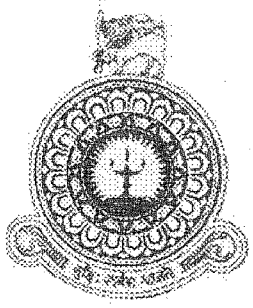

Application of molecular markers for genetic conservation and Breeding of *Camellia sinensis* L. (tea)

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ABSTRACT

Effective conservation and utilization of genetic resources of tea are essential for sustainability and increasing productivity of this valuable export plantation crop. No systemic assessments of genetic variability in tea have been carried out in Sri Lanka except for a few preliminary morphological or isozyme based studies. In the present study, DNA markers, Randomly Amplified Polymorphic DNAs (RAPDs) and Amplified Fragment Length Polymorphisms (AFLP) were applied to elucidate genetic relatedness of Sri Lankan tea (*Camellia sinensis* L.) genetic resources.

Twenty RAPD primers that produced consistent amplifications in pretesting were selected and used to generate 223 polymorphic loci. A considerable range of genetic diversity (genetic distances ranging between 0.17- 0.58) was observed with an average genetic distance of 0.37. In both 'TRI cultivars' and 'estate cultivars' not much diversity was found between (0.38) or within two groups (0.35 & 0.33 respectively). Two phenotypically distinct cultivars, 'Yabukita' and 'China' varied significantly from both 'TRI cultivars' and 'estate cultivars'. Furthermore, 'TRI 777', 'TRI 2016' and 'TRI 4006' cultivars demonstrated a higher degree of genetic divergence. 'TRI 777' showed closer relationship to 'estate cultivars' (0.39) than to 'TRI cultivars' (0.42). Cultivars 'TRI 2016', 'TRI 4006', 'TRI 777', 'China' and 'Yabukita' have been identified as progenitors that could be very effective in generating heterosis in the breeding programmes. The clear separation of 'TRI cultivars' and 'estate cultivars' into two distinct clusters in the dendrogram, confirmed most of their origins. The sub clusters within the main clusters clearly conformed parentages and historical pedigree records of tea germplasm accessions studied.

In the AFLP study, as revealed in the RAPD study, 'estate cultivars' were separated into two separate groups with HS10A and MT 18 separating from the rest. Distinct accession 'Yabukita', TRI 777, TRI 4071 and TRI 4079 also illustrated a closer affinity to 'Assam' and 'China' hybrids. Furthermore, in the AFLP study too, most of the 'TRI developed cultivars' clustered together confirming their similar origin of the ancestral type ASM 4/10. The clustering pattern of accessions TRI 4071, TRI 4079, TRI 777, TRI 3072, TRI 4052 and TRI 2043 was in agreement of their historical pedigrees as well as the findings of the present RAPD study.

Hence results generated from the AFLP study confirmed the results of RAPD study and pedigree data. Again, the sub clusters within the main clusters reflected the parentages and nicely confirmed historical pedigree data.

The major limitation for the application of DNA markers in tea is unavailability of tea-specific SSR primers. In the next part of this study, isolation and characterization of genomic SSR primers were attempted. In conventional method, cultivar TRI 2023 was used to construct a λ zap genomic library. Sequences generated from the genomic clones consisted with SSR motifs $(GAA)_5$ and $(CA)_5$. In the enriched method, cultivar TRI 2023 was used for the development of $(CA/GT)_n$ and $(CTT/GAA)_n$ enriched genomic libraries. Out of the sequences generated, 945 (84%) were mined for SSRs, where 339 (36%) were identified as containing SSR-positive sequences. Two hundred and seventy five of above 945 sequences consisted of perfect microsatellite repeat motifs (29.1%) and 252 (26.7%) were imperfect repeats. A total of 625 SSRs (331 perfect and 294 imperfect) were detected. Majority of the isolated perfect repeats were di- nucleotides (67%) followed by tri-nucleotides (27%). $(CA/GT)_n$ and $(CTT/GAA)_n$ were predominant in the genomic libraries. A total of 305 (157 perfect and 148 imperfect) genomic-SSR primer pairs (PPs) were developed.

Construction of detailed genetic linkage maps is a fundamental first step towards application of marker assisted breeding (MAB) in cultivar improvement programs. Although a practical genetic map is not available for tea, the potential of maker assisted selection is clear and strong. Thus, an attempt was made to construct a genetic linkage map of tea. F_1 full-sib population was developed according to a two-way pseudo-testcross strategy. Two parents and six F_1 progenies were first screened with 384 genomic and EST- SSR primer pairs, and 216 polymorphic primers were identified. Finally, 104 of the EST and 86 of the genomic SSR primers were used for construction of two genetic linkage maps. The male parent (TRI 2023) map was constructed with 83 EST- SSR and 97 genomic SSR loci. A total of 180 markers were grouped in 16 linkage groups (LGs) covering a total length of 1,227 cM with an average marker density of 6.8 cM/ marker. In the female parent (TRI 2043) map, 146 markers were clustered in 15 LGs spanning 1,018 cM with an average distance of 7.0 cM between adjacent markers. Fifteen LGs were consistent with the basic chromosome numbers of tea.