INTRODUCTION

Tea is the most popular and inexpensive beverage produced from young leaves of the commercially cultivated tea [*Camellia sinensis* (L.) O. Kuntze] plant. In North Bengal tea cultivation is practiced in Dooars and Terai region (Plate 1, figs. A&B), as well as Darjeeling hills. Total area under tea cultivation in North Bengal is approximately 1,10,000 ha producing 180 million kg of tea. Tea leaves, besides providing the most healthy drink, are now-a-days being exploited for other innovative aspects like extraction of active principles that give protection from cancer and other diseases (Chakraborty and Chakraborty, 1998). Therefore, the importance of this crop for human beings cannot be overemphasized.

Perennial habit of the tea plant, peculiar cultural conditions and warm humid climate of the tea growing areas are highly conducive for disease development (Hajra, 2001). A large number of pathogenic organisms are available in this ecological niche. Various diseases affect the crop by their indirect effect on bush health, but if both young and mature leaves are attacked, the quantity of harvest is reduced (Baby, 2001). Among the diseases of tea, brown blight is a foliar fungal disease (Ruan *et al*, 2000; Zee *et al*, 2003; Keith *et al*, 2000; Ponmorugan *et al*, 2007) caused by anamorph *Colletotrichum gloeosporioides* Penz. (Sacc.) [teleomorph *Glomerella cingulata* (Stoneman) Spauld.& Schrenk.]. The fungus is characterized by the conidia that readily form appressoria on germination (Plate 1, inset). Entry of the pathogen can be facilitated through a wound that may occur by insect damage, pruning or sunscorch. Most favourable conditions for occurrence of this disease is a humid atmosphere, with a minimum temperature of about 24°C, heavy rainfall and less hours of sunshine (Chakraborty *et al*, 2002). Small, oval, pale yellow spots first appear on leaves. Often the spots are surrounded by a narrow reddish halo (Plate 1, fig. D). As the spots grow and turn brown, tiny black dots become visible and eventually the dried tissue falls, leading to defoliation (Plate 1, fig. E).

The economic impact of the genus Colletotrichum has led to extensive studies on diverse aspects of its biology, such as host specificity (Correl *et al*, 2000; Freeman, 2000), cell biology of infection process (O'Connell *et al*, 2000), host-pathogen interaction (Prusky *et al*, 2000), genetic diversity (Freeman, 2000), cytogenetics of ascospores and conidia (Roca *et al*, 2003) and epidemiology (Forster and Adaskaveg, 1999; Timmer and Brown, 2000). Species of this genus have been used as models for

Plate 1 (A-D) : Tea gardens (A) Murti Tea Estate. (B) Sundew Tea Estate and (C & D) brown blight affected tea bush. Germinating conidia of *Glomerella cingulata* (inset).

studying infection strategies and host-parasite interactions (Perfect *et al*, 1999), the genetic basis of fungal symbiotic lifestyle (Rodrigues and Redman, 2000) and for developing disease forecasting systems (Monroe *et al*, 1997; Adaskaveg *et al*, 2001, 2002; Uddin *et al*, 2002). Out of all the Colletotrichum spp., *C. gloeosporioides* is by far the most predominant and major pathogen on a wide range of cultivated crops, particularly tropical perennial crops.

The hosts include plants from highly diverse families of dicots such as Rosaceae (*Fragaria sp.* and *Malus sylvestris*), Anacardiaceae (*Mangifera indica* and *Anacardium occidentale*) and Rutaceae (*Citrus spp.*). Monocot hosts are also from unrelated families such as Zingiberaceae (*Curcuma longa*), Gramineae (*Sorghum* sp.) and Musaceae (*Musa acuminata*). This unusually wide host range of the foliar fungal pathogen may indicate its adaptive capacity as well as highly advanced type of sexual reproduction, which is difficult to observe. *C.gloeosporioides* is a common saprobe and opportunistic invader of dead or damaged plant material, although there are many aggressive pathogenic forms, which are morphologically indistinguishable.

Many problems still remain in providing a workable taxonomy of this genus in spite of the work done on the molecular aspects of the fungus. Besides, the pathology of the systems involving *C. gloeosporioides* is complicated by the variability of its isolates with respect to pathogenicity, virulence, growth rate, morphology and other characters. In spite of these complications, several plant-pathogen systems involving *C. gloeosporioides* have beeen successfully used in elucidating the defence reactions in plants (Prusky *et al*, 2000; Wang *et al*, 2004). Therefore, tea - *G. cingulata* (teleomorph *C. gloeosporioides*) pathosystem was selected for the present study due to its economic importance and academic implications. Plant defense towards potential pathogens encompasses a wide array of mechanisms, some leading to the rapid reinforcement of preexisting structural barriers, others to the *de novo* synthesis of a large diversity of defense-related compounds via transcriptional activation of the corresponding genes. In recent years, numerous plant genes potentially involved in the pathogen defense response have been isolated. The antioxidant properties of tea have been largely attributed to the high amount of phenolics in tea leaves. Phenolics are also known to act as reactive oxygen species scavengers, thus extinguishing the harmful hydroxyl radicals (Grassmann *et al*, 2002). Induction of the PR (Pathogenesis-related) –proteins in the resistant interactions is well-documented (Van Loon, 1997, 1999; Jebakumar *et al*, 2001;

Chakraborty, 2005; Van Loon *et al*, 2006). A substantial body of evidence suggests the involvement of the various enzymes in resistance of plants to a plethora of pathogens. The defense gene products such as peroxidases (POX) and polyphenol oxidases (PPO) catalyze the formation of lignin and phenylalanine ammonia-lyase (PAL), which are involved in the synthesis of phytoalexins and phenolics (Karthikeyan *et al*, 2005). Pathogenesis-related proteins (PRps) such as β-1,3-glucanases (PR-2) and chitinases (PR-3, PR-4, PR-8, and PR-11) degrade the fungal cell wall and cause lysis of fungal cell walls. Furthermore, chitin and glucan oligomers released during degradation of the fungal cell wall by the action of lytic enzymes act as elicitors that elicit various defense mechanisms in plants (Karthikeyan *et al*, 2005). Peroxidase (POX) has also been proposed to be an important enzyme regulating host-pathogen interactions (Van Breusegem *et al*, 2001; Som and Chakraborty, 2003; Dowd and Johnson, 2005; Burkhanova *et al*, 2007). The involvement of each of these different enzymes at the various stages of pathogen infection calls for a thorough study at the ground level.

The utilization of the natural plant immunity response has long been a goal of modern biodynamic agriculture. Modern biotechnology has developed well-defined compounds that induce systemic resistance (Von Rad *et al*, 2005). One well characterized example is *S*-methyl benzo[1,2,3]thiadiazole-7-carbothioate (benzothiadiazole). It is a chemical analogue of salicylic acid, and as such, benzothiadiazole induces resistance through the SA-dependent pathway (Achuo *et al*, 2002; Cao and Jing, 2006). A number of commercial products such as BION® for benzothiadiazole and Elexa™ for chitosan (Sharatchandra *et al*, 2004) have been developed for broad range pathogen resistance. Aquaous solution of hydrogen peroxide has been patented by Larose and Abbot (1998) for use against a broad range of pests and pathogens on different crops. Besides, the evidence is accumulating that this radical induces protective mechanisms (Rizhsky *et al*, 2004; Kotchoni and Gachomo, 2006). Nitric oxide (NO), is a gaseous molecule that has been only recently proposed to be the key component in resistance mechanism of plants (Mur *et al*, 2005, 2006; Wang and Higgins, 2006).

Keeping in view the above mentioned points, present investigation was undertaken to elucidate the defense mechanism in tea plants against *G. cingulata* with the following objectives in mind : (a) to characterize the isolates of *G. cingulata* (b) to identify the varieties resistant and susceptible to *G.cingulata* (c) to determine the

disease reaction elicited by the cell wall and spore of *G.cingulata* (d) to analyse the soluble pathogen-induced proteins by SDS-PAGE in compatible and incompatible reactions (e) to find out the level of phenolics and diffusible compounds in tea plants following interaction with *G.cingulata*, (f) to assay the defense enzymes : peroxidase (POX), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), chitinase (CHT), β-1,3 glucanase (GLU) in constitutive and induced resistance (g) to analyse the isozymes of peroxidase and polyphenoloxidase in compatible and incompatible ractions (h) to induce resistance in tea plants using abiotic compounds and evaluate the level of defense enzymes therein.

In the beginning, a brief review of literature related to the area of this investigation, is presented.