



CHAPTER VI

SUMMARY

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Tea is an economically important beverage crop in the world. It is a woody perennial with a life span of more than 60 years. Hence, tea plantations often encounter several environmental stresses. Amidst all, drought is an important recurrent limiting factor to world tea cultivation which causes around 40% crop loss. Most of the tea growing areas in India and other countries are prone to drought. Owing to the fast climatic changes and water limitation, selection and/or development of cultivars that can withstand drought condition is an urgent need in modern agriculture. This study was undertaken for investigating the drought stress responses of tea genotypes with contrasting characters of drought tolerance at anatomical, physiobiochemical and molecular level in particular.

A drought experiment was conducted with two year-old tea seedlings of S.3/A3 (drought-susceptible) and TV-23 (drought-tolerant) cultivars under controlled greenhouse conditions (at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, $25 \pm 2^\circ\text{C}$ with 65-70% of relative humidity) in a completely randomized block design. 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. Oxidative metabolism was found as the prime defense mechanism of tea plants under drought stress which brings a cascade of physiobiochemical changes. The closing of stomata, elevated level 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. A higher level of membrane integrity was also observed in drought-tolerant cultivar under stress.

Construction of a cDNA library and analysis of cDNA clones through ESTs approach have provided several advantages in acquiring data and gathering information on many aspects of plant biology at molecular level. For the analyses of drought responsive transcriptome of tea roots, two intra-varietal and one inter-varietal SSH cDNA libraries were constructed. One standard full-length cDNA library was also

constructed using SMART protocol to be used as reference. A total of 3250 colonies randomly picked and sequenced which finally resulted 1701 drought induced (Genbank accessions: GH734203 to GH734851 of FSL1 and FSL2 libraries as well GT968791 to GT969386, GW316843 to GW317159 and GW315010-GW315149 of FSL3 library) and 811 standard full-length ESTs (Genbank accessions: GH623575 to GH624058 and HS389643 to HS389969). A total of 572 quality ESTs of the inter-varietal SSH library were produced 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. Amino acid, leucine was found as highest coded (9.37%) and methionine as least coded (1.98%) in the unigenes. There were 85 quality protein domains detected of which 74 found to be conserved on the basis of CDD of NCBI as well as 11 EST-SSRs were identified in the unigenes. A total of 76% drought induced unigenes were assigned to functional categories i.e biological process, cellular component and molecular function, as defined in *Arabidopsis* proteome which clearly revealed that 13.04% of genes were associated to stress. Comparison of tissue specific expression of transcripts i.e leaves and roots under drought suggested the involvement of different genes in each of these tissues.

A total of 346 standard full-length quality ESTs were collapsed into 207 unigenes comprising 58 contigs and 149 singlets. There were only 10 numbers of standard genes were found common with the drought induced unigenes. This result suggested the involvement of different genes under normal growth and drought stress of roots. Moreover, a comparative transcripts analysis under normal growth, winter dormancy stress and drought stress of leaves was also performed with the help of available ESTs in the public domain. It was found that drought stress has been more lethal to the growth and development of tea plants in comparison to winter dormancy stress.

Finally, a total of 123 drought associated genes were identified including well-known drought associated genes such as dehydrin, trehalose-6-phosphate synthase, Cu/Zn SOD, GR 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. This study provides a basis for studying drought tolerance mechanism of this important commercial crop for the first time which will also be a valuable resource for functional genomics study of woody plants in future.

In an attempt to identify new conserved miRNAs, previously known plant miRNAs were BLASTed against all the available ESTs and full length nucleotide sequences of tea. The sequences showing homolog no more than four mismatches were predicted for their fold back structures and passed through a series of filtration criteria, which finally led to the identification of 13 conserved miRNAs belonging to 9 miRNA families. A total of 37 potential target genes in *Arabidopsis* were identified subsequently for 7 miRNA families based on their sequence complementarity which encode transcription factors (8%), enzymes (30%) and transporters (14%) as well as other proteins involved in physiological and metabolic processes (48%). These findings will accelerate the way for further researches of miRNAs and their functions in tea.