INTRODUCTION

Plants are often simultaneously challenged by pathogens and insects capable of triggering an array of systemic responses that may be beneficial or detrimental to plant health and productivity. Inducible defenses in plants against pathogens and insect herbivores can be strongly influenced by the mix of signals generated by external biotic factors as well as by abiotic stresses such as drought, nutrient limitation, or high soil salinity. Our ability to capitalize on inducible defenses and utilize them optimally in agriculture depends, in part, upon a fundamental knowledge of their biochemical nature, and of the specificity and compatibility of the signaling systems that regulate their expression.

Plants protect themselves from stressful situations by changing their physiological conditions. This reaction is known as the defense response of higher plants and a series of proteins actively synthesized with this reaction is called defense-related proteins. In addition to specific defense responses, based on so called R-genes against certain strains of a pathogen (Ellis *et al.*, 2000), plants have broad spectrum defense responses which are pre-formed such as waxes or the responses that can be induced locally or systemically by biotic or abiotic agents in nature (Oostendorp *et al.*, 2001; Liu *et al.*, 2005). In due course plants have developed ingenious molecular strategies to defend themselves against the biotic and abiotic stresses they may be confronted with and the ability of plants to survive an attack by pathogens is generally related to the expression of defenses. However, even in those cases where a species is susceptible to a specific pathogen, it is likely that certain cultivars or developmental stages of the species successfully resist infection (Heath, 2000). Hence, it is not surprising that there are potentially multiple means by which plants detect the presence of pathogens and effectively respond to the infection (Hammerschmidt, 2003).

As a consequence to the threats from invasion by pathogenic microorganisms in the outside environment plants produce a series of proteins that exert direct antimicrobial activity. Such proteins are specifically termed antimicrobial proteins or AMPs which directly interfere with the growth, differentiation, the multiplication and/ or spread of microbial organisms (Broekaert *et al.*, 2000). By far the most studied group of AMPs, the pathogenesis-related (PR) proteins that are protective plant proteins specifically induced in pathological or related situations, was discovered through the pioneering works of Gianinazzi *et al.* (1970); Van Loon and Van Kamen (1970).

Information is emerging on how these AMPs interfere with growth of microorganisms, fungi in particular. Examples of some are, hydrolases such as PR-3 type chitinases, PR-2 type glucanases and possibly PR-4 type proteins, affect fungal growth by disturbing the integrity of their cell wall. Thionins, 2S albumins, lipid transfer proteins and puroindolines have been demonstrated to partially lyse artificial phospholipid vesicles and are therefore believed to interfere with the phospholipid bilayer of the microbial plasma membrane. Other, AMPs such as PR-5 type proteins and plant defensins are proposed to affect plasma membrane receptors. Defense-related proteins involved in the synthesis or transport of metabolites that are inhibitory to microorganisms, are not considered as AMPs, although expression of such genes often play a very similar role in host defense (Broekaert *et al.,* 2000). PRs are synthesized *de novo* and commonly found in plants responding hypersensitively to pathogen infection. The sequence similarities and serological relationships are the basis of the classification. PRs are constitutively present in tissues vulnerable to pathogen attack (Jangid *et al.*, 2004). They have been discovered in every plant species that has been studied so far, accumulate not only in response to microbial pathogens but also to nematodes, insects or herbivores, in healthy plants during flowering and senescence and can be present in virtually any organ. Most importantly, the occurrence of these novel proteins is not pathogen-specific but is determined by the type of reaction of the host plant, indicating that they are of host origin.

Pathogenesis-related proteins have been best described in the model plant tobacco and have progressively been extended to numerous other plant-pathogen combinations since its discovery. A great deal of research has focused on the isolation, characterization and regulation of expression of this unique class of defense proteins in a wide host range including tobacco, tomato, cucurbits, pepper stems, cotton, mustard, barley, wheat, rice, bean, groundnut, gram and many more. Yet, till date, there is no information in this line for tea. Tea which is cultivated under widely different geographical regions (Plate 1,figs. A & B) like any other plant suffers from a variety of damaging pests and fungal pathogens which prey on the leaves.

Plate 1 (figs. A & B). Tea plantations. [A] Hills and [B] plains.

The interactions between plants and potential pathogens maybe of the non-host type, compatible type or incompatible type. Non-host interactions occur when the attacked species does not belong to the specific host-range of the pathogen and the plant is not infected. Compatible interactions take place when the pathogen is able to multiply and spread through the host which is not able to adequately switch on its active defense mechanisms. In incompatible interactions the host recognizes the invading pathogen leading to a defense response which prevents the pathogen from infecting the plant any further usually leading to a hypersensitive reaction of the plant which impedes pathogen spread through the plant. This is followed by enhanced resistance in the uninfected parts of the plant against further pathogen attack, a phenomenon called 'induced' or 'acquired resistance'. During the hypersensitive response in plants many biochemical alterations are coordinately induced that can contribute to, or may even determine the resistance attained. PR-proteins may function as the first or last line of defense against pathogens and pests consequently representing a generalized plant defense response. PR-like proteins, which are expressed in apparently healthy tissues during normal plant growth, may have additional unsuspected roles in morphogenesis or symbiosis. Plants that are apt to intensively induce such proteins are certainly agriculturally valuable.

Tea is used as a drink made from the apical two leaves and a bud of *Camellia sinensis* (L.) O.Kuntze. The leaves contain in addition to the normal constituents, high levels of polyphenols (30-40%) and caffeine (5-6%). The beneficial health effects of tea are well known (Das *et al.*, 2005) and the terminal bud of tea contains the maximum caffeine, which contributes to the medicinal value of tea (Ramarethinam and Rajalakshmi, 2004). Tea is grown in more than 50 countries, mostly in plantation as a monocultural crop. It prefers a warm humid climate with well distributed rainfall and long sunshine hours. India with around 4,40,000 ha area under tea cultivation, is globally the largest producer and consumer of tea. Darjeeling produces the world's finest quality tea on the steep hill slopes of the eastern Himalayas up to an elevation of 2000 m (Chakraborty *et al.*, 2002a). Tea plants growing in and around the Darjeeling hills are seriously affected by pests and pathogens which is one of the acute problems of the tea industry. Of the various foliar fungal diseases 'Blister blight' (Plate 2), caused by the biotrophic fungal pathogen

Plate 2. Blister blight disease of tea.

Exobasidium vexans, is considered as one of the key factors in restricting the production of high-quality tea (Chandra Mouli, 2003). The pathogen attacks harvestable tender shoots, inflicting enormous yield loss (30-40%) and quality deterioration even below the 35% disease threshold (Radhakrishnan and Baby, 2004). *E. vexans* has no known alternate host and as such it completes its life cycle on tea itself. Under severe infection the affected leaves curl up. When the tender stem is attacked the entire shoot withers and falls. It has been noticed during appearance of blister blight that some of the tea bushes are not affected by the pathogen. This indicates that tea plants have an inherent immune system.

The aim of this discourse was to characterize the defense-related proteins in tea plants triggered by *E. vexans* and to elucidate the strategies of the defense mechanism in this host-parasite interaction. Biochemical and immunological characterization of the proteins which accumulate during compatible and incompatible interactions of tea plants against *E. vexans* was undertaken in the present investigation with the following major objectives,

(a) Biochemical analyses of proteins from tea varieties showing compatible and incompatible reaction towards *E. vexans*.

(b) Evaluation of defense related enzymes - chitinase, β-1,3-glucanase and peroxidase in tea plants following inoculation with *E. vexans*.

(c) Determination of time course accumulation of pathogenesis related (PR) proteins in tea – *E. vexans* interaction and assessment of their antimicrobial activities *in vitro*.

(d) Immunological characterization of pathogen-induced (PI) and pathogenesis-related (PR) proteins in tea varieties triggered by *E. vexans*.

(e) Analyses of PR-proteins in suspension cell cultures induced by *E. vexans* and abiotic elicitors.

(f) Induction of resistance in tea plants against *E. vexans* and associated changes in defense enzymes.

(g) Elucidation of defense responses of tea plants stimulated by *E. vexans* with special reference to antifungal phenolics.

(h) Immunocytochemical localization of the pathogen in compatible interactions and cellular localization of defense enzymes induced in leaf tissues and cell cultures of tea.

(i) Immunogold localization of *E. vexans*, chitinase and β-1,3glucanase in tea leaves.

At the onset, a brief review of literature on Pathogenesis-related (PR) proteins in conformity with the present work has been presented. In the following pages, the materials used and the methods applied to achieve the above objectives have been outlined along with a description of the results achieved.