SUMMARY

- 12 multicopper oxidases were identified from the *M. grisea* genome using bioinformatics tools.
- 2 out of the 12 multicopper oxidases were selected for further characterisation after expression profiling in normal and nitrogen starved conditions.
- MGG_08127.5 selected from normal condition was named and used as *Mg*Lac1 for further characterization.
- MGG_02876.5 selected from nitrogen starved condition was named and used as *Mg*Lac2 for further characterization.
- Heterologous expression of both *Mg*lac1 and *Mg*Lac2 was carried out in *S. cerevisiae.* The protein was purified using affinity chromatography.
- Both proteins were immunolocalised as secretory proteins with the polyclonal antibodies.
- Oxidation of ABTS and syringaldehyde, laccase specific substrates, were used to prove that both the proteins studied were laccase.
- The order of reaction of *Mg*Lac1 towards phenolics was in the order meta> ortho> para-substituted phenols.
- The order of reaction of *Mg*Lac2 towards phenolics was in the order para> ortho> meta- substituted phenols.
- The optimum pH for *Mg*Lac1 activity was found to be 4 - 5 and that of *Mg*Lac2 was 4 - 4.5.

- The maximum thermostability of both the laccases was found to be at 30^0C.
- The inhibition of *Mg*Lac1 was in the order Sodium azide > Cysteine > EDTA > Chloride.
- The inhibition of *Mg*Lac2 was in the order Cysteine > Sodium azide > EDTA > Chloride.
- Both the laccases showed dye decolorising activity.
- *Mg*Lac2 also showed DHN polymerization potential and ferroxidase activity.
- Antisense vectors were constructed under TrpC promoter in binary vector, harboring the gene for hygromycin resistance, under CaMV 35S promoter, as selectable marker.
- *Agrobacterium tumefaciens* mediated transformation was used to generate the *Mg*Lac1 and *Mg*Lac2 knockdown mutants. Transformants were selected on hygromycin (200µg/ml) and ~0.2-0.3% transformation efficiency was obtained.
- Random integration of T-DNA in the genome was observed in Southern blot analysis.
- In real time PCR, the expression of *MgLac1* in *MgLac1* knockdown transformants was upto ~88% less as compared to wild typeB157.
- *MgLac1* knockdown transformants were not affected in growth, sporulation melanin formation and appresorium formation and pathogenicity.
- *MgLac1* knockdown transformants were affected in growth in presence of EDTA, were able to grow better on higher concentrations NaCl, iron and copper. But they became sensitive to $CaCl_2$.

- In real time PCR, the expression of *MgLac2* in *MgLac2* knockdown mutant transformants was also upto ~88% less as compared to wild type B157.
- *MgLac2* knockdown transformants were affected in pathogenicity.
- *MgLac2* knockdown transformants were also affected in growth in presence of EDTA and became very sensitive to iron and copper.
- *MgLac2* knockdown transformants became hypersensitive to the cell-wall-degrading enzyme and became very sensitive to miconazole and SDS.
- Lipid mobilization from spore to appresorium was delayed in *MgLac2* knockdown transformants.
- Different levels of gene silencing were observed in the expression of other multicopper oxidases in *MgLac1* and *MgLac2* knockdown transformants
- The non pathogenic phenotype in the *MgLac2* knockdown transformants was due to silencing of *MGG_07771*.
- MGG_07771 is a cell wall/membrane associated protein having a probable role in *M. grisea* pathogenicity.