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Oscillating Transcriptome during Rice-*Magnaporthe* Interaction

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Abstract
Rice blast disease caused by the fungus, *Magnaporthe oryzae*, is one of the most devastating diseases of rice. Deciphering molecular mechanism of host-pathogen interactions is of great importance in devising disease management strategies. Transcription being the first step for gene regulation in eukaryotes, basic understanding of the transcriptome is *sine qua non* for devising effective management strategy. The availability of genome sequences of rice and *M. oryzae* has facilitated the process to a large extent. The current review summarizes recent understanding of rice-blast pathosystem, application of transcriptomics approaches to understand the interactions employing different platforms, major determinants in the interaction and possibility of using certain candidate for conditioning enhanced disease resistance (Effector Triggered Immunity and PAMP Triggered Immunity) and downstream signalling in rice. A better understanding of the interaction elements and effective strategies hold potential to reduce yield losses in rice caused by *M. oryzae*.

Introduction
Rice blast caused by *Magnaporthe oryzae* is one of the most serious diseases of rice, resulting severe yield losses (20-100%) across the globe (Khush and Jena, 2009; Sharma et al., 2012). The disease is widely distributed across 85 countries of the world and can be very devastating, when environmental conditions are conducive for disease development. It is extremely difficult to control rice blast disease; therefore, it poses a significant economic and humanitarian problem (Dean et al., 2005). The genome of the fungus *M. oryzae* is rich in repetitive segments and retro-transposons, which enable the fungus to alter pathogenicity or escape from host recognition by altering the effector molecules (Dean et al., 2005). It requires use of novel management strategies to be designed to manage this pathogen. One of the potential alternatives is employing new sources of host disease resistance against continuously evolving and geographically diverse pathogenic races. Pathogens purportedly evade this employed defence by releasing special effector molecules, aptly explained in *zig-zag* model of disease resistance (Jones and Dangl, 2006; Jacob et al., 2013). The blast fungus (*hemibiotropic*) affects all plant parts and appears at all stages of development, leading to death of plants. Major resistance genes (*R* genes) identified to play essential role fall into eight different classes of proteins, with additional subclasses defined on the basis of domain structures as well as their membrane topology (Sharma et al., 2014). The nucleotide binding site and leucine rich repeats (NBS-LRR) type form the largest class of R proteins that have either a coiled coil (CC) or a TOLL/interleukin 1 receptor (TIR) domain at the N-terminus. Each of the identified domains have structural and functional significance; the LRR domain that contains highly conserved segments (HCS) as well as variable segments (VS) is known to be involved in protein-protein or protein-ligand interactions (Matsushima and Miyashita, 2012). More than 700 NBS-LRR genes have been identified in the rice genome (Monosi et al., 2004; Singh et al., 2015) and current genome wide association studies indicate that majority of *R* genes are located on chromosome 11 of rice genome (Wang et al., 2014). The *R* genes are being widely used in breeding programs for protection against various diseases.

Rice-blast pathosystem in past few years has emerged as a model system to study host-pathogen interactions largely because of the availability of genomic sequences of both host as well as the pathogen. (IRGSP, 2005; Dean et al., 2005). A comprehensive and integrated database on rice blast: Genomic Resources of *Magnaporthe oryzae* (GROMO) has been developed for the research community (Thakur et al., 2009). Various studies on the molecular mechanisms of infection of rice with blast fungus, implicate many genes involved in plant defense and pathogen attack (Skamnioti and Gurr, 2009; Chen and Ronald, 2011; Valent and Khang, 2010; Wilson and Talbot, 2009). The emerging *omics* strategies like genomics, transcriptomics, proteomics and metabolomics have played a major role in understanding the interaction, better, like never before. Of these emerging technologies, transcriptomics plays a major role in understanding host- pathogen interaction, since these are the first evidences of expression profile in both host and pathogen.

Transcriptome refers to total set of transcripts in a given organism, or to the specific subset of transcripts present in a particular cell type at specified time frame. It is highly dynamic and changes continually. Transcripts of an organism can be measured based on the hybridization (microarray) and sequencing (RNASeq and HT/RL SAGE) technologies. Emerging high throughput sequencing techniques enable rapid acquisition of huge amounts of transcriptomic sequence data at relatively low costs (Wang et al., 2009). To date, microarray techniques have been

predominantly used for gene expression studies employing high quality annotation data. Sequencing technologies offer wider dynamic range, higher technical reproducibility, and better estimate of relative expression levels, compared to microarray technique (Fu et al., 2009; Marioni et al., 2008). The expression profiles of unannotated transcripts cannot be analyzed in microarray because the probes on chips are designed based on annotated data. HT SuperSAGE also requires gene structure information to convert tag counts to corresponding transcript expression levels. However, RNA-Seq can measure the expression levels of all transcripts without prior knowledge, and hence currently being extensively used for transcriptome profiling. Mixed transcriptome technique for simultaneous elucidation of the systems might further provide better insights. Transcriptomic studies thus provide glimpses to understand the interaction between rice and *M. oryzae* at whole genome level (Kawahara et al., 2012; Wang et al., 2014). Due to co-evolution processes, rice have also developed molecular mechanisms to suppress successful infection by pathogens, such as recognition of pathogens through receptors, generation of reactive oxygen species (ROS), expression of pathogenesis-related (PR) proteins, and accumulation of anti-microbial secondary defense compounds (Jwa et al., 2006). The transcriptome analysis thus, provide clues for understanding the rice immune response against blast fungus attack as well as how *M. oryzae* fungus counters host defence.

This article deals with the current understandings and advancement in interaction between rice-*M. oryzae* pathosystems with particular emphasis on the dynamics of transcriptomic profile in rice and *M. oryzae*. Several advancements related to the evolution of resistance genes in rice and avirulence genes in pathogenic races to understand selective forces acting on those loci have also been discussed.

Rice blast: the biggest challenge

The blast fungus was first described by Soong Ying-Shin in the 17th century (Ou, 1985). The rice blast fungus can be found in the literature under several names. *Pyricularia oryzae* was used to refer to the asexual stage of rice blast fungus, as it was found in the field. The rice pathogen was morphologically indistinguishable from pathogens of other hosts, and the entire group was defined under the name *Pyricularia (or Piricularia) grisea* (Rossman et al., 1990). The sexual stage of this fungus was named as *Magnaporthe grisea* until it was shown by phylogenetic analysis and inter-strain fertility tests that *Magnaporthe* isolates should be separated into species that infect *Digitaria* spp. (crab grass) *(M. grisea*), whereas *M. oryzae* collectively refers to the other characterized isolates, including the rice pathogen (Couch et al., 2005).The fungus *M. oryzae* (Couch and Kohn, 2002) is a hemi-biotrophic filamentous *Ascomycete* devastating rice blast pathogen (Kawasaki, 2004). Hemi-biotrophy is defined on the basis of the pathogen lifestyle that is initiated through biotrophic infection and later switching to necrotrophic growth. In biotrophic infection fungus invades living plant cells followed by necrotrophic infection where the pathogen kills the host cells, ahead of its infection and then invades them. Blast fungus has been long known to infect all the above ground parts of the plant. The most fatal form of the disease occurs when it infects the neck/panicle, which will fail to set seed (Ou, 1985). Interestingly, this fungus was shown to infect roots like a classical root pathogen, forming hyphapodia (Sesma and Osbourn, 2004). The sources of inoculum can be spores, crop residue or secondary hosts (Teng, 1994; Greer and Webster, 2001).

The blast fungus initiates disease cycle when the pyriformed, three-celled, asexual spore lands on a compatible plant host. The spore germinates to form a germ tube under optimal humidity. Appressorial formation begins when the tips of germ tubes swell and bend to leaf surface, known as 'hooking' which indicates recognition of the host (Howard and Valent, 1996). As the appressorium develops the cell wall becomes thinner at the host interface. This wall less region, called the appressorium pore, is surrounded by an 'O ring', which seals the appressorium to the host surface tightly. Melanin formation is critical for generation of enormous turgor pressure required to mechanically breach the plant cell wall. The turgor pressure in these appressoria which is around 80 times atmospheric pressure, is the highest known turgor pressure reported in a living organism. The fungus develops narrow primary invasive hyphae (IH) which subsequently develops into bulbous secondary IH. The invasive hyphae are enclosed in the extra-invasive hyphal membrane (EIHM) produced by plant cells. The hyphae move across the cells *via*. plasmodesmata and engulfs the surrounding cell. The fungus finally colonizes the host to form *eye-shaped* sporulating lesions, completing its life cycle (Figure 1).

Molecular basis of the rice blast disease development

Understanding the molecular basis of the biology of the biotrophic hyphae is an important step towards disease management. Effector proteins are usually synthesized in the invasive hyphae and secreted. Cytoplasmic effectors are secreted into specialized structure of biotrophic interfacial complex (BIC) before being translocated into plant cell cytoplasm. Apoplastic effectors are secreted into the space between the fungal cell wall and extra-invasive hyphal membrane. Few genes that impact biotrophic growth of the blast fungus have been identified, because extensive mutational analyses have mainly identified genes with a role in appressorium structure and function (Talbot, 2003). However, the exciting breakthrough in the field has been the identification of core effector proteins. Effector proteins are secreted by the pathogen into the host cell to manipulate the plant regulatory pathways (Slot and Knogge, 2002).

In cases where the plant has a resistance gene that recognizes the effector, the recognition triggers defense responses and arrests the pathogen growth. This is called the *gene-for-gene* resistance model (Flor, 1971). The rice blast system follows this model where the interaction of the host resistance (*R*) gene with the corresponding avirulence (*AVR*) gene confers resistance to the host. This interaction is very specific to a given *R*- gene. The absence of the corresponding *R*-gene or the *AVR*-gene renders the fungus



Figure 1. Typical phenotypic symptoms of rice blast disease on rice seedlings and leaf under field infection.

virulent (Jia et al., 2000). So far, 15 *M. oryzae* effector proteins have been characterized including nine Avr effectors (PWL1, PWL2, AvrPi-ta, AvrPiz-t, Avr-Pia, AvrPii, Avr-Pik/km/kp, Avr1-CO39, and ACE1) and six newly identified effectors (BAS1, BAS2, BAS3, BAS4, Slp1, and MC69) (Table 1). Identification of the fungal effectors has been a big challenge; since it has been difficult to enrich the biomass of the biotrophic hyphae in the conventional infection methods like spray inoculations and drop inoculations. Recently, a total of 851 *in planta* expressed genes encoding predicted effector proteins have been identified using a genome-wide transcriptome analysis of blast-infected rice leaves (Chen et al., 2013). Several other studies have focused on identifying infection-specific genes in the rice blast system using cDNA libraries and more recently whole genome microarray experiments. In the search for infection specific genes in *M. oryzae*, Takano et al. (2003) developed two cDNA libraries from mycelia grown in liquid cultures and conidia germinated for 8 h on an appressorium-inducing surface. Other factors like efficient nitrogen metabolism in fungi are considered pivotal to allow the fungus to adapt in different environmental conditions including infection of the host plants. Experiments conducted for identification of pathogen proteins responsible for the infection lead to either appressorial specific or *in planta* genes at later stages of infection. Jantasuriyarat et al. (2005) used an Expressed Sequence Tags (EST) sequencing approach to identify the molecular basis for defence responses. They studied resistant, partially resistant and susceptible interactions at 6 hpi and 24 hpi, representing the early stages of infection when the germ tubes were formed and when the appressorial penetration occur on the leaves, respectively. A total of 68,920 EST sequences were identified, of which only 4 sequences showed similarity to blast fungus sequences. Further, EST analysis of infected rice leaves at 74 and 120 hpi when the blast symptoms were visible lead to identification of 24.6% fungal sequences (Kim et al., 2001).

Understanding Rice-*M. oryzae* interaction

Understanding the innate mechanism underlying host-pathogen interactions helps devising strategies to manage diseases. *M. oryzae* is highly tractable and hence serves as a seminal model for plant pathological studies (Bhadauria et al., 2007). Molecular techniques like cDNA-AFLP using doubled haploid (DH) based bulked segregant analysis (BSA) led to the identification of genes that control rice blast (*Magnaporthe oryzae*). (Zheng et al., 2004). A transcript-derived fragment (TDF) was reported to be upregulated upon *M. oryzae* inoculation. Using modified SAGE technique (SuperSAGE), gene expression profiles of both the rice and blast fungus implicate *hydrophobin* gene to be most actively transcribed fungal gene in blast infected rice leaves (Matsumura et al., 2003). Expression profiles of two isogenic lines (G205 and G71) of rice in response to *M. oryzae* infection using cDNA microarray indicate expression of 2,200 expressed sequence tags (ESTs) and 998 genes were identified. Three annotated genes (casein kinase II alpha subunit gene, retrotransposon TOS17 insertion element gene and gene with unknown function) were found significantly induced in G205 only and other 35 genes exhibited differential expression in defence reactions, signal transduction, stress response, photosynthesis and sugar metabolism (Rao et al., 2002).

Table 1. Characterized effectors of *Magnaporthe oryzae*

Effector gene	*M. oryzae* isolate	Encoding protein	Gene function	Reference
MPG1	Guy11	Hydrophobin-like Protein, secreted protein	Role in conidial development and cell surface hydrophobicity	Talbot et al., 1993
PWL1	WGG-FA40	Glycine-rich, hydrophilic protein, secreted protein	Involves in resistance of weeping love grass	Kang et al., 1995; Sweigard et al., 1995
PWL2	Guy11	Glycine-rich, hydrophilic protein, secreted protein	Involves in resistance of weeping love grass	Kang et al., 1995; Sweigard et al., 1995
AvrPita	O-137	Secreted Zn-metallopeptidase protein	Interacts with *Pi-ta*	Orbach et al., 2000
ACE1	Guy11	Polyketide synthase / peptide synthetase	Interacts with *Pi33*	Bohnert et al., 2004
EMP1	70-15	Extracellular matrix protein 1, secreted pritein	Essential in appressorium formation and pathogenicity	Ahn et al., 2004
MHP1	70-15	Class II hydrophobin protein, secreted protein	Required for surface hydrophobicity	Kim et al., 2005
MSP1	70-15	Snodprot1 homolog protein, secreted protein	Essential in appressorium formation and pathogenicity	Jeong et al., 2007
Avr1-CO39	K76-79	Secreted protein	Interacts with *Pi-CO39*	Leong, 2008
AvrPiz-t	81278ZB15	Secreted protein	Interacts with *Piz-t*	Li et al., 2009
AvrPia	Ina168	Secreted protein	Interacts with *Pia*	Miki et al., 2009; Yoshida et al., 2009
AvrPii	Ina168	Secreted protein	Interacts with *Pii*	Yoshida et al., 2009
AvrPik/km/kp	Ina168	Secreted protein	Interacts with *Pik/km/kp*	Yoshida et al., 2009
BAS1	KV1	Secreted protein	Translocated into infected rice cells	Mosquera et al., 2009
BAS2	KV1	Secreted protein	Translocated into infected rice cells	Mosquera et al., 2009
BAS3	KV1	Secreted protein	Confined near to cell wall	Mosquera et al., 2009
BAS4	KV1	Secreted protein	Bounded with growing invasive hyphae	Mosquera et al., 2009
Slp1	Guy11	Secreted protein	Supresses rice basal resistance mechanism	Mentlak et al., 2012
MC69	Ina72	Secreted protein	Required for *M. oryzae* infection	Saitoh et al., 2012
MoCDIP1	Che86061 and KJ201	Secreted protein	Bcl-x1–mediated cell death suppression	Chen et al., 2013a
MoCDIP2	Che86061 and KJ201	Secreted protein	Bcl-x1–mediated cell death suppression	Chen et al., 2013a
MoCDIP3	Che86061 and KJ201	Secreted protein	Bcl-x1–mediated cell death suppression	Chen et al., 2013a
MoCDIP4	Che86061 and KJ201	Secreted protein	Bcl-x1–mediated cell death suppression	Chen et al., 2013a
MoCDIP5	Che86061 and KJ201	Secreted protein	Bcl-x1–mediated cell death suppression	Chen et al., 2013a
Iug6	98-06	Secreted protein	Supresses rice defence mechanism	Dong et al., 2015
Iug9	98-06	Secreted protein	Supresses rice defence mechanism	Dong et al., 2015

Till date, five pairs of *Avr* and *R* genes (*AvrPita* versus *Pita*, *AvrPik* versus *Pik*, *AvrPiz-t* versus *Piz-t*, *Avr-Pia* versus *Pia*, and *Avr1-CO39* versus *Pi-CO39*) have been intensively studied. Both direct and indirect interactions occur between these rice R proteins and blast Avr effectors. *AvrPita* and *Pita*, and *Avr-Pik* and *Pik* interact directly, while *AvrPiz-t* and *Piz-t* interact indirectly. The rice *R*-gene, *Pi-ta* and its corresponding *AVR-Pita* gene from the fungus were cloned and their interaction characterized (Bryan et al., 2000; Orbach et al., 2000). AVR-Pita protein appears to interact directly with Pi-ta protein and transient expression of this avirulence/effector protein in the cytoplasm of rice cells with Pi-ta triggers hypersensitive resistance (Jia et al., 2000). This suggests that the fungus delivers AVR-Pita protein into the cytoplasm of the rice cell. Although AVR-Pita appears to function as a protease inside rice cells, its role in the invasion process is not yet understood. To understand how AVR-Pita and other blast effectors function in promoting rice blast disease, it is first necessary to understand how the fungus co-opts normal plant cell processes for successful colonization of the host tissue.

Cloned blast resistance R-genes

Resistance mechanism in rice-*M. oryzae* pathosystem is governed by direct interaction as enunciated in the gene-for-gene concept (Flor, 1971). Accordingly, for each gene that confers avirulence to the pathogen there is a corresponding gene that confers resistance to the host, and vice-versa. Plant resistance (R) proteins recognize pathogen avirulence (Avr) determinants which in turn trigger signal transduction cascades that can lead to development of resistance by induction of defense responses resulting in the hypersensitive reaction at the site of infection. Although *R*-genes have been used in resistance breeding programs for a long time, they suffer from the disadvantage of being defeated by the co-evolving and variable nature of the pathogen. Hence, development of durably resistant cultivars for rice blast is a continuous and challenging process that demands a better understanding of the disease, especially when we consider the poor durability of many blast-resistant cultivars of rice, which have a typical field life of only 2-3 growing seasons before disease resistance is overcome. Till date, more than 100 *R*-genes has been mapped on rice genome, but only

twenty five genes have been cloned and characterized (Sharma et al., 2012). The cloned genes include, *Pib* (Wang et al., 1999), *Pita* (Bryan et al., 2000), *Pi54* (Sharma et al., 2005; Sharma et al., 2010), *Pi-9* (Qu et al., 2006), *Pid2* (Chen et al., 2006), *Pi2* and *Piz-t* (Zhou et al., 2006), *Pi-36* (Liu et al., 2007), *Pi-37* (Lin et al., 2007), *Pikm* (Ashikawa et al., 2008), *Pi5* (Lee et al., 2009), *Pid3* (Shang et al., 2009), *pi21* (Fukuoka et al., 2009), *Pit* (Hayashi et al., 2009), *Pb1* (Hayashi et al., 2010), *Pish* (Takahasi et al., 2010), *Pi-k* (Zhai et al., 2011), *Pik-p* (Yuan et al., 2011), *Pia* (Okuyama et al., 2011), *Pi54rh* (Das et al., 2012), *Pi25* (Chen et al.,2011), *Pi1*(Hua et al., 2012), *NLS-1*(Tang et al., 2011), *Pi54of* (Devanna et al., 2014) and *Pi35* (Fukuoka et al., 2014). Considering the requirement of broad spectrum resistance against highly variable and virulent strains of *M. oryzae,* new sources of resistance are required to develop blast resistant rice varieties. The availability of complete genome sequences of rice in public domain (rgp.dna.affrc.go.jp; www.genomics.org.cn) has been an important resource for the analysis of the genomes of different rice cultivars to develop improved rice varieties. This genome sequence information of rice will also be useful to facilitate rapid and efficient polymerase chain reaction (PCR) based allele mining approach. PCR based primer walking strategy has been used to isolate the useful alleles of cloned and functionally validated rice genes from a wide range of rice cultivars and wild species. The available sequence data of rice will also assist allele mining in other cereals like wheat which have conserved synteny at the genome level (Singh et al., 2007). Mining for allele of major *R*- genes will be important for giving rice breeders direct access to key alleles conferring resistance to biotic stresses. Allele mining for blast resistance has been reported from wild and cultivated species of rice. Readers can refer other exhaustive review on this topic (Sharma et al., 2012). Two major blast resistance genes *viz. Pi-ta* and *Pi54* have been studied extensively. *Pi-ta* alleles have been mined from various land races and different *Oryza* species. *Pi-ta* was conserved before the divergence of these *Oryza* species and there is dimorphic pattern of nucleotide polymorphism and low nucleotide diversity at the LRD region in both resistant and susceptible accessions (Wang et al., 2008; Yoshida and Miyashita, 2009; Lee et al., 2009). The allelic diversity of the *Pi-ta* in 58 US weedy rice accessions and Indian landraces was also reported (Lee et al., 2011; Thakur et al., 2013a). Similarly extensive allele mining has been reported for *Pi54*, *Pi9, Piz(t)* and *Pid3* from different rice germplasm sources (Shang et al., 2009; Liu et al., 2011; Kumari et al., 2013; Thakur et al., 2013b; Thakur et al., 2015).

Rice-*Magnaporthe oryzae* pathosystem

In recent past, considerable effort has been made to understand molecular mechanisms of *M. oryzae* infection towards rice plants. Based on those studies, a number of genes involved in rice blast disease has been cloned and characterized (Talbot, 1995; Hamer and Talbot, 1998; Xu, 2000; Balhadere and Talbot, 2001; Idnurm and Howlett, 2001). Attempts have also been made to explore the expression profiles of rice and *Magnaporthe oryzae* during both compatible and incompatible phases of interaction. These studies, though not yet comprehensive, have given a broad understanding about the probable molecular mechanisms during rice-*M. oryzae* interaction. However, available information on transcriptome analysis of rice and *M. oryzae* during infection process is present in scattered form and no efforts have been made to compile these findings together and draw specific conclusions.

Pre-colonization of rice blast fungus

Appressorium formation is a key process during *M. oryzae* infection on rice plants that leads to invasive growth of hyphae in host tissues. Naturally, conidium of *M. oryzae* is known to form appressorium on hydrophobic surface and several reports on gene expression of *M. oryzae* on hydrophobic surface have been reported (Beckerman and Ebbole, 1996; Fang and Dean, 2000; Kamakura et al., 2002). However, Lee and Dean (1993) have also shown that cAMP could induce appressorium formation even on hydrophilic membranes, if the required concentration (50 mM) of this compound is given to the sporulation. In a related study, Irie et al. (2003) reported many cAMP regulated genes in *M. oryzae*. Among the cAMP-induced, genes those related to sugar metabolism, nucleic acid metabolism, transcription factors and amino acid metabolisms have been annotated. As expected, many of them were also categorised in melanin synthesis genes that presumably involved in appressorium melanisation. High concentration of melanin in appressorium is very important in maintaining the required turgor pressure during rice plant infection. However, the appressorium formation in *M. oryzae* has a tight association with cell cycle regulation. Molecular mechanism of cell cycle mediated regulation of appressorium morphogenesis, controlled by a temperature-sensitive *MgNIMA* gene, has shown that this gene which encodes a protein kinase is essential for mitotic entry (Osmani et al., 1988). Moreover, autophagy is another process to indirectly regulate appressorium formation by conidial cell death, a programmed cell death in order to re-cycle the contents of the fungal spore which is governed by *MgATG8* gene that encodes a protein essential for autophagy process prior to rice plant infection (Veneault-Fourrey et al., 2006). For generation of very high turgor pressure inside appressorium, autophagy process and transportation of lipid bodies and glycogen to the developing appressorium can be readily observed (Thines et al., 2000). Subsequently, fatty acid metabolism in the appressorium is a complex process which requirs an orchestrated action of many triacylglycerol lipases necessary for penetration event during plant infection (Wang et al., 2007). Appressorium function is mainly regulated by two genes *MFP1* and *PEX6*. The *MFP1* gene, encoding a β-oxidation enzyme substantially affect virulence level, while *PEX6*, a peroxisomal biogenesis gene completely controls appressorium function and rice blast disease (Wang et al., 2007; Ramos-Pamplona et al., 2006). *PEX6* gene also helps in generation of Woronin bodies that is essential for proper development and functioning of appressoria by sealing the septal pores damaged in the process of autophagy. By this, a high cellular turgor is created and maintained inside the cell that is necessary for plant infection (Soundararajan et al., 2004). In addition, *Pth2* gene encodes a carnitine acetyl transferase that involves in

lipid mobilization across the peroxisomal membrane for further utilization during appressorium maturation and is necessary for penetration-hypha formation (Bhambra et al., 2006).

Highly conserved signaling networks that transfer signals from the environment to the nucleus play a crucial role in regulating host-pathogen interactions. Mitogen-activated protein kinase (MAPK) mediated signalling pathways have now been directly implicated in regulating infection-related development in various phytopathogenic fungi and highlighting the conservation of MAPK signalling as a regulatory component of fungal pathogenicity. In *M. oryzae*, three distinct MAPK mediated signalling pathways have been identified like *PMK1* for pathogenicity MAP kinase, *MPS1* for MAP kinase for penetration and sporulation and *OSM1* for osmo-regulated MAP kinase (Dixon et al., 1999; Xu et al., 1998; Xu, 2000). For normal appressorium formation, the activation of *Pmk1* gene is controlled by *Mst7* (MAPK kinase, MAPKK) and *Mst11* (MAPKK kinase, MAPKKK) genes and that was confirmed by gene replacement method using their mutated form in *M. oryzae*. *Mst50* a SAM containing protein that directly interacts with *Mst11* MAPKKK gene and mutant of this gene abolished appressorium formation and pathogenicity of *M. oryzae* (Park et al., 2006).

Post-colonization of rice blast fungus

In the rice- *M. oryzae* interaction study, several significant findings have been revealed at the stages of appressorium formation, conidial germination and other events like adhesion of conidia to the host surface, germ tube formation, turgor building inside the conidial cell, *etc*. (Howard et al., 1991; Sweigard et al., 1992; Talbot, 2003). All these events are essential for proper appressorium formation and subsequent development of blast disease in rice. Thus, it is necessary to explore the pathways and its regulation mechanisms during colonization of the fungus for actual understanding about the rice- *M. oryzae* pathosystem. Association of the effector molecules in case of *Pita-AvrPita*, *Piz-t-AvrPiz-t*, with the defense system of host plant has been well characterized in this system. The avirulence (*Avr*) protein, encoded by *AVR* gene is recognized by corresponding resistance (R) gene protein, leading to the race-specific recognition (de Wit, 2009). Several recently characterized effectors in rice blast fungus continue to follow the similar theme that most effectors are small novel secreted proteins and generally lack homology with known proteins. The mutant of *Avr* protein escapes detection by its interacting counterpart, *R* gene product and proceeds to a disease development process. In the host-pathogen interaction study of rice- *M. oryzae*, over 40 *Avr* genes have been identified (Kawahara et al., 2012) and among them nine *Avr* genes have been cloned. Among the cloned genes, all of the AVR genes except *ACE1* encode secreted proteins expressed in the invasive hyphae inside the host plant (Kawahara et al., 2012). *ACE1* is specifically expressed in the fungal appressoria and encodes PKS-NRPS protein (Bohnert et al., 2004). *Avr-Pita* and *Avr-Piz-t* are well characterized *Avr* genes of rice blast fungus. *Avr-Pita*, a family of genes (*Avr-Pita1*, *Avr-Pita2* and *Avr-Pita3*) encodes a putative neutral zinc metalloprotease (Jia et al., 2000; Khang et al., 2008). *Avr-Piz-t* acts to suppress pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) by inhibiting the ubiquitin ligase activity of the rice RING E3 ubiquitin ligase APIP6 (Park et al., 2012). *Pwl* effectors, another *Avr* gene family (*Pwl1*, *Pwl2*, *Pwl3* and *Pwl4*) are small, glycine-rich proteins that are present in this fungal pathogen (Kang et al., 1995). Though most of the *AVR* genes have been cloned from *M. oryzae* isolates from rice, *Avr1- CO39* was however cloned from a weeping love grass isolate (Peyyala and Farman, 2006). *Avr-Pia*, *Avr-Pik*/*km*/*kp*, and *Avr-Pii* were identified in a strain, Ina168 by using whole genome sequencing approach (Yoshida et al., 2009). The *M. oryzae* genome also encodes large suites of enzymes involved in secondary metabolism, including 23 polyketide synthases, several non-ribosomal peptide synthases and cytochrome P450 mono-oxygenases, consistent with the fungus having a significant capacity for secondary metabolite production. The precise function of such metabolites in pathogenesis is not well established, but interestingly one of the PKS-encoding genes *ACE1*, has been identified as an avirulence gene (de Wit et al., 2009). Therefore, it seems that secondary metabolites produced by the fungus play significant roles within the plant during establishment of disease. These effector molecules, including secondary metabolites, may be delivered from appressoria, mediated by transporters like ABC3, an ATP-binding cassette (ABC) type transporter (Sun et al., 2006). In addition, a few secreted proteins that are required for pathogenicity, *viz*. MPG1 (Talbot et al., 1993), EMP1 (Ahn et al., 2004), MHP1 (Kim et al., 2005), MSP1 (Jeong et al., 2007), MC69 (Saitoh et al., 2012), and Slp1 (Mentlak et al., 2012), and four biotrophy associated secreted proteins, BAS1 to BAS4 (Mosquera et al., 2009) have also been characterized. However, the majority of *M. oryzae* secreted proteins have not been experimentally validated for their functions in pathogenicity. Whether these predicted genes are expressed in infected rice plants remains largely unknown (Oh et al.,2008). Therefore, it is necessary to characterize all the predicted genes, especially those are expressed *in planta* to provide full insights into fungal pathogenesis.

Initiation of gene expression studies has been started with the advent of Sanger sequencing by decoding of cDNA and its libraries prepared from the rice blast fungus at different growth stages and during infection processes to rice plant (Takano et al., 2003). Initially, such studies were conducted by the help of differential display method. Using this method, Kim et al. (2000) characterized many defense-related genes expressed in rice against a treatment of *M. oryzae* elicitor. Suppression subtractive hybridization (SSH) is a rapid and effective method to isolate differentially expressed genes (Kim et al., 2001 and 2005; Xiong et al., 2001; Lu et al., 2004; Han et al., 2004). However, the high level of sequence redundancy in SSH libraries limits its ability to identify a large number of differentially expressed genes from rice infected by *M. oryzae*. The expressed sequence tag (EST) is another approach used after SSH to study a set of differentially expressed genes. It was used by Kim et al. (2001) and Jantasuriyarat et al. (2005) to identify defense transcripts on a large scale from rice. *In planta* expressed genes of *M. oryzae* were also identified in

the same study using an infected rice cDNA library (Kim et al., 2001). By employing this approach, a Class II hydrophobin MHP1 gene was found preferentially expressed inside host plant during late stages of infection and showed full involvement in virulence of rice blast fungus (Kim et al., 2005). Numa et al. (2009) sequenced 35,189 ESTs obtained from a highly destructive strain of rice blast fungus. These ESTs were mapped on *M. oryzae* genome and they validated only 4480 (~37 %) predicted genes of this fungus as protein coding genes. This study indicates that the evidence based gene prediction is essential for accurate gene-finding from any organism. A total 100 novel genes identified and reported in *M. oryzae* during a compatible rice and blast fungus interaction at later stages of infection using combined strategies of EST and SSH library analysis (Kim et al., 2010). Microarray and RNA-Seq based technologies overcome many of the limitations of using EST analysis and further take the gene expression studies at throughput level. Among microarray and RNA-Seq, the later technique scores many advantages over the earlier one (Table 2), but RNA-Seq is technically more demanding and more tedious than microarray technology (Figure 2).

Molecular analysis of Rice-*Magnaporthe* interaction

Transcriptome profile of Magnaporthe oryzae

Transcriptomics using microarray identified genes that were differentially expressed during infection-related morphogenesis of conidia and appressoria formation in *M. oryzae* (Tanako et al., 2003). Subsequently many studies have been conducted by various research groups to identify differentially expressed genes during compatible and incompatible rice- *M. oryzae* interactions (Table 3). The whole genome oligo microarray chip containing over 13,000 *M. oryzae* elements representing 10,176 predicted genes, during spore germination and appressorium formation on both an inductive hydrophobic surface and in response to cAMP (Oh et al., 2008). They suggested that *M. oryzae* employs a number of backup systems, such as functional redundancy and compensatory processes in order to protect appressorium formation from being de-regulated. Using microarray analysis, 262 fungal genes were found induced more than two fold during biotrophic invasion of *M. oryzae*. The numerous effector candidates identified and described distinctive *in planta* secretion patterns of *M. oryzae*, (*BAS*1-4 genes) which provides a valuable tool for assessing EIHM compartment integrity in individual invaded rice cells (Mosquera et al., 2009). To understand the conidiogenesis in *M. oryzae*, Kim and Lee (2012) measured global gene expression patterns in conidiation process using a whole genome oligonucleotide microarray. Approximately 4.42% and 4.08% genes were significantly up-regulated and down-regulated, respectively, during conidiation process, which shows that the process of conidiation is regulated both positively and negatively. However, the array based methods have several limitations. Dependency upon existing knowledge about genome sequence and high background levels owing to cross-hybridization are some main problems persistence with these technologies. These limitations have been overcome by tag-based methods that include serial analysis of gene expression (SAGE; Gowda et al., 2004) and massively parallel signature sequencing (MPSS; Nobuta et al., 2007). Irie et al. (2003) reported that about hundred statistically significant different tags were obtained from cAMP treated and non-treated SAGE libraries and equal numbers of the tags were induced and -repressed by cAMP. Using RL-SAGE libraries, Gowda et al. (2007) identified a large set of distinct tags (83,832) from the mixed tissues of rice and *M.oryzae* infected plants. They found that nucleotide conversions rates were high in the identified transcript tags and led to proportionate increase in the rate of mismatches in the tags prepared from the infected libraries. Many antisense transcripts were identified from rice and observed their enhanced expression in the infected rice leaves, considered as an evidence for the involvement of RNA variation and antisense transcript expression during plant-fungal interactions. An integrative approach by combining MPSS, robust long-SAGE (RL-SAGE) and oligoarray methods, Gowda et al. (2006) analyzed the mycelium and appressorium transcriptomes from *M .oryzae*. In this study, they identified a total of 2,430 mycelial genes and 1,886 appressorial genes. These differentially expressed

Table 2. Comparison of Microarray and RNA-Seq techniques in relation to transcriptome analysis.

Technology Specifications	Microarray	RNA Sequencing
Principle	Hybridization	High throughput sequencing
Resolution	Several to 100 bp	Single Base
Background Noise	High	Low
Trancriptome Data capture of a gene.	~20% of gene	Entire transcript
Dynamic range to quantify gene expression level.	Up to few hundred fold	>8,000 fold
Ability to distinguish between different isoforms.	Limited	Yes
Ability to distinguish allelic expression.	Limited	Yes
Required amount of RNA.	High	Low
For less abundant transcripts.	Less sensitive	More sensitive
Allele specific (SNP) expression.	No	Yes
Quantitation of splicing.	No	Yes
Novel alternative splicing and Novel genes	Limited	Yes

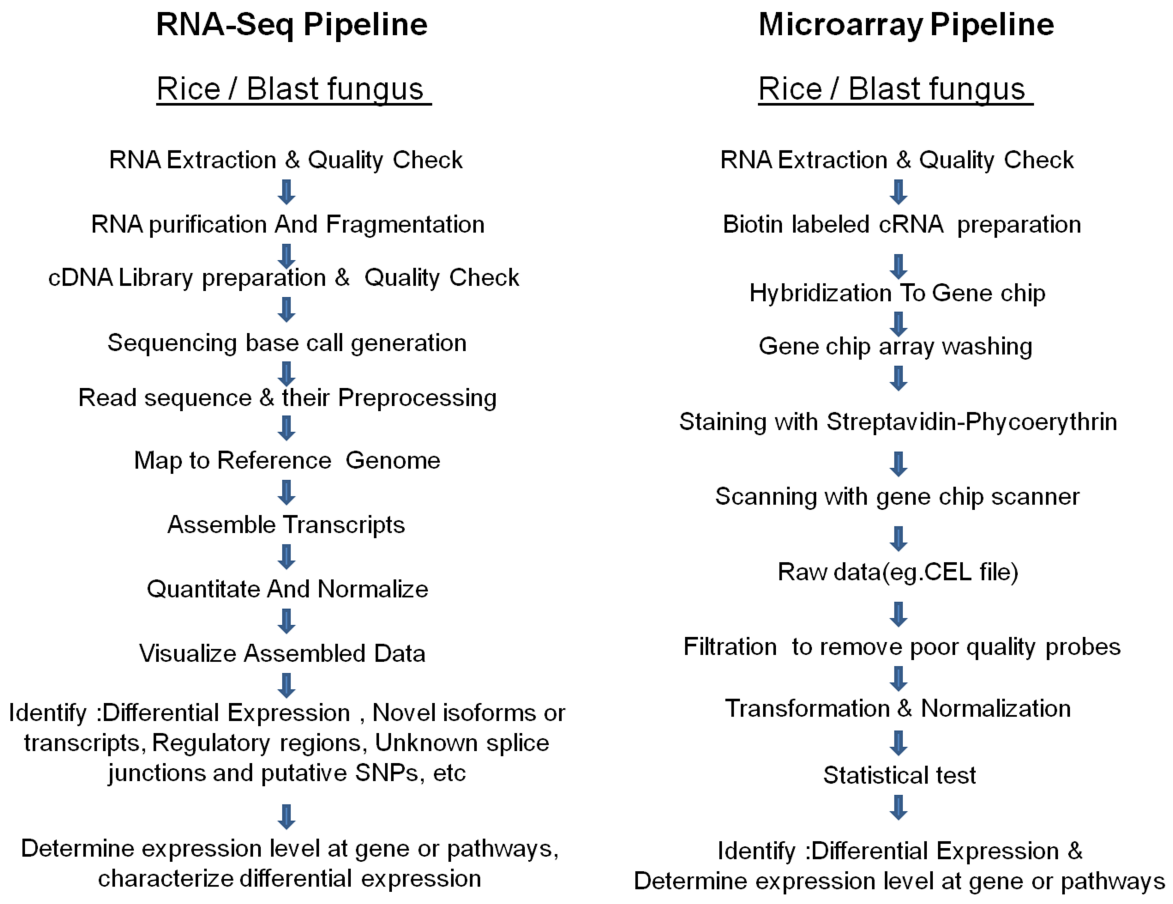

Figure 2. Comparative pipeline of microarray and RNA-Seq techniques.

Table 3. Different transcriptome studies in *M. oryzae* during its interaction with rice.

Time interval	Tissues	Technology	Reference
8 hi 24 hi	Appressoria Conidia	Microarray	Takano et al., 2003
24 hpi 96 hpi	Appressoria Mycelia	Microarray	Gowda et al., 2006
24 hpi 96 hpi	Appressoria Mycelia	MPSS	Gowda et al., 2006
96 hpi	Mycelia	RL-SAGE	Gowda et al., 2006
7 hi 12 hi	Conidia Appressoria	Microarray	Oh et al., 2008
36 hpi	Compatible interaction	Microarray	Mosquera et al., 2009
48 hpi	Conidia	Microarray	Kim and Lee, 2012
24 hpi	Compatible and Incompatible interaction	RNA-Seq	Kawahara et al., 2012
96 hpi	Compatible interaction	RL-SAGE	Chen et al., 2013
3, 6, 12, 24, 48 hpi	Incompatible and Compatible interaction	MPSS	Chen et al., 2013
6, 12, 24 hpi	Incompatible and Compatible interaction	SBS	Chen et al., 2013

transcripts, especially those specific to appressoria, represent a genomic resource useful for gaining a better understanding of the molecular basis of *M. oryzae* pathogenicity. A similar comparative transcriptome study was performed between rice and *M. oryzae* interaction in early and later interaction stages using microarray method (Wang et al., 2014). A total 608 genes were differentially expressed in response to compatible and incompatible interactions with *M. oryzae* fungus. Out of them 231 genes were highly induced in incompatible than compatible interaction. Most of the genes related to the metabolic process, response to stimuli, and cellular processes were highly expressed indicating their direct link with *M. oryzae* infection. These results suggest that a large number of both host and pathogen genes are required in the attack and counter-attack between host-pathogen in their incompatible interaction (Wang et al., 2014).

Advancement in sequencing technologies and its affordability has sorted out the problems related to the exiting methods used for mapping and quantifying transcriptomes. The whole genome transcriptome sequencing (RNA-Seq) is known to have a wider dynamic range, higher technical reproducibility, and provide a better estimate of absolute expression levels and is most commonly used technique for transcriptomic studies (Fu et al., 2009; Marioni et al., 2008). In next generation sequencing (NGS) technologies, RNA-Seq and high throughput-SuperSAGE (HT-SuperSAGE), provide almost similar sensitivity and accuracies of the measured expression levels, however HT-SuperSAGE is much more cost effective than RNA-Seq (Soanes et al., 2012). The later NGS technology has a major limitation as it require the prior information of gene structure to convert tag counts to corresponding transcript expression levels (Chen et al., 2013a). Using RNA-Seq, Kawahara et al. (2012) revealed the expression profiles of both rice and *M. oryzae* simultaneously in rice-infected leaf blades at the initial infection stage (24 hpi). They found that 16,048 rice and 889 fungal transcripts, including 432 and 17 unannotated transcripts, respectively, showed infection-responsive expression. Using two different types of fungal strains, they revealed the differential expression patterns between the compatible and incompatible interactions and also that drastic responsive reaction was common among the incompatible interaction of both rice and *M. oryzae* at the initial stages of infection. Recently, a total of 851 *in planta* expressed genes encoding predicted effector proteins have been identified by a genome-wide transcriptome analysis of *M. oryzae* infected rice leaves through RL-SAGE, MPSS and sequencing by synthesis (SBS) technologies (Chen et al., 2013a). In this study, they identified five novel effectors *MoCDIP1* to *MoCDIP5* from 42 *in planta*-expressed putative secreted proteins that induce plant cell death in rice. These genes are expressed during infection stages, especially 96 hpi in rice plant and share similar physiological phenotypes, such as the response to light, to inhibitors of calcium channel, and to Bcl-x1-mediated cell death suppression. Moreover, the CBD domain of MoCDIP4 protein served as PAMP elicitor that is a functionally conserved domain of the elicitor found among different plant-pathosystems. These results hypothesised that some of these cell death-inducing effectors may facilitate the colonization of *M. oryzae* during the late necrotrophic phase of the *M. oryzae* infection. Initially, several expression studies have been carried out using cDNAs libraries constructed from *M. oryzae* infected rice leaves to identify the interacting molecules between host and pathogen (Kim et al., 2001; Rauyaree et al., 2001). However, such studies are far from satisfactory to obtain a significant level of gene expression changes in *M. oryzae* during rice blast disease development (Chen et al., 2013a). This is possibly due to very less amount of expressed genes of invading fungus as compared to the level of indigenous expressing genes of host that present in the infected plant. Recent advances in genome sequencing technologies have led to a rapid discovery of numerous effectors in *M. oryzae* and have provided a wealth of information on their structure and function. In this pathogen, about 12% of the annotated genes (1546) coding for putative secreted proteins has been predicted from the reference genome of *M. oryzae* (Dean et al., 2005; Yoshida et al., 2009). Variability in effector molecules, especially in *M. oryzae* is mainly by their localization on unstable chromosomal regions and their linkage with transposable element. In several reports, this trend was experimentally observed and hypothesised that the presence of transposons nearby effectors, including *Avr* genes control the effectors to be gained or lost during course of evolution of the pathogen (Kang et al., 1995; Orbach et al., 2000; Yoshida et al., 2009; Singh et al., 2014). By analysis of transcriptome of a FJ81278 strain of *M. oryzae*, a total 256 candidate effectors were found through RNA-seq technique (Chen et al., 2013b). Moreover, 134 candidate effectors were identified from a genome of *M. oryzae* isolate 98-06 employing the same technique (Dong et al., 2015). By functional characterization of isolate specific genes, they also revealed that three genes, IUG6, IUG9 and IUG18 played critical role in pathogenicity of rice blast fungus. Therefore, it is essential to validate the candidate effectors and elucidate their role in rice- *M. oryzae* interaction.

Transcriptome profile of rice

Study of transcriptome revealing the levels of different transcripts and their probable functions in a given temporal and spatial conditions have been extensively studied in case of rice-*Magnaporthe* system. During the hemi-biotrophic interaction between rice and *M. oryzae*, the transcriptome of rice is subjected to stress, leading to constant fluctuations of transcripts levels during various phases of *M. oryzae* infection. In recent years many attempts have been made to explore the expression profiles of different rice lines during both compatible and incompatible rice-*M. oryzae* interactions (Table 4). These studies, though not yet comprehensive, have given a broad understanding about the probable molecular mechanisms during rice-*M. oryzae* interaction.

Transgenic approach helps understand the basic mechanism of disease resistance by over expressing the gene responsible for conditioning disease resistance. Analysis of rice transgenic lines over expressing TF, OsWRKY13 provided important cues of disease resistance

Table 4. Different transcriptome studies in rice during its interaction with *M. oryzae.*

Rice variety	Hours Post inoculation (hpi)	Technology	Reference
LTH, two NILs (IRBL18 and IRBL22)	24	Microarray	Wei et al., 2013
YY, NILs (R), NILs.(S) , LTH	0,12, 24, 36, 48, 60, 72, 96	Microarray	Huang et al.,2014
HR-12	6	Microarray	Vijayan et al., 2013
Nipponbare	84, 96, 120	Sequencing using ABI	Kim et al., 2011
NIL, H7R (resistant with Pi-k/Pir1) and H7S (susceptible)	0, 24	Microarray	Li et al., 2006
Nipponbare	72, 96	Microarray	Ribot et al., 2007
Transgenic rice TP309 (TP-Pi54) and non transgenic TP309	72	Microarray	Gupta et al. 2012
Jinheung	12, 24, 48, 72	Microarray	Wang et al., 2014
Nipponbare (carrying *Pia*)	24	RNAseq	Kawahara et al., 2012
Gigante Vercelli and Vialone Nano	24	RNAseq	Bagnaresi et al., 2012
LTH and its NIL (IRBL 12-M)	24, 48	Microarray	Kato et al., 2009

conditioning. The microarray analysis of two independent transgenic and uninfected rice lines (D11UM1-1 and D11UM7-2) overexpressing OsWRKY13 and also wild-type rice plants Mudanjiang 8, susceptible to *M. grisea*, identified a total of 460 and 445 upregulated and 478 and 605 down regulated genes specific to D11UM1-1 and D11UM7-2, respectively (Qiu et al., 2008). Microarray analysis in compatible and incompatible rice cultivars at 24 hpi suggests that the transcriptional profiles among these two interactions are mostly similar (Wei et al., 2013). Therefore, like in other systems, the incompatible and compatible rice-*M. oryzae* interactions differ mainly quantitatively (Wen et al, 2003; Lu et al., 2004). The WRKY47 transcription factor gene which showed significant upregulation during incompatible interaction was overexpressed in transgenic rice and the resulted plants could show increased resistance to rice blast fungus (Wei et al., 2013). In another RNA-seq experiment, gene ontology (GO) enrichment in compatible and incompatible interaction is similar, whereas the genes sets contributing to each GOs were dissimilar (Bagnaresi et al., 2012). On the contrary, there was a more drastic change in the transcriptome of rice in incompatible interaction compared with that of compatible (Kawahara et al., 2012). Together these studies indicate that the magnitude of change in the transcriptome of rice, during its interaction with *M. oryzae* is largely determined by the genotype of the rice plants and also probably the virulence nature of the blast fungus and the responsive reactions that are involved in the plant defense and pathogen attack are more active in the incompatible interaction (Kawahara, 2012). These correlations can be further supported with other studies also. In one such effort, 15,616 (30.0%) and 872 (5.2%) transcripts were found differentially expressed in the annotated rice and fungal transcripts, respectively (Kawahara et al., 2012). In our studies transcriptome analysis of the transgenic rice plants harbouring major rice blast resistance gene, *Pi54* identified a total of 1154 differentially expressed DEGs in TP-Pi54 plants (incompatible interaction) of which 587 were upregulated, whereas 567 genes were down regulated in case of incompatible interaction (TP-Pi54) in comparison to those in compatible interaction (TP309). Meta-analysis of the genes (FC≥2 and p value<.05) being upregulated in rice transcriptome 24 hours after infection with *M. oryzae* across different studies is demonstrated. Studies using RNA sequencing detected higher number of upregulated genes involved in Rice-*M. oryzae* interaction in comparison to those using microarray analysis. This shows the technological advantages of RNA- seq over microarray for transcriptome analysis. Overall we observed higher number of genes being significantly upregulated during incompatible interaction than compatible interaction in both microarray and RNA seq studies (Figure 3).

Reprogramming of cell wall associated genes

Rice cell wall is the first level of physical barrier for penetrating *M. oryzae* appressorium during early stage of infection. Callose (1, 3-β-glucan) is an important component of plant defense response. It is involved in the blockage of plasmodesmata and thereby acts as a physical barrier for the penetration of fungal mycelia into neighbouring plant cells (Beffa et al., 1996; Hammond-Kosack and Jones, 2000). Role of callose in rice blast resistance has already been reported in the case of blast resistance gene *Pi54* (Rai et al., 2011). Our own work reveals that the upregulation of two genes coding for callose biosynthesis was comparatively higher in the transgenic rice line TP-Pi54 in comparison to non transgenic control TP lines following *M. oryzae* infection (Gupta et al., 2012).

Phenylalanine ammonia lyase (PAL) is a first enzyme in the phenyl propanoid pathway. Previous study has reported the transcriptional activation of the phenylpropanoid pathway genes for various biotic stresses in different systems (Caldo et al., 2004). PAL is involved in phytoalexins and lignin biosynthesis (Dixon et al., 2001). The early accumulation of phenylpropanoids in case of rice- *M. oryzae* interaction has been an important determinant of resistance of rice to *M. oryzae* infection (Parker et al., 2009 and Wei et al., 2013). In resistance responses, the early activation of this pathway leads to production of both anti-microbial secondary metabolites as well as the precursors of lignin or suberin for cell-wall strengthening (Kawasaki et al., 2006). During compatible interaction between rice- *M.*

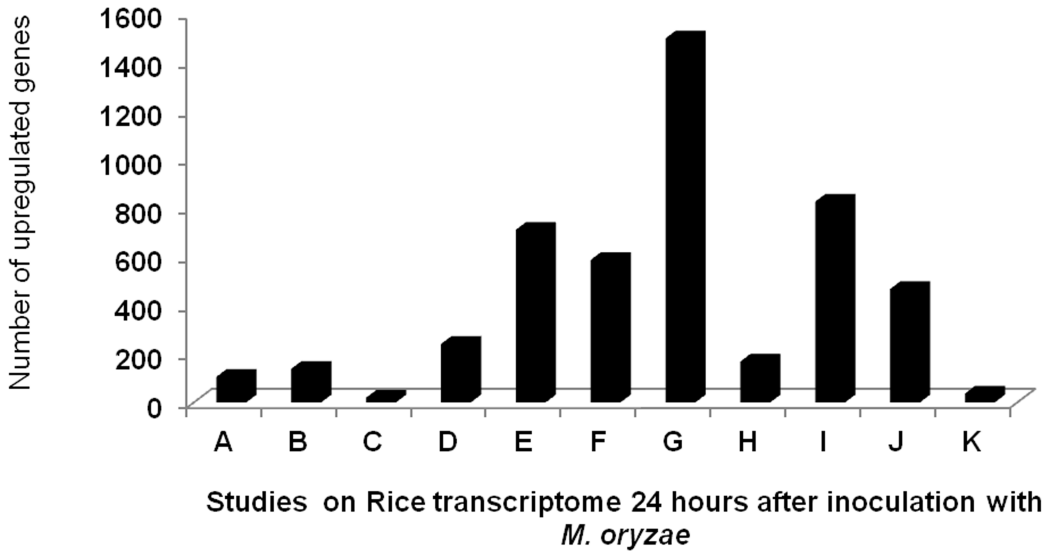

Figure 3. Comparative analysis of the genes upregulated in rice transcriptome after 24 h of infection with *M. oryzae* across. Incompatible interactions in rice line IRBL18 (A) and IRBL22 (B) (Wei et al., 2013); C: Compatible Interaction (Wei et al., 2013); D: After BTH treatment in rice plant (Shimono et al., 2007); E and F: Incompatible and compatible interaction (Bagnaresi et al., 2012); G and H: Incompatible and compatible interaction (Kawahara et al., 2012); I and J: Incompatible and compatible interaction (Priyanka et al., Unpublished); K: Compatible interaction (Vijayan et al., Unpublished).

grisea, the obvious accumulation these metabolites has been correlated with the absence of primary cell-wall thickening at the sites of fungal penetration (Huckelhoven, 2007). In this regard, previous studies, including our own study (unpublished data) reports that a class III peroxidase gene (LOC_Os07g48010), which was found to be 16.94-fold up-regulated in the transgenic line TP-Pi54 (Gupta et al., 2012) was earlier found to be involved in physiological functions like lignification and pathogen defence, and also in lignin biosynthesis (Quiroga et al., 2000; Almagro et al., 2009; Marjamaa et al., 2009). A similar trend in the expression profile of cell wall, beta-glucanase, and proteolysis related gene has been reported to be highly induced in response to *M. oryzae* infection (Bennett and Wallsgrove 1994; Wang et al., 2014).

Peroxidases and laccases are known to catalyze the polymerization of ROS-mediated lignin monomers and variation in their expression level may determine the structure of lignin formed. Beside the expression profile, the scale of expression might also determine the efficiency of lignifications. In one such analysis involving compatible interaction, our experimental results indicate the differential expression of peroxidase genes at different time intervals in response to *M. oryzae* infection. *M. oryzae* trigger early transcription of peroxidases and the number of peroxidase members expressed increases considerably with progress in infection.

Using the publicly available data we performed analysis for different functional categorisation of the upregulated genes (Figure 4). We observed significant differential regulation of cell wall related genes among different studies. The comparison of cell wall related genes being upregulated across different studies also shows that higher numbers of genes are upregulated in incompatible interaction compared to compatible interaction. This analysis indicates that during the initial phases of interaction, when *M. oryzae* penetrates in to the cell cytoplasm, rice plants enforce the defence mechanism by cell wall fortification.

Compromised photosynthesis and fluctuating primary metabolites

Photosynthesis is the source of energy for living organisms. The fungus *M. oryzae*, which infects rice, is the major competitor for photosynthates and it siphons out larger chunk of them leading to confrontation between the rice and *M. oryzae*. The fact that during the infection of rice with *M. oryzae* the rate of photosynthesis is compromised was known to scientific community way back 1993 (Bastiaans and Kropff, 1993). But, the molecular aspects of the process is being explored recently (Vergne et al., 2007; Ribot et al., 2008). During compatible rice- *M. oryzae*, most of the genes involved in photosynthesis were found be repressed, but this phenomenon is a common response of both susceptible and resistant rice plants (Vergne et al., 2007, Ribot et al., 2008). Similar findings by various authors and also our own studies have also revealed the down regulation of photosynthesis related genes post blast infection in rice (Vergne et al., 2007; Li et al., 2006; Yun et al., 2000; Bagnaresi et al., 2012; Vijayan et al, unpublished data). Li et al. (2006) further reported the upregulation of

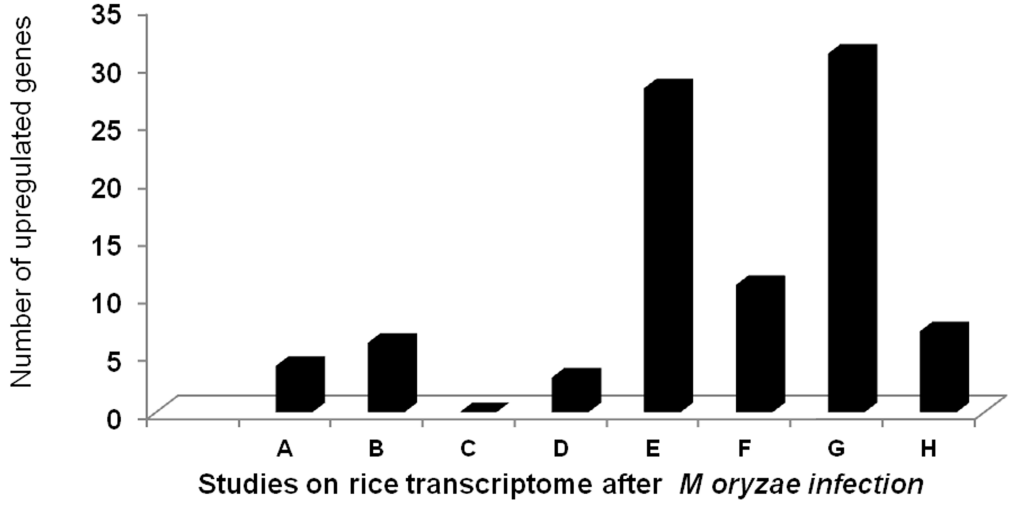

Figure 4. Comparative analysis of cell wall related genes upregulated in rice transcriptome after 24 h of infection with *M. oryzae*. Incompatible interactions in rice line IRBL18 (A) and IRBL22 (B) (Wei et al., 2013); C: Compatible Interaction (Wei et al., 2013); D: After BTH treatment in rice plant (Shimono et al., 2007); E and F: Incompatible and compatible interaction (Bagnaresi et al., 2012); G and H: Incompatible and compatible interaction (Kawahara et al., 2012).

peroxidase, NADH dehydrogenase subunit and protein synthesis-related genes in resistant interaction. Therefore, they concluded that those differentially expressed genes might represent a set of genes with different expression levels between incompatible and compatible disease reactions. Besides, many genes related to photosynthesis and metabolism were down regulated during rice-*M. grisea* interaction, signifying that energy metabolism in rice play an important role in its defense against blast fungus (Li et al., 2006).

Our study reveals the fluctuating expression of photosynthesis related genes between 12 hpi to 72 hpi (unpublished data). The initial down regulation of photosynthesis post pathogen invasion gives plants the much needed flexibility to divert the energy and resources to counter the invading pathogen (Bolton, 2009). Besides the findings reveal the increased expression of cell wall invertases in *M. oryzae* infected rice plants (Berger et al., 2004; Swarbrick et al., 2006). Cho et al. (2005) reported the up-regulations of rice *OsCIN1*, *OsCIN4*, and *OsCIN5*, post infecting with *M. grisea* and reported that these genes may play a role during a switch in metabolism to resist the invading *M. oryzae*.

Proteins play vital role in keeping plant cells viable and functional. Researchers have found that many genes related to protein synthesis were activated and protein degradation related genes were repressed in incompatible response with rice blast, suggesting that protein process might be associated with rice resistance (Li et al., 2006). In a compatible interaction between rice- *M. oryzae*, we also found the differential regulation of genes related to protein modification and degradation and they constituted around 10% of the total differentially expressed genes during early stage of infection (unpublished data). It is very much expected that infection of rice with *M. oryzae* triggers host of differentially expressed genes (DEGs) involved in host metabolism. Wang et al. (2014) analysed and found that, during incompatible interaction, GO terms associated host metabolic process showed a significant differential regulation of 35.37%, 30.19%, and 35.83% in clusters 1, 2, and 4, respectively. They suggested that infection with blast pathogen greatly alter the metabolic processes inside rice plants. Another study dealing with different time intervals (24-120 hpi) of an incompatible interaction also revealed the greater enrichment of transcripts related to carbon fixation,Glycolysis/gluconeogenesis and photosynthesis (Huang et al., 2014). Taken together, these data suggest that rice reprograms metabolic and biological processes related to energy metabolism in response to infection with *M. oryzae*.

Impaired hormonal balance and altered signalling

Plant hormonal pathways that are important regulators of defence-gene expression are Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) pathways. JA and ET pathways are involved in resistance response against necrotrophs while SA pathway is mainly involved in resistance to biotrophic and hemibiotrophic pathogens (Robert et al., 2011). There is considerable overlap between these pathways during different stress conditions. In Pathogen Triggered Immunity (PTI) response, JA-ET and SA pathways act synergistically. So blocking just one component can perturb overall plant stress response. Therefore, many pathogen effectors suppress the PTI response by interacting with different target proteins. In Effector Triggered Immunity (ETI) response, redundant activities of JA-ET and SA pathways are involved. So in the absence of SA signaling, JA-ET pathway contribute to maintain pathogen resistance in plants (Dodds et al., 2010).

Previous reports reveal that altered JA expression in rice plants acts as a powerful mediator of resistance against hemibiotroph *M. oryzae* and bacterial pathogen *Xanthomonas oryzae pv. oryzae* (Deng et al., 2012; Riemann et al., 2013; Tao et al., 2009; Xiao et al., 2009; Yamada et al., 2012 and Mei et al., 2006). The transcriptome analysis of rice- *M. oryzae* in resistant near isogenic line and susceptible control showed upregulation of enzymes involved in JA biosynthesis (Wei et al., 2013). Similarly, Wang et al. (2014) reported the role of ET and JA in resistance response of rice leaves infected with incompatible *M. oryzae.* During incompatible rice- *M. oryzae* interaction, upregulation of five genes related with ethylene, as well as the genes related with salicylic acid (SA) and jasmonic acid (JA) signaling were reported at 48 hpi (Wang et al., 2014). They also reported the induction of those genes related to JA and SA signaling, oxidation-reduction as well as calcium regulation, MAP kinase, and phosphoinositides. Beside this, our study also confirms the early induction of ET biosynthesis genes and in general gradual increase in the number DEGs related to ABA, auxin, cytokinin, SA, and GA from 24 hpi (Vijayan et al., unpublished data). Together these data indicate a major reprogramming of hormonal pathways and cell signaling after pathogen infection at 24 hpi. Incompatible interaction with *M. oryzae* could also activate ET emissions earlier than compatible interaction (Iwai et al., 2006). Therefore, these results confirm that during early phase of fungal infection there is drastic reprogramming of genes related to hormonal pathways involved in defence mechanism against rice blast fungus.

Plant signal perception and activation of downstream responses is crucial during innate immunity. Cellular events which are common in ETI and PTI mediated response in rice after of *M. oryzae* infection is well deciphered in rice. These include depolarization of plasma membrane, activity of ion channel is modified and is burst of metabolism occurs which leads to generation of reactive oxygen species (ROX) and antimicrobial compounds, mitogen activated protein kinase (MAPK) cascades or calcium dependent protein kinase (CDPKs) are activated, pathogen responsive genes are transcribed and lignin and callose are deposited at plant cell wall (Dodds et al., 2010; Meng., 2013). Other important molecules involved in defence signalling include chitin elicitor binding protein (CEBiP), chitin elicitor receptor kinase (OsCERK1), receptor-like kinases (RLKs), and wall-associated kinases (WAK). Genes involved in signaling pathways within rice after *M. oryzae* infection are highly upregulated in near isogenic line carrying blast resistance gene *Pi9* (Wei et al., 2013) and transgenic line carrying blast resistance gene *Pi54* compared to susceptible control (Gupta et al., 2012) . This shows ETI mediated response to be quantitatively stronger during resistance response than during susceptible interaction (Jones et al., 2006, Dodds et al., 2010, Tao et al., 2003, Wei et al., 2013). Rice CEBiP is characterized to be involved in sensing and binding of chitin oligosaccharides. Post elicitor binding, CEBiP forms a hetero-oligomers with OsCERK1, and this complex through transphosphorylation might trigger downstream signalling cascade (Kaku et al., 2006; Shimizu et al., 2010). The transcriptome analysis of during rice infection with *M. oryzae* has revealed that *CEBiP* and *OsCERK1* genes were highly upregulated during incompatible interaction in comparison to compatible reaction and also additional OsLysM-RLK genes were found induced only in incompatible interaction (Bagnaresi et al., 2012).

In signaling pathways receptor kinase play important role and are upregulated in near isogenic line carrying blast resistance gene *Pi9* (Wei et al., 2013). Receptor kinase category includes receptor like kinase, receptor like cytoplasmic kinase and wall associated kinase which are responsible for internal and external signal perception. (Wei et al., 2013). Wall associated kinases (WAKs) are known to perceive the fungal cell wall associated oligogalacturonides during pathogen infection, and trigger innate immune response in plants (Brutus et al., 2010). The RNA- seq analysis of resistant cv. GV and the susceptible cv. VN showed significant upregulation of 15 WAK transcripts in GV and only 4 WAKs in VN (Bagnaresi et al., 2012). Through microarray analysis, very high upregulation of *OsWAKY71* and *OsWAK25* has been reported during incompatible rice interaction with *M. oryzae* (Wei et al., 2013). OsWAK 25 which is reported to enhance resistance to bacterial pathogen *Xanthomonas oryzae pv. oryzae* (Seo et al., 2011) was also found upregulated in near isogenic line carrying blast resistance gene *Pi9* (Wei et al., 2013). A putative WAK family gene was also found down regulated in compatible disease response during our analysis (Vijayan et al., 2013). Together these data suggest that signal perception and the activation of downstream signalling cascade involves a complex set of signalling molecules and is a vital in providing resistance during rice incompatible reaction with *M. oryzae.*

MAP kinases are the signalling molecules involve in signaling downstream of receptor kinase involved in biosynthesis of photoalexin camalexin in *Arabidopsis* (Ahuja et al., 2012). Four MAPK transcripts including isoforms of MAP3K.3 and MAP3K.1 were found upregulated in resistant line GV but not in susceptible. In an interconnecting signalling cascade, MAPKs OsMPK3, MAPKK OsMKK4 and OsMPK6 were found to play a key role in amplifying chitin elicitor signal for defense responses (Hamel et al., 2006).

Transcription factors in Rice M. oryzae interaction

Transcription factors play a pivotal role in arming the plants with the required phenotypic plasticity which is very critical under various biotic and abiotic stresses. During rice-*M.oryzae* interaction there has been an extensive reprogramming of their respective transcriptomes leading to the adaptive plasticity of plants or establishing compatible interaction by fungus in highly variable environments. This plasticity is mainly achieved by network of transcription factors like *WRKY, NAC, Dof* Zinc finger, *MAD* box and *bZIP* (Gupta et al., 2012). Transcriptome analysis during interaction of rice with *M. oryzae* has revealed that transcripts coding for WRKY family TFs are most commonly reprogrammed and they are crucial regulators of disease resistance (Eulgem, 2005; Ryu et al., 2006; Shimono et al., 2007; Delteil et al., 2012; Bagnaresi

et al., 2012; Kawahara et al., 2012; Wei et al., 2013). Among the WRKY family, transcripts coding for *WRKY22*, *WRKY30*, WRKY45, *WRKY45-2*, *WRKY47*, *WRKY53*, *WRKY55/WRKY31* and *WRKY104/WRKY89* are the positive regulators of rice resistance to *M. oryzae*, whereas those coding for *WRKY13*, *WRKY28, WRKY42* and *WRKY76* are negative regulators (Shimono et al., 2007; Cheng and Wang, 2014; Chujo et al., 2013; Qiu et al., 2008; Cheng et al., 2015). Using RNA-seq and microarray approaches, significant upregulation of WRKY family TFs has been reported during rice- *M. oryzae* interaction at 24 hpi (Shimono et al., 2007; Gupta et al., 2011; Kawahara et al., 2012; Bagnaresi et al., 2012; Yokatani et al., 2013; Vijayan et al., 2013; Chujo et al., 2007; Zhang et al., 2008; Wei et al., 2013) .WRKY gene activate several pathogenesis related genes (Chujo et al., 2007 and Peng et al., 2010). Higher number of WRKY were upregulated in blast resistant genotype GV and near isogenic line carrying blast resistance gene *Pi9* compared to susceptible control (Bagnaresi et al., 2012,Wei et al., 2013). These studies show that WRKY as an important regulator in rice blast resistance.

The other important TF gene family genes coding for ethylene response factors (ERF) are differentially regulated by various signalling molecules like ethylene, abscisic, salicylic, jasmonic, gibberellic acids and their interactions during *M. oryzae* interaction with rice. (Sakuma et al., 2002, Grennan, 2008). Besides these, *OsDREB* and *NAC4* TF were also induced during the early phase of infection at 12 and 24 hpi (Kaneda et al., 2007; Kawahara et al., 2012; Vijayan et al., 2013). The expression of *DREB* TF can be attributed to the finding that DREB helps in overcoming the rice susceptibility to blast caused by drought and cold stress (Koga et al., 2004a). The MYB and MAD box TF genes too have been reported to be induced during infection of blast fungus *Magnaporthe* during both compatible and incompatible interactions (Gupta et al., 2011; Vijayan et al., 2013; Wang et al., 2014). These TFs play an important role in activation of plant defense signaling (Ramalingam et al., 2003; Rasmussen et al., 2012). The microarray and Differential Display-PCR analysis have revealed the induction of AP2/EREBP family TF genes, which is required for ethylene responsiveness during rice- *M. oryzae* (Kim et al., 2000; Wei et al., 2013). NAC family transcription factors play a positive role in rice plant defense response. *OsNAC4,* OsNAC6 have been found upregulated in many of the incompatible interaction with *OsNAC* being induced at the early stage of infection (Mosquerel et al., 2009; Gupta et al., 2012; Kawahara et al., 2012). This report signifies the role of *Os NAC4*, which plays an important role in the initial hypersensitive cell death in rice plants after the recognition of flagellin in rice cells (Kaneda et al., 2007).

Our Meta analysis of transcription factors coding genes indicated upregulation of this category of genes in different studies (A to H) and also shows that higher number of genes coding for TF was found upregulated in the incompatible interaction when compared to compatible interaction (Figure 5).

Secondary metabolites and pathogenesis response genes

Secondary metabolites play an important role in PAMPs and Effector Triggered Immunity (PTI/ ETI). Rice have developed various defense techniques like variety of pathogens receptors, generation of reactive oxygen species (ROS) and use of anti-microbial secondary metabolites such as phytoalexins (Jwa et al., 2006). The rice reactive oxygen species (ROS) are involved in cross-linking and host cell wall enforcement during *M. oryzae* infection. ROS levels are well related with *M. oryzae* infection in rice (Chi et al., 2009; Mittler et al., 2004). Further, levels of peroxidase and glutathione-S-transferase

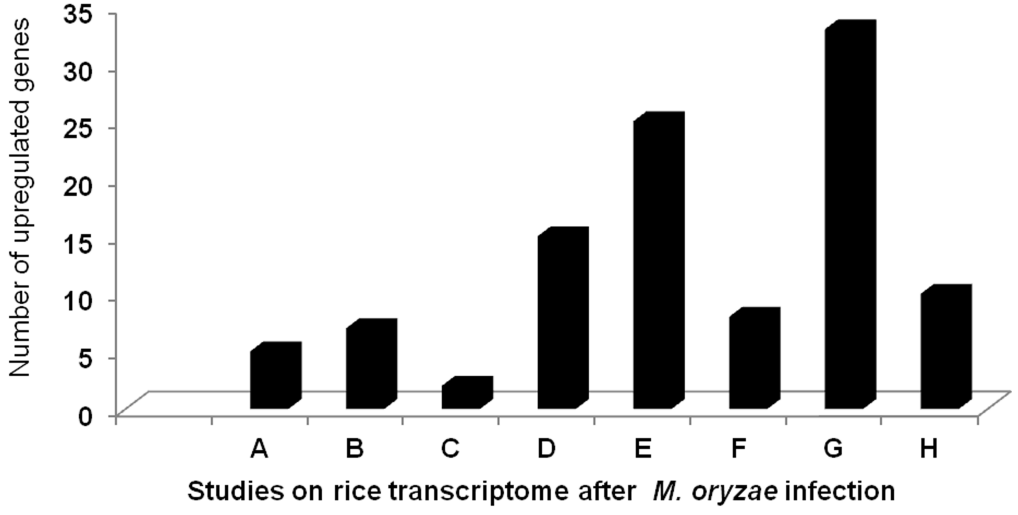

Figure 5. Comparative analysis of transcription factor related genes upregulated in rice transcriptome after 24 h infection with *M. oryzae*. Incompatible interactions in rice line IRBL18 (A) and IRBL22 (B) (Wei et al., 2013); C: Compatible Interaction (Wei et al., 2013); D: After BTH treatment in rice plant (Shimono et al., 2007); E and F: Incompatible and compatible interaction (Bagnaresi et al., 2012); G and H: Incompatible and compatible interaction (Kawahara et al., 2012).

were comparatively more up-regulated at 48 hpi than at 12 hpi suggesting increased level of ROS scavengers in infected tissues at later stage of infection (Wang, 2014). These findings suggest elevation of ROS in *M.oryzae* infected tissues. The two peroxidases detected in an early stage of infection suggest that ROS signalling is activated in the early stage of *M. oryzae* infections (Wang et al., 2014). On the other hand, transcriptome analysis has also revealed the upregulation of transcripts coding for ROS detoxifying enzymes like peroxidases and mono-oxygenases during only incompatible interactions (Qiu et al., 2008; Bagnaresi et al., 2012). Out of the 20 induced ROS homeostasis genes, two transcripts were coding for putative glutaredoxin family proteins (Qiu et al., 2008).

Malate is known to serve as a substrate for NADPH production, which in turn is utilized by NADPH oxidase for the generation of ROS intermediates. NADP-ME (Malic enzyme) activity, which coverts malate into NADPH has been found reduced in susceptible host-pathogen interactions (Parker et al., 2009). The recent study performed in our lab also indicates a similar trend with the down regulation of NADP-ME at the early stages after *M. oryzae* infection, but subsequent up regulation at later stages of infection in rice (Vijayan, unpublished data). The late up regulation of a NADP-ME gene suggests the delayed induction of these ROS producing genes in susceptible host genotypes.

We also performed a meta analysis of genes upregulated at 24 hpi and the findings show significant differential regulation of respiratory burst related genes (peroxidises and glutathione S-transferase) in the incompatible interactions when compared to the compatible interaction among different studies (Figure 6).

Up regulation of secondary metabolite expression is a common trend in response to stress (Bennett and Wallsgrove, 1994). Down regulation of carotenoids has been previously reported as a result of *M. oryzae* infection (Bagnaresi et al., 2013). The repression of genes of the carotenoid biosynthesis pathway might suggest the redirection of substrates to produce defensive constituents of isoprenoid pathway probably in response to pathogen infection (Boba et al., 2011).

Secondary metabolism genes were found upregulated in resistant and susceptible rice line after *M. oryzae* infection. In phenylalanine and shikimate biosynthesis as well as downstream of phenylpropanoid biosynthesis, large number of enzymes show upregulation in resistant near isogenic line (NIL) carrying blast resistance gene *Pi9* (Wei et al., 2013) and transgenic line carrying blast resistance gene *Pi54* (Gupta et al., 2012). Phenyl propanoid pathway plays an important role as phenylpropanoids are important antimicrobial compound. This pathway is involved in synthesizing lignin and phytoalexin that prevent invasion of pathogen in host. To counteract *M. oryzae* infection, rice leaves accumulate diterpene phytoalexins phytocassanes, oryzalexins and momilactones (Okada,2011; Dillon et al. 1997; Umemura et al., 2003). The important genes in diterpene phytoalexin, momilactone and phytocassanases biosynthesis pathways were upregulated in blast resistant genotype GV after *M. oryzae* infection (Bagnaresi et al., 2012). Similarly diterpene phytoalexin biosynthetic (DPB) geneOsKSL8 (LOC_Os11g28530; oryzalexin S synthesis) shows higher expression in incompatible interaction than in compatible reaction after *M. oryzae* infection (Bagnaresi et al., 2012). These results therefore highlight the importance of diterpene phytoalexin biosynthesis genes during rice-*M. oryzae* interaction.

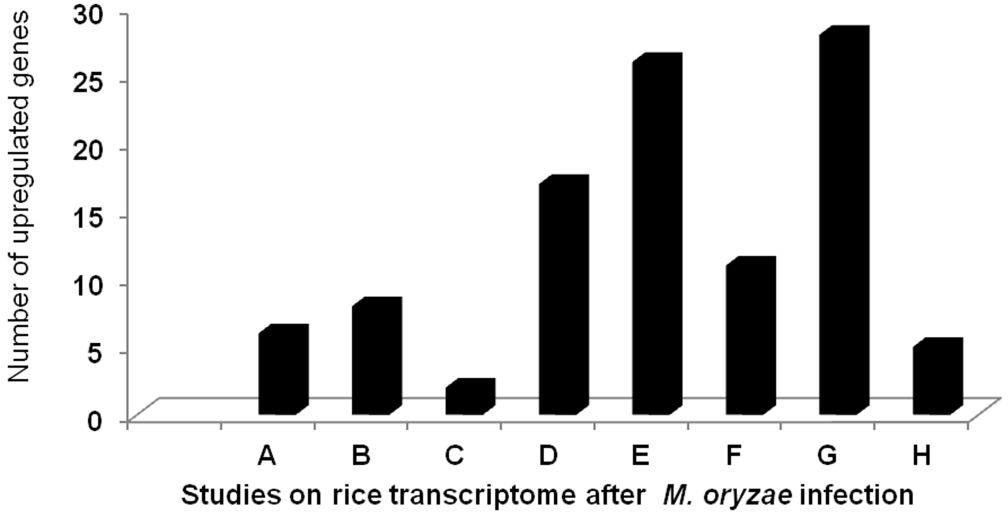

Figure 6. Comparative analysis respiratory burst related genes found in different studies related to the transcriptomes of *M. oryzae* - rice interaction after 24 h of infection. Incompatible interactions in rice line IRBL18 (A) and IRBL22 (B) (Wei et al., 2013); C: Compatible Interaction (Wei et al., 2013); D: After BTH treatment in rice plant (Shimono et al., 2007); E and F: Incompatible and compatible interaction (Bagnaresi et al., 2012); G and H: Incompatible and compatible interaction (Kawahara et al., 2012).

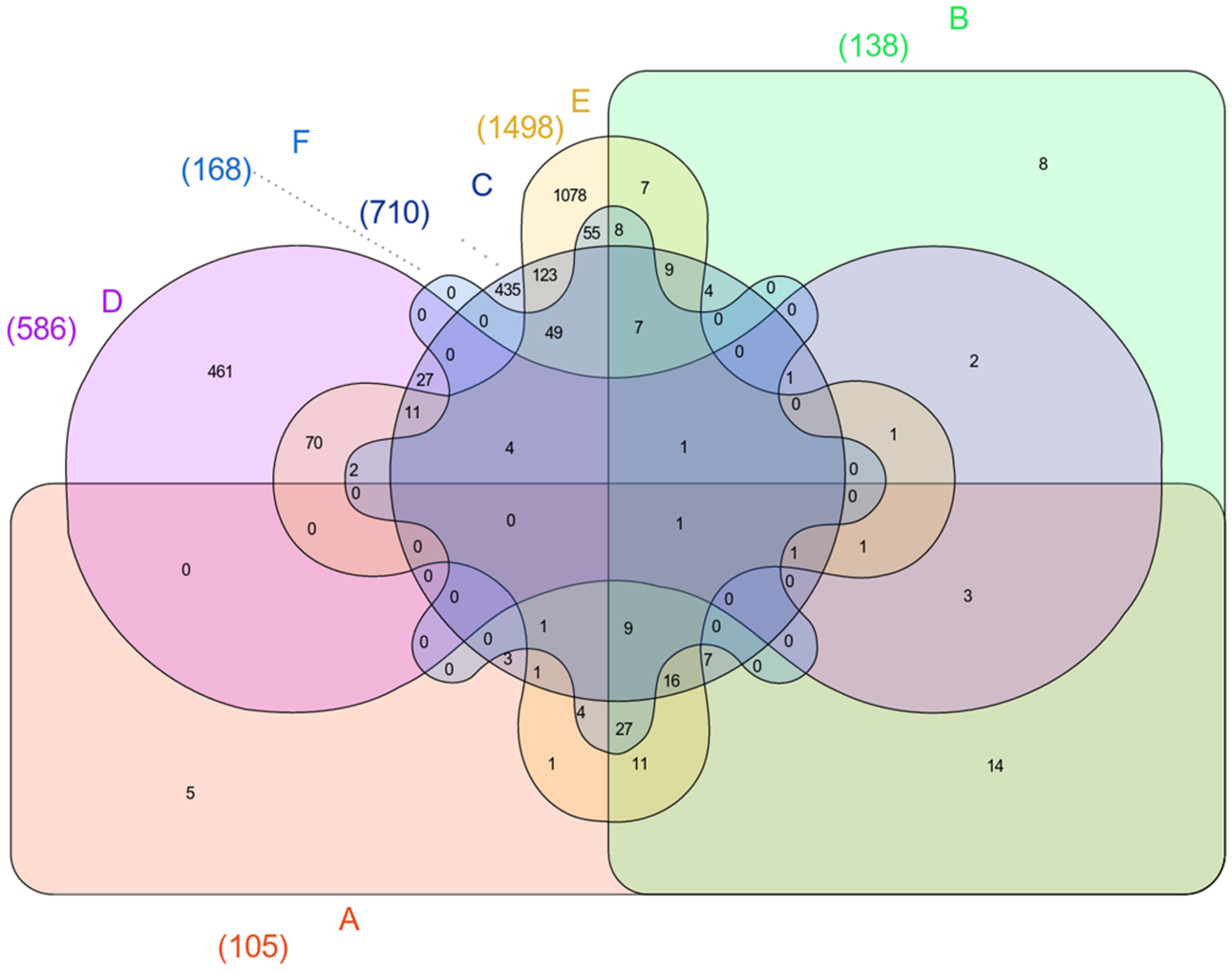

Figure 7. Venn diagram to compare upregulated genes across different studies on rice transcriptome 24 hours after *M. oryzae* inoculation. Incompatible interactions in rice line IRBL18 (A) and IRBL22 (B) (Wei et al., 2013); C and D: Incompatible and compatible interaction (Bagnaresi et al., 2012); E and F: Incompatible and compatible interaction (Kawahara et al., 2012).

Pathogenesis related (PR) genes are those, which are involved in the process of plant defense response and in suppressing pathogen infection. The commonly known PR genes are thaumatin-like proteins and chitinases (Sels et al., 2008). In a resistance interaction, plant immune signaling was triggered rapidly through the recognition of rice receptors by their elicitors/effectors, and PR genes were more rapidly accumulated in incompatible than in compatible interaction (Wang et al., 2014; Kawahara et al., 2012. Transcriptional expression analysis of PR genes also confirmed that an early defense response rapidly occurred in the incompatible interaction at 12 hpi, with high accumulation at 48 hpi (Wang et al., 2014). Similarly, Kawahara et al., 2012 found the higher level of expression of PR genes like PR1, PR10, POX22 and PR2 family transcripts like GH17 during incompatible rice- *M. oryzae* interaction. Moreover, their study reveals the essentiality of fungal penetration for the activation of defense PR genes.

The upregulated genes found in above eight interactions was compared using InteractiVenn (www.interactivenn.net). The findings of this analysis show that there is no common gene observed across these different studies. But when we compared the data from upregulated genes of six interaction studies, it was observed that a single gene coding for cytochrome P450 (LOC_Os03g12500.1) is found to be common among these interactions (Figure 7). Therefore, the cytochrome P450 genes, found to be common among the six studies has a critical role to play during rice- *M. oryzae* interaction. Hence, the application of transcriptome data has huge potential in the identification of novel disease response genes of rice and also novel effector and *Avr* genes of *Magnaporthe oryzae* (Vergne et al., 2007; Chen et al., 2013a). The whole genome transcriptome analysis also helps in understanding the molecular mechanism of rice resistance and *Magnaporthe oryzae* pathogenicity.

Conclusions

Rice-*M.oryzae* interaction has emerged as a model plant-pathosystem over the years. Sequence information of both rice and *M. oryzae* genomes have largely contributed to this development. Transcriptome analysis during rice- *M. oryzae* interaction reveals differentially expressed transcripts at a given phase of infection process. These transcripts provide important information for identification, cloning and characterization of underlying genes for the functional correlation. Interestingly, transcriptome profile of rice during compatible and incompatible interactions differs only quantitatively , otherwise the set of genes differentially regulated remain more or less similar in both the cases, cytochrome P450 being one such molecule. Moreover, different resistant rice lines activate significantly different set of genes in response to *M. oryzae* infection. At the initial stages of infection there is a perturbance in the transcripts related to the respiratory burst, cell wall associated genes, early signalling genes in case of rice and those related to cellular metabolism, conidiation and appressorium formation in case of *Magnaporthe oryzae*. At later stages of infection transcripts coding for effectors are found with elevated expression. Besides effector molecules, transcripts related TF, kinases, hormones, photosynthesis, secondary metabolite and defence response gene were found up-regulated. The transcriptome analysis has a great potential to the identification of novel defence responsive genes and the genes mediating the resistance response in case of rice and also effector and *Avr*- genes contributing to the pathogenesis in case of *M. oryzae*. The transcriptome being the first step in gene regulation provide important cues for understanding the arms race between the host and the pathogen. A sustainable strategy for disease control can only be devised once we understand the metabolic and regulatory pathways involved in host-pathogen interaction.

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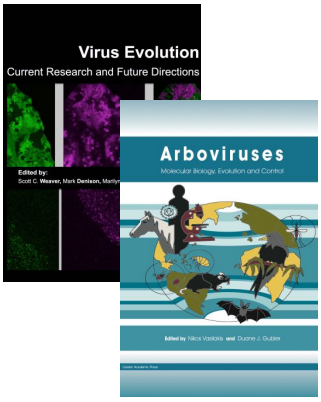

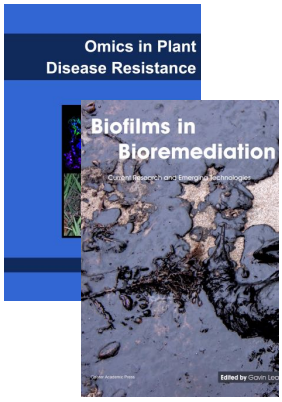

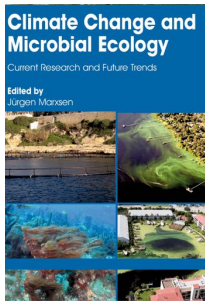

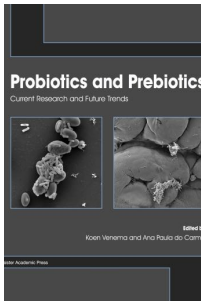

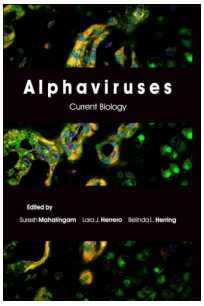



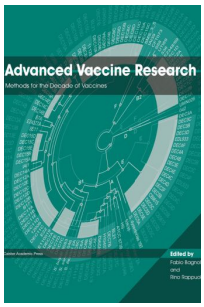

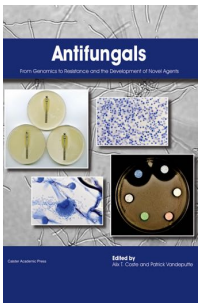

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