Chapter 2

Review of literature

2.1. Overview

Rice blast is a serious fungal disease of cultivated rice, which can lead to epidemics throughout the regions where rice is grown and consumed as staple diet (Baker et al., 1997). Rice blast is caused by the ascomycetous fungus *Magnaporthe oryzae*, formerly known as *Magnaporthe grisea* (Couch and Kohn, 2002). This filamentous, hemibiotrophic fungus causes disease in many species of the grass (Poaceae family) including wheat, millet and barley, although most of the strains infect only a limited number of grass species. Rice blast fungus has developed morphogenetic and biochemical specialization to its pathogenic lifestyle which makes it highly efficient and devastating fungal pathogen (Talbot, 2003).

Rice is a major staple food for more than half of the global human population but, the global rice production is severely reduced by the blast disease (Jantasuriyarat et al., 2005) . Rice contributes almost 23% of the total calories consumed by human population globally and is particularly important crop in Asia, where nearly 92% of rice is grown and consumed (Wilson and Talbot, 2009). Yield losses of 10%–30% have been reported in blast affected rice crops, although regional epidemics have been reported to cause losses up to 80% when predisposing conditions favor severe disease development (Odile et al., 2010; Piotti et al., 2005). According to the estimates, rice blast disease causes losses of rice crop that is adequate to feed 60 million people (Dean et al., 2005). Between 2001 and 2005, rice blast epidemics have occurred in China, Japan, Korea and Vietnam and 5.7 million hectares of rice were destroyed in China alone (Wilson and Talbot, 2009). International Rice Research Institute (IRRI) reports more than 266,000 tons of rice lost every year in India, which was about 0.8% of the total yield. In addition to rice, *M. oryzae* causes disease in finger millet (*Eleusine coracana*), which is an important food crop in India.

Rice blast fungus can cause leaf blast, which kills seedlings, or neck and panicle blast, which damages the plants during the seed-setting stage, or can infect through roots (Sesma and Osbourn, 2004; Viaud, 2002). The disease spreads frequently under moist conditions with humidity ranging from 80% -100% and temperatures ranging from 25-30°C (Talbot, 2003). The most economically viable and environment friendly practice to manage rice blast disease is host resistance. However, the fungus has developed ability to overcome plant resistance quickly, and most of the cultivars typically become ineffective after 2–3 years (Ou, 1980; Zeigler et al., 1994). Detailed molecular analysis of rice blast disease is highly significant and economically important area, as it offers the avenues of developing novel strategies for durable disease control (Skamnioti and Gurr, 2007).

(Galhano and Talbot, 2011)

Figure. 1. Blast infection in various parts of rice plants A. Rice seedlings, disease symptoms appear as necrotic lesions initially, which subsequently become larger and coalesce. **B**. Mature plants, the infection spreads to the panicle, causing severe yield losses. **C**. Neck blast, observed at nodes and the neck area near the panicle.

2.2. Phylogeny

Magnaporthe oryzae is a member of the *Magnaporthe grisea* complex, which contains at least two biological species that are genetically different and do not interbreed (Couch and Kohn, 2002).

Magnaporthe oryzae (earlier named *Pyricularia oryzae* by Cavara in 1891, *Pyricularia grisea* and *Magnaporthe grisea* by Rossman in 1990) causes the rice blast disease as well as foliar diseases of wheat and barley (Choi et al., 2013).

Scientific classification

Kingdom:	Fungi
Phylum:	Ascomycota
Class:	Sordariomycetes
Order:	Magnaporthales
Family:	Magnaporthaceae
Genus:	*Magnaporthe*
Species:	*Magnaporthe grisea* (T. T. Hebert, M. E. Barr 1977) ***Magnaporthe oryzae* (B. C. Couch 2002)** *Magnaporthe poae* (Landsch. & N. Jacks. 1989) *Magnaporthe rhizophila* (D. B. Scott & Deacon 1983) *Magnaporthe salvinii* (R. A. Krause & R. K. Webster 1972)

2.3. *Magnaporthe oryzae* as a model phytopathogen

Due to a number of attributes *M. oryzae* has emerged as an excellent model phytopathogen (Talbot, 2003). The fungus can grow away from its host (rice) in standard growth medium (Valent and Chumley, 1991). Complete genome sequence of *M. oryzae* has become available, therefore providing the genomic resources to understand genetic basis of pathogenesis by this phytopathogen (Dean et al., 2005). The genome sequence of two rice subspecies, *indica* and *japonica*, have been published (Goff, 2002; Yu et al., 2002). The sequence availability of rice and *M. oryzae* makes it the first plant host and fungal pathogen combination available for the study of host pathogen interactions at genetic level.

M. oryzae can be easily transformed using a number of selectable markers, including antibiotics such as hygromycin B, bialaphos, and sulfonylurea (Talbot and Foster, 2001; Valent and Chumley, 1991). The initial stages of plant penetration which involves development of appressorium can be carried out on synthetic hydrophobic glass and plastic surfaces (Talbot, 2003). *M. oryzae* transformation is reliable and adequate though not as efficient as yeast transformation (Talbot, 2003). *Agrobacterium tumefaciens* mediated transformation of *M. oryzae* has shown promise of improved transformation frequencies (Rho et al., 2001). Targeted gene replacement approach is generally used to study gene function, where 1 kb of flanking DNA on either side of a gene of interest is used for homologous recombination (Talbot and Foster, 2001). *In vitro* transposon mutagenesis offers another efficient means of carrying out gene disruption at a high throughput (Hamer et al., 2001).

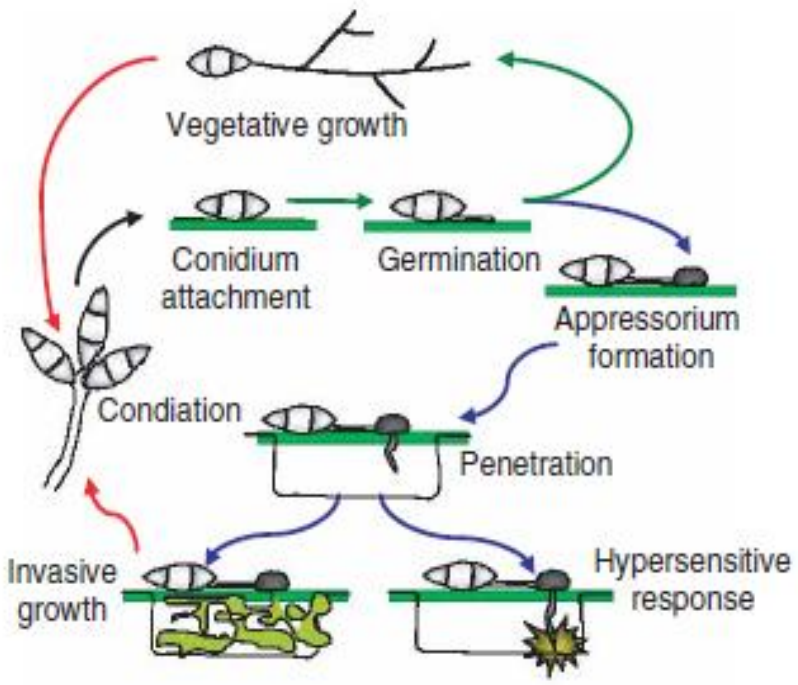
M. oryzae is a heterothallic ascomycete with two mating types *MAT1-1* and *MAT1-2*. When two fertile isolates of opposite mating type are paired together on an agar medium at 20^{o}C, sexual fruiting bodies known as perithecia are developed within 18-21 days (Valent et al.,

1991). Each perithecium contains numerous asci, and each ascus contains accospores as a result of meiotic division followed by mitotic division. Detailed analysis of segregation patterns of genetic markers can be an important tool for the determination of genetic basis of phenotypic traits (Talbot, 2003).

2.4. The infection cycle

Rice blast infection starts when an asexual spore lands on the hydrophobic surface of a rice leaf, the spore attaches itself to the leaf surface by release of an adhesive (Hamer et al., 1988). Within two hours of landing on the rice leaf, a polarized germ tube emerges from one of the apical cells of the three celled conidia. The germ tube normally extends for only a short distance (15–30 µm) before swelling at tip and attaching to the leaf surface. This phase of infection is known as hooking, which is followed by development of appressorium (Talbot, 2003). Generally appressorial development in rice blast fungus takes place on hard and hydrophobic surfaces in the absence of any exogenous nutrients (Dean, 1997). Appressorial differentiation normally takes place within 6-10 h and it becomes darkly pigmented due to formation of a distinct layer of melanin. Glycerol concentration builds up within the appressorium to generate an enormous turgor pressure of around 80 bars (8.0 MPa). This turgor leads to a mechanical pressure which forces a penetration peg through the leaf cuticle into the plant tissue (Howard et al., 1991). The fungal hyphae spread through the plant tissue forming the dark disease lesions. Under favourable conditions like high humidity, the fungus inside the plant tissue sporulates profusely. The disease spreads rapidly to adjacent rice plants due to water droplets and wind (Wilson and Talbot, 2009).

M. oryzae has also been demonstrated to infect underground parts of host plants via formation of hyphopodia (Sesma and Osbourn, 2004) . Thus, fungal pathogens have efficiently adapted to the ecological niches they occupy to infect host plants.



(Jeon et al., 2007)

Figure. 2. Infection cycle of *M oryzae*. Depending on the environmental cues, asexual spores follow either normal vegetative growth or may undergo morphological changes to cause infection. During infection, a specialized infection structure known as appressorium is developed, which helps penetration peg to break the leaf surface barrier and cause infectious growth. Inside the plant tissue, more conidia are produced to reinitiate the infection cycle.

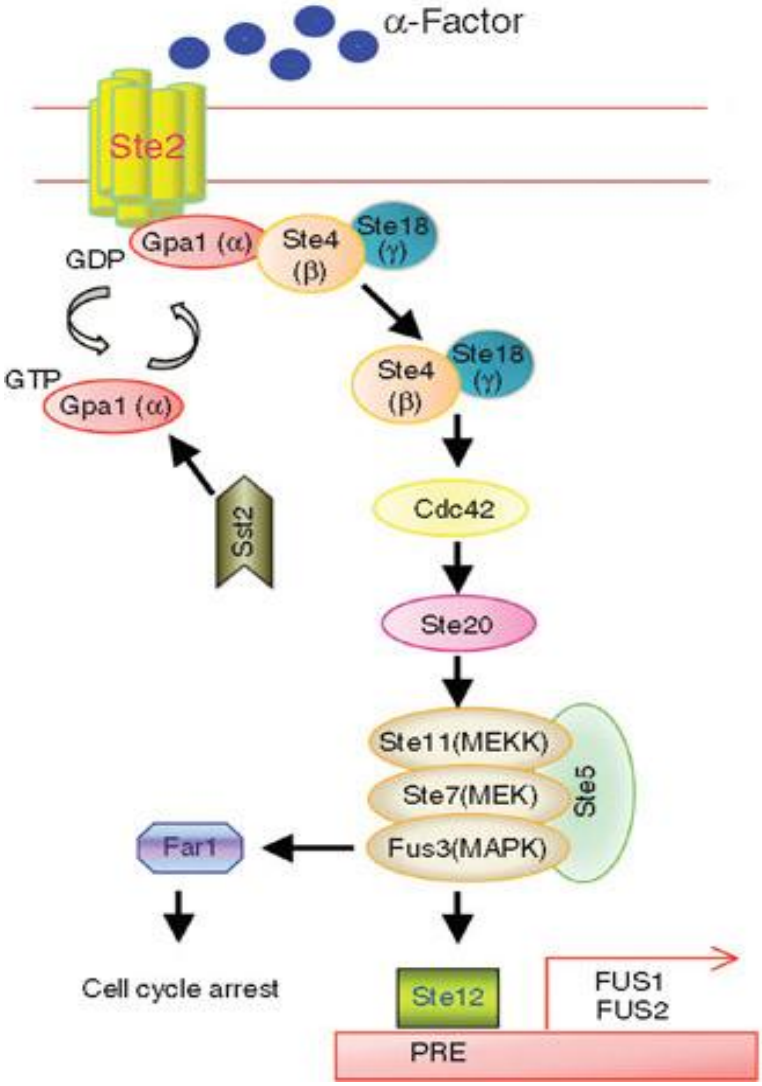
2.5. *M. oryzae* genome analysis

Rice blast fungus was the first plant pathogenic fungal genome to be sequenced and published (Dean et al., 2005). The approach to study biology of the rice blast disease has revolutionized due to availability of the *M. oryzae* genome sequence. The first *M. oryzae* strain to be sequenced was 70-15 and it was found to possess more than 13,000 genes in its 40 MB haploid genome. Analysis of genome sequence of *M. oryzae* has revealed the presence of a large number of repetitive elements such as transposons and retrotransposons (Fudal et al., 2005). Emergence of new races of the rice blast fungus has been a hindrance to durable resistance, thus affecting rice productivity. Transposable elements can be one of the causal agents of genetic variability in *M. oryzae* (Kang, 2001; Orbach et al., 2000). It has been demonstrated that a Pot3 element insertion in the promoter of the *AVR-Pita* gene, has led to a gain of virulence on cultivar Yashiro-moshi (Kang et al., 2001). It shows that transposons have the potential to bring about changes in the virulence of *M. oryzae.* Pot2 transposable element has been identified from *Magnaporthe oryzae*. The element has 43-bp perfect terminal inverted repeats (TIRs) and 16-bp direct repeats within the TIRs (Kachroo et al., 1995). Repetitive-PCR based on Pot2 has been widely employed to study pathogenic and the genetic diversity of the isolates of *M. oryzae* obtained from different geographical locations.

M. oryzae genome also encodes a large repertoire of predicted G protein-coupled receptors GPCRs (Dean et al., 2005). Out of the 61 previously unidentified GPCRs that have been found in *M. oryzae*, 12 of these contain a conserved fungi-specific extracellular membrane-spanning (CFeM) domain at the amino terminus (Kulkarni et al., 2003). Functional analysis of pth11 (CFEM domain containing protein) was carried out and it was found to be required for pathogenicity (DeZwaan et al., 1999).

2.6. Sexual reproduction in yeast

In *S. cerevisiae,* cells can exist as either haploid or diploid form. Haploid cells are present as α and a-cells which are the two opposite mating type forms. The opposite mating type cells (α or a) can mate by fusion and consequently form a diploid cell (Gustin et al., 1998). At the molecular level, this process requires pheromones, receptors, a cascade of MAP kinase proteins, transcription factors and effector proteins. *MAT*α cells release a small peptide mating pheromone α-factor, which acts on *MAT*a cells, similarly *MAT*a cells also release a-factor pheromone, that act on *MAT*α cells. Mating is initiated when a mating pheromone binds to the receptor on opposite mating type cell and the release of stimulatory Gβ subunits. The liberated Gβ associates with the Ste5 scaffold protein and a MAPKK kinase protein Ste11. Ste11 activates Ste7. Ste7 in turn phosphorylates and activates Fus3 and Kss1, which are partially redundant MAPKs involved in regulation of mating process (Kusari et al., 2004) . Fus3 regulates the mating process by phosphorylating downstream proteins including Ste12 and Far1. Ste12 being a transcription factor regulates expression of genes involved in mating whereas Far1 is involved in cell cycle arrest during mating (Errede and Ammerer, 1989; Kusari et al., 2004; Peter and Herskowitz, 1994). Far1 is inhibitor of cyclin dependent kinase CDC28 that leads to cell cycle arrest during mating in *S. cerevisiae* (Peter and Herskowitz, 1994).



(Dong et al., 2010)

Figure. 3. Pheromone signaling pathway in yeast. Yeast α-factor binds to ste2 receptor, leading to GDP–GTP exchange on the Gα subunit (GPa1). The Gβγ dimer (Ste4 and Ste18) gets dissociated from the G protein and activates a MAP kinase cascade. Fus3 (MAPK) activates the transcription factor Ste12, which in turn induces the expression of genes required for yeast mating such as FUS1 and FUS2. In addition, Fus3 also activates Far1 to induce cell cycle arrest before mating would start.

2.7. Sexual reproduction in *M. oryzae*

M. oryzae is a hermaphrodite, heterothallic ascomycetous fungus which reproduces mostly asexually in nature. Sexual reproduction is possible *in vitro* between two strains of opposite mating types. Out of the two strains, at least one should be female-fertile strain , which means it should have ability to form perithecia (Saleh et al., 2012) . One gene with two different alleles namely MAT1-1 and MAT1-2 controls its mating type during mating (Notteghem and Silue, 1992). In nature, the different host specific strains of *M. oryzae* show distinct differences in fertility (Notteghem and Silué, 1992). Rice pathogenic strains are predominantly infertile and very few fertile strains have been recovered from the field (Kato and Yamaguchi, 1982). Guy11, a *MAT1-2* strain from French Guyana is the most commonly studied strain of *M. oryzae* and has been an important strain for genetic studies (Leung et al., 1988). Efforts have been made to generate laboratory strains of *M. oryzae* that can be genetically crossed, therefore many fertile strains of *M. oryzae* have been developed in laboratory (Ellingboe et al., 1990; Valent et al., 1991).

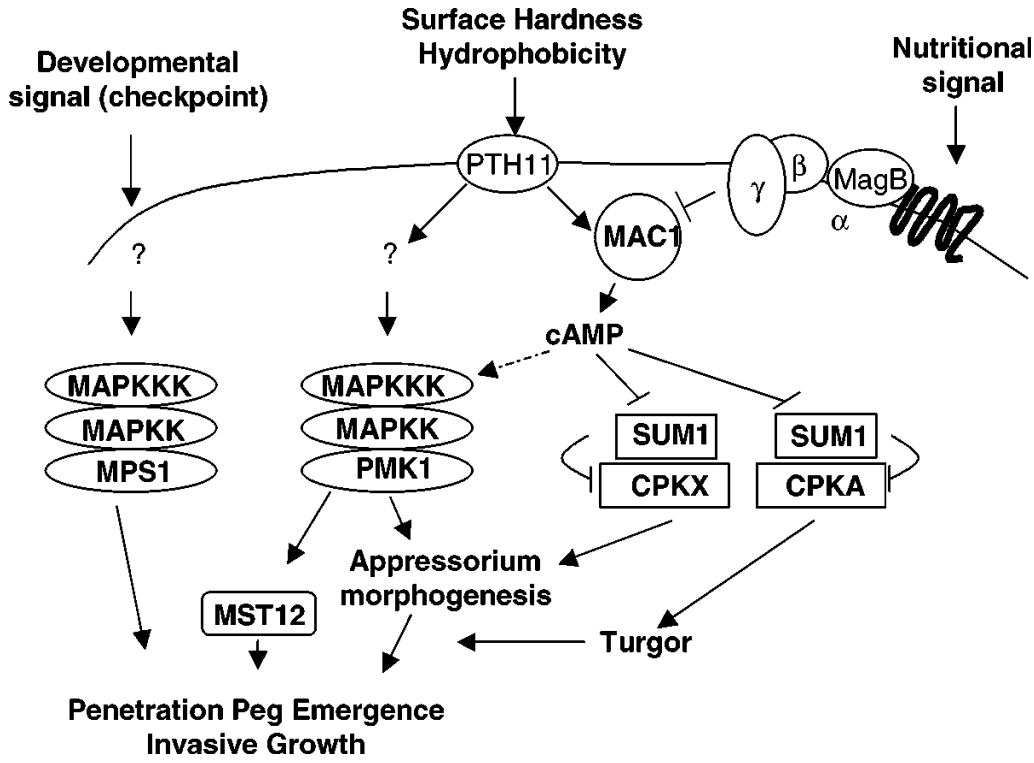
2.8. Cyclic AMP signaling during appressorium development

Cyclic AMP (cAMP) is a widely known secondary messenger and has been reported to induce morphogenesis in a variety of fungi. In case of the rice blast fungus, a cyclic AMP response pathway is believed to be triggered during early stages of infection as it has been reported that Δ*mac1* mutants, which are unable to synthesize cAMP, are not able to infect plants due to inability to form appressoria (Choi and Dean, 1997). It has also been demonstrated that the exogenous addition of cAMP to germinating conidia induces appressorium development on

noninductive hydrophilic surfaces, indicating the significance of cAMP mediated signaling during plant infection (Lee and Dean, 1993).

cAMP is believed to exert its effect via activation of cAMP-dependent protein kinase A (PKA) and binding of cAMP to inactive enzyme causes the release of a catalytic subunit of the protein kinase A, CPKA. This catalytic subunit migrates into the nucleus to activate target proteins by phosphorylation (Kronstad, 1997). The cAMP signal in the rice blast fungus is elicited in response to surface hydrophobicity when germ tube lands on plant or artificial surface (Dean, 1997). MPG1, a hydrophobin secreted from conidia of *M. oryzae* establishes its attachment to the host leaf surface and MPG1 deletion mutants are weakly pathogenic due to inability to form appressoria. (Talbot et al., 1993). This indicates that surface attachment is essential for the regulation of appressorium development. Appressorium formation in the Δ*mpg1* mutants can be restored by application of exogenous cAMP (Soanes et al., 2002).

PTH11 is a membrane protein containing nine membrane spanning domains and a cytoplasmic domain (DeZwaan et al., 1999). Δ*pth11* mutants are severely impaired in appressorium development even on hydrophobic surfaces. The appressorium defect in Δ*pth11* mutants can also be restored by application of exogenous cAMP. It suggests that *PTH11* may have some role in regulation of cAMP signaling even though there is no direct evidence to support that *PTH11* has a sensory role in *M. oryzae* (Zhao et al., 2007).



(Talbot, 2003)

Figure. 4. Signal transduction involved in infection related development. Surface signals like hydrophobicity are perceived by the receptor protein PTH11 leading to activation of MAC1 adenylate cyclase. MAC1 produces cAMP, and response pathway regulates signalling during plant infection. The PMK1 MAPK signalling is involved in appressorium morphogenesis and plant penetration via the MST12. MPS1 signalling is responsible for penetration peg formation that leads to invasive growth.

2.9. G protein and MAPK signaling in *S. cerevisiae* and *M. oryzae*

Analysis of the *M. oryzae* genome show a large number of putative G protein-coupled receptors (GPCR), however no receptor protein kinases genes are likely to be present (Kulkarni et al., 2005). The GPCRs that are likely to be present in *M. oryzae* genome include putative homologs of S. *cerevisiae* Ste2 and Ste3. Deletion mutants of putative homologs of Ste2 and Ste3 are not affected in appressorial development or plant infection (Zhao et al., 2007). There are almost twelve putative GPCRs in *M. oryzae* genome which contain the unique CFEM domain. One such GPCR, PTH11, has been reported to be involved in surface recognition during early stages of infection (Dean et al., 2005; DeZwaan et al., 1999).

Heterotrimeric G proteins are named so due to their ability to bind guanine nucleotides and presence of three different subunits α, β and γ. Heterotrimeric G proteins interact with a seven-transmembrane GPCR at the cell membrane. When bound to GTP, Gα subunit gets activated and interacts freely with effector proteins. However, Gα subunit also possesses intrinsic GTPase activity by which it can readily convert from active to inactive form (Bölker, 1998). MAGB in rice blast fungus encodes a Gα subunit and *magB* mutants are non pathogenic due to inability to form appressoria. However the appressorial defect can be restored by exogenous application of cAMP, suggesting that MAGB may be involved in regulation of cAMP signal in the rice blast fungus (Liu and Dean, 1997).

Mitogen activated proteins (MAPK) are involved in transduction of extracellular signals involved during various growth and differentiation processes (Gustin et al., 1998). During signal transduction, the MAP kinases (MAPK) are activated by MAPK kinases (MAPKK) that are in turn activated by MAP kinases (MAPKKK). These MAKKK-MAPKK-MAPK cascades are generally highly conserved in eukaryotic organisms and have been comprehensively studied

in many organisms. Five MAPK pathways have been reported in *S. cerevisiae* which control mating, high osmolarity response, maintenance of cellular integrity, filamentous growth, and ascospore formation (Dohlman, 2002; Gustin et al., 1998). Yeast pheromone response pathway also known as mating pathway is the best understood MAPK pathway among all eukaryotes (Gustin et al., 1998) . This pathway is initiated when a mating pheromone (α or a) binds to a receptor (STE2 or STE3) and promotes release of activating β subunit. The free Gβ directly links to Ste5 (a scaffold protein), Ste20 and also activates the Ste11 (MAPKKK). Ste11 phosphorylates and activates the Ste7 (MAPKK) and Ste7 in turn activates Fus3 and Kss1 by phosporylation. Some of the pheromone response pathway proteins including Ste20, Ste11, Ste7, and Kss1 have been demonstrated to be involved in filamentous growth in yeast (Cherkasova et al., 2003). *M. oryzae* MST7 and MST11 are homologous to the *S. cerevisiae* STE7 and STE11 respectively. The mst7 and mst11 deletion mutants have been reported to be defective in appressorium development and plant infection (Zhao et al., 2005). Direct interaction between MST7 and PMK1 has been only detected during appressorium development and MAPK-docking site of Mst7 is indispensible for its binding and activation to downstream kinase PMK1 (Zhao and Xu, 2007)

Fus3 regulates the mating process by phosphorylating downstream proteins including Ste12 and Far1 (Kusari et al., 2004). PMK1 (Pathogenicity MAP kinase1) is a homolog of *S. cerevisiae* FUS3 and it has been reported to be involved in regulation of appressorium development and plant infection (Xu and Hamer, 1996). PMK1 has been demonstrated to rescue the mating defect in a fus3 kss1 double mutant and shows in vitro kinase activity. Δpmk1 deletion mutants normally fail to form appressoria but appressorium development can be restored in presence of exogenous cAMP. Homologous proteins of PMK1 have been

characterized in various plant pathogens independently. It has been found that these MAPK proteins are involved in appressorium development in all four appressorium forming fungi characterized so far. The other three fungi include *Cochliobolus heterostrophus*, *Colletotrichum lagenarium* and *Pyrenophora teres* (Lev et al., 1999; Ruiz-Roldan et al., 2001; Takano et al., 2000).

Ste12 is a transcription factor which regulates expression of genes involved in mating process in *S. cerevisiae* (Errede and Ammerer, 1989). MST12, a homolog for *S. cerevisiae* STE12, has been functionally characterized in *M. oryzae.* Gene deletion mutants of MST12 have been found to be non-pathogenic even though they could form appressoria (Park et al., 2002). The defect in pathogenesis lies in appressorium function rather than appressorim formation because Δ*mst12* mutant appressoria are unable to form penetration peg, which is important for pathogen entry into host. Therefore, it can be concluded that PMK1 regulates target proteins involved in the appressorium development as well as subsequent stages of invasive growth.

2.10. FAR1 in *S. cerevisiae* and other fungi

Cell synchronization is prerequisite during mating of two opposite mating type cells in *S. cerevisiae.* During mating, the cell cycle arrest at G1 phase is caused due to inactivation of Cdc28-Cln2 complex by FAR1 (Mendenhall, 1998). FAR1was originally identified in a mutant screening for growth of *S. cerevisiae* cells in presence of mating pheromone. FARI stands for factor arrest resistant, based on mutants showing resistance towards pheromone mediated cell cycle arrest. The protein is composed of 830 amino acids with a molecular mass of 95 kDa. Cdc28-Cln activity is required for passage through Start phase during cell cycle, FAR1 inhibits Cdc28-Cln activity causing the cell cycle arrest (Chang and Herskowitz, 1990). FARl is usually

expressed in haploid cells only and never in diploid cells as they do not undergo the mating pheromone response. FARl expression undergoes frequent changes during different stages of cell cycle, with high levels of mRNA from early G2 to Start phase and very low levels from Start to S-phase (McKinney et al., 1993). Far1 expression under uninduced conditions is inversely correlated to the expression pattern of its downstream targets for inhibition, Cln1and Cln2. It has been demonstrated that when cell cycle is arrested with microtubule inhibitor nocodazole, there is accumulation of high levels of Far1. FARl transcription is induced by four to five folds in presence of the mating pheromone α-factor and Stel2 transcription factor has been reported to regulate this induction (Chang and Herskowitz, 1990). Overexpression of FAR1 in *S. cerevisiae* leads to increase in the mRNA and protein levels but cannot induce cell cycle arrest unless the cells are also exposed to mating pheromone (Busti et al., 2012). Enhanced FAR1 expression is therefore essential but not sufficient for the pheromone-induced cell division arrest and Far1 phosphorylation by Fus3 is compulsory for the inhibitory action.

Gene disruption of a predicted homolog for *S. cerevisiae* FAR1 in *Candida albicans* has been demonstrated to inhibit pheromone-mediated cell cycle arrest and block the pheromone mediated morphological changes. Transcriptional profiling of the *C. albicans* FAR1 overexpression shows an enhanced response of the pheromone-induced genes (Côte and Whiteway, 2008). FAR1 has not been identified and functionally characterized in any of the filamentous fungi till date therefore it would be interesting to know if it plays any role in pathogenesis and filamentous growth in *M. oryzae.*

2.11. Interspecific pheromone response in fungi

In *S. cerevisiae,* there are two mating type cells known as a and α cells, with MATa and MATα genotypes. MATα cells secrete α-factor pheromone, a diffusible peptide consisting of 13 residue with amino acid sequence WHWLQLKPGQPMY. MATa cells secrete a-factor, a 12 residue peptide with amino acid sequence YIIKGVFWDPAC. α-factor is recognized by STE2 receptor in MATa cells, inducing cell cycle arrest, whereas, a-factor is recognized by STE3 receptor in MATα cells (Naider and Becker, 2004). α-factor was isolated from medium of MATα cell cultures as tridecapeptide pheromone acting on MATa cells (Stötzler and Duntze, 1976). The peptide has been reported to be encoded by two genes MFα1 and MFα2 (Kurjan and Herskowitz, 1982). The peptide sequence of α-factor has been chemically synthesized and was found to demonstrate properties similar to natural pheromone (Ciejek et al., 1977; Masui et al., 1977). Another peptide pheromone, a-factor is covalently attached to a farnesyl group, therefore it has been found difficult to purify or synthesize chemically.

It has been shown that the yeast α-factor and the human hypothalamic decapeptide gonadotropin-releasing hormone (GnRH) have sequence similarities suggesting evolutionary relatedness (Naider and Becker, 1986). The tridecapeptide mating pheromone, α-factor shows extensive sequence homology with the decapeptide gonadotropin releasing hormone (GnRH). It has been reported that α- factor specifically binds to rat pituitary GnRH receptors thereby stimulating the release of luteinizing hormone (LH) from cultured gonadotrophs (Loumaye et al., 1982).

The α-factor pheromone of *S. cerevisiae* has activity in closely related yeast species like *Saccharomyces kluyveri*. *S. kluyveri* a-cells show cell cycle arrest and exhibit morphological changes in response to *S. cerevisiae* α-factor but the concentration of α-factor has to be

increased fourfold to elicit response equivalent to *S. kluyveri* α-factor. Similarly, *S. kluyveri* α-factor (α-k-factor) induced cell cycle arrest in *S. cerevisiae* at a concentration 25 times higher than required for response by *S. kluyveri* a cells (McCullough and Herskowitz, 1979). These results suggest that there is interspecific pheromone response between different fungi however the effective concentration for such responses is higher.

S. cerevisiae α-factor pheromone has been demonstrated to inhibit appressorium development in a *MAT1-2* strains in *M. oryzae* but not in *MAT1-1* strains (Beckerman et al., 1997*)*. 300 mM of α-factor causes almost 95% inhibition of appressorium formation of *MAT1-2* strains, which is nearly 10^4 fold higher than the concentration of α-factor required to induce cell cycle arrest in *S. cerevisiae* (Beckerman et al., 1997; Raths et al., 1988).However, appressorium development was restored with addition of cyclic adenosine 3',5'monophosphate (cAMP). It indicates that α-factor and *M. oryzae* interaction is somehow inhibiting accumulation of cAMP inside the cells (Beckerman et al., 1997).