1. REVIEW OF LITERATURE

1.1 Rice as a staple diet and its economic significance

Rice cultivation is the main source of livelihood for about one fifth of the world's population. Asia is the largest producer of rice with 90% rice cultivation. It has more than 200 million rice farms mostly small farms less than one hectare. Majority of rural population in this region depend on rice farming for their economy. In Asian developing countries rice is associated with food security and closely linked to political security. Increase in rice demand and price have caused civic unrest in several developing countries. To keep rice prices stable and affordable, IRRI (International Rice Research Institute) estimates an additional 8-10 million tons of rice need to be produced every year (http://irri.org/). There are over 80 reported rice diseases and some of them are major limitation to rice yield in different rice ecosystems (Mew, 1991). The challenge, above anything else, is to produce this additional rice in limited land area in more efficient, environment friendly production systems. Thus, adaptation to climate change and resistance to diseases, remain critical to rice production.

1.2 Plant pathogens of rice

Sheath blight, bacterial blight, rice blast, yellow mottle virus, sheath rot, bakanae, brown spot, narrow brown spot, bacterial leaf streak and grassy stunt are the common diseases which affect rice cultivars (Fig.1). Among these the most prevalent are sheath blight, bacterial blight and rice blast. The microbial pathogens which cause these

diseases are *Rhizoctonia solani*, *Xanthomonas oryzae* and *Magnaporthe oryzae* respectively. Sheath blight symptoms are usually observed from tillering to milk stage in a rice crop. Bacterial blight affects the rice plant at the seedling stage where infected leaves turn greyish green and roll up. In later stages of the disease the leaves turn yellow to straw coloured and wilt, and then gradually die. Rice blast fungus attacks almost all parts of the plant; the collar, which can ultimately kill the entire leaf blade; the leaf, which can be completely infected to give a burnt appearance; the stem, which turns blackish and breaks easily; or the panicle where, in severe cases the panicles fall over. It was also reported that, under laboratory conditions this pathogen can also infect the roots with specialised infection structures known as hyphopodia (Sesma and Osbourn, 2004).

1.3 Rice Blast Disease and symptoms

Rice blast is perhaps the most widely distributed plant disease, as it occurs in 85 countries world-wide (Ou, 1972) and causes 70-80% of crop loss during an epidemic season (Ou, 1985; Bonman *et al*., 1991). Blast was first reported in Asia more than three centuries ago and was probably first recorded as *rice fever disease* in China in 1637 and was later described as *imochi-byo* in Japan in 1704, and as *brusone* in Italy in 1828 (IRRI, 2002). During 2003, in India, rice blast was responsible for losses of more than 266,000 tons of rice. In Japan, the disease affects approximately 865,000 hectares of rice fields each year. In the Philippines, rice fields suffer more than 50% yield losses each year because of rice blast (IRRI 2003). Rice blast fungus also affects non-rice cereals like wheat, barley and millets. The disease has caused huge economic losses to wheat and barley cultivars in Brazil, where as equally affected the millet cultivars in east and


A.
B.
C.

southern Africa. Thus, Rice Blast disease is still a major concern all over the world. *Magnaporthe* can infect rice from seedling stage to maturity. Infection results in lesions on leaf, node, stem, panicles and grain. The disease is known as leaf blast, nodal blast, panicle blast, stem blast or seed blast depending upon the site of infection. The lesions begin as small whitish or greyish spots. As the infection establishes, the spots widen to eye shaped lesions with grey or white centers and narrow brown or reddish brown borders. As the lesion matures the affected area develop greyish cottony centers due to sporulation of the fungus (Fig. 2). On susceptible cultivars, lesions may initially appear gray-green and water-soaked with a darker green border and they expand rapidly to several centimeters in length. Older lesions often become light tan in colour with necrotic borders. On resistant cultivars, lesions often remain resistricted to 1-2 mm and brown to dark brown in colour.

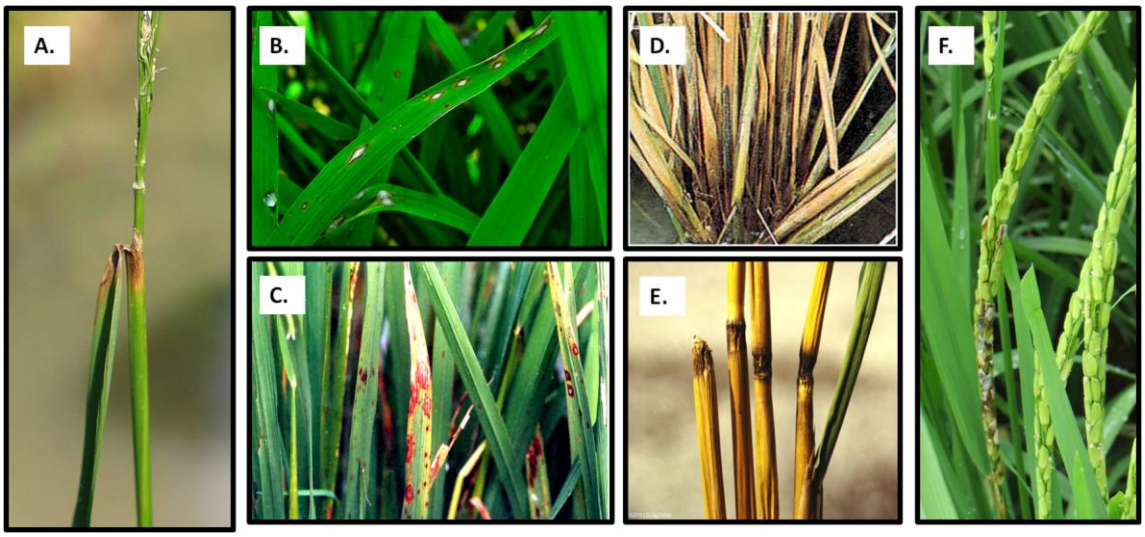
1.4 Rice blast pathogen *Magnaporthe oryzae*

Magnaporthe oryzae is an ascomycetous fungus, belonging to the family Magnaportheceae. It has a heterothallic lifestyle with two mating types MAT1-1 and MAT1-2. The asci are found within specialized structures called perithecia. Asci can be dissected to liberate the ascospores, which are arranged as octads (four pairs of spores produced by meiosis followed by mitosis). In either case the segregation patterns of genetic markers can be readily followed and the genetic basis of phenotypic traits can be determined (Talbot, 2003). The sexual, or teleomorphic, stage of *M. oryzae* can be produced in the laboratory with opposite mating types when paired, but is not common in the field. The asexual stage of *M. oryzae* is described by the name

Pyricularia oryzae (previously named as *P. grisea*) and it is the most common spore form of the fungus. These spores called conidia are produced abundantly on lesions and in culture on specialised stalks, called conidiophores. The conidia are usually three-celled, and produced at the tip of conidiophores (TeBeest *et al*., 2007). The mycelium of *M. oryzae* is septate. *M. oryzae* is a haploid organism having 7 chromosomes and a small genome of 40 Mb. *M. oryzae* is an extremely effective plant pathogen as it can reproduce both sexually and asexually. Their effectiveness accounts to the presence of specialised infectious structures known as appressoria that infect aerial tissues and hyphopodia that can infect root tissues (Ebbole *et al*., 2007; Sesma and Osbourn, 2004).

1.4.1 Infection cycle of *Magnaporthe oryzae*

M. oryzae infects as a spore that produces lesions or spots on parts of the rice plant such as the leaf, leaf collar, panicle, culm and culm nodes. The pathogen establishes itself on its host through a series of developmental processes starting from the germination of conidia till the invasive hyphal development and asexual conidiation. Plant diseases are often severe during periods of warm temperatures and high moisture. The dispersed conidium attaches to the hydrophobic surface of the host, germinates and forms a dome shaped infection structure known as the appressorium. The mature appressorium is darkly pigmented due to its melanised cell wall. The appressorium generates enormous turgor pressure so as to penetrate the rice cuticular surface (Talbot *et al*., 2003). Melanin is essential for appressorium turgor generation and mutants that do not synthesise melanin are inefficient in penetration. The penetration is followed by the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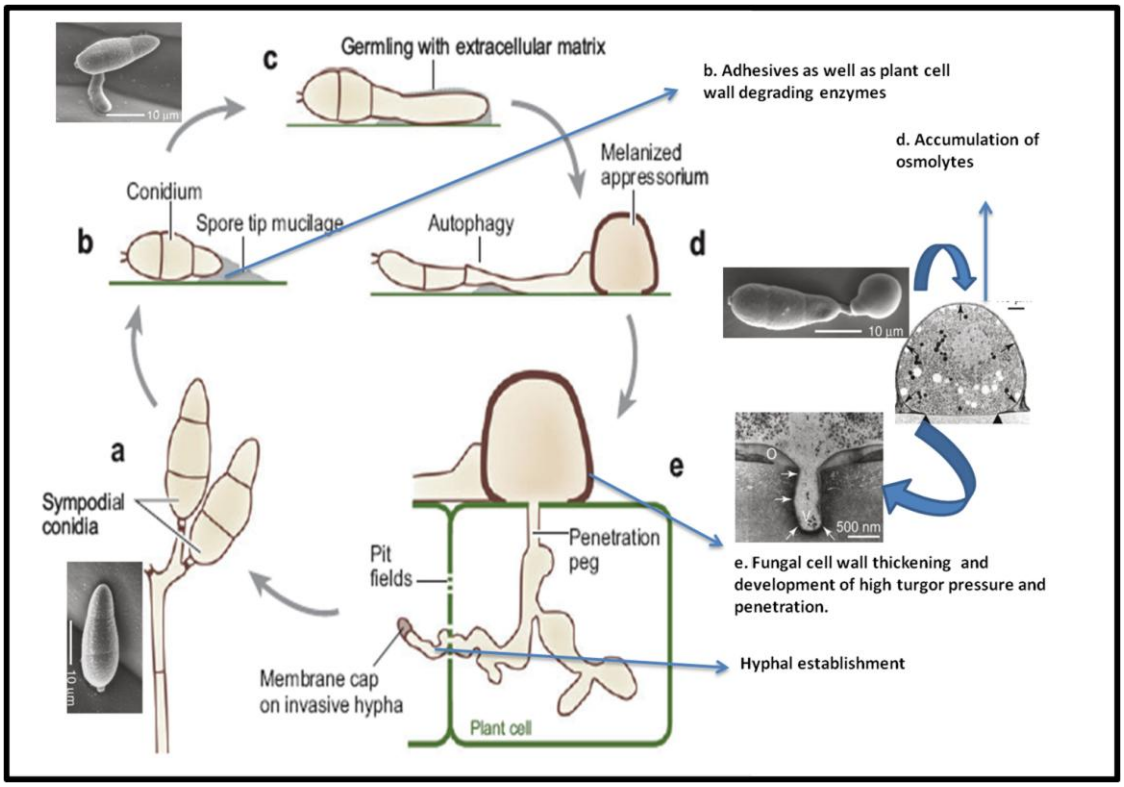
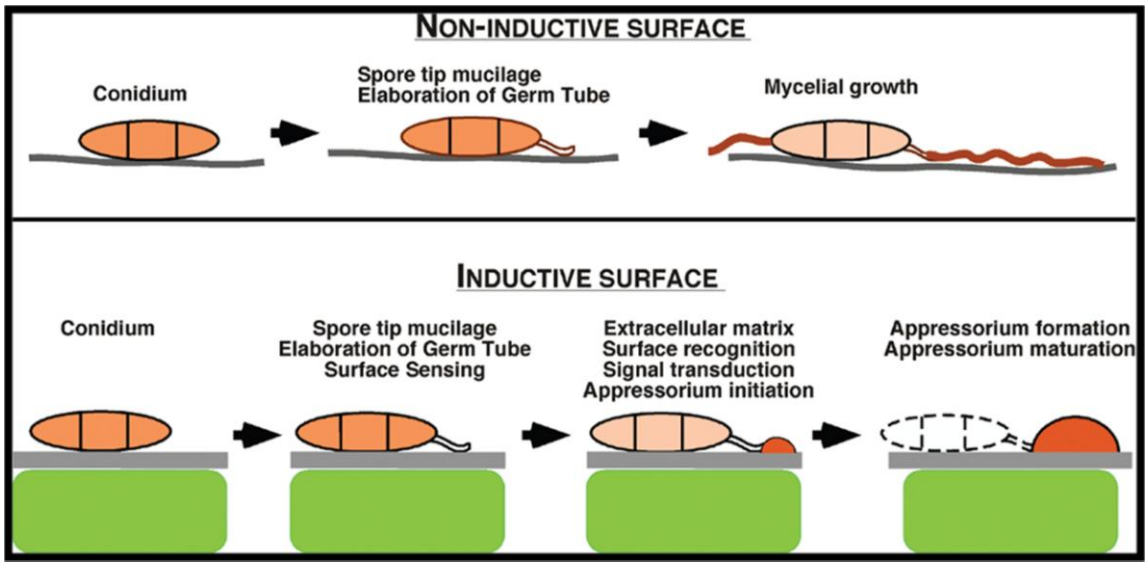
establishment of the invasive hyphae invading the plant tissue causing necrosis (Fig. 3). The conidia produced on these lesions are spread around to infect new areas (Ebbole *et al.*, 1996).

1.5 Regulation of Signalling and metabolic processes initiating pathogenesis

1.5.1 Formation of the infection structure appressorium

The morphogenetic events leading to the formation of the infection structure in *M. oryzae* initiate the moment the conidia adhere to the plant surface (Fig.4). As soon as the conidia land on the host, the signals perceived from the host surface and its environment initiate the germination of the conidia followed by the germ tube formation. The germ tube is highly distinct from the vegetative hyphae and with proper signals available grows only for a short length before its differentiation into the specialised infection structure, the appressorium. Morphogenesis of the appressorium depends on the signals received by the germ tube. A complex genetic reprogramming is initiated to develop this essential pathogenic structure. However, if the signals are not perceived in a timely manner, the germ tube remains undifferentiated and later enters into a vegetative life cycle in the presence of enough nutrients, or growth gets arrested as soon as the intrinsic nutrients are exhausted. Formation of appressorium depends on a number of environmental cues like hydrophobicity, hardness of contact surface, nutrient starvation and presence of plant cutin monomers. The major determinants of pathogenicity of the fungus at this stage are adhesins which adheres the conidia and the germ tube to the host surface, and cutinases secreted by the pathogen followed by cutin monomer induced


c
Germling with extracellular matrix
b. Adhesives as well as plant cell wall degrading enzymes
d. Accumulation of osmolytes
Melanized appressorium
Conidium
Spore tip mucilage
b
Autophagy
d
10 µm
a
Sympodial conidia
e
Penetration peg
Pit fields
500 nm
e. Fungal cell wall thickening and development of high turgor pressure and penetration.
Hyphal establishment
Membrane cap on invasive hypha
Plant cell


NON-INDUCTIVE SURFACE
Conidium
Spore tip mucilage
Elaboration of Germ Tube
Mycelial growth
INDUCTIVE SURFACE
Conidium
Spore tip mucilage
Elaboration of Germ Tube
Surface Sensing
Extracellular matrix
Surface recognition
Signal transduction
Appressorium initiation
Appressorium formation
Appressorium maturation

hydrophobin expression which provides hydrophobicity to the fungal surface (Talbot *et al*., 1996). Cutinase2 in *M. oryzae* was found to be needed for proper germ tube as well as appressorium formation, but was not needed for adhesion and appressorial maturation (Skamnioti and Gurr, 2007). The *M. oryzae* Class I hydrophobin MPG1 is required for surface recognition and initiation of appressorium formation. It was observed that nitrogen starvation and the expression of *NUT1* and *NPR1* is required for the proper functioning of *MPG1* hydrophobin (Beckerman and Ebbole, 1996). The synchronisation of a number of pathways is needed for environmental cue perception, formation of the appressorium, and the establishment of the fungus inside the host. Maturation of the appressorium requires the cAMP mediated activation of cPKA pathway and the PMK1 MAP Kinase pathway (Xu *et al*., 1997; Xu and Hamer, 1996). The appressorial development is accompanied by a series of metabolic processes. Trehalose and glycogen reserves in the conidia are rapidly degraded during the conidial germination, while the lipid bodies in the conidia are translocated to the developing appressoria. The mature appressoria accumulate huge quantities of osmolytes mainly glycerol up to about 3 M. The cell wall of the appressorium thickens enormously and also develops a melanised layer to aid in turgor generation within the appressorium. About 8 MPa of turgor pressure is generated inside the appressorium to facilitate the penetration of the fungus in the host (Wang *et al.*, 2005). Storage reserve metabolism immensely contributes to the hyphal development, penetration and further fungal development. For instance, multi-functional fatty acid β-oxidation protein Mfp1 and carnitine acetyl transferase, which is responsible for transport of acetyl CoA across the mitochondrial and/or peroxisomal membrane, are critical for virulence (Wang *et al*., 2005; Bhambra *et al*., 2006; Ramos-Pamplona and Naqvi, 2006).

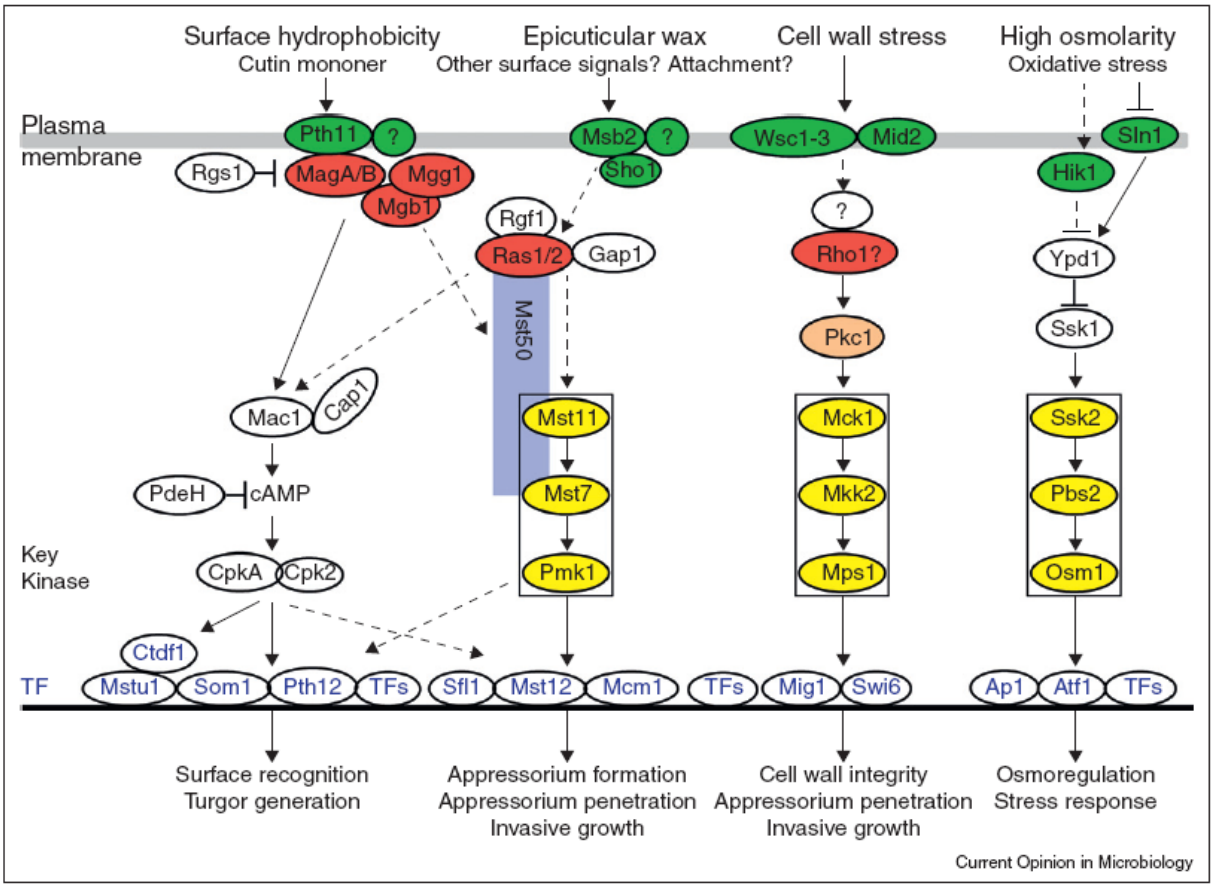
1.5.2 Nutrient uptake strategies of the hemibiotrophic pathogen

Even though fungal phytopathogens have the ability to respond to available carbon and nitrogen sources in the plant host, as infection develops, the pathogen also experiences a nitrogen-starved environment at the start of the infection cycle (Snoeijers *et al*, 2000; Wilson *et al*., 2012). The pre-penetration phase utilises the conidial reserves for nutrition. However, as soon as the fungal pathogen enters the plant the nutritional uptake from the host plant begins. The initial biotrophic phase involves nutrient uptake from the apoplast, while the later necrotic phase involves degradation of the host tissue for nutrient absorption. The Tor signalling pathway as well as the nitrogen and carbon catabolite repression inactivation enables the fungus to sense nutrient deprivation and utilise alternative nitrogen and carbon sources like xylose and nitrate available from the host tissue. Fungi utilise a wide variety of nitrogen compounds for nutrition. The fungus takes up ammonium and glucose as favourable nutrient sources. The signal of ammonium availability controls many important processes like mating, secondary metabolism and hyphal growth. The whole processes of nutrient uptake and metabolism is governed by a set of regulatory genes, eventually bringing about the expression of metabolic and transport genes (Rutherford, 2011). PKA signalling is found to be activated in response to ammonium availability under nitrogen starved conditions in *S. cerevisiae* (Van-Nuland *et al*., 2006). PKA acts as a central player in nutrient sensing through both the classical G-protein pathway and the RAS or c-AMP independent signalling in *S. cerevisiae (*Fuller and Rhodes, 2011). Pathogenic fungi have evolved so as to adapt to the dynamic nutrient needs during pathogenesis. These features include

several virulence factors, toxins, cell wall degrading enzymes, and other mechanisms which allow direct contact of the fungus with its host and utilise the host for the survival benefits of the pathogen (Divon and Fluhr, 2007).

1.6 MAP Kinases in *M. oryzae*

The first to be reported as well as the most studied MAP kinase in *M. oryzae* is *PMK1*, a homologue of *Fus3*/*Kss1* in yeast. It was shown to be important for appressorium formation (Thines *et al*., 2000). *PMK1* mutants were responsive to cAMP and is therefore, considered to act downstream of cAMP. PMK1 signalling is not involved in vegetative growth as well as in sexual or asexual reproduction, but is needed for infectious hyphal growth after penetration. Several upstream components of the PMK1 pathway, including the MEKK Mst11 and the MEK Mst7 and a Ste50 homolog, Mst50, have been characterized (Park *et al*., 2006). The MPS1 MAPK is essential for conidiation, appressorial penetration, and plant infection in *M. oryzae*. The *mps1* deletion mutant is significantly reduced in aerial hyphal growth and conidiation, but it displays no obvious changes in the growth rate. In contrast to PMK1, MPS1 is dispensable for appressorium formation (Zhao *et al*., 2007). Appressoria formed by the *mps1* mutant fail to penetrate and develop infectious hyphae but still elicit plant defense responses. Vegetative hyphae of this mutant have a weakened cell wall, undergo autolysis in aging colonies and are hypersensitive to cell wall lytic enzymes (Xu *et al*., 1998). MAP kinase *OSM1* mutation in *M.oryzae* was dispensable for infection, but rendered the fungus hypersensitive to stress conditions (Dixon *et al*., 1999).

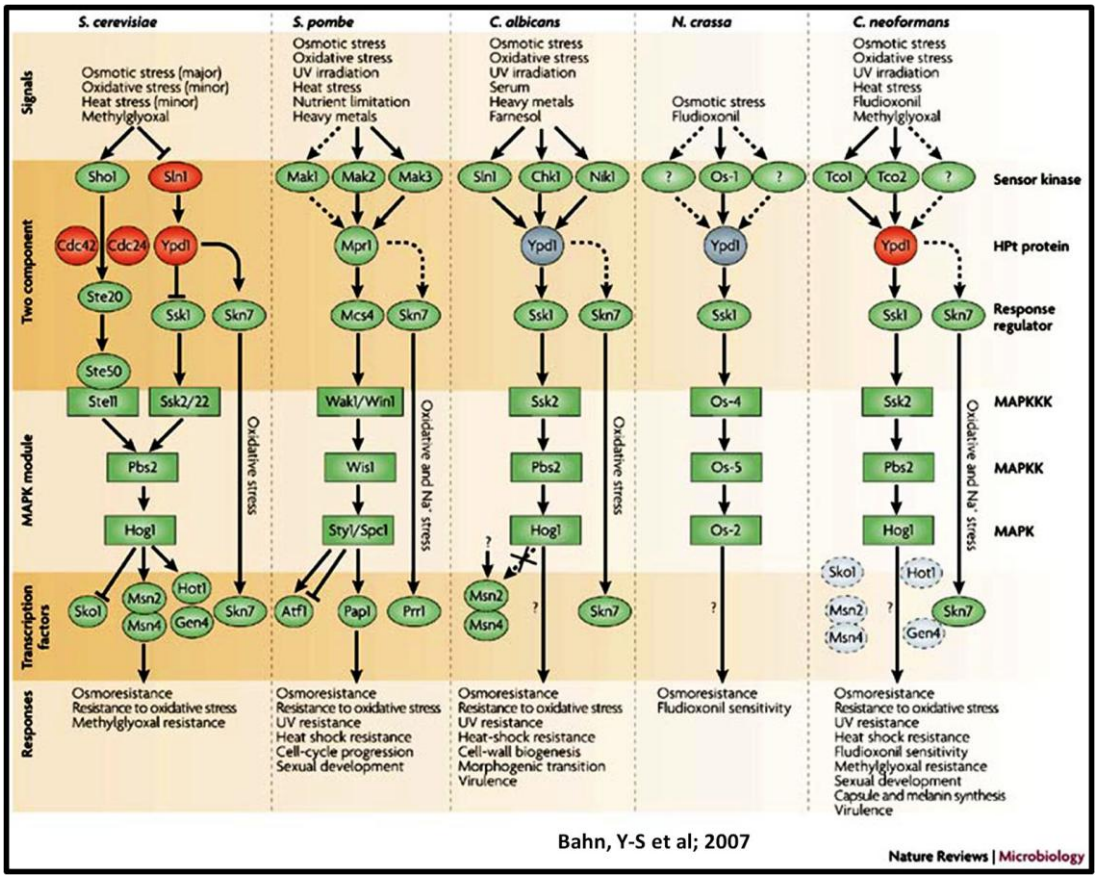

Surface hydrophobicity
Cutin mononer
Epicuticular wax
Other surface signals? Attachment?
Cell wall stress
High osmolarity
Oxidative stress
Plasma membrane
Pth11
?
Msb2
?
Sho1
Wsc1-3
Mid2
Sln1
Hik1
Rgs1
MagA/B
Mgg1
Mgb1
Rgf1
Ras1/2
Gap1
Mst50
?
Rho1?
Ypd1
Ssk1
Pkc1
Mac1
Cap1
Mst11
Mck1
Ssk2
PdeH
cAMP
Mst7
Mkk2
Pbs2
Key Kinase
CpkA
Cpk2
Pmk1
Mps1
Osm1
Ctdf1
TF
Mstu1
Som1
Pth12
TFs
Sfl1
Mst12
Mcm1
TFs
Mig1
Swi6
Ap1
Atf1
TFs
Surface recognition
Turgor generation
Appressorium formation
Appressorium penetration
Invasive growth
Cell wall integrity
Appressorium penetration
Invasive growth
Osmoregulation
Stress response
Current Opinion in Microbiology

1.7 Stress activated pathways in filamentous fungi

The rapid stress signalling and associated gene expression is responsible for the stress survival of micro organisms within their hostile environment. Stress-activated protein kinase (SAPK) pathways are the ones generally involved in stress-signalling. These pathways are universally found in all eukaryotes and prokaryotes, including eukaryotic microorganisms such as fungi. These pathways consist of a stress response protein kinase (SAPK) that activates a phosphorelay cascade. Once this kinase cascade is activated, the SAPK phosphorylates a range of cytoplasmic and nuclear proteins, which determine the appropriate response, leading to activation of genes necessary for stress survival. These stress related pathways seems to be conserved in fungi, but the mechanisms of stress signalling have evolved differently in different fungi and have diverged (Deborah *et al*., 2010) (Fig.5). Thus, it is seen that the mechanisms for relaying stress signals to the SAPK pathways in different yeasts *S. cerevisiae* and *S. pombe* have diverged significantly. Besides, there is growing evidence that the role and regulation of the *C. albicans* SAPK pathway differs from that of these model yeasts (Kaba *et al*., 2013). However, despite such differences, one universal mechanism by which stress signals are sensed and relayed to the SAPK modules in these fungi involves a two component signal transduction pathway.

1.8 Two Component Systems for Signal Transduction

Two component pathways are widely seen in bacteria to respond to environmental

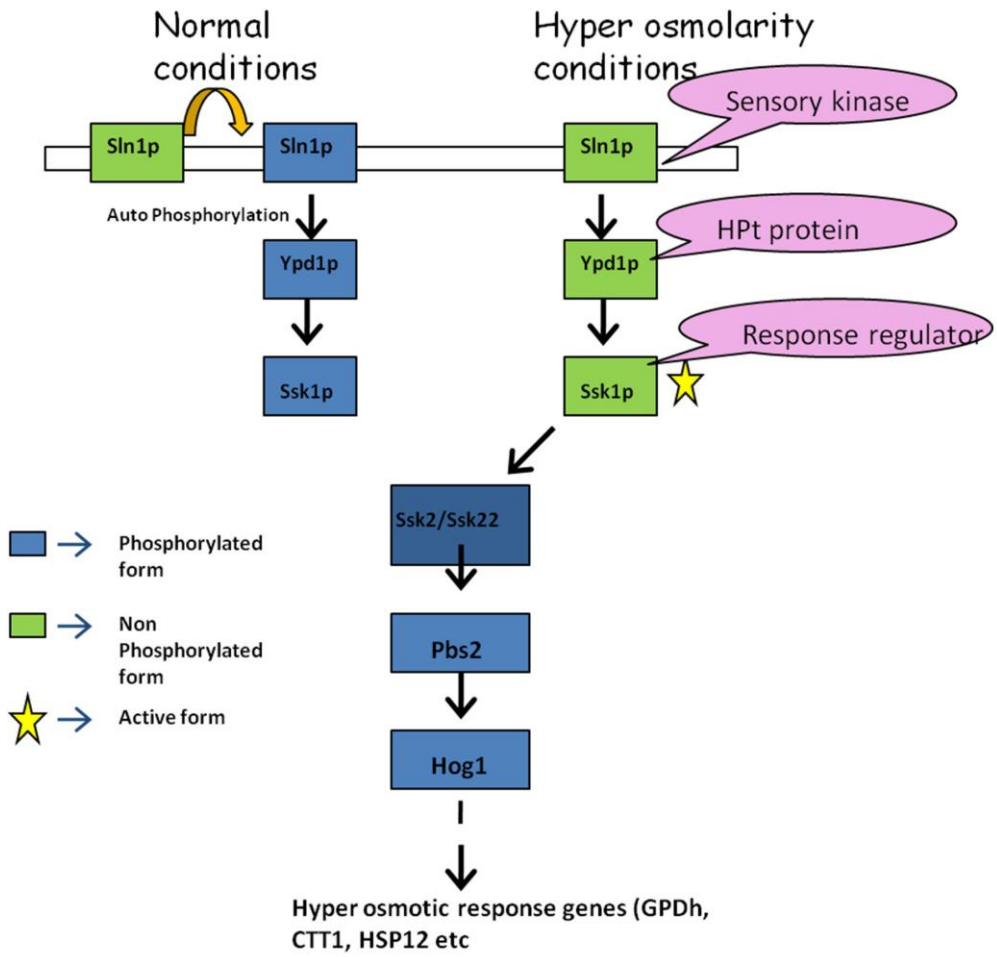

S. cerevisiae
S. pombe
C. albicans
N. crassa
C. neoformans
Signals
Two component
MAPK module
Transcription factors
Responses
Osmotic stress (major)
Oxidative stress (minor)
Heat stress (minor)
Methylglyoxal
Osmotic stress
Oxidative stress
UV irradiation
Heat stress
Nutrient limitation
Heavy metals
Osmotic stress
Oxidative stress
UV irradiation
Serum
Heavy metals
Farnesol
Osmotic stress
Fludioxonil
Osmotic stress
Oxidative stress
UV irradiation
Heat stress
Fludioxonil
Methylglyoxal
Sho1
Sln1
Cdc42
Cdc24
Ypd1
Ste20
Ste50
Ste11
Ssk1
Skn7
Ssk2/22
Pbs2
Hog1
Sko1
Msn2
Msn4
Hot1
Gen4
Skn7
Oxidative stress
Mak1
Mak2
Mak3
Mpr1
Mcs4
Skn7
Wak1/Win1
Wis1
Styl/Spc1
Atf1
Pap1
Prr1
Oxidative and Na+ stress
Sln1
Chk1
Nik1
Ypd1
Ssk1
Skn7
Ssk2
Pbs2
Hog1
Msn2
Msn4
Skn7
Oxidative stress
?
Os-1
Ypd1
Ssk1
Os-4
Os-5
Os-2
Tco1
Tco2
Ypd1
Ssk1
Skn7
Ssk2
Pbs2
Hog1
Sko1
Msn2
Msn4
Hot1
Gen4
Skn7
Oxidative and Na+ stress
Sensor kinase
HPt protein
Response regulator
MAPKKK
MAPKK
MAPK
Osmoresistance
Resistance to oxidative stress
Methylglyoxal resistance
Osmoresistance
Resistance to oxidative stress
UV resistance
Heat shock resistance
Cell-cycle progression
Sexual development
Osmoresistance
Resistance to oxidative stress
UV resistance
Heat-shock resistance
Cell-wall biogenesis
Morphogenic transition
Virulence
Osmoresistance
Fludioxonil sensitivity
Osmoresistance
Resistance to oxidative stress
UV resistance
Heat shock resistance
Fludioxonil sensitivity
Methylglyoxal resistance
Sexual development
Capsule and melanin synthesis
Virulence
Bahn, Y-S et al; 2007
Nature Reviews | Microbiology

signals (Egger *et al*., 1997). Upon activation, a sensor histidine kinase is autophosphorylated on a conserved histidine residue, which in turn phosphorylates a conserved aspartate residue in the receiver domain of a response regulator protein, which then triggers an appropriate response. Unlike the other signalling pathways, Histidine-aspartic acid phosphorelay is the key feature of universally displayed two component systems. A typical two component system involves an external cue sensing sensory kinase which is a histidine kinase and a response regulator. As a response to external signals, MAP kinase (MAPK) is activated (phosphorylated) by a MAP kinase kinase (MEK), which in turn is activated by a MAP kinase kinase kinase (MEKK). Active MAPK then can be translocated to the nucleus where it interacts with various proteins, particularly transcription factors to direct new gene expression (Bahn, 2008). One of the most-characterised stress signal transduction pathways in eukaryotes is the MAP (mitogen-activated protein) kinase pathways regulated by 'Two component system' (TCS). Two component systems have been well studied in bacterial systems and have a scope for investigation in depth in higher eukaryotes. The two component system can be considered as a master regulator which governs the signalling of a number of downstream pathways which regulate cell motility, cell wall integrity, cell cycle progressions, antibiotic resistance and its response to different type of environmental stress including cold stress, hyper osmotic stress, oxidative stress and many more.

1.8.1 TCS in fungal system

The TCS was first reported in eukaryotes in plants by the discovery of *ETR1* Histidine kinase (Chang *et al*., 1993) and in fungi by the discovery of *SLN1* in yeast (Ota and Varshavsky, 1993). The first breakthrough in the field of eukaryotic TCS was the finding of SLN1-YPD1-SSK1 pathway in *S. cerevisiae* (Posas *et al*., 1996); (Fig.6). The Histidine phosphotransfer in yeast has been widely studied. The TCS-SAPK in Yeast has been implicated in a number of stress responses including those to osmotic, oxidative, cold, methylglyoxal and also some antifungal response (Saito and Posas, 2012)**.** TCS in fungi appear to be conserved, but show divergence in the course of evolution in the mechanism of signalling by which stress signals are sensed and relayed to SAPK modules in species of higher fungi. Typical instance of such divergence are seen in the model yeasts *S. cerevisiae, S. pombe and Candida albicans* (Bahn, 2008; Enjalbert, 2006). The TCS of *S. cerevisiae* is known to have a single sensory histidine kinase (HK), a single histidine phosphotransferase (HPT) and a single response regulator (RR), responsible for the regulation of the downstream SAPK, HOG1 pathway (Maeda, 1994; Posas, 1996). This stress activated MAPK initiate appropriate response against osmotic, oxidative and temperature stress (Hohmann, 2002; Ikner, 2005). *S. pombe* downstream SAPK Sty1 shows much divergence as *S. pombe* harbours multiple HKs with diverse sub-domains which assign different functions to individual signalling modules depending on the sub-domains present. *S. pombe* TCS not only responds to different types of stress, but also controls differentiation and morphology of cells (Smith, 2010; Millar, 1995). While the *C. albicans* SAPK *CaHOG1* showed some similar functionality of that of *Sty1*, at the same time it provided new insights into the possible divergent functions. CaHOG1

was attributed to functions like iron sensitivity and availability, morphogenetic switching and chlamydospore formation (Smith *et al*., 2010; Su *et al*., 2013; and Kaba *et al*., 2013). Thus the divergence of the signalling cascade in higher fungi enables better



adaptation and survival. Recently there has been a lot of research in filamentous fungi on the 'Two component system' and its evolution. Studies show that with comparatively large number of Histidine kinases, the TCS in filamentous fungi has evolved to cope with different stresses through different histidine kinases. The validation of these studies is to be genetically confirmed so that the TCS networking becomes much clearer.

1.8.2 Two component systems in filamentous fungi

Filamentous fungi harbour more hybrid sensor kinases than yeast species studided. Fungal hybrid sensor kinases have been phylogenetically classified into 11 groups (Catlett *et al*., 2003). Many of these groups contain HKs that are highly conserved in filamentous fungi. Other groups are more divergent and contain gene families that have evolved and expanded within species and few orthologues between species. These groupings suggest that some HK genes are necessary for vital functions shared by almost all ascomycetes (e.g., osmosensing), while others may have evolved to adapt to specific aspects of pathogenic lifestyle. Filamentous fungi generally have a far greater number of histidine kinases than yeasts. *Neurospora crassa, Aspergillus nidulans,* and *Aspergillus fumigatus* have 11, 15 and 14 hybrid sensor kinases, respectively. Ascomycetes (Euascomycetes) contain even larger numbers of hybrid histidine kinases: 16 HKs in *Gibberella moniliformis*, 21 HKs in *Cochliobolus heterostrophus* and 20 HKs in *Botryotinia fuckeliana* (Catlett *et al*., 2003). Particularly, sensor kinases studied in filamentous fungi are involved mainly in morphogenetic

functions and cellular differentiation but not in stress sensing, in addition to its role in osmo-sensing. Therefore, stress sensing hybrid histidine kinase associated TCS in filamentous fungi remain to be further elucidated (Bahn, 2008).

1.9 Importance of two component system and stress activated MAP Kinases in pathogenicity of the phytopathogens

Functional analysis of several pathogenic fungal two component systems has revealed roles for these signal transduction pathways in osmotic and oxidative stress responses, fungicide resistance, phase transition, dimorphism, biofilm formation and quorum sensing, secondary metabolite production, virulence factor regulation, conidiation, cell wall maintenance, hyphal morphogenesis, and mating and asexual development (Li *et al*., 2010). Moreover, two component pathways are vital determinants of pathogenicity in animal and plant pathogens, such as *C. albicans*, *C. neoformans*, *A. fumigatus*, *C. heterotrophus*, *Gibberella zeae* (Alex *et al*., 1998; Pott *et al*., 2000; Bahn *et al*., 2006; Oide *et al*., 2010), *Fusarium oxysporum* (Rispail *et al*., 2010), *Botrytis cinerea* (Oshima *et al*., 2002; Viaud *et al*., 2006) and *Alternaria brassiciola* (Cho *et al*., 2009; Dry *et al*., 2004).

1.10 Cross-talks involving the two component system regulated SAPKs and other MAP Kinases and other key signalling pathways

Evidence for cross talk between different fungal MAPK cascades and between MAPK and other key signaling pathways, such as the cAMP-PKA and the target of rapamycin (TOR) pathway is seen in fungal systems. In *C. albicans* reduced Tor1 signaling lowers Hog1 phosphorylation via Hog1 phosphatases and activate *BRG1* expression resulting in hyphal elongation (Su *et al*., 2013). Cross talks of Hog1 with other pathways, especially the CWI pathway has been extensively studied in fungi under different stress conditions like hyper-osmotic stress, oxidative stress and zymolase treatment (Fuchs and Mylonakis, 2009)

1.11 Involvement of histidine kinase signalling in light regulated expression

Among the abiotic factors influencing the virulence of *M. oryzae* very little is known about the influence of light. Light is known to regulate growth, pigmentation, sporulation and development of perithecia in other filamentous fungi (Schmoll *et al*., 2010). Thus, the importance of light as a vital determinant of virulence in pathogens is now being appreciated. Photo-oxidation is one of the major stresses experienced by both plants and plant pathogens. But not much is known about photo oxidative stress responses in *M. oryzae*. Incidental formation of singlet oxygen ($^1O_2$), a highly reactive oxygen species, occurs in photosynthetic as well as non-photosynthetic cells by the simultaneous presence of light, oxygen and photo-sensitisers, which causes photo-oxidative stress to the cells. Phytochromes are a widespread family of red/far-red photo-receptors, which sense the quantity and quality of light in photosynthetic as well as non-

photosynthetic organisms, and transduce the signal for responding to the ambient light conditions. A typical (R/FR) phytochrome consists of a conserved N-terminal PAS-GAF-PHY tridomain photo-sensory core, which is combined with a C-terminal catalytic domain with a histidine kinase (HK) or histidine kinase-related domain (HKRD). Such light regulated histidine kinase phytochromes have been reported in *N. crassa* as well as *A. nidulans. Neurospora* PHY-2 is capable of binding either biliverdin or phycocyanobilin with a photo-cycle *in vitro*. In *A. nidulans* red light conditions led to enhanced sexual development in the *fphA* null mutant.

1.12 Histidine kinases and its downstream components in *M. oryzae*

In the *M. oryzae* genome there are ten Histidine kinases, one Histidine phosphotransferase and three response regulators. A mitogen activated protein kinase (MAPK) encoding gene *OSM1 was* isolated from *M. oryzae* and was shown to encode a functional homolog of *HIGH-OSMOLARITY GLYCEROL HOG1*, which encodes a MAP kinase that regulates cellular turgor in yeast. Although cellular turgor is controlled by OSM1 during the response to external osmotic shock, appressorium turgor is unaltered in Δ*osm1* mutants and the mutants were fully pathogenic (Dixon *et al*., 1999). In another study, *HiK1* the group III histidine kinase in *M. oryzae* is involved in antifungal response and does not affect pathogenicity (Motoyama *et al*., 2005). Subsequently, they examined the putative response regulators in *M. oryzae* Kita 1 strain. MoSSK1 was attributed a role in osmotic stress response and antifungal sensitivity, while MoSKN7 and MoRIM15 were implicated in full virulence (Motoyama *et al*., 2008). MoSLN1 the ortholog of Sln1

in *M. oryzae* was highly responsible for hyper osmotic survival response irrespective of the salt or sugar stress, cell wall integrity, oxidative stress response and pathogenicity (Zhang *et al*., (2010)). The histidine phosphotransferase knock-out in *M. oryzae* referred to as Δ*Moypd1* in 70-15 is viable but fails to form conidia on standard medium. *In planta* assays of Δ*Moypd1* in 70-15 on wounded rice leaves showed that it was incapable of growing within the plant. Jacob *et al* (2015) showed that the mutants were non-pathogenic and sensitive to osmotic stress and resistant to the phenylpyrrole antifungal agent fludioxonil (Jacob et al., 2015). However, resistance to fludioxonil may only be a small part of the diverse cues to which MoYpd1 (MGG_07173) responds. For instance, MoYpd1 is known to transfer signals from two HKs, MoSln1 and MoHik1. MoYpd1 being the only histidine phosphotransferase, may be activated by signals from other histidine kinases and relay them to the downstream response regulators; thus it may play an important role in developmental processes distinct from pathogenicity. Even though, there has been much research in *Magnaporthe* as well as other filamentous fungi to elucidate the function of TCS components in stress response little has been done in the light of targeting the TCS in field isolates most of which have gradually acquired resistance to some of these fungicides. Since the pathway provides response for multiple environmental stresses, it is ideal to look at different stress response at the same time to identify potential antifungal targets in resistant variants.

1.13 The TCS as new target for novel fungicides

The studies of two-component proteins in fungal pathogens have revealed the critical functions of these proteins in adaptation to stress, regulation of virulence factors, and sensitivity to antifungal drugs, highlighting the importance of these signalling pathways in fungal pathogenesis. The involvement of two-component pathways in bacterial and fungal pathogenesis has generated significant interest in using these pathways as targets for antimicrobial drug development. Since, TCS are found in all major fungal pathogens, drugs targeting these factors may yield broad spectrum molecules. Research efforts have thus far centred on the histidine kinase proteins; however, the HPT and RR domains are also suitable targets, since they are absent from animal genomes.

In the present study, we attempted to study the TCS by particularly analysing the only Histidine Phosphotransferase in *M. oryzae* B157 and dissect its role as a member of the TCS in sporulation, pathogenicity, cell wall integrity, stress management, light response and antifungal response. We also analysed the global transcriptome of the *MoHPT1* knock-down transformant to understand the regulation of *MoHPT1,* to identify its possible downstream effectors and understand its role in pathogenicity.