INTRODUCTION

Tea is the oldest and non-alcoholic caffeine containing beverage producing from the young shoot tips (two and a bud) of tea plant [*Camellia sinensis* (L.) O. Kuntze]. The genus *Camellia* belongs to the family Theaceae. The tea plant in its natural condition is a perennial evergreen shrub or a small tree (woody perennial) grows over a wide area from 35° S to 42° N latitude and attained the heights from 1-12 meter. Under cultivation, tea bushes are pruned to a manageable height and tender shoots are regularly plucked in tea industry. Tea is grown in more than 50 countries, mostly in plantation as a monoculture crop. It prefers a warm humid climate with well distributed rainfall and long sunshine hours. The tea leaves contain in addition to the normal constituents high levels of polyphenols (30-40%) and caffeine (2-5%). The beneficial health effects of tea are well known (Das *et al.*, 2005), and the terminal bud of tea contains the maximum caffeine, which contribute to the medical value of tea (Ramarethinam and Rajalakshmi, 2004). Tea is indigenous to India and is an area where the country can take a lot of pride. India is the world's largest tea producer followed by China. India accounted for 27.4% of world output, followed by China (24.6%), Sri Lanka (9.75%), and Kenya (9.4%). Total area under tea cultivation in India is 5.12 lakh hectares, which generates income and livelihood for nearly 20 million people in the country.

The production in 2007 was a record high of 955 million kg, up by 27 million kg compared to 2006. 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.*, 2002). It exports bio-organic tea over 40 percent of its total production to different foreign countries like France, Germany, United Kingdom and United States of America. In order to retain its 28% share of international trade and to meet the increasing domestic demand, India needs to improve the productivity of tea substantially. The northeastern state of Assam is considered the heart of India's tea industry with the state accounting for about 55% of the country's total annual tea production. So, it urgently needs of an integrated all embracing strategy so as to place the Indian tea industry on a sound and firm footing and at the same time to achieve long-term objectives for the development of elite tea clones with better quality, flavour and higher yield by doing research giving stress on genetic diversity,

germplasm conservation, breeding, crop nutrition, biological control of pests and diseases. Tea is made from the tender leaves of the different cultivars of the three species based on morphological parameters *viz. Camellia sinensis* (L.) O. Kuntze (China type), *Camellia sinensis* var. *assamica* (Masters) (Assam type) and *Camellia sinensis* sub sp. *lasiocalyx* (Planchon MS.) (Cambod or Southern type). All the three varieties of tea (*Camellia sinensis*) are highly cross-pollinated and intercrossable without any reproduction barrier, so the existing population is a mixture of three categories of tea (Wight, 1962 and Banerjee, 1992).

Assam type was developed in India and China type in China; they were subsequently introduced in to Japan. Indo-China region being a primary center of origin, India harbors a large tea biodiversity. The diversity further widens as various scientists have developed a number of region specific, high yielding cultivars in different parts of the country by introgression of some important genes from the related wild species *viz-* TV-series, UPASI-series, and other common cultivars. Unfortunately, some important traits such as abiotic stress resistant, caffeine deficiency, resistant to pathogens (fungal and bacterial) and quality-yield appear to be absent from the gene pools of tea cultivars. In order to select the right crossing combinations, it is important for plant breeders to have knowledge about relationships between genotypes that may be used in new crosses, and about total genetic diversity available in germplasm. Conventionally the tea breeders often select the parent on the basis of a few parameters *i.e.* morphology, yield and biochemical descriptors without considering any genetic basis, however these parameters are highly dependent on environmental conditions. So, plant breeders urgently need some of the unchangeable genetic markers of the varieties, which can be used for the development of new improved varieties in all respects.

Breeders can employ DNA based molecular markers because they are effective at evaluating genetic diversity among the species and cultivars and the data are easier to obtain than classical morpho-anatomical descriptors. For characterization of genome (fingerprinting): a variety of molecular marker techniques are available, which are either hybridization based or PCR based. These markers are RFLP, AFLP, RAPD, SSR, ISSR, *etc.* The advantage of molecular markers is that they are least affected by environmental factors (Varghese *et al.*, 1998) and are almost unlimited in number. They also offer a possibility to observe the genome directly, and thus

eliminate the shortcomings inherent in a phenotype observation. Molecular markers highlighted important differences in the partitioning of diversity within and among population. These include AFLP based marker analysis (Paul *et al.*, 1997), RFLP based fingerprinting (Matsumoto *et al.*, 1994; Devarumath *et al.*, 2002), RAPD profiles (Waugh and Powell, 1992; Wu *et al.*, 1994; Lee *et al.*, 1995; Wachira *et al.*, 1995, 1997; Weir *et al.*, 1997; Kaundun *et al.*, 2000; Hsiao *et al.*, 2001; Kaundun and Park, 2002; Hamik, 2003; Luo *et al.*, 2004; Roy, 2004; Li *et al.*, 2005; Roy and Chakraborty, 2007; Liang *et al.*, 2007; Cheng-Wen *et al.*, 2008), ISSR based analysis (Hsiao *et al.*, 2001; Mondal, 2002; Devarumath *et al.*, 2002, and Liang *et al.*, 2007). The CAPS markers have also been used in tea characterization (Kaundun and Matsumoto, 2003). These markers are useful for characterization, estimation of genetic relatedness and determination of genetic diversity among the tea germplasm. Comparison between RAPD, AFLP and SSR markers in different plants species has revealed that co-dominant SSRs detect the highest level of polymorphism per locus and it surveys the hyper-variable microsatellite regions of the genome, hence they have higher information content (Powell *et al.*, 1996; Maughan *et al.*, 1996). Sometimes, AFLP and RAPD despite their dominant nature can estimate relationship with a high resolution due to their multilocus approach (Powell *et al.*, 1996; Russell *et al.*, 1997; Teulat *et al.*, 2000). A number of DNA based genetic markers are now available for the effective quantification of genetic variation in plant species. The genetic diversity and phylogenetic relationship among the commercial cultigens, therefore, deserve investigation.

The introgression of pest and disease resistance genes from related wild species into modern tea cultivars over the past several decades has been done which broadened the germplasm base of the cultigens and showing resistance against targeted pathogen. But, it is not as satisfactory as it was desired. There are several major diseases of tea plant that threatens production such as red rot, blister blight, root rot, caused by several pathogens. The pathogen infects only the economically important, tender shoots which lead to enormous crop loss, estimated up to 50% depending on the severity of infection. Blister blight incited by a fungal pathogen *Exobasidium vexans* Massee is the most important leaf disease in India affecting both the quality and productivity of tea (causes 35-50% crop loss). The protections against pest and pathogens have been achieved (40-68%) by using some conventional

fungicide and pesticides but it hampered the export quality. So we need high yielding, disease resistant, quality full cultivars for more production to promote export. In order to optimize disease and pest management strategies it is important to understand the genetic capabilities of tea germplasm to defend itself against diseases. Resistant/tolerant cultivars can be screened from the available tea germplasm on the basis of some genetical parameters such as immunity of a plant against pathogen with respect to plant's own defense system. Plant has its own defense strategies to combat the pathogenic infection by producing pathogenesis-related protein/enzyme (PR-proteins).

So, elite tea clone can be identified on the basis of the chitinase gene structure- a PR-protein gene. Many attempts have already been made to analysis the crop plant germplasm on the basis of chitinase gene but not in tea plant. Plant chitinases belong to a family of pathogenesis-related (PR) proteins, which are over-expressed by plants in response to a pathogen attack (Boller *et al.*, 1983; Giazinazzi, 1987; Legrand *et al.*, 1987; Collinge *et al.*, 1993). Chitinases catalyze the hydrolysis of the β-1,4 linked N-acetylglucosamine polymers that form chitin chains, a major component of fungal cell walls and is involved in the inducible defenses of plants. Those with lysozyme activity also cleave ß-1,4 linkages between GlcNAc and *N*-acetylmuramic acid. They are present in a broad range of organisms including bacteria, fungi, plants, and animals. Recently eleven families of PR proteins (PR-1 to PR-11) were recognized and classified in different plant species (tobacco and tomato, cucumber and parsley (Van Loon *et al.*, 1985, 1994, 1997, 1999).

Plant chitinases and lysozymes are likely to have arisen from one coancestor by divergent evolution (Monzingo *et al.*, 1996). Some researchers are trying to address the question whether manipulation of defense signaling pathways, either through genetic engineering or through application of defense signal-mimicking plant protectants, will boost the plant's immunity to potential invaders or will be a burden in crop protection strategies. Researchers have tried to isolate different chitinase gene from different plant species to use it in crop improvement program in order to increase the plant's own immune system (Schlumbaum *et al.*, 1986; Metraux and John, 1989; Samac *et al.*, 1990; Roby *et al.*, 1991; Broglie *et al.*, 1991; Huynh *et al.*, 1992; Datta *et al.*,1999; Passrinho and De Vries, 2002; Sekeli *et al.*, 2003; Sharma and Kumar, 2005; Eilenberg *et al.*, 2006). Intensive studies showed that

numerous genes involved in these processes have been identified (Hammnd-Kosack and Jones, 1996). Defense response genes are induced in both incompatible and compatible plant-pathogen interactions. However, mRNA accumulation for many plant defense genes is more rapid during interactions involving a plant expressing resistance to a particular pathogen (Christensen *et al.*, 2002; Davis *et al.*, 2002). Polyphenol oxidases (PPO) are nuclear coded enzymes of almost ubiquitous distribution in plants (Mayer and Harel, 1979; Mayer, 1987). Pritsh *et al.* (2000) studied the pattern of transcripts accumulation of six-typical defense response genes, POX (peroxidase), PR-1, PR-2 (β-1,3-glucanase), PR-3 (chitinase), PR-4 & PR-5 (thaumatin like protein) in spray-inoculated panicles of both the susceptible cv. Wheatson and the resistant cv. Sumai3 of wheat. POX transcripts accumulation has been studied by Collinge and Boller (2001) in potato upon infection with *Phytophthora infestans.*

Defense proteins (PRs) related transcript accumulation have been studied in several plant species by many researchers throughout the world to characterize and interaction between host and pathogens (Cary *et al.,* 1992; Hunt *et al.,* 1993; Newmann *et al.,* 1993; Joy *et al.,* 1995; Dry and Robinson, 1994; Sherman *et al.,* 1995; Boss *et al.*, 1995; Thipyapong *et al.,* 1995; Thipyapong and Steffms, 1997; Van Gelder *et al.,* 1997; Fossdal *et al.,* 2001; McFadden *et al.,* 2001; Yu and Muehlbauer., 2001; Christensen *et al.*, 2002; Mohammadi and Kazemi, 2002; Constabel *et al.,* 1995, 2000; Li and Steffens 2002).

Chitinases are enzymes that hydrolyse ß-1,4-*N*-acetyl-D-glucosamine (GlcNAc) linkages. Those with lysozyme activity also cleave ß-1,4 linkages between GlcNAc and *N*-acetylmuramic acid. They are present in a broad range of organisms including bacteria, fungi, plants, and animals. According to their primary structure, chitinases are divided into seven classes (classes I–VII). Plant chitinases and lysozymes are likely to have arisen from one coancestor by divergent evolution (Monzingo *et al.*, 1996). The protein genealogy of chitinases shows that class I and class II chitinase genes evolved from the same ancestral gene (Shinshi *et al.*, 1990; Araki and Torikata, 1995). Moreover, a basic class II chitinase is a putative ancestor of basic class I and acidic class II chitinase genes (Ohme-Takagi *et al.*, 1998). It has also been proposed that chitinases in class IV, which are phylogenetically related to class I and II chitinases (Araki and Torikata, 1995; Hamel *et al.*, 1997; Gomez

et al., 2002), evolved from a class I chitinase gene by four deletions in the coding sequence (Araki and Torikata, 1995). Wounding of plant tissues has been shown to induce complex molecular responses many of which (caffeine) are considered plant defense response (Aneja and Gianfagna, 2001; Leon *et at.*, 2001; Cheong *et al.*, 2002). Wounding can induce formation of jasmonates and ethylene, each of which are capable of independently inducing signal transduction pathways leading to resistance against plant pests. Jasmonic acid and related compound methyl jasmonate are part of the octadecanoid pathway in plants (Strassner *et al.*, 2002; Wasternack and Hause, 2002) and in some incidences contribute to resistance to insects (McConn *et al.*, 1997; Baldwin, 1998) and pathogen (Park *et al.*, 2001). Jasmonates have been shown to function as regulators of many different plant defense genes (McConn *et al.*, 1997; Park *et al.*, 2001). Ethylene is known to induce specific subsets of pathogenesis-related proteins in many plant species (Kitajima and Sato, 1999; Thomma *et al.*, 1999). The effects of ethylene have been associated with resistance to many different plant pathogens (Abeles *et al.*, 1971; Thomma *et al.*, 1999; Park *et al.*, 2001) and insects (Abeles *et al.*, 1971; O'Donnell *et al.*, 1996; Argandona *et al.*, 2001; Bailey *et al.*, 2005).

In response to pathogens, plants synthesize two classes of chitinases with either basic or acidic isoelectric points. These two classes appear to be targeted to different parts of the cell, and there is evidence that they are differentially regulated. Acidic chitinase is found in the intercellular space of infected plants and plants treated with salicylic acid or necrotizing salt solution. Basic chitinase accumulates in the central vacuole and is systematically induced by ethylene, a stress related hormone (Broglie *et al.*, 1986; Mauch and Staehelin, 1989; Metraux and John, 1989; Samac *et al.*, 1990).

The aim of this discourse was

(a) To characterize the tea genotypes at molecular level using DNA based marker in order to elucidate the genetic diversity and phylogenetic relationships among the tea cultivars.

(b) To obtain fundamental information on callus growth and development in tea clones *in vitro* and regeneration of plantlets

(c) To study the somatic embryogenesis and regeneration of plantlets from the cotyledonary tissues in relation to improvement of genetic traits.

(d) To know the genomic constitution of tea cultivars at the chromosomal level both *in vitro* and *in vivo* conditions

(e) To generate partial sequence information of caffeine synthase gene of tea clones

(f) To determine transcript accumulation of chitinase gene specific mRNA during induced systemic resistance by abiotic elicitor

(g) To quantify chitinase specific mRNAs through cDNAs using reverse transcriptase-PCR reaction (RT-PCR)

(h) To amplify chitinase gene specific DNA fragment by PCR, T/A Cloning and sequencing of tea clone

At the onset, a brief review of literature on DNA-based molecular markers for fingerprinting the tea genome, genomic constitution at the chromosome level, tea tissue culture, partial genetic information of caffeine synthase gene and chitinase gene in conformity with the present investigation has been presented.