1. INTRODUCTION

Rice is the corner stone of global food security. It is cultivated in an area of 163 M ha globally with a production of 719 million tonnes of paddy (FAO, 2012). Rice is grown in over 100 countries and is the life-line of the Asia-Pacific region where 56% of humanity lives, produces and consumed more than 90% of the world's rice. India is a second largest rice producing country in the world (Maclean *et al.*, 2002). The average annual production of India is 99 million tonnes which is about 21.5% of global rice production (Viraktamath et. al., 2010, Chand, 2013). Rice occupies one-quarter of the total cropped area, contributes about 40 to 43% of total food grain production in India and continues to play a vital role in the national food and livelihood security system. However, productivity of rice is only 2.1 tonnes/ha which is lower than world's average productivity of 2.9 tonnes/ha (FAO, 2009). An estimated demand of rice in India will be 126.14 million tonnes by 2029-30 (Goyal and Singh, 2002).

Rice pathogens

Rice is known to be attacked by many pests and diseases which results in severe loss in world agronomy. The major diseases are caused by bacteria, fungi and viruses. Bacterial diseases include bacterial blight, bacterial leaf streak, and bacterial sheath rot (Ou, 1985). Among the viral diseases of rice, tungro, grassy stunt, ragged stunt,

orange leaf (in Asia), hoja blanca (America), stripe and dwarf virus (in temperate Asia) are most prevalent. The blast, sheath blight, brown spot, narrow brown leaf spot, sheath rot and leaf scald are major fungal diseases. Among these, rice blast is a major threat causing 30% loss per year worldwide (Talbot, 2004).

Rice blast

Rice blast is earmarked as a major limiting factor in global rice production because of its wide distribution and destructiveness. Outbreak of this disease is a serious and recurrent problem in all rice growing regions of the world and imposes a serious threat to food security worldwide. Blast was first reported in Asia more than three centuries ago. It was 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). The causative organism of blast disease is a filamentous ascomycete fungus *Magnaporthe oryzae*, which was recently defined as a new species, separate from *Magnaporthe grisea*, based on multi-locus genealogy and mating experiments. It can infect all parts of the rice plant from seedling stage to maturity causing lesions on leaf, node, stem, panicle, grain and even root (Figure1B) and it is known as leaf blast, node blast, panicle blast, stem blast or seed blast depending upon the site of infection (Figure 1A). Due its severity of infection, worldwide distribution and a potential

threat that can cause crop failure, rice blast has been ranked among the most important of all the plant diseases.

Biology of pathogen- *Magnaporthe oryzae*

M. oryzae is a filamentous heterothallic ascomycete fungus, reproduces both by sexual and asexual means. The sexual cycle of *M. oryzae* has not been seen in nature, but some strains undergo sexual crosses in the laboratory (Yaegashi and Udagawa, 1978). Fungus produces sexual spores (ascospores) called asci which are found within specialized structures called perithecia. The mycelium of *M. oryzae* is septate with a single nucleus in each cell. It produces hyaline, fusiform shaped (spindle-shaped with tapering ends) ascospores with three septa and are unitunicate. This fungus is heterothallic with a bipolar mating system (mating controlled by two different alleles at a single locus namely MAT1-1 and MAT1-2) with additional genes controlling the sexual cycle.

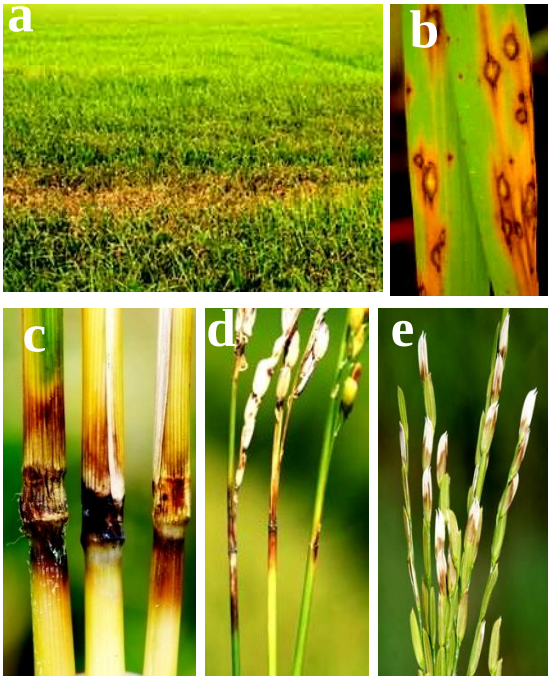
The asexual mode of propogation includes the production of three celled spores (conidia), on specialized stalks (conidiophores) present abundantly as lesions on leaf surface. When a conidium lands on the host leaf surface, it forms a germ tube that leads to formation of a specialized dome shaped infectious structure known as appressorium. Appressorium generates high turgor pressure which facilitates

Figure 1: Rice blast symptoms

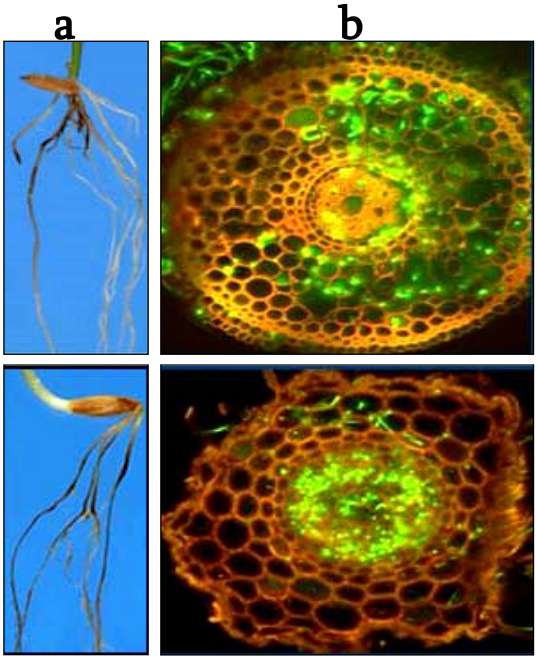
A. Blast infected rice plants. a. Blast infected rice field, b. Foliar Lesions, c. Node blast, d. Neck blast and e. Panicle blast. The lesions appear as large brown spots with gray or whitish centre and brown margins.

B. Root infection by rice blast. a. *M. oryzae* penetrates the stele. b. Confocal imaging of radial sections of a two-week-old rice and barley seedlings infected with GFP-tagged *M. oryzae*. Figures adapted from Rice Research Station, LSU Agricultural Center and Sesma *et al.*, 2004.

A.



B.



penetration into the leaf tissue. After penetration, the primary infection hyphae grow rapidly and proliferate within susceptible tissues.

During infection, environmental adaptation and stress responses are significant for the plant pathogen, as it has to mount effective responses to counteract the defenses of its host and adapt to available nutrients in the host niche. This adaptation is frequently achieved through post-translational modifications of many proteins. For example, de-acetylation and phosphorylation of Twl, drive translocation of Twl (twilight) protein from cytoplasm to nucleus upon photo induction which has a key role in conidiation and pathogenesis of *M. oryzae*. Twl coordinates developmental (asexual reproduction and *in planta* growth), metabolic (carbon/nitrogen homeostasis), and environmental cues (light, ROS levels) in the blast fungus during its adaptation and establishment within the host plants (Deng *et al.*, 2015).

Post-translational modification

Posttranslational modifications of proteins play critical role in cellular adaptation of all organisms as well as their growth, division, differentiation, development and pathogenicity. These modifications change the properties of a protein, for example, by proteolytic cleavage or by the addition of a modifying group to one or more amino acid residues. They determine the activity, state, stability, localization, and/or their

interactions with other proteins. Such modifications include phosphorylation, ubiquitination, sumoylation, glycosylation, and methylation. For example; glycosylation of fungal cell surface mannoproteins fundamentally influences the shape of cell wall and interactions with the hosts. (Latge, 2010, Lengeler *et al.*, 2008). In pathogenic fungi, *O*-linked mannosylation is required for virulence. A number of proteins functions are regulated by the covalent attachment of polypeptide modifications, the best known examples are ubiquitin and ubiquitin-related modifiers. Ubiquitin-mediated modification, which is known to be highly selective, plays a key role in nutrient assimilation, fungal development and pathogenicity of *M. oryzae* (Oh *et al*, 2012). Like ubiquitination, sumoylation is highly conserved, reversible post-translational modification that involves the conjugation of polypeptide to target proteins which is an understudied research area, especially in fungal pathogen. However, the mechanisms by which sumoylation of specific target proteins control these cellular processes and hence fungal virulence remain to be elucidated.

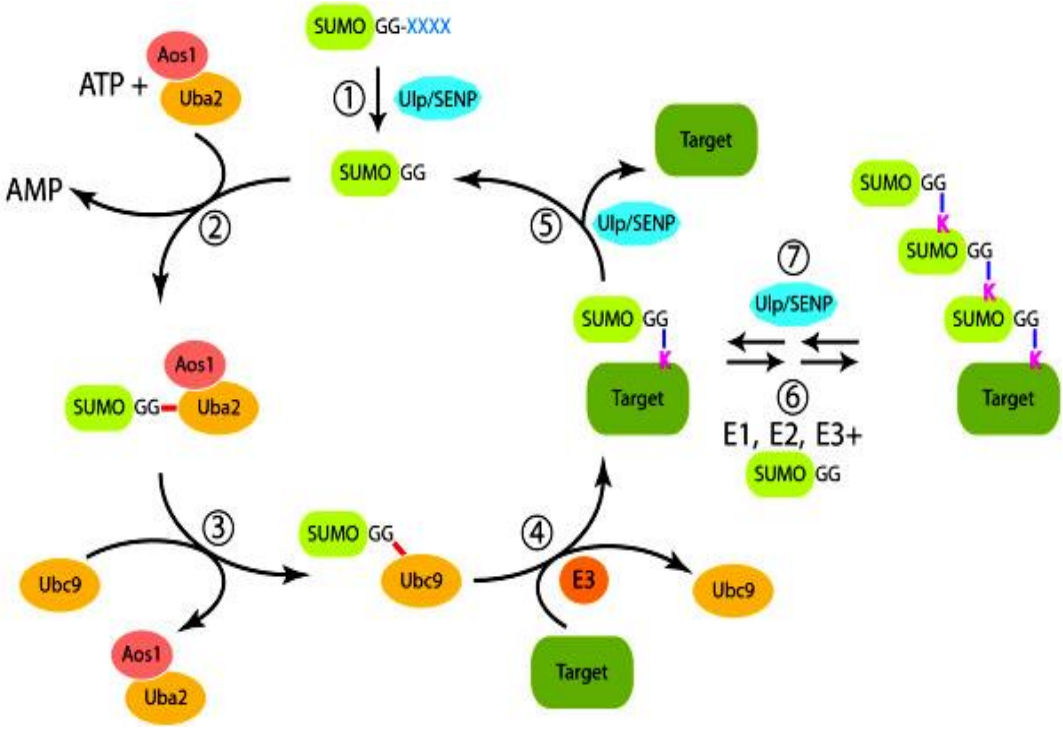
Sumoylation

SUMO is 'small ubiquitin-like modifier', covalently attached to proteins to modulate their activity and is involved in many eukaryotic cellular signaling pathways including cell-cycle regulation, transcription, nucleocytoplasmic transport, DNA

replication and chromosome dynamics (Xhemalce *et al.*, 2007, Fukagawa, 2001, Mahajan., 1997, Bachant, 2002, Hardeland, 2002). SUMO proteins are synthesized as inactive precursors, which must first undergo a C-terminal cleavage mediated by a family of sentrin/SUMO-specific protease (SENP) enzymes. This cleavage exposes a di-glycine motif, which is available for subsequent activation and conjugation. In each conjugation cycle, SUMO is activated in an ATP-dependent manner by E1 activating enzyme consisting of heterodimer Aos1-Uba2. The Aos1 subunit uses ATP to adenylate the SUMO C-terminus, which is then followed by release of AMP and the parallel formation of a thioester bond between SUMO and the catalytic cysteine in the Uba2 subunit. In the second reaction SUMO is trans-esterified to cysteine of the E2 conjugating enzyme Ubc9. Finally, SUMO is covalently attached to lysine residues of the target protein through the isopeptide bond between the terminal glycine residue and the ε-amino group of a lysine residue in the target protein by SUMO E3 ligase (Hay, 2005; Ulrich, 2005; Wilkinson and Henley, 2010). Sumoylation is a highly dynamic process that can be reversed by deconjugating enzymes such as the SENP enzymes (Yeh, 2009) (Figure 2). The balance between SUMO-modified and unmodified proteins is tightly regulated at multiple levels by the action of multiple factors which impinge on both the sumoylation and desumoylation pathways. Sumoylation has been studied in nonpathogenic yeasts like *S. cerevisiae* (Biggins *et*

Figure 2: SUMO conjugation pathway

SUMO proteins undergo post-translational maturation, catalyzed by Ulp/SENPs, to reveal a C-terminal di-glycine motif (Step 1). Mature SUMO undergoes ATP-dependent activation, resulting in a thiolester linkage between the C-terminal di-glycine and their activating enzyme, Uba2/Aos1 (Step 2). The thiolester is transferred to their conjugating enzyme, Ubc9 (Step 3). Ubc9 acts in concert with SUMO ligases/E3 enzymes to form an isopeptide linkage between the SUMO C-terminus and an ε-amino group of a lysine within the target protein (Step 4). SUMOs can be removed from conjugated species by the action of Ulp/SENPs (Step 5). In some cases, SUMO chains can be formed through linkage of additional SUMO moieties to previously conjugated SUMOs (Step 6). While it is possible that multiple Ulp/SENPs may disassemble SUMO chains (Step 7), members of the Ulp2 family appear to be specialized for this reaction (Dasso, 2008).


SUMO GG-XXXX
Aos1
ATP +
Uba2
①
Ulp/SENP
AMP
SUMO GG
②
Target
⑤
Ulp/SENP
SUMO GG
K
SUMO GG
K
SUMO GG
K
Target
⑦
Ulp/SENP
SUMO GG
Target
⑥
E1, E2, E3+
SUMO GG
Aos1
SUMO GG
Uba2
③
SUMO GG
④
Ubc9
Ubc9
E3
Ubc9
Aos1
Uba2
Target

al., 2001), *S. pombe* (Tanaka *et al*., 1999) and in human pathogens like *C. albicans* (Leach *et al*., 2010) and *A. nidulans* (Wong *et al*., 2008).

Rational of the research

As outlined above, studies revealed that sumoylation has been implicated in wide variety of processes and is crucial for the development and virulence in pathogenic and nonpathogenic organisms. However, an insight into the regulation of cellular proteins by sumoylation and its impact on virulence is lacking in phytopathogenic fungi. Therefore it becomes important to know the role of sumoylation in the development and pathogenicity of *M. oryzae*. The objective of this research is to investigate the role of SUMO in the development and pathogenicity of rice blast fungus *M. oryzae*.

Objectives:

1. Molecular characterization of Small Ubiquitin-like Modifier (*MoSUMO*) gene in *Magnaporthe oryzae.*

2. Functional complementation of *MoSUMO* in *S. cerevisiae*

3. Subcellular localization of MoSUMO.

4. Identification of sumoylated proteins in *M. oryzae.*