CHAPTER 2: Transcriptome of *M. oryzae* Histidine Phosphotransferase *MoHPT1* knock-down reveals significant functional role of two component systems in establishment of infection.

4.0 ABSTRACT

M. oryzae Histidine phosphotransferase *MoHPT1* (*MoYPD1*) knock-down resulted in increased sensitivity to various stresses like oxidative stress and cell wall stress. The transcriptome analysis of the *MoHPT1* knock-down transformant without stress showed 146 genes differentially expressed as compared to wild type. Up-regulated genes included transporters, nutrient acquisition and metabolism genes, oxidative stress related genes and fungal cell wall degrading genes, suggesting their importance in nutritive stress and pathogenicity. Down-regulated genes included those required for light response, detoxification mechanism, amino acid metabolism, secondary metabolism and secretory proteins like endoglucanases, laccases and polysaccharide deacetylases. Oxidative stress sensitivity of the *MoHPT1* knock-down transformant encouraged us to analyse their transcriptome under oxidative stress. A majority of the genes which were differentially expressed under oxidative stress were the same as those observed in WT vs RA6 without stress, but with a higher fold changes in the transcript levels. There were about 52 genes which were only expressed in the oxidative stress condition. Our results identified the genes regulated by *M. oryzae* *MoHPT1* to affect oxidative stress response and pathogenicity.

4.1 INTRODUCTION: Functional role of TCS in virulence and development

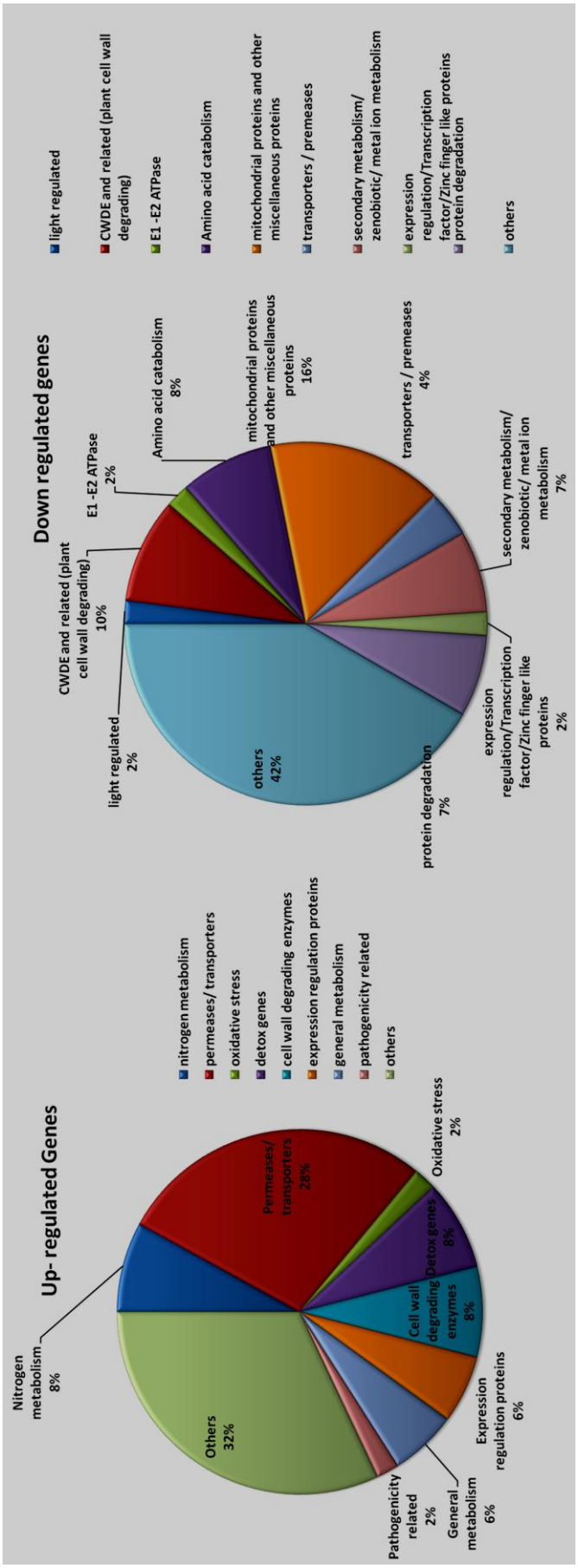
Magnaporthe oryzae has emerged as a model plant pathogen owing to its hemibiotrophic lifestyle and its amenability to genetic studies. Every plant pathogen whether a biotroph or necrotroph has to overcome the host defence barriers in order to establish itself. Production of Reactive oxygen species (ROS) is one of the first responses of the host to limit the spread of the pathogen. Ability to overcome oxidative stress is therefore necessary for virulence in phytopathogens. Oxidative stress response is mediated through the stress activated MAP kinases in fungi (SAPK) and other eukaryotes. These SAPKs are activated by regulatory signalling components which include environmental cue sensors and response regulators. In fungi, this signalling is performed by the sensory histidine kinase, the intermediate histidine phosphotransferase and response regulator commonly referred to as the 'Two Component System' (TCS). In pathogenic fungi like *C. neoformans, A. fumigatus, C. heterotrophus, G. zeae,* and *C. albicans* they have been associated with virulence (Alex *et al.*, 1998; Pott *et al.*, 2000; Bahn *et al.*, 2006; Oide *et al.*, 2010). But understanding the biology of TCS in pathogenic fungi has been difficult since the downstream targets are not known. We observed that the TCS in *M. oryzae* is involved in the oxidative stress response through MoHOG1 by silencing *MoHPT1*, the *M. oryzae* histidine phosphotransferase. Our studies as mentioned in chapter 1showed that *MoHPT1* is also involved in light response and cell wall integrity maintenance. Global expression analysis is an effective and powerful method which can also give quantitative information on transcriptional expression providing an over all preview into the biology of a particular stage or condition. A comparative transcriptome analysis of *MoHPT1* knock-down transformant was performed to identify

differentially expressed genes. We demonstrate the importance of *MoHPT1* in development, stress regulation and pathogenicity, by studying *MoHPT1* at 60% of its normal expression levels. Our study reveals that *MoHPT1* regulates genes important for oxidative stress survival, light response, fungal cell wall development and pathogenicity.

4.2 RESULT: Investigation of *MoHPT1* regulated stress response by global expression analysis

4.2.1 Differential expression of genes in the knock-down transformant RA6 compared to wild type B157

The comprehensive gene expression profiles of the *MoHPT1* regulated genes in *M. oryzae* were analysed by comparing the transcriptome of *MoHPT1* knock-down transformants to the wild type. 50 genes were significantly up-regulated while 96 genes showed significant down-regulation (p-value <= 0.01). Up-regulated genes were categorised into different classes according to their predicted functions, namely, nitrogen metabolism, transporters and permeases, detoxification genes involved in response to oxidative stress and xenobiotics, cell wall degrading enzymes, transcription factors and also some minor classes (Fig.27). However, the down-regulated genes predominantly included plant cell wall degrading genes, amino acid catabolism genes, secondary metabolism genes, light perception and regulation genes, protein degradation, and transporters. These results were further validated by qRT-PCR. The validation experiments were performed in triplicates and analysed statistically. The primers used


Up- regulated Genes
Nitrogen metabolism 8%
Others 32%
Pathogenicity related 2%
General metabolism 6%
Expression regulation proteins 6%
Cell wall degrading enzymes 8%
Detox genes 8%
Oxidative stress 2%
Permeases/ transporters 28%
nitrogen metabolism
permeases/ transporters
oxidative stress
detox genes
cell wall degrading enzymes
expression regulation proteins
general metabolism
pathogenicity related
others
Down regulated genes
CWDE and related (plant cell wall degrading) 10%
E1 -E2 ATPase 2%
Amino acid catabolism 8%
mitochondrial proteins and other miscellaneous proteins 16%
transporters / premeases 4%
secondary metabolism/ zenobiotic/ metal ion metabolism 7%
expression regulation/Transcription factor/Zinc finger like proteins 2%
protein degradation 7%
others 42%
light regulated 2%
light regulated
CWDE and related (plant cell wall degrading)
E1 -E2 ATPase
Amino acid catabolism
mitochondrial proteins and other miscellaneous proteins
transporters / premeases
secondary metabolism/ zenobiotic/ metal ion metabolism
expression regulation/Transcription factor/Zinc finger like proteins
protein degradation
others

are given in Appendix 3, Table 2.

4.2.1.1 Up-regulation of genes involved in nutrition and metabolism

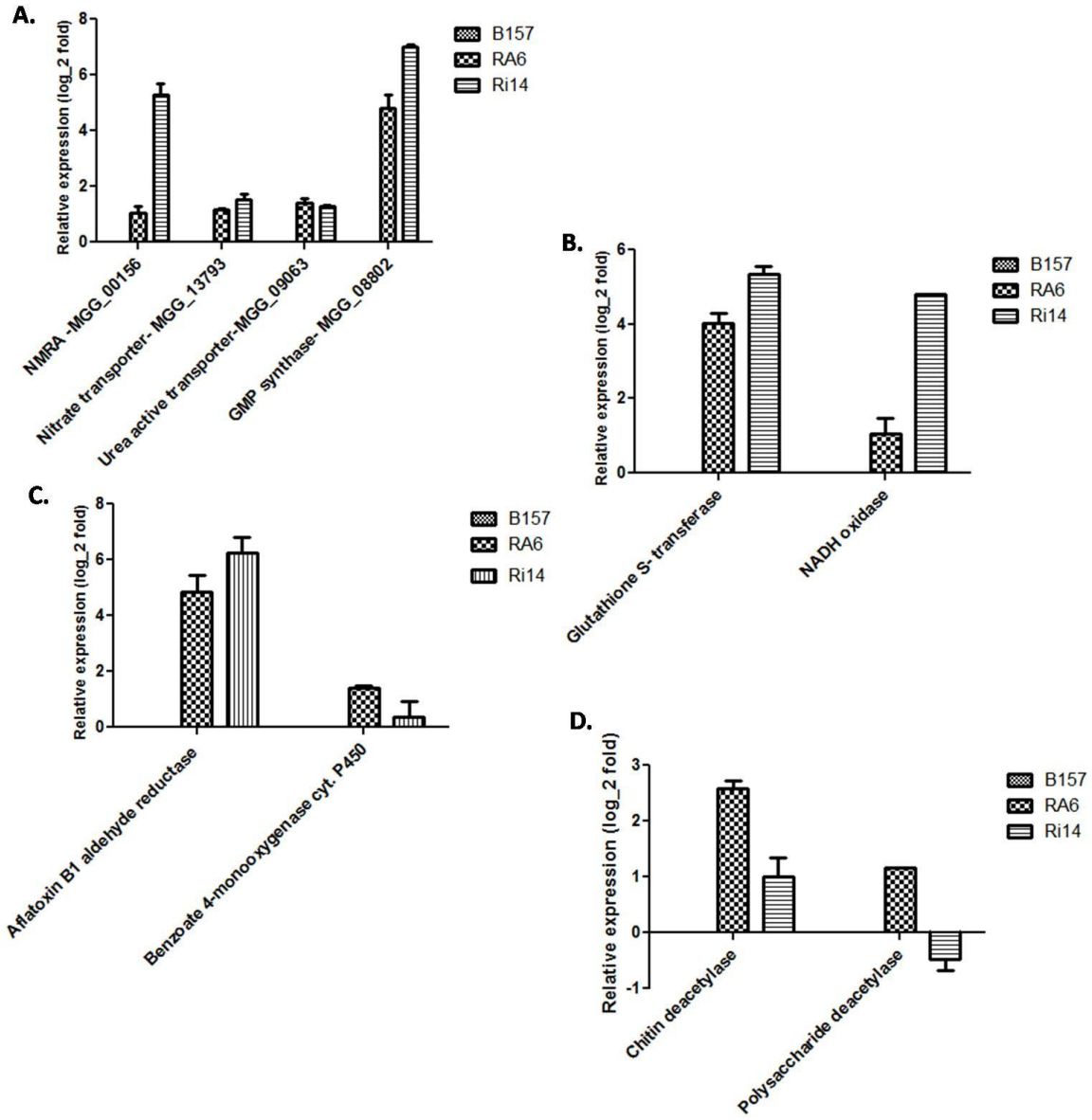
Transcriptome analysis of the *MoHPT1* knock-down transformant RA6 revealed a large set of nutritionally important genes that was differentially expressed. The majority of genes showing higher expression coded for amino acid transporters, sugar transporters, purine and pyrimidine transporters and metabolism genes, allantoin metabolism genes, urea metabolism genes and other transporters. These genes are generally found to be up-regulated in plant pathogenic fungi during the biotrophic phase (Divon & Fluhr, 2007). GMP synthase MGG_08802 was found to be up–regulated with a log_2 fold 9.15. The purine metabolism gene MGG_08801 was expressed only in RA6. The NMR-like protein MGG_00156 (*NMR1*), nitrate transporter MGG_13793, putative amino acid permease MGG_00289, transporters like high affinity nicotinic acid transporter MGG_02119 and sugar transporter MGG_07980 were up-regulated, suggesting a starvation like condition in the knock-down transformant. The urea metabolism genes (MGG_09063, MGG_15535) as well as genes for oligopeptide transport and other MFS transporters are also seen to be up-regulated. The increased transcript levels of some of these genes were validated using gene specific primers (Fig. 28). The up-regulation of these genes in *MoHPT1* knock-down transformant suggests that *MoHPT1* may be negatively regulating nutrition processes, since the down-regulation of *MoHPT1* is up-regulating the expression of these genes.

4.2.1. 2 *MoHPT1* regulates detoxification mechanism and fungal cell wall remodelling genes

In this study, oxidative stress and detoxification enzymes related genes like glutathione S-transferase MGG_09138, NADH oxidase MGG_03823 and benzoate monoxygenase cytochrome-P450 gene MGG_01924, aflatoxin B1 aldehyde reductase MGG_08519 and N- acetyl transferase MGG_10704 showed higher expression levels in the *MoHPT1* knock-down transformant**,** suggesting a critical role of *MoHPT1* in protection against the host defences. Other up-regulated genes also included some fungal cell wall remodelling enzymes like peptidoglycan binding domain containing protein MGG_10023, chitin deacetylase MGG_05828 and hydrolases like cutinase MGG_05798, polysaccharide deacetylase MGG_01922 which are required at specific developmental stages. The validation of the study is shown in Fig.28.

4.2.1.3 Differential expression of genes important for transcription regulation

The *MoHPT1* knock-down differentially expressed some regulatory genes which included transcription factors (TFs) and SAM dependent methyl transferases. The knock-down of *MoHPT1* up-regulated two fungal specific transcription factors namely MGG_10694 and MGG_05829; and a methyl transferase MGG_09874. The down-regulated genes had a Zn LITAF TF MGG_07815 and a SAM methyltransferase MGG_04036. The two component regulation of the downstream stress regulated genes is brought about by the activation of transcription factors or other genes involved in


A.
Relative expression (log_2 fold)
B157
RA6
Ri14
NMRA -MGG_00156
Nitrate transporter- MGG_13793
Urea active transporter-MGG_09063
GMP synthase- MGG_08802
B.
Relative expression (log_2 fold)
B157
RA6
Ri14
Glutathione S- transferase
NADH oxidase
C.
Relative expression (log_2 fold)
B157
RA6
Ri14
Aflatoxin B1 aldehyde reductase
Benzoate 4-monooxygenase cyt. P450
D.
Relative expression (log_2 fold)
B157
RA6
Ri14
Chitin deacetylase
Polysaccharide deacetylase

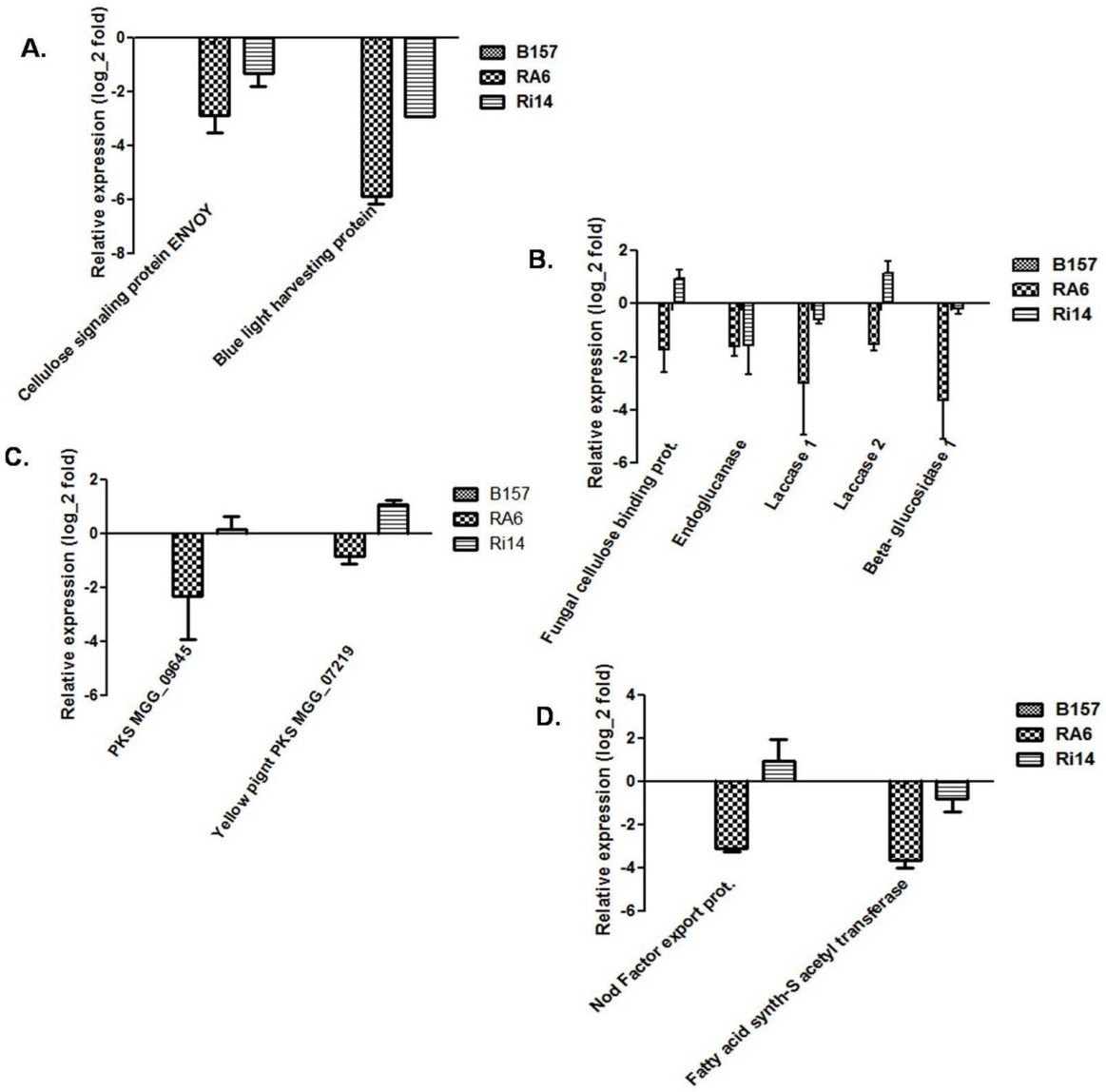
regulation of gene expression. In most cases response regulators themselves are the effector transcription factors or they activate other transcription factors which lead to the response. The up- and down- regulation of these transcription factors and methyl transferases appears to be of great importance in stress response and adaptation.

4.2.1.4 Down-regulation of amino acid metabolism genes in *MoHPT1* knock-down

Amino acid metabolism is key for recycling nitrogen resources and therefore, is important at all developmental stages of the fungus. A group of amino acid catabolism genes were down regulated in the transcriptome of the *MoHPT1* knock-down transformant RA6. Majority of the down regulated amino acid catabolism genes had a mitochondrial target signal. Most of these were involved in leucine catabolism. Others included genes involved in tyrosine, tryptophan and glutamine catabolism.

4.2.1.5 *MoHPT1* regulate plant cell wall degrading enzymes important for pathogenicity

Enzymes like ligninases, pectate lyases, laccases and some glycoside hydrolases of the families 7, 10, 5, 2, 11, 31, 67 etc. act on plant cell walls (Adams, 2004). A set of 8 such down-regulated genes which might be important for both development and pathogenicity (Fig. 29B) were seen to be differentially expressed. Two laccases, MGG_11608 and MGG_13464, were seen to be highly down-regulated. The down-regulation of laccases was validated by qRT-PCR. Other down-regulated genes included


A.
Relative expression (log_2 fold)
0
-2
-4
-6
-8
B157
RA6
Ri14
Cellulose signaling protein ENVOY
Blue light harvesting protein
B.
Relative expression (log_2 fold)
2
0
-2
-4
-6
B157
RA6
Ri14
Fungal cellulose binding prot.
Endoglucanase
Laccase 1
Laccase 2
Beta- glucosidase 1
C.
Relative expression (log_2 fold)
2
0
-2
-4
-6
B157
RA6
Ri14
PKS MGG_09645
Yellow pigmt PKS MGG_07219
D.
Relative expression (log_2 fold)
4
2
0
-2
-4
-6
B157
RA6
Ri14
Nod Factor export prot.
Fatty acid synthr-S acetyl transferase

those encoding endoglucanases (MGG_11774 & MGG_05364: GH61) and fungal glycoside hydrolase family 61 proteins (MGG_13622, MGG_07631) (Fig.29B) (www.cazy.org)

4.2.1.6 *MoHPT1* regulates the expression of light regulated genes

Light regulated genes, namely blue light harvesting protein (MGG_03002) and the PAS/lov domain containing *Envoy* gene encoding a cellulose signalling protein (MGG_01041) were significantly down-regulated (Fig. 29.A) Histidine kinases with PAS domains and phytochrome domains are well known for light regulated response as well as circadian responses in prokaryotes and plant systems (Taylor and Zhulin, 1999; Mizuno, 2004). The transcriptome data suggested a strong regulation of light regulated genes through *MoHPT1*.

4.2.1.7 *MoHPT1* knock-down affects secondary metabolism and xenobiotic response

The knock-down of *MoHPT1* caused a down-regulation some of the genes in secondary metabolism (Fig. 29C). Polyketide synthases like MGG_07219 and MGG_09645 and genes involved in aflatoxin and trichothecene biosynthesis namely MGG_09945 averantin oxidoreductase and MGG_03375 isotrichodermin C-15 hydroxylase were highly affected. Few studies have been done to investigate the role of secondary metabolites including mycotoxins and their relation to pathogenicity. Other genes involved in xenobiotic responses which were found down-regulated due to *MoHPT1* knock-down were MGG_09225, MGG_06222 cobW domain-containing

protein (involved in Zinc metabolism) and MGG_07227, putative cadmium resistance gene.

4.2.1.8 *MoHPT1* knock-down affects protein degradation

The analysis of RA6 showed that the knock-down of *MoHPT1* led to a down-regulation of several protein degrading genes, including some proteases and peptidases such as MGG_09817 minor extracellular protease vpr , MGG_09818 Aspartate protease, S8 and A4 peptidases like MGG_16831, MGG_08429, MGG_00311, MGG_17123 and F-box protein MGG_09708. These down-regulated genes may be involved in protein homeostasis.

4.2.2 Differential expression of genes in RA6 compared to wild type B157 under oxidative stress conditions

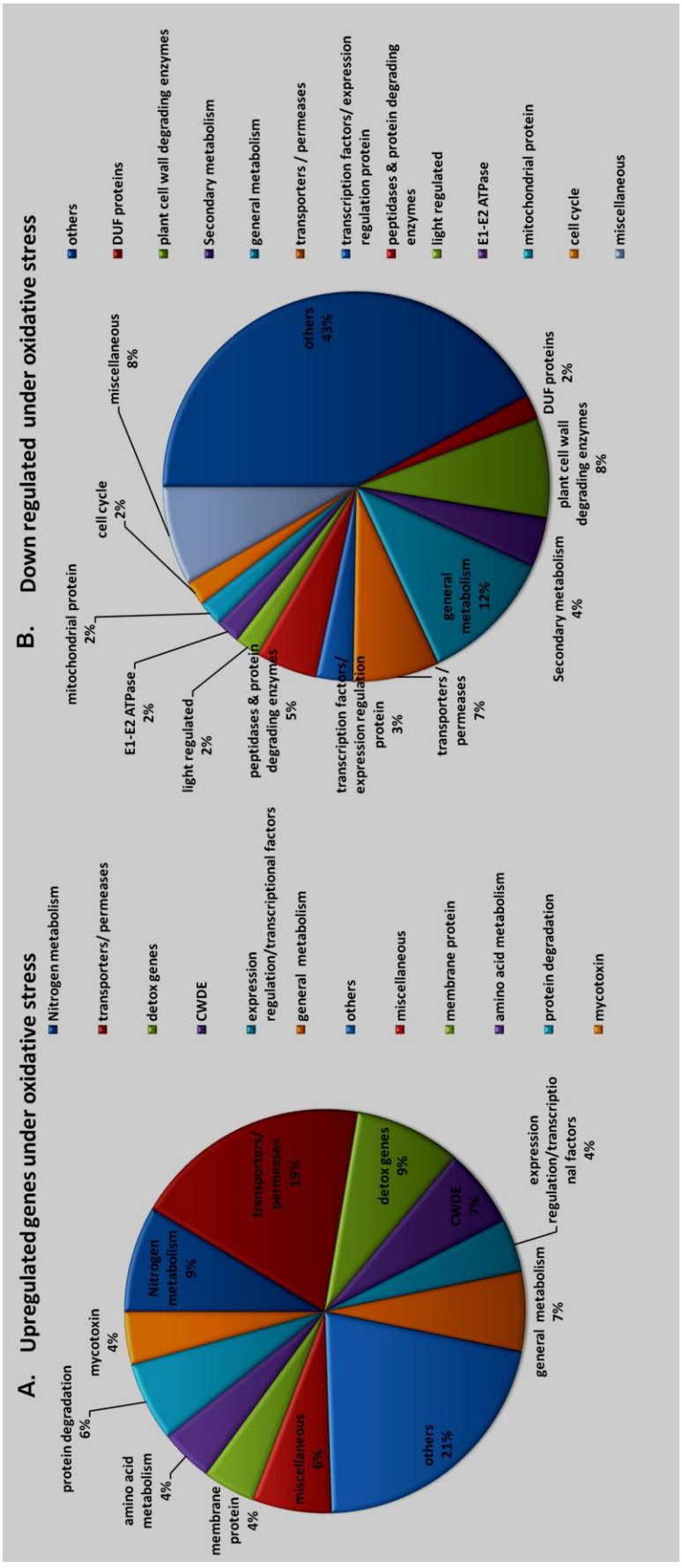
The comparative expression analysis of histidine phosphotransferase knock-down transformant in *M. oryzae* revealed several groups of genes which suggest the role *MoHPT1* in xenobiotic stress and oxidative stress. To investigate it further, a differential expression analysis of *MoHPT1* down-regulated transformant was done under oxidative stress conditions.

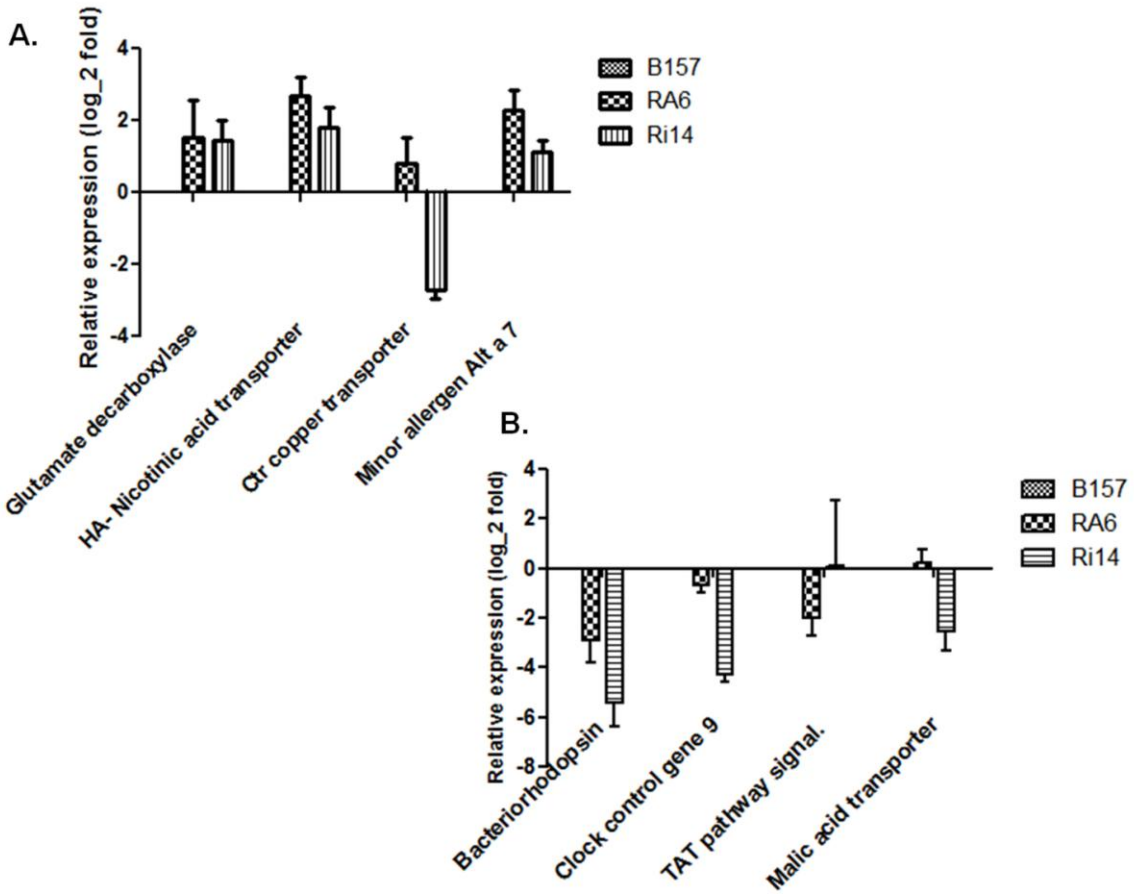
47 genes were significantly up-regulated while 97 genes showed significant down-regulation (p-value <= 0.01). Among the differentially expressed genes, 52 genes were unique to the oxidative stress condition. Up-regulated genes were categorized into different classes according to their predicted functions. Some new classes of genes were identified, namely genes for membrane proteins, amino acid

metabolism, protein degradation, mycotoxin in the up-regulated dataset; and cell cycle regulation genes in the down-regulated dataset. Other classes in the oxidative stress treated up-regulated dataset included nitrogen metabolism, transporters and permeases, detoxification genes involved in response to oxidative stress and xenobiotics, cell wall degrading enzymes and transcription factors (Fig.30). The set of down-regulated genes under oxidative stress conditions predominantly included genes required for plant cell wall degradation, secondary metabolism, light perception and regulation; protein degradation, transcription factors, E1-E2 ATPases, mitochondrial proteins and transporters. These results were further validated by qRT-PCR (Fig. 31). The validation by qRT-PCR was performed in triplicate and analysed statistically.

4.2.2.1 Genes which were differentially expressed only in Oxidative stress conditions

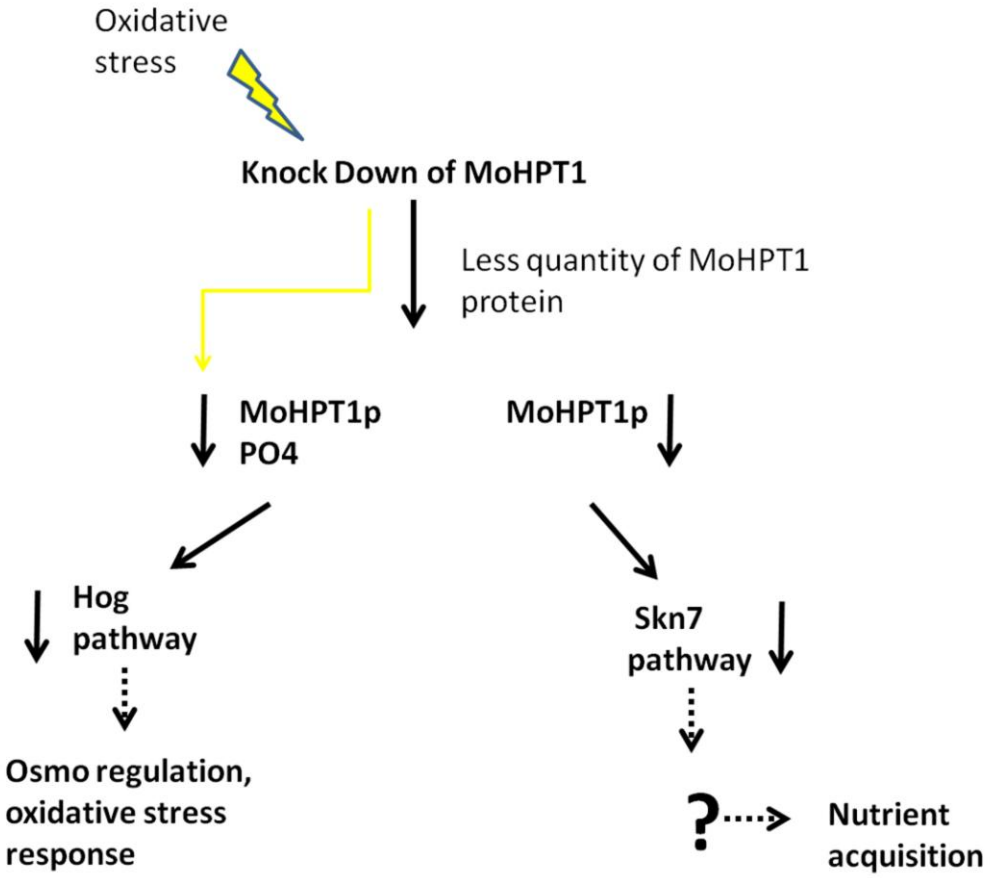
Some of the genes were differentially expressed only in oxidative stress treated *MoHPT1* knock-down transformant. Up-regulation of genes involved in mycotoxin production, amino acid metabolism, protein degradation and some membrane proteins were seen only in oxidative stress treated samples. Membrane proteins like MGG_05386 an integral membrane protein and MGG_03367 a GPI anchored protein had a fold change of about 5.1 and 5.0 respectively, while mycotoxin producing genes like MGG_09351 Aspergillopepsin and MGG_01569 Minor allergen Alt a7 showed a fold change of about 4.3 and 4.9 respectively. Amino acid metabolism genes and protein


A. Upregulated genes under oxidative stress
Nitrogen metabolism
transporters/ permeases
detox genes
CWDE
expression regulation/transcriptional factors
general metabolism
others
miscellaneous
membrane protein
amino acid metabolism
protein degradation
mycotoxin
protein degradation 6%
mycotoxin 4%
Nitrogen metabolism 9%
transporters/ permeases 19%
detox genes 9%
CWDE 7%
expression regulation/transcriptio nal factors 4%
general metabolism 7%
others 21%
miscellaneous 6%
membrane protein 4%
amino acid metabolism 4%
B. Down regulated under oxidative stress
others
DUF proteins
plant cell wall degrading enzymes
Secondary metabolism
general metabolism
transporters / permeases
transcription factors/ expression regulation protein
peptidases & protein degrading enzymes
light regulated
E1-E2 ATPase
mitochondrial protein
cell cycle
miscellaneous
mitochondrial protein 2%
cell cycle 2%
miscellaneous 8%
E1-E2 ATPase 2%
light regulated 2%
peptidases & protein degrading enzymes 5%
transcription factors/ expression regulation protein 3%
transporters / permeases 7%
general metabolism 12%
Secondary metabolism 4%
plant cell wall degrading enzymes 8%
DUF proteins 2%
others 43%


A.
Relative expression (log_2 fold)
4
2
0
-2
-4
B157
RA6
Ri14
Glutamate decarboxylase
HA- Nicotinic acid transporter
Ctr copper transporter
Minor allergen Alt a 7
B.
Relative expression (log_2 fold)
4
2
0
-2
-4
-6
-8
B157
RA6
Ri14
Bacteriorhodopsin
Clock control gene 9
TAT pathway signal.
Malic acid transporter

degradation genes were also found to be highly expressed in the *MoHPT1* knock-down transformant. Some other interesting genes which were found up-regulated include MGG_02095T0 NMRA like protein, MGG_10510 Ribonuclease T2, MGG_05484 AP2 complex Clathrin adapter complex, MGG_06026T0 PAS_3 domain, MGG_08801T exopolyphosphatase and MGG_08800T0 CAIB/BAIF family enzyme.

Down-regulated genes which were differentially expressed only in oxidative stress treated *MoHPT1* knock-down transformant, included a NimA interacting protein TinC (MGG_09383), a HET-C protein MGG_09107, and a TAT protein (twin arginine translocation pathway gene MGG_00743T0 with a log_2 fold change -2.3, -2.6, and -2.7 respectively. Other interesting genes which are seen up-regulated are fungal transcription factor MGG_10422, fatty acid synthase S-acetyl transferase MGG_14831, malic acid transporter MGG_05116, lipase MGG_02543 and an oxoprolinase MGG_00771. Two light regulated genes MGG_00195 clock-controlled-9 protein and MGG_09015 Red light sensory protein was also found to be down-regulated with a fold change of -2.4 and -2.2 respectively. We also compared the two datasets; both oxidative stress treated and without oxidative stress, so as to analyse the difference in expression levels of the genes which are commonly found up-regulated or down- regulated in both the datasets. There was no significant difference found in the expression levels of these genes, indicating that the differential expression of these genes is solely because of the knock-down of *MoHPT1* and there is no influence of oxidative stress on these genes.


Oxidative stress
Knock Down of MoHPT1
Less quantity of MoHPT1 protein
MoHPT1p PO4
MoHPT1p
Hog pathway
Skn7 pathway
Osmo regulation, oxidative stress response
?
Nutrient acquisition

4.3 DISCUSSION

In Chapter 1 we had discussed that *MoHPT1* knock-down reduces sporulation and pathogenicity. It also renders the fungus highly sensitive to oxidative stress and cell wall stress and reduces light induced expression of PAS Histidine kinases. We hypothesised that the down-regulation of *MoHPT1* would down-regulate the mechanisms initiated by both of the core response regulators under normal as well as under oxidative stress conditions (Fig. 32).

The further investigation by differential expression analysis of the *MoHPT1* knock-down transformant revealed a set of genes which might be directly or indirectly regulated by *MoHPT1* in *M. oryzae*. The up-regulated data set identified a group of genes involved in nutrition acquisition both in oxidative stress treated and untreated samples. Most of these genes were differentially expressed in both the experiments. But, the oxidative stress treated samples showed slightly greater differential expression than the untreated dataset. Genes encoding GMP synthase, glutamine tRNA amidotransferase, carbamyl phosphate synthase, nitrogen metabolism repressor NMR and other metabolite transporters are seen up-regulated generally in pre-penetration as well as biotrophic phase, when the fungus derives nutrients from the plant apoplast (Divon and Fluhr, 2007). The up-regulation of these genes in the RA6 transformant suggests that these genes are probably repressed by *MoHPT1* under normal conditions. Importance of nitrogen and nutrition acquisition on disease development and gene expression has been discussed in detail in pathogenic bacteria and in pathogenic fungi (Snoeijers *et al.*, 2000; Solomon *et al.*, 2003). During the biotrophic invasion, the plant generates ROS and also releases other compounds like uric acid and GABA, as a defense mechanism. These are either detoxified by the fungus or used as a nutrient

source. The detoxification mechanism genes which were seen up-regulated in both oxidative stress treated as well as untreated conditions suggests that *MoHPT1* may repress these genes under normal conditions, implying that this repressive role of *MoHPT1* may be of significance during its transition from biotrophic phase to necrotic phase of host invasion.

As discussed in Chapter 1, *MoHPT1* was found to respond to light and also influence the expression of the PAS domain containing Histidine kinases in *M. oryzae*. The transcriptome data suggested strong regulation of light regulated genes through *MoHPT1*. Among ten of the histidine kinases present in *Magnaporthe* six have PAS domain. PAS domain containing proteins are well known for their role in sensory modules in response to oxygen tension, redox potential and light intensity. One of the histidine kinases MGG_12377 is also a phytochrome. The light regulated genes which were found differentially expressed in oxidative stress treated and untreated transcriptome of the knock-down transformants included MGG_01041 (Envoy), MGG_03002 (blue light harvesting protein), MGG_00195 clock control gene 9 and MGG_09015 (red light sensory protein). It was striking to observe the down-regulation of the alternative TPS1 (MGG_00195) under oxidative stress conditions, which has 80% homology to *N. crassa* ccg-9. The gene was reported as a new alternative trehalose synthase and was shown to be regulated by blue light (Shinohara *et al*., 2002). Since there are six HKs in *M. oryzae* with PAS domains, the data set was explored for more blue–red light regulated genes. The validation experiments confirmed that in all the transformants where *MoHPT1* was down-regulated, these light regulated genes (MGG_00195, MGG_03002, MGG_09015, and MGG_01041) were also differentially down- regulated. The differential expression of blue and red light regulated genes in RNAi *MoHPT1* transformants suggested the role of *MoHPT1*

in fungal light regulated development and probably in pathogenicity. The circadian clock regulates the expression of *Hpt1* and the transcriptional control of the SAPK pathway in *N. crassa* (Lamb *et al.*, 2011). Earlier it was shown,that dark and light cycle is important for sporulation; blue light inhibits conidiophore formation, while blue and red light both control asexual spore release in *M. oryzae* (Lee *et al*., 2006). In other filamentous fungi, *N. crassa* and *T. reesei* blue light is also required for cell wall remodelling, mating and carbon metabolism functions (Gruber, 2012; Schmoll, 2010; Schmoll, 2012). Taking the observations from the analysis, *M. oryzae* might harbour a set of genes which can be co-ordinately regulated by light and might be indispensable for its developmental processes as well as during stress responses.

The early invasion of plant pathogenesis involves hypersensitive response like ROS from the plant which checks the pathogen from spreading within the host. Further the host might also produce antifungal compounds as a part of its defence mechanism. Thus to establish biotrophically, the fungus has to overcome these defence mechanisms of the host. Two component systems have been earlier reported to be involved in detoxification mechanisms. Bacterial two component regulatory systems like TodS/TodT and StyS/StyR regulate the catabolism of aromatic compounds (Lacal *et al.*, 2006). Glutathione S-transferase, NADH oxidase and other detoxification enzymes which were found differentially up-regulated indicate that *MoHPT1* negatively regulates these genes and such a regulation may be of importance once the pathogen has advanced into the necrotrophic phase.

The decreased expression of plant cell wall degrading genes in the *MoHPT1* knock-down RA6 was observed in both the oxidative stress treated and untreated datasets. As discussed in chapter 1, the decrease of the laccase expression levels was also observed in RA6 transcriptome. Moreover, the decrease of other plant cell wall

degrading genes like endoglucanases, polysaccharide deacetylase and fungal cellulose binding proteins may explain the inability of the fungus to infect the host. In compatible plant pathogen interactions of a hemibiotroph, plant cell wall degrading ability of the fungus is very important because to attain necrotrophy the pathogen must evade the plant tissue and utilise the host for its nutritional benefits. The pathogen secretes enzymes which would dissolve the host cell wall and also at the same time utilise those breakdown products as a nutrient source (Divon and Fluhr, 2007). The blue light regulated Envoy is also known to regulate cellulase gene transcription in response to cellulose under the influence of light in *Trichoderma* (Schmoll *et al*., 2005). It was also seen that some of the fungal cell wall remodelling genes are differentially up-regulated as well. The simultaneous regulation of both the plant cell wall degrading enzymes and the fungal cell wall enzymes for the developmental cell wall maintenance is necessary for an effective host invasion. The differential expression of these genes after *MoHPT1* knock-down suggests an important role for *MoHPT1* during pathogenesis.

Heavy ROS can oxidise and damage the fungal proteins and pose a challenge for survival of the fungus. Therefore proteostasis needs to be maintained in the organism during such an environmental stress. Repair and removal of damaged proteins is equally important as the expression of the proteins required for survival. In eukaryotes it is also seen that during oxidative stress the 20s proteosomal system is activated so as to maintain this homeostasis. Moreover, protein degradation can be also associated with mechanisms which involve nutrient uptake from the host. The differential expression of genes required for protein degradation in both oxidative stress treated and untreated data sets suggests *MoHPT1* to be involved in maintaining stress responsive protein homeostasis.

Secondary metabolites in filamentous fungi are often produced to assist the pathogen during host invasion. Secondary metabolic pathways which lead to fungal toxin production are as important as other virulence factors for necrotic pathogens. These toxins are either small peptides or other secondary metabolites, which induce programmed cell death (PCD) in the host. The differential expression of genes involved in secondary metabolism in the *MoHPT1* knock-down transformant suggests another role for MoHPT1 in pathogenesis. There have been few attempts made in *Aspergillus, Alternaria, Cochliobolus* and *Fusarium* to study such toxins and secondary metabolites and their role in virulence (Jonathan and Jacques, 2001; Desjardins and Hohn, 1997). Most of the toxins studied are known to aid pathogenicity, but are not sole determinants of pathogenicity. Similarly in *M. oryzae MoHPT1* mediated expression of these toxins might aid pathogenic invasion of the host.

Considering all the observations together, all these pleiotropic effects shown by *MoHPT1* may involve many HKs rather than one. The differential transcriptome analysis of the *MoHPT1* knock-down identifies genes which might be responsible for stress adaptation under xenobiotic stress and oxidative stress. The differential expression of genes involved in nutrition acquisition, metabolism and metabolite transporters, cell wall remodelling genes, plant cell wall degrading genes and secondary metabolic genes also suggests that *MoHPT1* might be crucial for host invasion. This study is a prelude to the previously unexplored effectors of Histidine phosphotransferase signalling during environmental stress response and advancement of pathogenicity of *M. oryzae*. The differential transcriptome analysis of *MoHPT1* strengthens our hypothesis that *MoHPT1* is indeed indispensable for successful host invasion in *M. oryzae*. Owing to this multifunctional importance of *MoHPT1* in

overall development of the fungus, *MoHPT1* is probably indispensable to *M. oryzae* B157 and therefore can be an excellent target for novel fungicide development.