



CHAPTER V

GENERAL DISCUSSION

GENERAL DISCUSSION

Tea is an economically important plantation crop with a life span of 60 years. It grows around three million ha of land world-wide (Alkan et al. 2009). For the last several decades, conventional breeding and propagation techniques were successful for the development of improved tea varieties. However, due to the limitation of these techniques for the development of cultivars with high yield and quality, application of biotechnology becomes an alternative approach (Mondal 2007). Different biotechnological applications have already been adopted in tea for the selection and/or development of quality cultivars such as micropropagation, cell and organ culture, transgenic production, DNA fingerprinting as well as functional genomics (Mondal et al. 2002).

Tea plant is a woody perennial, and hence, encounters a large number of environmental stresses throughout its entire life span. Since most of the world's tea growing areas are prone to drought, tea plant is often subjected to water deficit stress. Drought is the most important recurrent limiting factor of tea cultivation in India and other tea growing countries which incurred around 40% crop loss (Jain 1999). Drought is defined as the absence of adequate water necessary for normal plants growth and development. Water is basically important component of the metabolism of all living organisms, facilitating many vital biological reactions by being a solvent, a transport medium and evaporative coolant. In plants, water provides the necessary energy to drive photosynthesis through autolysis by yielding electrons. Drought stress affects severely the growth, crop yield and various morphological, anatomical, physiological and biochemical processes of tea plantations (Lu 1992, Upadhyaya et al. 2008). The changes basically take part as a mechanism of drought adaptation and/or tolerance. The drought tolerance capability differs from plant to plant depending on the age and genotypes of tea cultivars (Burgess and Carr 1996). Moreover, different environmental stresses to tea plant may result in similar responses at the cellular and molecular level. This is due to the fact that the impacts of the stressors trigger similar strains and downstream signal transduction chains (Urano et al. 2010).

Plants have acquired various stress tolerance mechanisms involving physiological and biochemical changes that result in adaptive or morphological changes (Urano et al. 2010). Drought tolerance is a complex trait, expression of which depends on the action and interaction of different morphological, physiological and biochemical characters (Beck et al. 2007). The level of drought stress correlates with the degree of changes in these characters which provides the scope for identifying drought tolerant cultivars and the study of tolerance mechanism at molecular level thereof. During drought, plants maintain the internal water potential, turgor and water uptake by increasing the level of osmolytes, either by uptake of soil solutes or by synthesis of metabolic solutes (Zhu 2002) as well as maintain the toxic oxygen species level through anti-oxidative mechanisms. For the biosynthesis of these solutes and compounds, numbers of genes and genetic pathways get activated. Hence, a detail knowledge of physiological and biochemical changes under drought provides the scope of correlation with transcriptional analysis, and considerably broaden the prospect of understanding the lying mechanism of tolerance. Understanding the mechanisms of signal perception, transduction and downstream regulation of cellular pathways that are involved in drought stress responses provide valuable information of stress tolerance mechanism for the development of drought tolerant cultivars (Cruz de Carvalho 2008). Understanding the responses of tea plants to their environment in terms of adaptability and performance is of paramount importance for selecting and/or developing cultivars that can withstand the unwanted environmental changes. However, except few physiological and biochemical investigations, no comprehensive analysis of drought stress responses of tea plants have been reported. Hence, present investigation of anatomical, physiological, biochemical and transcriptional changes under drought stress was undertaken in tolerant (TV-23) and susceptible (S.3/A3) cultivars (Konwar 2004) for understanding the drought stress responses of tea plants.

Two year-old vegetatively propagated well-rooted tea seedlings (~ 36 inch height) of S.3/A3 and TV-23 cultivars were planted in earthen pots (12 inch dia) under controlled greenhouse condition (at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, $25 \pm 2^\circ\text{C}$ with 65-70% relative humidity) in a completely randomized block design for drought stress imposition. Drought stress was induced by withholding water and subsequently severe stress level was determined on the basis of soil moisture content and

physiological parameters. On average 7% of soil moisture content ($-1.2 \text{ Mpa} \pm 0.20$), $8.73 \mu\text{mol m}^{-2} \text{ s}^{-1}$ of photosynthesis rate and $0.42 \text{ mmol m}^{-2} \text{ s}^{-1}$ of stomatal conductance, on 21st d of drought stress induction various experiments were performed. Pigments such as chlorophyll-a (chl-a), chl-b carotenoids and RWC were found decreased in both the cultivar; however the decrease was more rapid in S.3/A3. The quantity of protein, proline, TSS, RS, phenolics, AA and ABA were enhanced more in TV-23 whereas hydrogen peroxide (H_2O_2), superoxide anion, lipid peroxidation and electrolyte leakage were increased rapidly in S.3/A3. In between tissues, the enhancement of protein, proline, superoxide anion, AA content, lipid peroxidation and electrolyte leakage were found more in leaves as well as TSS, RS, phenolics, ABA and H_2O_2 content were found more in roots of each cultivar. The activities of SOD, POX, APX and CAT were more enhanced in S.3/A3 except the activities of POX in leaves as well as CAT and SOD in roots. A decrease in starch content was also recorded in both tissues of each cultivar which was more pronounced in roots of TV-23. There are many reports that underline the intimate relationship between enhanced antioxidative enzyme activities and increased level of drought tolerance in several crop species including rice and wheat (Guo et al. 2006, Khanna-Chopra and Selote 2007). Various cellular organelles membrane damage was observed under drought stress. The damages were more severe in susceptible cultivar which proves its inefficiency in drought tolerance. Hence, observations of the damage to cell membranes, such as crista of mitochondria, thylakoids of plastid and vacuolar membranes, provides valuable information on the ability of a plant to withstand stress (Utrillas and Alegre 1997). Thus, present investigation concluded that oxidative metabolism is the prime defense mechanism under drought stress of tea which brings a cascade of biochemical changes. The closing of stomata, increasing amount of ABA and biochemical as well as rapid synthesis of antioxidative enzymes play an important role in tolerant cultivar to cope up under drought stress. Importantly, leaves were found more vulnerable to oxidative damage in comparison to roots in both tolerant and susceptible cultivars.

For the analysis of differentially expressed transcriptome under drought stress, three SSH libraries were constructed between 21 d drought induced and control plants roots of each TV-23 and S.3/A3 cultivars as well as in between them taking drought induced TV-23 plant's roots as 'tester' and S.3/A3 plants roots as 'driver'. A

total of 572 quality ESTs were generated from the inter-varietal SSH library which gave rise to 246 unigenes, containing 54 contigs and 192 singlets. Average length of the unigenes was of 528 bp with an average GC percentage of 44.28 and coding GC percentage 44.46. There were genes of molecular chaperone, heat shock proteins, transcription factors, transporters and enzymes as well as other functional proteins. The amino acid, leucine was found as highest coded (9.37%) and methionine as least coded (1.98%) in the unigenes. There was 85 quality protein domains detected of which 74 were found to be conserved on the basis of Conserved Domain Database of NCBI as well as 11 simple sequence repeats identified in the unigenes. GO analysis of unigenes as defined in *Arabidopsis* proteome clearly showed that 13.04% of genes were associated to stress. The comparison of tissue specific expression of transcripts under drought suggested the involvement of different genes in leaves and roots. Moreover, a standard cDNA library was also constructed from roots using SMART technology. A total of 346 full-length ESTs were generated which gave rise to 207 unigenes comprising 58 contigs and 149 singlets. There were only 10 numbers of reference genes found to be redundant in drought induced unigenes. This result suggested the involvement of different genes under normal growth and drought stress of roots. Comparative analyses of transcripts showed that drought stress is more lethal to the growth and development of tea plants in comparison to winter dormancy stress. A total of 123 drought associated genes were finally identified including well-known drought associated genes such as dehydrin, trehalose-6-phosphate synthase, Cu/Zn SOD, glutathione reductase etc. Interestingly, there were candidate genes of ubiquitin-proteasome and glutathione metabolism pathways as well as numbers of genes associated to sugar metabolism and transportation. The results strongly suggested the pivotal role of these pathways in drought tolerance mechanism of tea roots. Therefore, this study provides a basis for studying drought tolerance mechanism of this important commercial crop which will also be a valuable resource for functional genomics study of woody plants in future.

Genomes of higher eukaryotes encode diverse non-coding RNAs, particularly 20 to 30 nt regulatory RNAs (Zhu et al. 2008). They bind to the complementary sites on target mRNAs and repress post-transcriptional gene expression (Nagaraju 2008). It has been well-demonstrated that miRNAs play critical roles in diverse biological processes such as development, cellular differentiation, cell-cycle control, apoptosis

and oncogenesis (Sunkar et al. 2007). Due to the advantages of low cost and high efficiency, computer based miRNA identification methods have received more and more attention in recent years. Sequence and structure homologies are the main theory behind the computer-based approach for miRNA prediction. Since, tea genome sequences is not available, both EST and nucleotide databases were mined for miRNA identification. A total of 13 conserved miRNAs were identified belonging to 9 miRNA families where miR414 family has 5 members and the rest have single member in each. Average length of precursor sequence was 248 nt; however a majority of them (62%) have 65-200 nt. The identified tea miRNAs were found conserved in diverse plant species from monocotyledonous to dicotyledonous plants. These results suggested that different miRNAs might evolve at different rates not only within the same plant species, but also in different ones. Target identification of miRNAs is important to know their functional roles. Predicting miRNA targets in plants is much easier due to the high and significant complementarities to miRNA-mRNA targets. A total of 37 target genes were identified for the 9 miRNA families. The target genes were of transcription factors (8%), enzymes (30%), transporters (14%) and others involving various physiological and metabolic processes (48%). Improved understanding of molecular mechanisms of miRNAs in tea plants will help in better understanding of post-transcriptional gene silencing in response to drought and other stresses and in the development of more precise techniques.