SUMMARY

FUNCTIONAL ANALYSIS OF PATHOGENICITY RELATED GENE(S) IN *MAGNAPORTHE ORYZAE*

The present study revealed the significance of **S**mall **U**biquitin like **MO**difier (*MoSUMO*) in rice blast fungus *Magnaporthe oryzae* and its role in the regulation of vegetative growth, conidiation, appressorium development and pathogenicity.

A single hit of highly conserved 110 amino acid protein MoSUMO was investigated in *M. oryzae* using *Saccharomyces cerevisiae* SUMO (SMT3) as the query sequence, have conserved diglycine motif, critical for SUMO conjugation and also a conserved Lys residue present within a sumoylation consensus site which has the potential to form poly-SUMO chains.

Since MoSUMO showed 53% identity with SMT3, a complementation of MoSUMO was carried out in *smt3* mutant of *S. cerevisiae* which displayed the restoration of defective phenotype of *smt3* mutant cells including growth and nuclear segregation. The importance of diglycine (GG) motif of MoSUMO was tested with site directed mutangenesis method in *smt3* mutant cells by transforming *MoSUMOgg* expression construct (pYes-*MoSUMOgg*) showing critical role in sumoylation pathway.

Characterization of *MoSUMO* gene in *M. oryzae* was performed by targeted deletion of *MoSUMO* using split marker approach. *M. oryzae* mutant lacking the *MoSUMO* was viable and showed dramatically reduced vegetative growth, conidiation, appressorium development and pathogenicity. Complementation of *ΔMosumo* with the wild type *MoSUMO* gene restored its defects.

Depletion of *MoSUMO* causes defects in nuclei and septation in hyphae and during developmental stages of appressoria. Most of the vegetative cells had abnormal distribution of nuclei, indicating nuclear segregation defect indicating that MoSUMO might have cell cycle regulated targets which are involved in nuclear segregation and septation of the cell.

In addition, *ΔMosumo* exhibits aberrant chitin deposition in developmental stages of conidia. The examination in developmental stages, conidia of both strains, wild type and *ΔMosumo* were allowed to form appressoria on hydrophobic surface and stained with CFW. Interestingly, the abnormal chitin deposition, irregular shape and septation in germ tube were observed in *ΔMosumo.*

To investigate the occurrence of abnormal phenotype of chitin and septation, *in silico* analysis was carried out with chitin synthases and septins since biosynthesis of chitin was catalyzed by chitin synthases (CHS) which were characterized in *M.*

oryzae previously. The analysis revealed that three chitin synthases Chs1p, Chs2p, Chs7p and four septins Sep3p, Sep10p, Sep11p, Sep12p had potential sumoylation sites which confirmed that chitin synthases and septins might be the probable targets of MoSUMO.

An indirect immunolocalisation in hyphae and conidia was carried out using raised polyclonal MoSUMO antibody indicating that MoSUMO has septal and nuclear targets. This is more clear by live cell imaging of GFP tagged MoSUMO strain which was studied at each developmental stage. The time lapse imaging of MoSUMO expression from conidia to the development of appressoria was examined at 0hr, 2hr, 3hr, 4hr and 7hr on artificial hydrophobic surface and found that GFP signal was observed throughout the conidia, but intense GFP signal was observed in appressoria. The expression of MoSUMO was profusely found during infection after 12hpi in host tissue. This suggests that several cellular proteins might be the target of MoSUMO.

Co-localization of MoSUMO, nuclear proteins and septal proteins was studied by simultaneous staining of hyphae of GFP::MoSUMO fusion strain with DAPI and CFW and observed that GFP fluorescence was overlapping at the nuclear region and at the septal region. Similarly, colocalization of MoSUMO and actins was carried out by staining the conidia of localization transformants with rhodamine

phalloidin. The MoSUMO and actins localization was observed at the corresponding sites. The sumoylation prediction analysis also revealed that Actin1 and Arp related protein 5 had sumoylation consensus sequence which might be the MoSUMO targets.

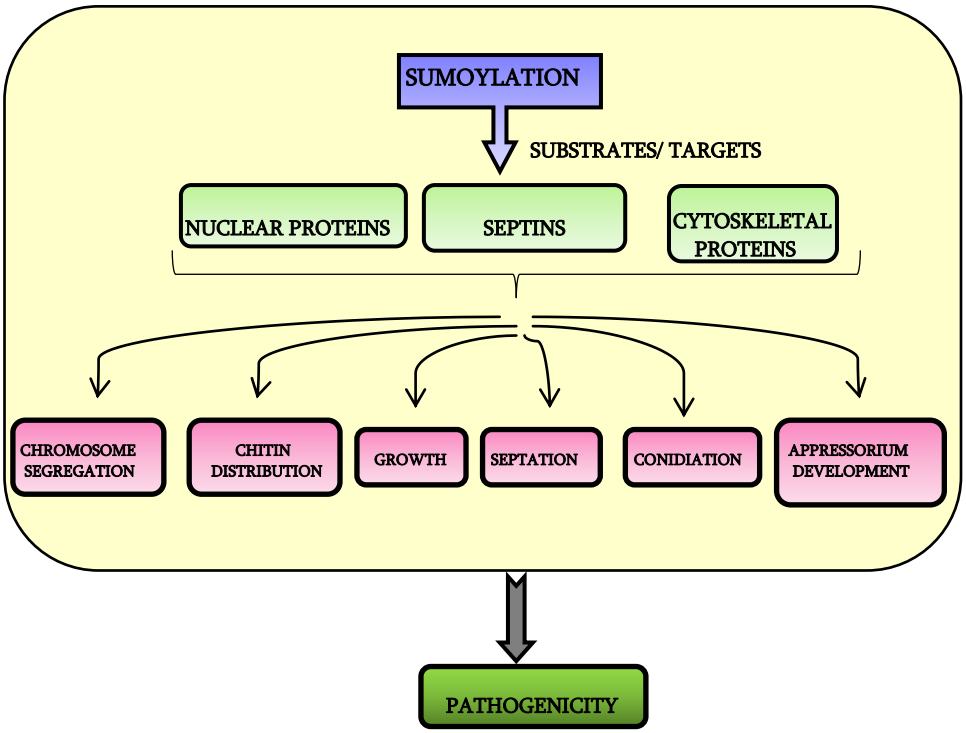
The investigation of differential protein expression of wild type B157 and *ΔMosumo* was achieved using two dimensional polyacrylamide gel electrophoresis (2DGE) and isolated four protein spots which were absent in *ΔMosumo*. The identification of these peptides was carried out by MALDI-TOF/TOF spectroscopy. The proteins identified are MGG_06958 (HSP like protein), MGG_14729 (mitochondrial ribosomal protein subunit S18), MGG_15192 (conserved hypothetical protein) and MGG_10292 (conserved hypothetical protein) which were predicted as potential MoSUMO targets by *in silico* analysis.

The present investigation highlighted that, the deletion of MoSUMO leads to the failure in development and pathogenicity of the rice blast fungus. In addition, it showed nuclear segregation and imperfections in septation suggesting its role in cell cycle. Localization study also confirmed that MoSUMO and/or MoSUMO targets present at septal and nuclear region. Collectively, these findings confirm that the proteins which are involved in the regulation of development and pathogenicity might be absent or affected at respective stage due to inactivation of

sumoylation. The findings from present investigation shall greatly facilitate the study of identified targets, enabling to know other sumoylation targets and their role in development and pathogenicity of the fungus. Understanding of sumoylation in host-pathogen interaction may have profound impact on controlling diseases in economically important crops.

Figure 30: Schematic representation of role of sumoylation in pathogenicity of rice blast fungus

The sumoylation has nuclear, septal and cytoskeletal proteins as substrates/targets which plays a key role in the developmental processes contributing a role in pathogenicity of the rice blast fungus. This picture is modified from Leach et al., 2012.


SUMOYLATION
SUBSTRATES/ TARGETS
NUCLEAR PROTEINS
SEPTINS
CYTOSKELETAL PROTEINS
CHROMOSOME SEGREGATION
CHITIN DISTRIBUTION
GROWTH
SEPTATION
CONIDIATION
APPRESSORIUM DEVELOPMENT
PATHOGENICITY