Agri Gene 1 (2016) 143–150

Contents lists available at ScienceDirect

Agri Gene

journal homepage: www.elsevier.com/locate/aggene

# Comparative transcriptomics of rice and exploitation of target genes for blast infection

Varshika Singh [a], Vinay Sharma [b,*], Pramod Katara [c]

[a] Department of Bioscience and Biotechnology, Banasthali University, P.O. Banasthali Vidyapith, Rajasthan 304 022, India
[b] Department of Bioscience & Biotechnology, Banasthali University, P.O. Banasthali Vidyapith, Rajasthan 304 022, India
[c] Center of Bioinformatics, University of Allahabad, Allahabad 211002, India

ARTICLE INFO

*Article history:*
Received 6 April 2016
Received in revised form 21 August 2016
Accepted 25 August 2016
Available online 27 August 2016

*Keywords:*
Rice blast
Bioinformatics
Microarray
plant-pathogen interaction
gene ontology

ABSTRACT

Rice is a major cereal crop and serves as staple food for a large part of the human population. Rice blast is a very important disease that attacks rice and is found in every region where rice is grown. Here we identified differentially expressed genes during different time intervals of the blast infection. Our results show that at 24 hpi almost half of the identified genes (174 of 224) are under expressed, then at 36 hpi only 26 genes out of 278 identified genes were down regulated. Then for 2nd, 4th and 6th day mostly differentially expressed genes remained up regulated. Also, the most significant gene ontology terms identified for these genes were diterpenoid metabolic process, diterpene phytoalexin metabolic process and phytoalexin metabolic process, isoprenoid metabolic process, terpenoid metabolic process and lipid metabolic process. This study has led to a more comprehensive data for understanding rice defense response.



## 1. Introduction

Rice is a very important staple food for a large part of world's population. The rice crop is affected by various abiotic (unfavorable soil, wound, temperature, flooding, etc.) and biotic (microbes) stresses resulting in huge yield losses. Among the biotic stresses, blast disease caused by *Magnaporthe oryzae* results in 10 to 30% of total loss in rice production each year (Khush and Jena, 2009). The ascomycete hemibiotrophic fungus *Magnaporthe oryzae* is known to cause infection in >50 monocot species (Ou, 1980). *Magnaporthe oryzae* causes gray leaf spot disease in St. Augustine grass and perennial ryegrass (Smiley et al., 1996), and rice blast disease in rice (Yorionori and Thurston, 1974). The pathogen infects rice plant at all growth stages affecting leaves, nodes, collars, panicles and roots resulting in total loss of the rice grain (Das et al., 2012). Rice blast is a major factor influencing stable rice production and food security in many rice-growing countries in Asia and Africa (Liu et al., 2010). Each year about 50% of rice yield is lost in eastern Indian upland regions alone (Khush and Jena, 2009). *Magnaporthe oryzae* secrete effector proteins to attack host defenses and cellular processes and invade rice cells. The hemibiotrophic fungus combines both biotrophic and necrotrophic growth during infection (Marcel et al., 2010).

The rice–*Magnaporthe* interaction has emerged as a model system for the analysis of plant–pathogen interactions because of its economic significance, genetic tractability and availability of genomic resources in these species (Das et al., 2012). Many rice genes involved in defense responses and fungal genes involved in pathogenicity have been identified in the past (Kim et al., 2000; Jantasuriyarat et al., 2005; Li et al., 2006; Liu et al., 2010; Kawahara et al., 2012). Using resistant rice varieties has been the most effective and economical method to control rice blast (Liu et al., 2011). Thus identification of novel blast defense and resistance genes is important for controlling blast disease. The main objectives of the present study were to investigate and compare, the global gene expression changes that occur in response to infection by *Magnaporthe oryzae* in rice and to classify the identified differentially expressed genes on the basis of gene ontology terms.

## 2. Materials and methods

### 2.1. Dataset collection

For the analysis of differential gene expression during different stages of blast infection, we downloaded the microarray gene expression data from gene expression omnibus (GEO) database at NCBI (Table 1). To get realistic analysis we selected time series data in such

*Abbreviations:* hpi, hours post infection; RMA, Robust multi-array analysis; SEA, Singular Enrichment Analysis.

* Corresponding author.
*E-mail addresses:* varshika13singh@gmail.com (V. Singh), vinaysharma30@yahoo.co.uk (V. Sharma), pmkatara@gmail.com (P. Katara).

http://dx.doi.org/10.1016/j.aggene.2016.08.004
2352-2151/

**Table 1**
The datasets retrieved from GEO.

| S·No. | GSE ID | Description |
|---|---|---|
| 1 | GSE8518 | Rice leaf sheath at 36 h after inoculation rice with blast fungus *Magnaporthe oryzae* (Mosquera et al., 2009). |
| 2 | GSE16470 | Infected rice culture at 24 and 48 h after inoculation. |
| 3 | GSE30941 | Infected and noninfected rice leaves at 24 h post inoculation with *Magnaporthe oryzae* (Abbruscato et al., 2012). |
| 4 | GSE18361 | Infected (with *Magnaporthe oryzae*) and noninfected roots of rice at 2, 4, and 6 days post-inoculation (Marcel et al., 2010). |

a combination so that a wide range of infectious conditions could be covered.

### 2.2. Data analysis

Microarray data were analyzed with bioconductor packages using R. Robust multi-array analysis (RMA, Irizarry et al., 2003) in the affy Bioconductor package was used for affymetrix data (CEL files) normalization. Functions for normalizing two-color agilent data are available in package limma; lowess (or loess) function was used. Then for detection of differentially expressed genes we used limma package for both agilent and affymetrix datasets. We filtered differentially expressed genes having at least 2 fold expression changes and *P* value ≤0.05. Further, we used SEA tool from agriGO to search GO annotations and functionally classify these differentially expressed rice genes.

## 3. Results

### 3.1. Identification of affected genes

During the plant–pathogen interaction, the perception of the pathogen by the plant is accompanied by the induction of a large number of genes in the plant. The identification of over- or under expressed genes is one of the most widely used types of analysis to screen for potential significant genes.

In the first dataset GSE8518, we obtained 278 differentially expressed probesets in the infected rice leaf sheath after 36 h post inoculation (hpi) which changed at least 2 fold gene expression and had *P* value < 0.05. Out of 278 expressed probesets only 26 were down regulated (Fig. 1A). Thus most of the rice genes affected at this time period showed over expression. In the second dataset GSE16470, we made a contrast for 24 hpi data with 48 hpi data with *Magnaporthe oryzae* inoculation and identified 91 differentially expressed probesets in this dataset. However most of them corresponded to control probesets and did not correspond to genes and hence we obtained 18 probesets in the Venn diagram (Fig. 1 B) and all of them were found to be down regulated. The other identified six probesets showing up regulation did not match any known genes during further GO analysis by agriGo SEA tool. Hence we can say that only 18 genes are commonly expressed at both these time intervals and all are under expressed. In the third dataset GSE18361, the comparisons were made for 2nd, 4th and 6th day post inoculation with fungus and mock inoculated rice tissue. For 2nd day (i.e. 48 hpi) we identified 1107 differentially expressed genes, out of which 111 were down regulated. For 4th day we identified 152 differentially expressed genes, out of which only 6 were down regulated. For 6th day analysis we found 339 differentially expressed genes with 35 being down regulated. Lastly in the fourth dataset GSE30941, we found 224 probesets to be differentially expressed in this dataset with 174 of these under down regulation. The data was collected 24 h after inoculation with different strains of *Magnaporthe.* Almost half of the genes were down regulated in this condition.

### 3.2. GO classification of differentially expressed genes

Using agriGO SEA tool each probeset/gene was assigned either one or more of the three main gene ontologies (GO); biological process, cellular components and molecular function. GO terms allow to

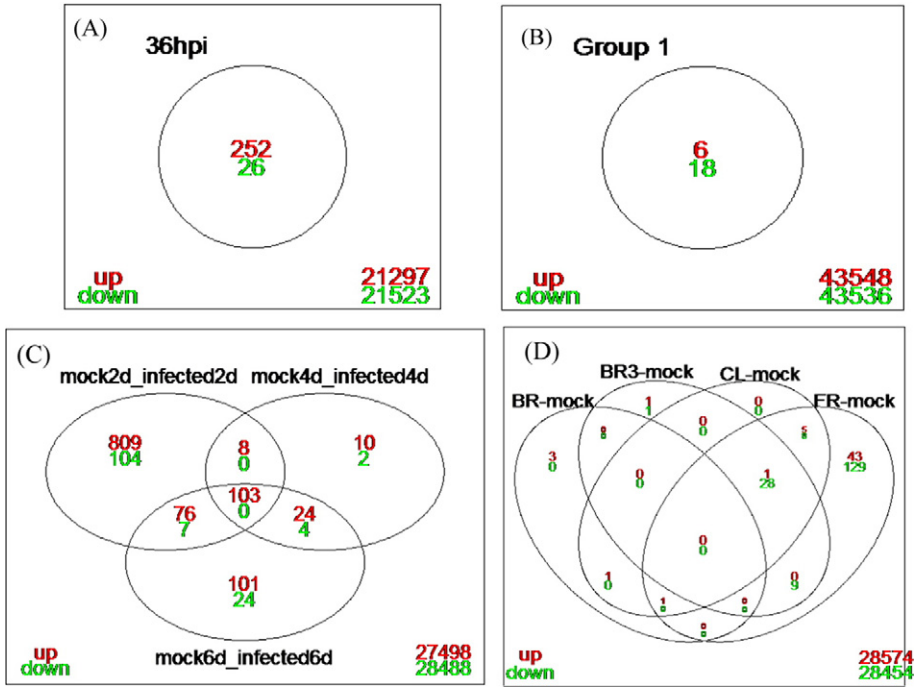


**Fig. 1.** Venn diagrams representing differentially identified genes in various datasets. The up and down values at downside of each Venn diagram represents the total number of genes identified to be up or down regulated without log fold and *P* value restriction. (A) Venn diagram representing results of dataset GSE8518; data comparing mock inoculated data at 36 hpi with infected plat tissue data. (B) Venn diagram for dataset GSE16470; contrasts were made for 24 hpi data with 48 hpi data. (C) Venn diagram for dataset GSE18361; comparisons were made for 2nd, 4th and 6th day post inoculation with fungus and mock inoculated rice tissue. (D) Venn diagram for dataset GSE30941; comparisons made for rice tissues inoculated with different strains of (*Magnaporthe oryzae* FR13 (represented as FR-mock) and CL367 (represented as CL-mock)), non-adapted strain BR32 (represented as BR3-mock) and *Magnaporthe grisea* strain BR29 (represented as BR-mock) and mock inoculated rice tissue at 24 hpi.