1. Introduction

Outbreaks of rice blast disease are a serious and recurrent problem in all rice-growing regions of the world, and the disease is extremely difficult to control. Rice blast, caused by the fungus *Magnaporthe oryzae*, is therefore a significant economic and humanitarian problem. It is estimated that each year enough rice is destroyed by rice blast disease to feed 60 million people.

Infections occur when fungal spores land and attach themselves to leaves using a special adhesive released from the tip of each spore. The germinating spore develops an appressorium, a specialized infection cell, which generates enormous turgor pressure (up to 8MPa) that ruptures the leaf cuticle, allowing invasion of the underlying leaf tissue. Subsequent colonization of the leaf produces disease lesions from which the fungus sporulates and spreads to new plants. When rice blast infects young rice seedlings, whole plants often die, whereas spread of the disease to the stems, nodes or panicle of older plants results in nearly total loss of the rice grain. Different host-limited forms of *M. oryzae* also infect a broad range of grass species including wheat, barley and millet. Recent reports have shown that the fungus has the capacity to infect plant roots.

M. oryzae outbreaks are controlled through the application of potentially hazardous fungicides and the use of resistant cultivars. The fungus has been able to develop resistance to many of these cultivars, because it is highly variable. An understanding of early events of the infection is of paramount importance if durable control measures are to be developed.

The genome of a rice pathogenic strain of *M. oryzae*, 70-15, was sequenced through a whole-genome shotgun approach. It has seven chromosomes. The total length of all sequence contigs is 39.5 (Mb). Within the *M. oryzae* genome, 12,841 genes are predicted with protein products, ~80% of which are hypothetical proteins and ~20% are predicted proteins. It has a large and complex secretome, 739 proteins are predicted to be secretory (Dean *et al*., 2005).

The secretory proteins can be classified as:

i. Enzymes for degradation of the plant cell wall and cuticle.

ii. Proteins with carbohydrate substrate-binding domains, with a role in attachment and colonization of plant tissue.

iii. Pathogen effector proteins which fungus secretes directly into host plant cells to perturb host cell signalling or suppress the plant innate immune system.

Plant cell wall and cuticle degrading proteins are cutinases, cellulase, xylanases, laccases etc. Several of these genes are significantly up-regulated during infection-related development.

Biological role of fungal laccases are uncertain, they could be involved in:

(i) Melanin polymerization

(ii) Lignin degradation

(iii) Oxidation – reduction of plant toxins

Laccase is a copper- containing enzyme that catalyzes the oxidation of a phenolic substrate by coupling it to the reduction of oxygen to water.

Laccases have been shown to be an important virulence factor in many diseases caused by fungus, e.g. *Cryphonectria parasitica* (severe chestnut blight), *Gaeumannomyces graminis* (severe root disease), *Cryptococcus neoformans* (laccase knock out mutants are non pathogenic). High laccase activity in culture filtrate of *M. oryzae*, 24 hours after spore germination), was detected (Iyer and Chattoo., 2003), suggesting that it might play a role during the infection of rice plant.

The present work focuses on the identification and characterisation of two laccases from *M. oryzae.*

Objectives:

1. Identification of putative laccase gene(s) in *Magnaporthe oryzae.*
2. Silencing of laccase gene(s) in *Magnaporthe oryzae.*
3. Characterisation of antisense transformants.
4. Heterologous expression, purification and characterisation of laccase(s).