1. Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze.) occupies an important position among the plantation crops due to its popularity. Among the beverages tea is one of the low caffeine containing plant. Tea has stimulating and good health effects. Flavan 3-ols of green tea has health-promoting effects (Chacko *et al*, 2010). Tea leaves contain four different compounds like (–)-epigallocatechin, (–)-epicatechin, (+)-gallocatechin, and (+)-catechin along with their derivatives which has positive effects on human health as reported by Jigisha *et al.* 2012; Macedo *et al.*, 2012; Azzahra *et al.* 2012; Punyasiria *et al.* 2004).

Tea originated in southwest of China over 4000-5000 years ago (Mondal *et al.*, 2004; Chen and Lin, 2015). In 2017, about 47 countries have been reported to grow tea for commercial production (https://ratetea.com/region.php). Tea plants are cultivated between 42° north to 35° south latitude. Tea plants thrive well in, humid climate, well drained acidic soil (pH- 4.5 to 5.5) well distributed rainfall and long sunshine hours. Three major tea growing areas of India are Northeast India, Northwest India and South India. Northeast Indian tea growing areas belong to states of Assam, West Bengal, Bihar, Tripura, Sikkim, Manipur, Nagaland, Meghalaya, Arunachal Pradesh, and Mizoram. Southern region include three states (Tamil Nadu, Kerala and Karnataka) and Northwest region include two states Himachal Pradesh and Uttarakhand (Sharma *et al.*, 2010; Bhardwaj *et al.*, 2014).

Plants generally initiate various defense reactions to protect it. Some of these are production of phytoalexines, antimicrobial proteins, reactive oxygen species etc. when they are infected by any of the pathogens. Successful defense reactions do not allow the infection to proceed. Successful defense depends upon several factors. If the reactions occur within a very short time the plants can protect themselves and the plants

are considered to be resistant plants. However, if the defense reactions do not occur or occur at a later stage, the infection process, proceed successfully and plants are said to be susceptible plants (Somssich and Hahlbrock, 1998; Sharma *et al.*, 2012; Leon and Montesano, 2013).

Fungal infection is one of most destructive of 'biotic stress' affecting tea plants. Like many other plants, tea plants are also susceptible to several fungal pathogens. Some of the diseases like blister blight, grey blight, brown blight and leaf spot are of serious nature and caused by fungal pathogens such as *Exobasidium vexans, Pestalotiopsis theae, Colletotrichum camelliae* and *Curvularia eragrostidis* respectively. Tea plants are also exposed to some other fungal pathogens like *Lasiodiplodia theobromae, Corticum theae, Fusarium oxysporum, Rhizoctonia bataticola, Fusarium solani, etc.* (Sarmah *et al.* 2016; Saha *et al.*, 2001; Sarmah, 1960; Naglot *et al.*, 2015; Liu *et al.*, 2017).

Several scientists have shown that defense response may be initiated by exogenous application of some biotic and abiotic inducers. During the last twenty years several such inducers have been shown to induce defense (systemic acquired resistance) in a variety of plants (Ghosh and Purkayastha, 2003; Kaur and Kolte, 2001; Justyna and Ewa, 2013; Ghosh, 2015). When a plant is infected by pathogens, a large number of genes that are involved in various metabolic activities, signal transduction, transcriptional regulation, and defense responses are activated (De Vos *et al.,* 2005). The regulation of defense-related genes is one of the important defense mechanism that is used by plants against biotic and abiotic agents (Edreva, 2005).

Although, the molecular interaction between fungus and plant is yet to be elucidated in details but only comprehensive approaches of transcriptome and proteome analysis have become available (Campo *et al.*, 2004; Tan *et al.*, 2015; Thatcher *et al.*, 2016). Differential expression of messenger RNAs has provided intriguing results in different host-pathogen interactions. High degree of variability has been detected between "*Fusarium verticillioides* and susceptible maize lines infection" and

"*Fusarium verticillioides* and resistant maize lines infection". Although similar functional categories of genes were involved in the response to infection in resistant and susceptible maize genotypes, in the susceptible line, the genes were qualitatively induced from a basal level and responded specifically to pathogen infection. In the resistant line, the defense-related genes assayed were transcribed at high level before infection and provided basic defense to the fungus (Lanubile *et al.*, 2010 & 2012).

Some of the major challenges of the present day cultivation of tea plants include susceptibility of the tea plants to harmful pests and fungal diseases. In the present study, from the fields it has been experienced that brown blight caused by *Colletotrichum camelliae* and leaf spot disease caused by *Curvularia eragrostidis* and *Lasiodiplodia theobroamae* are three important diseases to be taken into consideration for successful cultivation of tea in the sub-Himalayan West Bengal, the present study area (Figs. 1.1, 1.2 & 1.3).

A separate term 'induced systemic resistance' (ISR) was proposed by Kloepper *et al.*, in 1992. ISR differs mechanically from SAR (Systemic acquired resistance). Plant growth promoting rhizobacteria (PGPR) trigger plant-mediated resistance mechanism also called induced systemic resistance (ISR), which can suppress the disease by suppressing the causal foliar pathogen (Dube, 2001; Beneduzi *et al.*, 2012).

Plant enzymes are involved in defense reactions and in some cases successfully protect plants against plant pathogens. In cases where enzymes are produced at a later stage or the enzymes cannot be produced up to a threshold level required for resistance, results to susceptibility. Some of those enzymes are oxidative enzymes such as phenylalanine ammonia- lyase (PAL) and polyphenol oxodase (PPO), which catalyse the formation of lignin as well as other oxidative phenols which ultimately contribute to the formation of defense barriers making the plant cell structure inaccessible to the pathogen (Avdiushko *et al.*, 1993). In response to pathogen invasion in plants, Phenylalanine ammonialyase (PAL) frequently increase. Increased activity, in the phenylpropanoid pathway,

help in synthesis of defense-related compounds like lignin, flavonoid, phytoalexin, signalling molecules and salicylic acid (Mandal *et al.*, 2010; Duan *et al.*, 2014; Le Roy *et al.*, 2016; Boba *et al.*, 2017).

Pathogenesis related proteins (PR-proteins) are also assumed to play an important role in defense of plants. Two PR-proteins such as plant hydrolases, β-1,3-glucanase and chitinase are of special importance. 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 is related with the defense reaction in plants. Peroxidase detoxifies the reactive oxygen species (Higa *et al.*, 2001). Its activity is changed under various environmental stresses such as temperature, salts and heavy metals and also by air pollution (Kiwan and Lee, 2003; Langebartels *et al.*, 2002; Das and Roychoudhury, 2014; Liu *et al.*, 2015).

Understanding the molecular responses associated with host defence mechanism in tea is very important for better management of the crop production. Since the whole tea genome sequence is not yet deciphered, very little information is known about the genes and genetic regulations associated with tea stress responses. The molecular basis of induction of some transcripts of the some defence-related enzymes has been thought to be studied for validation of the semi-quantitative estimation of the transcripts of some defence related enzymes. For performance of these following objectives were thought to be fulfilled in the present dissertation.

Objectives:

1. Control of disease by different abiotic inducers and disease assessment.
2. Induction of defence-related enzymes in tea by abiotic inducers.
3. Molecular identification of defense related genes of tea.

4. Analysis of selected gene specific transcript accumulation in induced tea plants.
5. *In vitro* control of pathogenic microorganisms by botanicals and biocontrol agents.

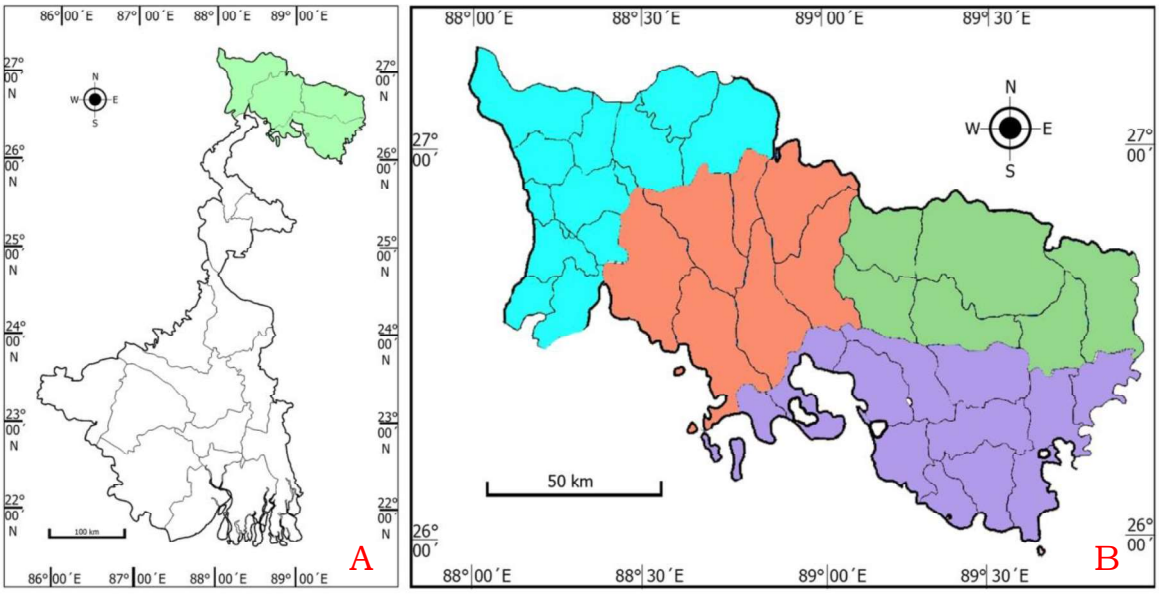

Fig. 1.1: (A) Map of West Bengal. (B) Four districts of northern part of West Bengal (The present study area) where tea is grown as an important cash crop.

Fig. 1.2: Different views of Gayaganga Tea State, Siliguri; (A) Ten years old tea plants. (B) Nursery for raising clonal tea plants. (C) Nursery for raising tea plants from seeds. (D) 25 years old tea plants Gayaganga Tea State, Siliguri.

Fig. 1.3: Naturally infected tea plants. (A) & (B) Brown blight of tea. (C) Leaf spot disease of tea.