and direct approach for estimating the genetic diversity of *M. grisea* isolates and a powerful tool for studying *M. grisea* genetics.

Reddy *et al.* (2000) used fluorescent *Pseudomonads* isolated from rice seedlings to screen their antagonistic ability and siderophore mediated antibiosis under *in vitro* conditions against *Magnaporthe grisea* and *Rhizoctonia solani* pathogens. Among ten isolates, strain P.f 003 gave significantly higher inhibition of mycelial growth of *M. grisea* and *R. solani*. Strains of P.f 001, P.f 003, P.f 005 and P.f 007 produced siderophores when grown on Fe deficient and Fe fortified King's B medium. They tested these strains again for their *in vitro* antagonistic activity against *M. grisea* and *R. solani* on King's B media with or without FeCl3. Their results showed that all strains significantly reduced the

growth of *M. grisea* and *R. solani* with FeCl3 in the media compared to without $Fecl_3$. Finally, they concluded that strain P.f 003 had a superior activity compared to other evaluated strains.

Nakayashiki *et al.* (2001) identified a novel Ty3/Gypsy retrotransposon, named Pyret, in the plant pathogenic fungus *Magnaporthe grisea* (anamorph *Pyricularia oryzae*). Pyret-related elements were distributed in a wide range of *Pyricularia* isolates from various gramineous plants. The Pyret element was 7250 bp in length with a 475 bp LTR and one conceptual ORF. The ORF contained seven nonsense mutations in the reading frame, indicating that the Pyret clone was lightly degenerate. Comparative domain analysis among retroelements revealed that Pyret exhibits an extra domain (WCCH domain) beyond the basic components of LTR retrotransposons. The WCCH domain consisted of ~300 amino acids and was located downstream of the nucleocapsid domain. The WCCH domain was so named because it contained two repeats of a characteristic amino acid sequence, W-X2-C-X4-C-X2-H-X3-K. A WCCH motif-like sequence was found in the precoat protein of some Gemini viruses, viral RNA-dependent RNA polymerase and also in an Arabidopsis protein of unknown function. Interestingly, detailed sequence analysis of the GAG protein revealed that Pyret, as well as some other chromodomain-containing LTR retrotransposons, displayed significant sequence homology with members of the gammaretroviruses (MLV-related retroviruses) in the capsid and nucleocapsid domains. This suggested that chromodomain- containing LTR retrotransposons and gamma retroviruses may share a common ancestor with the GAG protein.

Vila *et al.* (2001) prepared a purified antifungal protein (AFP) from *Aspergillus giganteus* which exhibited potent antifungal activity against the phytopathogenic fungi *Magnaporthe grisea* and *Fusarium moniliforme*, as well as the oomycete pathogen *Phytophthora infestans.* Under conditions of total inhibition of fungal growth, no toxicity of AFP