SUMMARY

Brown blight caused by *Glomerella cingulata* was studied with reference to damage on tea leaves, which was assessed with the help of Scanning Electron Microscopy. Three isolates of *G. cingulata* (GC-1, GC-2 and GC-3) were compared with respect to their characters like spore and appressoria size, growth rate, perennating structures, sporulation amount and patterns as well as protein content in different media. Spore production was always best when the organism was grown in Oat Meal Agar. The isolate GC-1 was found to be best sporulating and showed formation of perithecia-like structures. Comparison of SDS-PAGE profiles of total soluble proteins of different isolates indicated that this isolate (GC-1) was superior, for which polyclonal antibody was raised. Immunodiffusion, PTA-ELISA, dot blot and Western blot of the mycelial protein of *G. cingulata* revealed that the PAb recognized numerous proteins, of which the 24 kDa protein gave the strongest signal. Besides, the reaction was the strongest for GC-1 isolate in all the immunological studies performed.

Screening of the different tea varieties against each isolate of *G. cingulata* separately performed by detached leaf revealed that GC-1 was the most virulent. Immunological techniques like PTA-ELISA and dot blot were also used to confirm the results that showed differential response towards the pathogen, revealing that some varieties especially T-17/1/54 , TV-22 and CP-1/1 were most susceptible, while others like TV-30, BS/7A/76 and TS-449 were highly resistant.

A brief study on the events of spore germination on the tea leaves of resistant (TV-30) and susceptible (TV-22) varieties revealed that there was lower percentage of appressoria formation and lesser germ tube length on the dorsal and ventral surfaces of TV-30 than on the surface of TV-22. The diffusates collected from the surface of TV-30 were more fungitoxic than the same collected from TV-22 leaf surface. Cell wall extract of *G. cingulata* elicited antifungal compounds to a greater extent than the spore. Glycoprotein nature of the cell wall extract was confirmed and a single protein band of 66 kDa was identified.

Quantitative reduction in the total protein in *G. cingulata* inoculated tea leaf tissues were observed mainly in the susceptible varieties, while in the resistant ones there were no significant changes. Resolution on SDS-PAGE revealed some additional bands of high molecular weight (97.4 and 94.3kDa) in the resistant varieties. Besides, protein preparation from leaves of susceptible varieties following inoculation with *G. cingulata*

yielded 40 kDa protein by probing with PAb of *G. cingulata*, indicated as pathogen-induced protein.

Among the antifungal compounds, total and ortho-dihydroxy phenols increased compared to the healthy control in the resistant varieties on inoculation with *G. cingulata* in contrast to the susceptible varieties. The time course accumulation of enzymes involved in phenolic biosynthesis such as phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), polyphenol oxidase (PPO) and peroxidase (POX) was followed for all the varieties under the present study. PAL activity increased several fold as early as 24 hours after inoculation in the resistant varieties, while for TAL the same occured 72 hours after inoculation POX indicated that its crucial role was played 48 hours after inoculation, when its high activity was correlated with resistance. But at 24 hours after inoculation high activity was associated with susceptibility. Analysis of isozyme patterns showed that the particular peroxizyme associated with the constitutive resistance was found to be of R_m= 0.11. On the other hand, PPO was involved in the resistance only 72 hours after inoculation and the isozyme associated with resistance was of R_m= 0.55.

Three inducers, viz. hydrogen peroxide (active oxygen radical), sodium nitroprusside (nitric oxide donor) and benzothiadiazole (SAR inducer) were evaluated on three varieties (TV-30, TV-22 and T-17/1/54) in association with time course accumulation of five selective enzymes [peroxidase (POX), ascorbate peroxidase (APX), catalase (CAT), chitinase (CHT) and β-1,3-glucanase (βGLU)].

Application of H_2O_2 led to a gradual increase in the activity of five enzymes (POX, CAT, APX, CHT and βGLU) especially in the susceptible plants. Inoculation with pathogen further increased the level of these enzymes. Similar results were revealed when the tea plants were treated with sodium nitroprusside. However, increase in CAT was more pronounced in this case. Both these inducers provided moderate protection to the tea plants against brown blight. However, BTH protected tea plants to a much higher level than the other two, which was associated with suppression of CAT and APX activity and increase in the level of POX, CHT and βGLU. Isoperoxidase with R_m=0.11 was active in the induced resistance following BTH treatment. Besides, in the BTH-induced plants 29 kDa band could be noticed in Western blot analysis probing with PAb of chitinase. Immunolocalization of chitinase and β-1,3-glucanase (defense enzymes) in tea leaf tissues of BTH-induced tea plants were confirmed using FITC and RITC conjugates which were evident in the mesophyll tissues.