2. *INTRODUCTION*

Rice (*Oryza sativa*) is the primary staple food for more than half of the world's population and is cultivated across 122 countries worldwide. Besides its agronomic importance, rice is a model monocot and has small genome size of ~430 Mb contained in 12 chromosomes compared to other major cereal crops. Even though rice is one of the most important food crops in the world both economically and nutritionally, it encounters a number of biotic and abiotic stresses which leads to huge crop losses. Rice is vulnerable to both fungal (mainly *Magnaporthe oryzae*) and bacterial (mainly *Xanthomonas oryzae*) diseases and there are over 85 reported rice diseases (Mew, 1991).

Rice blast disease caused by *Magnaporthe oryzae* continues to be a serious and recurring problem in all rice growing regions across the world.

The first case of blast disease was reported in the United States in 1876, and since then has been identified in over 85 rice producing countries worldwide (Wang *et al.*, 2014). *M. oryzae* is part of a species complex which can cause disease in a variety of grasses and related species, including crops such as barley, wheat and millet (Couch *et al.*, 2005). Pathogens including *M. oryzae* have evolved various strategies to overcome different barriers which they encounter during infection of their hosts. The rice blast fungus attacks rice plants at all stages of development and can infect leaves, stems, nodes, panicles and roots.

Foliar infection occurs by formation of a dome-shaped infection structure called the appressorium, which upon maturation generates turgor pressure by accumulating high concentrations of compatible solutes such as glycerol (De Jong *et al.*, 1997) and is important for breaching the rice cuticle; thereby the fungal hyphae ramify through the plant tissue and grow within the host cells. The fungus sporulates profusely from disease lesions under conditions of high humidity, allowing the disease to spread rapidly to adjacent rice plants by wind and dewdrop splash (Talbot, 2003).

M. oryzae also causes systemic plant invasion through root infection and has been shown to undergo different developmental events which are typical of root infecting pathogens (Sesma and Osbourn, 2004). Root infection is initiated by the formation of hyphopodium (penetration structure) which leads to the formation of infection pegs. Upon penetration, intra and intercellular growth leads to invasion of epidermal and cortical layer of the root. Once inside the root, the fungus spreads from the cortical cells through the endodermis and into the stele (vascular tissue) leading to systemic infection and causing classic disease symptoms in aerial tissues. It has also been demonstrated that specific gene-for gene type disease resistance mechanisms occur in the roots (Sesma and Osbourn, 2004).

Over the years, various resistant cultivars have been used for resistance breeding, leading to development of new resistant cultivars; but these new cultivars too will become susceptible to new virulent strains of the fungus in few years. Races of *M. oryzae* differ between countries and also in different locations within a country. Also reaction of cultivars differ from season to season (Ou, 1980). It has been shown through reactions of cultivars using different fungal races that some cultivars are resistant, some are susceptible and many are in between these two extremes, suggesting involvement of various resistance (*R*) genes of the host and avirulence (*avr*) genes of the pathogen in these interactions (Ou, 1980). When corresponding *R* and *avr* genes are present in both the host and pathogen, the host shows disease resistance. If either of *R* and *avr* is inactive or absent, the host is susceptible to the disease.

The extreme variability of the fungus enables it to overcome the resistance of the cultivars. Considering the poor durability of many blast-resistant cultivars of rice, which have a typical field life of only 2-3 growing seasons before disease resistance is overcome (Ou, 1980, Zeigler *et al.*, 1994), and increasing energy costs which affect fungicide and fertiliser prices, there is a need for better understanding of rice blast disease to combat this deadly crop destroyer (Ribota *et al.*, 2008). Rice blast control strategies that can be deployed as part of an

environmentally sustainable plan for increasing the efficiency of cereal cultivation are therefore urgently required (Wilson and Talbot, 2009). Such strategies will depend on a better understanding of the disease process.

The development of spores leading to appressorium formation is initiated through recognition of environmental cues and is mediated by cross-talk between signal transduction pathways within the cell. In the past two decades, studies on signalling pathways, which include the Mitogen Activated Protein Kinase (MAPK) signalling cascade and signalling pathways dependent on secondary messengers like Ca^{2+} (Nguyen *et al.*, 2008) and cAMP (Zhang *et al.*, 2010; Lee and Dean, 1993), which regulate various stages of the *M. oryzae* infection cycle, have been initiated. Although the cell cycle and signal transduction pathways tightly regulate *M. oryzae* development and infection, studies of how metal ions affect these developmental pathways have been largely limited to calcium signalling. The ability to grow, divide, respond to cell wall stress, sporulate and establish infection are complex but critical processes in *M. oryzae* for its colonisation and establishment in the host as a successful pathogen. Magnesium being a co-factor for a wide range of enzymes is important in a variety of biochemical processes. Mg^{2+} is utilised by twice as many metalloenzymes as zinc (Andreini *et al.*, 2008). Free Mg^{2+} is essential for stabilising cell membrane, cell wall (Asbell and Eagon, 1996; Zimelis and Jackson, 1973; Prescott *et al.*, 1988; Trofimova *et al.*, 2010) and ribosomes. It is essential for neutralising the negatively charged phosphate groups of nucleic acids (Wolf and Cittadini, 2003), DNA repair, and is indispensable for DNA replication fidelity. Mg^{2+} regulates electrolyte transport across the cell membrane (Wolf and Cittadini, 2003), as well as activity of the sodium potassium pump (Na/K-ATPase) and the calcium pump (Ca-ATPase) (Pasternak *et al.*, 2010). In the fission yeast *Schizosaccharomyces pombe* and the budding yeast *Kluyveromyces fragilis*, intracellular levels of Mg^{2+} regulate the timing of cell cycle progression (Walker and Duffus, 1980). Among pathogens, Mg^{2+} is also required for germ

tube formation in *Candida albicans* vegetative cells and consequently affects its morphogenesis and pathogenicity (Walker *et al.*, 1984). Regulation of intracellular concentration of Mg^{2+} is achieved by three mechanisms: uptake systems, efflux from the cell and sequestration within organelles (Lim *et al.*, 2011). However, the relation between Mg^{2+} concentrations and morphogenesis has not been investigated in fungal plant pathogens, including *M. oryzae*.

The molecular identity, function and regulation of Mg^{2+} transporters have been studied extensively to understand the basis of Mg^{2+} homeostasis in eukaryotic cells. The **CorA** (or **Metal Ion Transporter**, **MIT**) superfamily is an important group of Mg^{2+} transporters in both prokaryotes and eukaryotes (Lim *et al.*, 2011). Despite divergent primary protein sequence, the CorA Mg^{2+} transporters are characterised by two or three conserved transmembrane domains near the carboxy terminus, one of which is followed by the conserved motif **(F/W) GMN** (Graschopf *et al.*, 2001) that is essential for Mg^{2+} transport. In *Salmonella typhimurium* and *Escherichia coli*, three proteins (CorA, MgtA, and MgtB) have been shown to be involved in Mg^{2+} transport across the plasma membrane (Graschopf *et al.*, 2001). Magnesium uptake by CorA is essential for viability of *Helicobacter pylori* (Pfeiffer *et al.*, 2002).

Eukaryotic CorA proteins have diversified in function, facilitating both Mg^{2+} uptake and distribution between sub-cellular compartments. *Saccharomyces cerevisiae* **Alr1** is the first characterised Mg^{2+} transport system in eukaryotes and is distantly related to the bacterial CorA Mg^{2+} transporter family (Graschopf *et al.*, 2001). Subsequently a second CorA protein, **Alr2**, was identified in *S. cerevisiae*. Alr1 and Alr2 are present on the plasma membrane; loss-of-function mutations in Alr1 result in reduced Mg^{2+} uptake and growth defects restorable by external Mg^{2+} supplementation (Graschopf *et al.*, 2001; MacDiarmid and Gardner, 1998). Alr2 makes only a minor contribution to Mg^{2+} homeostasis, due to low

expression and activity (Pasternak *et al.*, 2010). The Alr1 clade of CorA proteins includes a subgroup represented by **Mnr2**, a vacuolar membrane protein required for access to intracellular magnesium stores (Knoop *et al.*, 2005). Another subfamily includes the yeast **Mrs2** protein, which supplies Mg^{2+} to the mitochondrial matrix (Schindl *et al.*, 2007).

In *Arabidopsis thaliana*, a family of 10 **Mg**$^{2+}$ **t**ransporters (***MGT***) homologous to the yeast *MRS2* gene and to the CorA family in bacteria has been identified, most of which have been shown to be expressed in a range of plant tissues (Li *et al.*, 2001). One of the transporters, AtMgt5, has shown to be localised in mitochondria facilitating Mg^{2+} trafficking between the cytosol and mitochondria and essential for pollen development in *Arabidopsis* (Li *et al.*, 2008; Chen *et al.*, 2009).

In rice, there are nine members of the *OsMRS2* family, which belongs to five clades (A-E), out of which four of the nine members possess the ability to transport Mg^{2+}. Genes belonging to clade A encode the chloroplast localised Mg^{2+} transporter in plants (Saito *et al.*, 2013). Up-regulation of a magnesium transporter, *OsMGT1*, has been shown to be required for conferring aluminium resistance in rice by increasing Mg^{2+} concentration within the cell (Chen *et al.*, 2012).

Considering its diverse metabolic roles, magnesium is indispensable for cellular functioning. However, the regulation and role of CorA Mg^{2+} transporters in development and pathogenicity of *M. oryzae* are still unexplored. In view of the diverse roles of Mg^{2+} ions, understanding the regulation of Mg^{2+} in *M. oryzae* is of considerable interest. In the present study we identified the *M. oryzae* orthologues of *S. cerevisiae ALR1*, Mgg_08843 (*MoALR2*) and Mgg_09884 (*MoMNR2*) and studied these CorA Mg^{2+} transporters with respect to fungal development and progression of the infection cycle.