Summary

- MoSKP1 gene was identified as MGG_04978 gene accession number and amplified by using Polymerase Chain Reaction (PCR), cloned into 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.