Summary

- *MoSKP1* gene was identified as MGG_04978 gene accession number and amplified by using Polymerase Chain Reaction (PCR), cloned in to a cloning vector pBSKS+ by means of blunt end ligation.
- *MoSKP1* gene was cloned in yeast expression vector pYES2 and interspecific gene complementation experiment was performed. *S. pombe skp1A7* mutant was utilized for complementation study. Mutant phenotype of *S. pombe* Skp1 was found to be largely restored in the complemented strain of *S. pombe Skp1A7-MoSkp1*.
- Total deletion of *MoSKP1* was found to be lethal to the fungus as found from the attempt made to disrupt the *MoSKP1* gene and no true disruptant could be generated.
- Various silencing constructs (RNAi-*MoSKP1*, antisense-*MoSKP1*) and over expression construct have been generated for *MoSKP1* gene. *Magnaporthe oryzae* B157 fungal strain protoplast transformation was optimized. The knock down transformants were selected on hygromycin (200µg/ml) and monoconidial isolation was done for each lines of transformants.
- RNAi and antisense transformants of *MoSKP1* were confirmed by Southern blot analysis, western blot analysis and small RNA enrichment and *MoSKP1* siRNA detection by Northern blot analysis. Real time PCR analysis of RNAi *MoSKP1* and antisense *MoSKP1* transformants confirmed the silencing of MoSkp1 in the mutant.
- The silencing efficiency was found to be higher in RNAi-*MoSKP1* transformants than the antisense-*MoSKP1* transformants. The transcript levels of *MoSKP1* RNAi transformants vary from 15% to 40% whereas in *MoSKP1* antisense transformants it goes up to 60%.

- Knock down *MoSKP1 M. oryzae* B157 transformants showed reduced growth, less sporulation, defective appressoria development, elongated germ tube germination and apparently mutant were unable to infect rice leaves.
- Total protein ubiquitination pattern was estimated by ubiquitinated protein enrichment assay and reduction of ubiquitinated protein was observed in R6 *MoSKP1*-RNAi transformant.
- Yeast two hybrid assay was performed to see the interaction ability of MoSkp1 with one of the probable target MoFrp1 MGG_06351.5 (a hypothetical protein) and the interaction was further confirmed by pull down assay and co-immunoprecipitation assay.
- Immunolocalisation study revealed the presence of MoSkp1 in the hyphae, spore, developing germ tube and in the appressoria.
- The *MoSKP1* knock down transformants were defective in cell wall integrity confirmed by growing *MoSKP1*RNAi transformants in the presence of cell wall disrupting agent like Congo Red, Caffeine and Calcofluor White.
- Over expression of *MoSKP1* increases the efficiency of appressoria development and consequently infectivity.
- Expression profiling of *MoSKP1* in wild type *M. oryzae* suggests that MoSkp1 protein level increases after release from S-phase arrested state.
- *MoSKP1* RNAi transformants were severely affected in sporulation and development. Number of spore on each conidiophore was reduced and spores were morphologically defective.

- Total protein pull down assay and peptide mass fingerprinting confirms that MoSkp1 is a component of E3 ubiquitin complex SCF scon-3 and indicate the probability to interact with RNA polymerase II large subunit.
- Two dimensional gel electrophoresis experiment with R6 *MoSKP1* transformants showed the reduction in protein expression compared to wild type strain *M. oryzae* B157.
- Bioinformatics prediction of phosphorylation sites in MoSkp1 was confirmed by performing phosphorylation and dephosphorylation assay followed by Iso-electric focusing and western blot analysis.
- Comparison of *MoSKP1* knock down with hydroxyurea treated wild type *M. oryzae* B157 strain confirmed the cell cycle defect in the *MoSKP1* RNAi transformants.