Introduction

Tea is the most widely consumed hot beverage in the world, the habit of tea drinking being well established for more than half of the world's population. Tea is made from the young leaves and unopened leaf buds of the tea plant, *Camellia sinensis* (L.) O. Kuntze. The centre of origin of the tea plant is considered to be near the source of the Irrawady river and further north (Kato, 1989). In India, tea is one of the most important cultivated crops, its cultivation being spread over 3,96,000 hectres of land divided into 2 distinct regions - the North Indian tea belt located between 22-27° North and South Indian tea belt located at 7° North. North East India produces 75% of the total Indian tea in 3 different land scapes (Jain, 1991). Darjeeling produces the world's finest quality teas in the steep hill slopes of Eastern Himalayas upto an elevation of 2000 m. The extensive riverine flat plains at the base of Himalayas are the tea districts of the Terai and Dooars. Brahmaputra valley of Assam is located 100 m. above sea level and is the largest flood plains of the world growing tea which accounts for more than half of Indian production. Tea is also grown in the slopes of Nilgiris and Annamalai hills of Peninsular South India.

A number of fungal pathogens cause foliar diseases of tea which assume extreme importance economically as even slight damages to the leaves reduces the quality and quantity of tea production. A commonly occurring pathogen on tea leaves in all tea growing areas is *Glomerella cingulata* (Stoneman) Spauld and Schrenk, causing brown blight disease (Agnihothrudu, 1995). The fungus generally gains entrance through a wound or into tissues that in some other way have been weakened (Baxter, 1974; Bertus, 1974; Dickens and Cook, 1989). The disease patches usually start on the margin of the leaves and spread in wards. When two or more patches occur side by side whole leaf may be affected. The edges of the patches are sharply defined and mostly marked with a delicate concentric zonation. The colour on the upper surface is yellowish to chocolate brown at first gradually changing to grey from centre outwards. Minute black, scattered dots which are the fructifications, appear on both sides of the diseased patch. The affected portion of the leaf finally turns over and shrivels up (Plate I, figs. A & B).

Plants respond to infection by a pathogen in a number of ways which are triggered by the initial recognition phenomenon. The initial recognition triggers the activation of immune system of plants, which though different from that of animals, is functionally similar. The immune system in plants involves several mechanisms, some of which are known to us but

many of them are still unknown. The complexities of the interactions that affect the selection of parasites and allow their establishment and survival among host cells is manifest in the frequency and variability of cell surface antigens. Some intriguing research work suggests that antigenic similarities between host and pathogen may be a prerequisite for compatible reaction or in other words successful establishment of the pathogen in its host depends on some kind of molecular similarities between the two partners (DeVay and Adler, 1976; Chakraborty and Purkayastha, 1983; Heide and Swedegaard - Peterson, 1985 ; Alba and DeVay, 1985 ; Chakraborty, 1988 ; Purkayastha, 1989 ; Protsenko and Lady Zhenskaya, 1989 ; Chakraborty and Saha, 1994 b, Chakraborty *et.al.*, 1995).

An important area of immunological studies of diseases involves the use of pathogen antiserum for detection of infection in the host beginning from the very early stages of host pathogen interaction. Disease detection by immunological means though of common use in viral diseases is only gaining ground in case of fungal diseases (Mohan, 1988; Sundaram et.al., 1991; Lyons and White, 1992; Linfeld, 1993; Holtz *et.al.*, 1994; Beckman *et.al.* 1994; Chakraborty *et. al.*, 1996, Wakeham and White, 1996). The recent diagnostic techniques for pathogen detection include enzyme linked immunosorbent assay, dot immunobinding assay, radioimmunosorbent assay, dip-stick immuno-assay, immunofluorescence and immunoenzymatic staining. Though significant advances have been made in the development of rapid, sensitive assays for fungi in recent years, commercially available techniques are limited to a few pathogens and diseases (Hansen and Wick, 1993). Such detection techniques makes it possible to detect the microquantities of the pathogen within a few hours of infection; this is definitely much more advantageous than the conventional techniques involving pathogen isolation, visible symptoms and microscopy. This has tremendous potential for plant disease control measures since detection of a pathogen at the initial stages of infection can lead to formulation of control measures before much harm has been done.

Since such immunological studies on brown blight disease of tea caused by *G.cingulata* are lacking, and, considering the importance of such studies, this study has been undertaken. The main objectives of this study are as follows: (a) to screen all available tea varieties (Tocklai, Darjeeling and UPASI) for resistance to *Glomerella cingulata* ; (b) to extract antigens from mycelial and cell wall preparations of *G.cingulata* ; healthy, naturally infected and artificially inoculated tea leaves ; non host species and non pathogen; (c) to raise antisera against antigen preparations from tea leaves, mycelia

and cell wall of *G.cingulata* as well as *F.oxysporum*, a non pathogen ; (d) to detect serological cross reactivity between isolates of *G.cingulata* and tea varieties following agar gel double diffusion, immunoelectrophoresis and enzyme linked immunosorbent assay; (e) optimization of the sensitivity of ELISA using antisera raised against both mycelial and cell wall preparations of *G.cingulata* ; (f) to detect infection in tea leaves of different varieties by ELISA ; (g) to determine the earliest period after inoculation with *G.cingulata* when detection can be done with ELISA and to quantify the fungal biomass in the tissues; (h) to purify the antigenic protein from the crude mycelial extract of *G.cingulata*; (i) to evaluate the antiserum raised against partially purified antigen of *G.cingulata*; (j) to characterize the chemical nature of cell wall of *G.cingulata* ; (k) to determine the cellular location of cross reactive antigens in mycelia and conidia of *G.cingulata* and tea leaf tissues.

Before going into details of the present work a brief review in conformity with this study has been presented in the following pages.

Plate I (figs.A&B). Tea plants (TV - 18) grown in the Phytopathological Experimental Garden , naturally infected with *Glomerella cingulata*