Molecular analysis of pathogenesis in *Magnaporthe oryzae*

VARSHA C. MOHANAN

SUMMARY

- *MoHPT1 M. oryzae* Histidine phosphotransferase was found to be phylogenetically closely related to the *N.crassa* Histidine phosphotransferase.
- *in silico* analysis of *MoHPT1* suggested the presence of two different transcripts of the *MoHPT1* gene and also suggested a probable phosphorylation site at the N-terminal apart from the conventional histidine phosphorylation site.
- *MoHPT1,* as predicted, expressed dual transcripts but the novel transcript was expressed only under light induced conditions.
- Immunolocalisation studies showed that the protein was expressed in the fungal hyphae, appressoria and nucleus.
- The transcript expression of *MoHPT1* was found to increase under stress conditions.
- Further investigation of *MoHPT1* was carried out using RNAi technology since the disruption stratergies failed to give a disruptant in *M. oryzae* B157.
- The genomic sequence analysis of B157 revealed several mutations in some of the Histidine Kinases.
- TAIL-PCR of the RNAi trans*formant* RA6, which showed the lowest expression of *MoHPT1,* indicated that the RNAi construct had been integrated between a WD23 repeat gene and a Sec gene. The transcript levels of these genes w*e*re found to be unaltered in the RNAi transformant.

- The *MoHPT1* knock-down transformants showed lower number of spores, but the percentage of appressorium formation from the total spores produced was comparable to the wild type.
- The *MoHPT1* transformants could not infect the rice host efficiently. The *in planta* infection studies showed reduced infection, while the detached leaf wounded infection assays showed that the pathogen could initiate infection but could not attain necrotrophy.
- The *MoHPT1* RNAi transformants obtained were sensitive to osmotic, oxidative and cell wall stress as the downstream Hog pathway was also affected. However neither B157 wild type nor the *MoHPT1* knock-down transformants showed resistance to antifungal agents like phenylpyrrolles and dicarboximides.
- The western analysis of wild type showed that Hog1 phosphorylation is altered under different stress conditions. The protein levels of the *MoHPT1* RNAi transformants showed that the phosphorylated Hog1 is decreased in the knock-down transformants, while total Hog1 protein levels remained the same.
- The transcript levels of the TCS genes, *HOG1* and its effector *GPDH* under different stress conditions suggested that *MoHPT1* has a greater role to play under oxidative stress than under osmotic stress conditions.
- The expression of Histidine Kinases with PAS domains under light induced conditions indicates that *MoHPT1* regulates the expression of these histidine kinases.
- *MoHPT1* knock-down affected laccase expression and activity, justifiying the previous observations that Histidine kinases influence laccase activity in *M. oryzae.*
- The co-immunoprecipitation experiments showed immunoprecipitated proteins

upon silver staining but they could not be purified owing to low concentrations.

- The differential expression analysis of the RNAi transformant RA6 revealed about 146 genes were differentially expressed in RA6 when compared to wild type.
- All the differentially expressed genes were classified according to their functional domains and analysed. The majority of these genes were those which are expressed during the biotrophic and necrotic phase of pathogen invasion.
- The most distinct classes of differentially expressed genes in the knock-down transformant were nutrient acquisition and metabolism genes, light regulated genes, secondary metabolism genes and cell wall degrading enzymes.
- The differential expression analysis under oxidative stress conditions revealed 144 genes differentially expressed in RA6. 52 genes out of them were specifically seen under the oxidative stress conditions. The other distinct classes differentially expressed in oxidative stress conditions were similar to those without stress. The classification and analysis of these genes suggested the role of *MoHPT1* in stress adaption and pathogenicity.