Chapter VI

General summary
Plants are continuously exposed to a variety of environmental conditions, abiotic (high salinity, drought, heavy metals pollution, extreme temperatures etc) or biotic (pathogens, pests), that limit their growth and productivity. Such conditions are commonly referred to as environmental stress. When a plant is exposed to abiotic stress, the expression of many genes is altered to induce protection against the negative effects of the stress. It has now become clear that increased protection involves a complex regulatory network that mediates morphological, physiological, biochemical and molecular changes. Understanding such changes has been of key importance for breeding plant resistance to abiotic stress. Breeding crop varieties with improved performance under suboptimal growing conditions is now one of the ambitious, but crucial objectives in modern plant biotechnology.

Tea yield is greatly influenced by weather, and especially by droughts, which causes irreparable losses because tea is a rainfed crop and irrigation is seldom used in tea plantations. Under these circumstances, the tea industry in India is clearly vulnerable to predicted climate changes, and subsequently greater economic, social, and environmental problems. On a limited and local level drought can be combated with irrigation which is subject to availability of sufficient water. But with global climatic change water is increasingly becoming scarce. Therefore on a large scale fighting drought with irrigation is impossible. Hence the alternative is to find out and develop such plants which have genetically controlled inherent tolerance to drought. Against this background the emphasis of the present study was to explore functional genomics for molecular breeding to improve water deficit stress tolerance in tea. As a contribution to the development and implementation of MAS approach in tea, the objective of this research was to develop functional markers and gene targeted markers which will facilitate MAS for drought tolerance. The concept here was to study whole genome transcriptome polymorphisms between drought tolerant and susceptible genotypes and use transcript derived fragments and candidate genes identified from differential gene expression study to assess stress tolerance mechanisms in the land races or wild lines that show elevated tolerance.
The availability of in-house germplasm of tea and relevant information formed the foundation for initial part of the work. From the earlier studies, done at Tocklai Experimental Station, it was found that TV23 can be considered as drought tolerant and S.3A/3 as drought susceptible cultivar. A comparison of drought tolerance between these two cultivars (TV23 and S.3A/3) was conducted to establish their contrasting characters by carrying out induced drought stress experiment in control condition. The changes in relative water content, leaf water potential, transpiration rate, water use efficiency, photosynthesis rate and stomatal conductance were measured for progressive drought stress till permanent wilting of the plants of both cultivars were observed. From this experiment, it was concluded that tolerance or susceptibility to drought has distinct genetic basis with TV23 being more tolerant than S.3A/3. Based on physiological data (basically WUE), the extent of drought induction was divided into two stages - before wilting (BW) and wilting (W). The BW and W samples were used for whole genome transcript profiling experiments (cDNA AFLP and SSH). As drought stress is multigenic in nature and the gene functioning would vary with the type of organs considered, the present work focuses on the genes differentially expressed during ‘BW’ and ‘W’ stage of drought in 3rd and 4th leaves of the plant.

The cDNA AFLP technique was employed to identify differentially expressed transcripts/genes in the tolerant cultivar (TV23), whose expression is responsive to drought stress. The genetic differences between the two cultivars at different stages of drought induction were thus visualized as polymorphisms in the transcriptome. 108 TDFs were identified as differentially expressed in tolerant cultivar, out of which 89 sequences could be obtained. Fifty nine of them showed homology in the public databases, while 30 TDFs did not show significant matches. Functional ontology based on molecular processes showed genes related to carbohydrate metabolism, stress response, protein modification process and translation associated with drought response. Functional ontology based on molecular function showed that genes having antioxidant activity, calcium ion binding, RNA binding were up-regulated more in tolerant cultivar. On the basis of cellular component, genes related to plastid, ribosome, plasma membrane, vacuole and
mitochondria were found to be up-regulated. The study also suggested that raffinose oligosaccharide pathway (RFOs) is up-regulated during drought stress in tea.

In the second approach, PCR based SSH technique was used to identify transcripts/genes responsive to drought stress. Four SSH libraries were constructed from samples collected at BW and W stage. Library analysis showed several transcripts specific to BW stage of TV23 and they appear to be much more important as these are the early responsive genes. The analysis showed presence of MAP Kinase, serine/threonine kinase, phospholipid signaling and calcium mediated signaling up-regulated during drought stress. KEGG based pathway analysis showed presence of flavonoid biosynthesis, phenylpropanoid biosynthesis, tyrosine metabolism, tryptophan metabolism, lysine biosynthesis, proline metabolism, glutathione metabolism and amino sugar and nucleotide metabolism related enzymes which were associated with drought response. Gene ontology analysis identified genes having hydrolase activity, peptidase activity, (cinnamoyl CoA) reductase activity, GTP binding activity, (amine) oxidase activity, (diaminopimelate) decarboxylase activity and zinc ion binding activity. The TV23 transcriptome also showed transcripts that encode proteins involved in the protection of cells from the effects of reactive oxygen species. But it is difficult to conclude that the mechanism of drought-tolerance in tea is limited to only transcriptional up-regulation of these genes. There is also a possibility that post transcriptional modification may play a crucial role in drought tolerance mechanisms in tea. The purpose of this study was to compare two contrasting tea cultivars and to generate a resource to initiate gene-by-gene analysis for the trait. The genes identified on the basis of SSH experiment corroborate with the results from similar studies on other plants.

Drought tolerance is a combined effort of many loci resulting in adaptation at physiological as well as molecular levels which greatly depend on genetic × environment (G × E) interactions. Tea plants can grow up to a 10-15 m tall tree (C. assamica) or a 5-8 m tall shrub (C. sinensis), if they are allowed to grow uninterrupted. However, in commercial plantations, tea is maintained (by pruning) as a bush of about 1 m tall, having a flat-topped foliage canopy with
a depth of about 0.6 m. Thus, in normal field condition, the tea plant is always under stress. There is a probability that a gene which was found as drought stress induced in pot experiment in control condition may show constitutive expression in field or vice-versa. Since field drought is a slow process and the plants go through an adaptive process in contrast to the drastic condition of rapid dehydration in case of pot experiments, the validation of expression of the identified genes was done under field conditions using a parent progeny combination (TS-463). Now, the aim was to identify genes and physiological functions that are central to the establishment of drought tolerance in tea. This study was done to get an overview of correlation and/or association between transcript abundance (of selected drought induced genes) and physiological parameters during field drought condition. In the present study drought tolerant progenies showed symptoms of drought stress earlier than susceptible progenies but survived longer than the susceptible progenies. It is inferred that the tolerant progenies are able to sense water stress much earlier and the morphological effects thus seen, is part of their adaptive preparation for the forthcoming stress. The DT/TS-463 progenies were able to maintain WUE upto 70th days of water deficit stress (DWS) whereas DS/TS-463 progenies showed nil WUE after 45th DWS. The selected drought induced genes showed four types of expression pattern at three stages of water deficit stress. Most of the genes in DT/TS-463 exhibited III and IV expression pattern. Pattern IV includes genes (dehydren, ABA ripening protein, glutathione peroxidase, cinnamoyl CoA reductase, calmodulin binding protein) which showed higher expression at before wilting stage. The expression of these genes may be related with the ability of DT/TS-463 to maintain turgor at low soil water content along with WUE. Enhance ROS scavenging which results in an improved detoxification system also seems to provide tolerance to DT/TS-463 progenies with the higher expression of genes such as glutathione peroxidase and glutathione S-transferase. Effort was made through statistical clustering analysis, to see if physiological traits related to water stress and its components were correlated with molecular traits related to gene expression and transcript accumulation in the third and fourth leaves of tea. It was observed that physiological parameter like WUE formed a close group with genes such as calmodulin binding protein, drm3, hexose
transporter, hydrogen peroxide induced protein, ACC oxidase, lipase, ethylene responsive transcription factor and diaminopimelate decarboxylase. This means that for tolerance during wilting stage, the above mentioned genes and WUE can be the potential candidate markers. The PCA analysis (bi-plot) separated DT/TS-463/BW from DT/TS-463/W. Thus, between the germplasm there is a high variability of the studied trait as they are separated on the basis of time-points during drought and this variability is genetically controlled.

The present work generated an EST database which includes genes that were strongly expressed in response to drought in tolerant cultivar. Detailed characterization of several genes, including putative novel genes and genes of unknown function, which may be involved in specific processes, will help to unravel the fine networks underlying drought tolerance in tea. Mere understanding of the genes associated with drought tolerance is not enough; their mechanism of functioning particularly at different time point and in different organs is equally important. It can be inferred that the present work made substantial contribution in this direction. In conclusion, it can be opined that the present work contributed to revealing the genetic components and their mode of functioning which trigger a cascade of diverse biochemical reaction culminating in drought tolerance. The five progenies identified from the present work and the information generated can be used in future breeding programme with the objective of developing drought tolerant tea cultivars in aid of tea industry.