

Chapter 6

CONCLUSION

- ❖ Fifteen different rice cultivars from different regions of North Bengal and Sikkim were collected and their seed morphological diversity, germination ability, growth rate, total protein content and were determined for initial screening.
- ❖ Percent Disease Index was measured following natural infection with brown spot disease. Occurrence of the disease was much higher in local cultivars viz. Black nuniya, Brimful and Champasari. Accumulation of defence enzymes such a PAL, POX, CHT and GLU and total phenol was also found to be in much lesser quantity in these cultivars. Hence they were selected for further experimental purpose.
- ❖ Brown spot causing pathogen was isolated and following completion of Koch's postulate.
- ❖ Growth characters and spore morphology of the pathogen was studied.
- ❖ Pathogenecity test of the three susceptible rice cultivars was studied using two techniques- detached leaf and whole plant inoculation technique. Both the techniques gave similar result where it was observed that Black nuniya was highly susceptible followed by Brimful and Champasari.
- ❖ Immuno detection of foliar fungal pathogens in healthy and artificially inoculated leaf tissues was carried out using PTA-ELISA as well as Dot immunobinding assay. The occurrence of infection was seen as early as 24 hrs and increased by time. Greater colour intensity was noted in Black Nuniya followed by Brimful and Champasari with the IgG which showed susceptible reaction to the pathogen in pathogenecity tests.
- ❖ Polyclonal antibody was raised separately against mycelial antigen of the fungal pathogen. These antibodies were used for serological characterization of the fungal pathogens by PTA-ELISA, Dot-blot, Western blot and Indirect immunofluorescence of mycelia and spore. Western blot analyses using polyclonal antibody of *D. oryzae* revealed that the PAb could show different levels of homologous reactions with the antigens of *D. oryzae* respectively. Sharp and intense bands were produced on the nitrocellulose membrane after enzymatic reaction with NBT BCIP. Efficacy of polyclonal antibodies raised against the

mycelial antigen was further tested with the help of indirect immuno fluorescence of young mycelia of *D. oryzae*. The mycelia treated with PAbs and labeled with FITC showed apple green fluorescence.

- ❖ Molecular detection of foliar fungal pathogen *D. oryzae* (R1.DO.01) was carried out using 18S rDNA sequencing using ITS1/ITS4 primers. The BLAST query of the 18S rDNA sequence of the isolates against GenBank database confirmed the identity of the isolate R1.DO.01 as *D. oryzae*. The sequences have been deposited to NCBI, Genbank database under the accession number KT768092.
- ❖ A multiple sequence alignment of ITS gene sequences of the above sequenced isolate was also conducted. Phylogenetic analysis of the isolate was carried out with the Extype strain sequences obtained from NCBI Genbank Database which showed maximum homology with the respective isolate
- ❖ Association of Arbuscular Mycorrhizal Fungi (AMF) in rice cultivars were observed and it was found that *Glomus* was the most dominant genera in the rice rhizosphere followed by *Gigaspora* and *Scutellospora*.
- ❖ Characterization of the species belonging to genus *Glomus* and *Gigaspora* was done. *Rhizophagus fasciculatus* was found to be most abundant in the rice rhizosphere, they were isolated and mass multiplied in maize plants.
- ❖ Histopathology and root colonization with AMF in rice cultivars was studied.
- ❖ Ten selected PGPR and six PGPF were evaluated for their antagonistic effect against the fungal pathogen and it was recorded that these bio inoculants could easily prevent the growth of fungal pathogen *in vitro*.
- ❖ Ten selected PGPR were screened for plant growth promotion and changes in biochemical activity. Among all the bacterial isolates *B. altitudinus* (NAIMCC-B01485) showed better results than the others.
- ❖ Percent Disease Index and defence enzyme activity of PAL,POX,CHT and GLU following treatment and pathogen infection was also measured and it was found that maximum activity was shown by treated samples in comparison to their control.
- ❖ HPLC analysis of rice leaf samples of rice cultivar Black nuniya treated with *B. altitudinus* (NAIMCC-B01485) showing lowest PDI% was also done which clearly

indicated the increased accumulation of Phytocassanes in treated samples than the control.

- ❖ Six selected PGPF were also screened for its role in plant growth and changes in biochemical activity following pathogen challenge in rice cultivars. *T. harzianum* (NAIMCC-F-03288) treated plants showed the maximum enhancement in the growth and biochemical parameters in comparison to the control.
- ❖ Disease suppression and increase in the level of all four defence enzyme viz. PAL, POX, CHT and GLU was also observed. Rice cultivars treated with *T. harzianum* (NAIMCC-F-03288) showed better results in all the cultivars.
- ❖ HPLC analysis of Phytocassanes was done for Black nuniya treated with *T. harzianum* (NAIMCC-F-03288). Enhancement of the compound in the treated samples was clearly visible which proves its role in suppression of the disease.
- ❖ Rice cultivars were treated with *R. fasciculatus* and challenged with the pathogen. Considerable increase in the height and biochemical activity was observed.
- ❖ A decrease of Percent Disease Index in the treated samples in comparison to the control was observed and considerable increase of defence enzymes was also observed in treated samples.
- ❖ Growth promotion and biochemical changes was observed following dual and combined application of PGPR (*B. altitudinus*, NAIMCC-B01485), AMF (*R. fasciculatus*) and (PGPF *T. harzianum* ,NAIMCC-F-03288). Enhancement in the growth and biochemical activities was observed in all treated samples in comparison to their control sets. Combined application gave better results than the dual ones.
- ❖ Decrease in disease incidence and increase in the level of defense enzymes was observed in treated samples. Combined application gave better results than the dual application. Isozymes of peroxidase were also checked using native PAGE and it was revealed that appearance of new peroxyzyme was seen in infected leaf samples.
- ❖ Cellular localization of two important defence enzymes – Glucanase and Chitinase was studied using indirect immunofluorescence. Expressions of these enzymes were noted in treated leaf sections, confirming earlier results obtained.

- ❖ HPLC analysis of phenolics in the leaf samples of rice cultivar Black nuniya treated with PGPR (*B. altitudinus*, NAIMCC-B01485), AMF (*R. fasciculatus*) and (PGPF *T. harzianum* ,NAIMCC-F-03288) was also done. Increase in the level of phytoalexin in comparison to the control was clearly seen. Presence of phenols such as Galleicacid, Ferulic acid, Salicylic acid and Phloroglucinol was observed in both control and treated samples but intensity increased in treated infected samples. Hence it was evident that Galleic acid, Ferulic acid, Salicylic acid and Phloroglucinol play important role in the defence of rice plants against brown spot pathogen.
- ❖ HPLC analysis of Phytocassanes was done for Black nuniya treated with PGPR (*B. altitudinus*, NAIMCC-B01485), AMF (*R. fasciculatus*) and (PGPF *T. harzianum* , NAIMCC-F-03288). Enhancement of the compound in the treated samples was clearly visible which proves its role in suppression of the disease.
- ❖ The application of bio inoculants promotes growth and bio primes the rice plant against brown spot pathogen by up-regulation of defence activities. These findings could be helpful in protecting rice plants against fungal pathogens and improving its health status that would in turn provide quality yield.