Introduction

Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze] is a cash crop and occupies an important position among agro-products exported from India. Among the plantation crops tea is also important in view of its worldwide popularity as beverage. Tea originated in China in the 27th century BC (Yamanishi, 1991). Although globally about fifty countries are growing tea, but about a dozen can be regarded as the major producers. The countries substantially contributing to global production include India, China, Sri Lanka, Kenya, Russia, Japan, Argentina and Uganda. Tea plant requires moderately humid climate, high land, well-drained soil having acidic pH in the range 4.5 to 5.5 and more than 2% organic matter and long sunshine hours. Presently, they are grown from Georgia 43^{0}N latitude to Nelson (south Island) in New Zealand 42^{0}S latitude and from sea level to 2300m above mean sea level. Tea, cultivated near the equator produces almost the same yield every month, but further from equator, harvest in winter gradually declines and at latitude beyond about 160, there is complete winter dormancy, the length of dormant period increases progressively with increasing distance from equator (Barua, 1989). The tea leaves containing polyphenol, epigallocatechin gallate and an enzyme polyphenol oxidase are considered to be powerful antioxidant (Weisberger, 1999; Edwin *et al.,* 2002). Regular intake of tea may reduce the risk of cancer and coronary heart diseases (Edwin *et al.,* 2002).

Tea plants are cultivated in three distinct zones in India viz. 'Western India', 'South India' and 'Himachal Pradesh Uttaranchal' (Fig.1). Tea plants are exposed to a number of pathogens, mostly fungi that cause diseases in tea. Among the fungal diseases, the important diseases are blister blight, gray blight, brown blight, black rot and diplodia disease caused by *Exobasidium vexans, Pestalotiopsis theae, Colletotrichum camelliae, Corticium invisum and Lasiodiplodia theobromae* (Pat.) Griffon & Mauble (=*Botryodiplodia theobromae* Pat.) respectively. Diplodia disease is one of the common diseases of tea in sub-Himalayan West Bengal. The symptoms

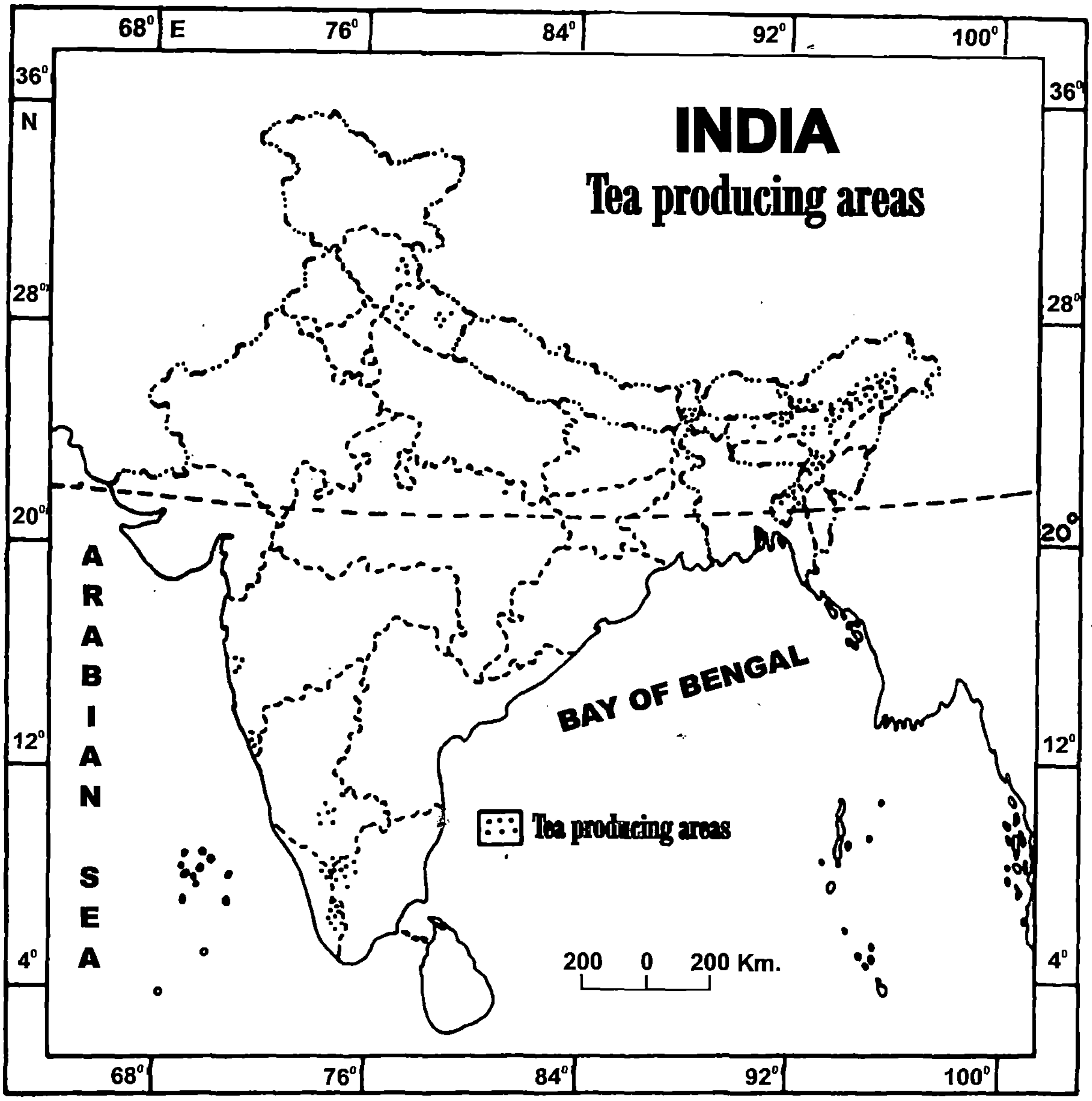

Fig.1: Tea growing states of India

appear as dark brown necrotic spots. The fungus forms dark-coloured, two celled conidia for its propagation. The pathogen, *Lasiodiplodia theobromae* can attack the tea plant, young or old at any parts (Sarmah, 1960). *Lasiodiplodia theobromae* is a homothallic ascomycete and produce dormant conidia that have aerobic respiratory system containing cytochrome-a, heme-a, and cytochrome-c oxidase activity in the mitochondria (Brambl *et al.*, 1978).

Higher plants have the ability to initiate various defense reactions such as the production of phytoalexines, antimicrobial proteins, reactive oxygen species etc. when they are infected by pathogens. These reactions do not allow the infection to proceed, if the reactions occur in a timely manner. However, if the defense reaction occur too late or are suppressed, the infection process will proceed successfully (Somssich and Hahlbrock, 1998). Hence, it is important for plants to detect infecting pathogens effectively and deliver such information intracellularly or intercellularly to activate their defense machinary (Shibuya and Minami, 2001). Management of disease is possible by inducing plant defense response by exogenous application of certain biotic and abiotic inducers, and phyto-extracts in order to provide protection against pathogens. Extensive research work has been performed for the establishment of systemic acquired resistance (SAR) by the application of a variety of biotic and abiotic inducers, and phyto-extracts (Rasmussen *et al.,* 1991; Cohen *et al.*, 1993; Kessmann *et al.* 1994a,b; Maurhofer *et al.,* 1994; O'Donnell *et al.,* 1996; Ryals *et al.,* 1996; Kaku *et al.,* 1997; Smith-Beaker *et al.* 1998; Siegrist *et al.* 2000; Klessig *et al.* 2000; Meena *et al.* 2001; Higa *et al.* 2001; Kaur and Kolte 2001; Paul and Sharma, 2002; Ghosh and Purkayastha, 2003). Plant growth promoting rhizobacteria (PGPR) can suppress the disease caused by foliar pathogen by triggering plant-mediated resistance mechanism called induced systemic resistance, so called ISR (Dube, 2001). Systemic resistance induced by rhizobacteria differs mechanically from SAR, it is designated by a separate term ISR proposed by Kloepper *et al.*, 1992.

Many plant enzymes are involved in defense reactions against plant pathogens. These includes oxidative enzymes such as phenylalanine ammonia-lyase (PAL) and polyphenol oxodase (PPO), which catalyse the formation of lignin appear as dark brown necrotic spots. The fungus forms dark-coloured, two celled conidia for its propagation. The pathogen, *Lasiodiplodia theobromae* can attack the tea plant, young or old at any parts (Sarmah, 1960). *Lasiodiplodia theobromae* is a homothallic ascomycete and produce dormant conidia that have aerobic respiratory system containing cytochrome-a, heme-a, and cytochrome-c oxidase activity in the mitochondria (Brambl *et al.*, 1978).

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Many plant enzymes are involved in defense reactions against plant pathogens. These includes oxidative enzymes such as phenylalanine ammonia-lyase (PAL) and polyphenol oxodase (PPO), which catalyse the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for the pathogen to the plant cell structure (Avdiushko *et al.*, 1993).

Phenylalanine ammonialyase (PAL) frequently increase in plants responding to pathogen invasion. It is generally assumed that the resulting increased activity in the phenylpropanoid pathway lead to the synthesis of defense-related compounds such as lignin and flavonoid phytoalexin as well as the signalling molecule, salicylic acid (Heath, 2002).

Among PR-proteins, two plant hydrolases, β-1,3-glucanase and chitinase are given special importance by the workers because many pathogenic fungi contain β-1,3-glucans and chitin as major structural cell wall components (Wessels and Sietsma, 1981). Several authors (Mauch *et al.*, 1988; Arlorio *et al.*, 1992; Bishop *et al.*, 2000) have demonstrated the activity of β-1,3-glucanase and chitinase to degrade fungal wall components *in vitro*, resulting in growth inhibition of fungi.

Peroxidase activity is changed under various environmental stresses such as heavy metals, salts, temperature (Kiwan and Lee, 2003) and air pollution (Lee *et al.*, 2000). Peroxidase is related with the defense reaction in plants, lead to the detoxification of the reactive oxygen species (Higa *et al.*, 2001).

The reduction of inoculum density or disease producing activity of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of environment, host or antagonist, or by mass introduction of one or more antagonists (Prasad and Rangeswaran, 1999). A lot of work has been done on potential antagonists against different pathogens (Biswas, 1999; Mukhopadhyay *et al.*, 2001; D'souza *et al.*, 2001; Boff *et al.*, 2002; Helbig, 2002). It is known that out of 2,50,000 higher plant species which are believed to exist on earth, only relatively few have been thoroughly studied for their therapeutic potential (Deans and Svoboda, 1990). Plant products comprise a rich storehouse of biochemicals that could be tapped for use as pesticides. The total number of plant chemicals exceeds 4,00,000 are secondary metabolites whose major role in the plants is defensive (Narasimhan and Masilamani, 2002). Now efforts have been made to evolve eco-friendly strategy for disease management. In this regard, a new strategy of chemically mediated disease management based on chemicals which activate the plant's own defense system, in addition to antagonistic microorganisms and botanicals for direct control of diseases have been shown by Kaur and Kolte, 2001; Sharma *et al.,* 2001; Paul and Sharma, 2002.

A pathogen and its host share antigens, which play an important role in the determination of compatible interaction. The absence of the common antigens leads to incompatible interaction. Even within the compatible interactions the degree of compatibility might be determined by the sharing of the common antigens (Dasgupta *et al.*, 2005).

At the onset of the present study it was considered to determine pathogenicity of *L. theobromae* in different elite seed varieties of tea commonly cultivated in north east India (Fig. 2 & Plate I) following conventional and serological techniques. Although several inducer chemicals, antagonistic microorganisms and botanicals are known from different plants through literature but little information is available for controlling the diplodia disease in tea caused by *Lasiodiplodia theobromae.* Hence, in the present study environment-friendly strategies have been taken into consideration for control of diplodia disease in tea. Therefore, the basic objectives of this study are:

- To determine the pathogenicity of *Lasiodiplodia theobromae* in tea varieties.
- Studies on physiological characteristics of the fungus.
- Induction of defense-related enzymes by biotic and abiotic inducers, and phyto-extracts.
- Control of the disease using biocontrol agents, botanicals and abiotic inducers.

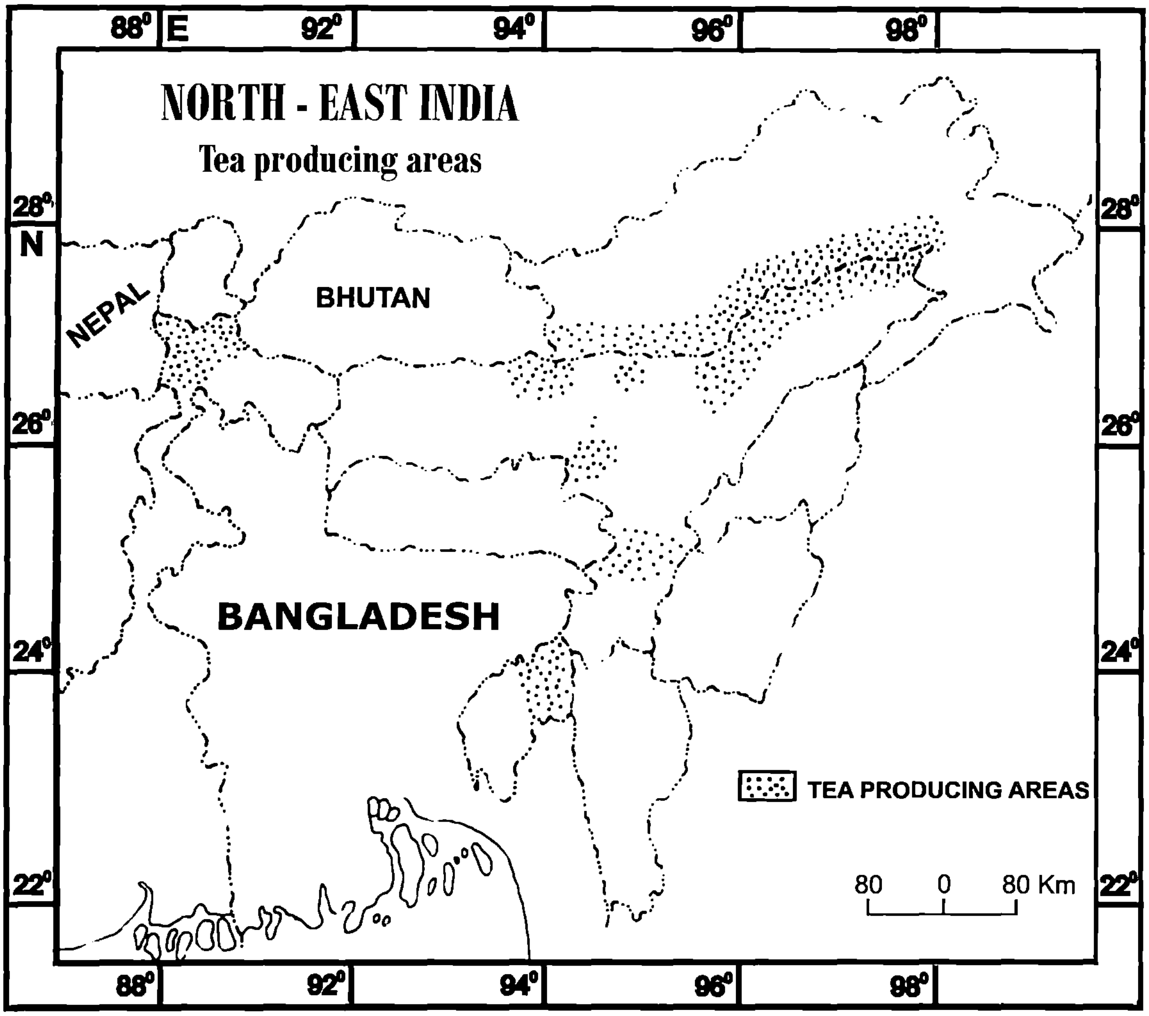

Fig.2: Tea growing regions in north-east India

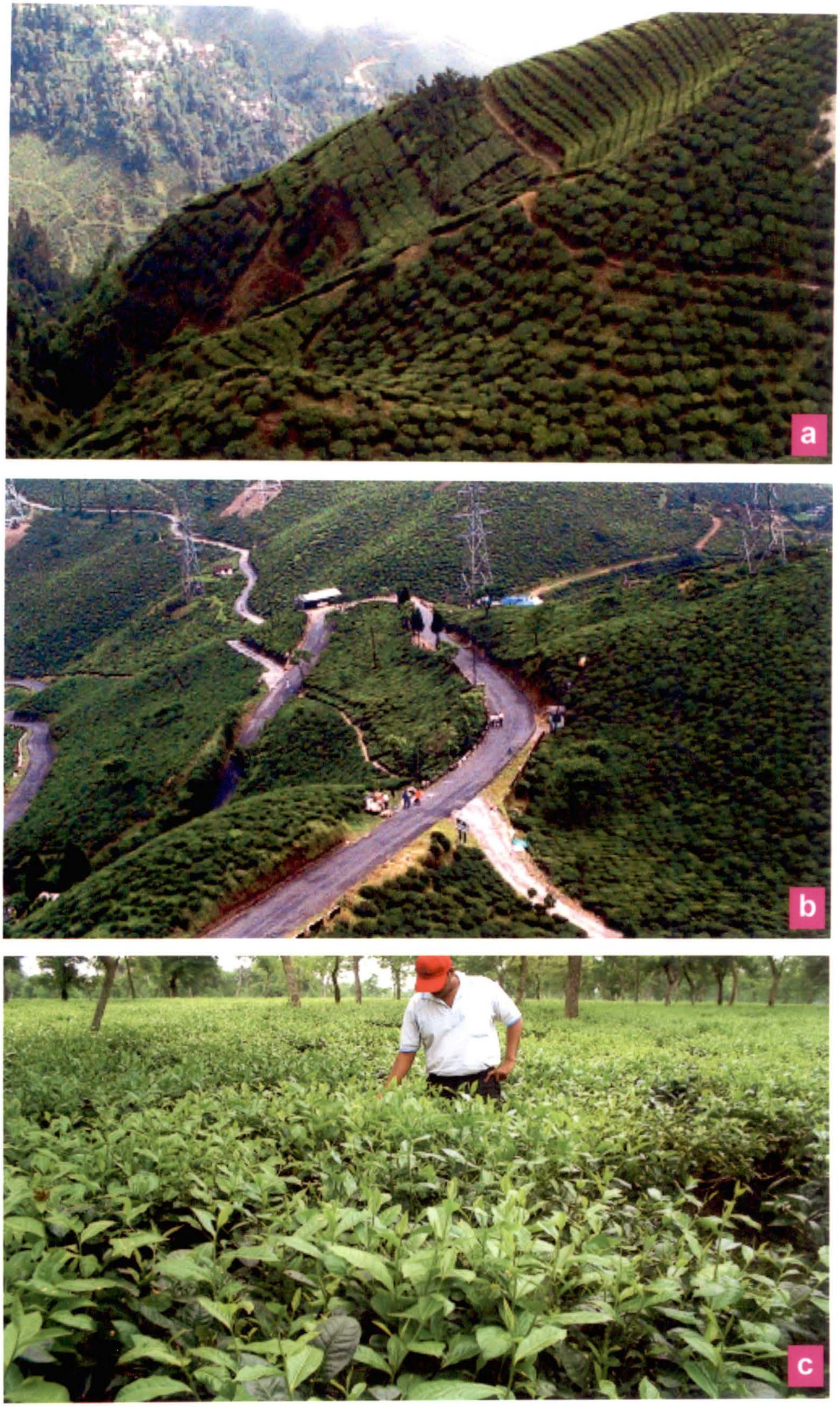

PLATE I

Tea plantations.

fig. a : Tea garden at Kurseong, Darjeeling, West Bengal.

fig. b : Tea garden at Mirik, Darjeeling, West Bengal.

fig. c : Taipoo Tea Estate, Kharibari, Siliguri, West Bengal.