Chapter 6

Conclusions
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Present study was focusing upon two main objectives, identification of sequence based markers, *i.e.* microsatellite markers and their utilization of genetic diversity and phylogenetic analysis in tea because only a limited numbers (15 Nos. only) of such markers exists in tea. In an another objective, highly polymorphic microsatellite markers identified during this study were compared with AFLP markers for genetic diversity analysis to find out at what extent and which markers system proved to be better for future markers based studies and genetic improvement of tea. Considering, the heterogeneous nature of tea, cpDNA loci have also been cloned and sequenced in multiple accessions for major varietal types (China, Assam, Cambod) and selected five other related *Camellia* species to find out the species/ varietal specific markers.

Genetic analysis in tea has been hindered due to the highly cross-pollinated complex genome and lack of sufficient number of sequenced based markers. In the present study, 116 novel microsatellite markers have been identified and validated in tea. Fifty two have been developed from the (GA)n enriched genomic libraries of tea and named as Tea Enriched Genomic MicroSatellite (TEGMS) markers (Bhardwaj *et al.*, 2010; communicated). Another set of 61 novel microsatellite markers have been developed from mining and prediction of unigenes from publicly available expressed sequence and named as Tea Unigene derived MicroSatellite (TUGMS) markers. These markers have successfully characterized and validated for genetic diversity and phylogenetic analysis in selected accessions of beverage tea and related *Camellia* sps. and therefore demonstrated their immense use in various genotyping, genetics, genomics and breeding studies in tea. One hundred sixteen novel markers developed and experimentally validated will be enriching the limited existing microsatellite markers resource in tea (Sharma & Bhardwaj *et al.*, 2009). Further, functional categorization of these TUGMS markers corresponded to many genes with biological, cellular and molecular functions; and also recorded the allelic variation in tea, hence offers an opportunity to investigate the consequences of SSR polymorphism on gene functions. In conclusion, novel sequence based TEGMS and TUGMS markers would reduce the cost and facilitate genetic diversity assessment, gene mapping and marker-aided selection in tea. For the first time, comparatively larger set of tea specific microsatellite markers have been identified in the present study.
Comparison of AFLP and microsatellite markers for the first time in tea for genetic diversity in commercially important 53 Indian tea germplasm demonstrated the utility of SSR markers in revealing heterogeneous nature in tea. However, considering the genome size of crop (which is 4.0 GB), larger numbers of such markers and other sequence based markers needs to be identified in tea. AFLP with high multiples ratio also found to be good markers but unable to distinguishing some of the closely related accessions.

The sequence comparisons of chloroplast genome highlight the species specific regions in the cultivated and wild species. Markers (In/dels; SNPs) specificity in the genome of tea and related wild species will helpful in characterizing the gene flow in the commercial cultivars and will also be helpful in identifying important commercial cultivars with wild species for identification of parental groups for the future breeding programme in tea.

The findings of this study can be a good source of molecular markers and markers based information for future tea breeders because it eliminates the limitations and shortcomings of selection methodology based on morphological, biochemical, and physiological data, which are easily influenced by environmental conditions and the age of the plant. Detailed information about the level of genetic diversity using AFLP and SSR marker systems in the selected standard, quality, and high-yielding tea accessions would be useful in proper identification, management, utilization, and strategizing in future tea breeding programs at the regional and national levels.