S.L.J Tea Sci. 70(1), 42-53, 2005, *Printed in Sri Lanka*

Studying Genetic Relationships among Tea (*Camellia sinensis* L.) Cultivars in Sri Lanka Using RAPD Markers

[1]K M Mewan, [1]A C Liyanage[1], [2]J M D T Everard, [1]M T K Gunasekare and [3]E H Karunanayake
([1]Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka
[2] Coconut Research Institute of Sri Lanka, Lunuwila, Sri Lanka
[3] Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Colombo 03, Sri Lanka.)

ABSTRACT

Genetic variability is one of the key factors, which decide the success of a plant-breeding programme. Therefore, it is important for the plant breeder to identify the sources of genetic variability in the available germplasm and thereafter, exploit the variability, which promotes the effective conservation and sustainable utilization of plant genetic resources. Molecular makers offer several new possibilities for accessing genetic variability. In the present study, genetic relatedness of 39 Sri Lankan tea (*Camellia sinensis* L.) cultivars was assessed by Random Amplified Polymorphic DNA (RAPD) marker technique.

Twenty RAPD primers that produced consistent amplifications were selected and used for this study to generate 223 polymorphic markers. 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 selections' not much diversity was found between (0.38) or within two groups (0.35 and 0.33 respectively). Two phenotypically distinct cultivars, 'Yabukita' and 'China' varied significantly from both 'TRI cultivars' and 'Estate selections'. Furthermore, 'TRI 777', 'TRI 2016' and 'TRI 4006' cultivars demonstrated a higher degree of genetic divergence. 'TRI 777' showed closer relationship to estate selections (0.39) than to TRI developed cultivars (0.42). Furthermore, although 'TRI 777' clustered with 'China' and 'Yabukita', it was considerably genetically different from both 'Yabukita' (0.45) and 'China' (0.46). 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.

While the clear separation of 'TRI cultivars' and 'Estate selections' into two distinct clusters in the dendrogram confirmed most of their origins, the sub clusters within the main clusters conformed nicely with their parentages and historical pedigree records.

Key words: *Camellia sinensis* L., Conservation, Diversity, Molecular markers, RAPD, Tea.

INTRODUCTION

Plant breeding refers to the genetic improvement of a plant as required by the people concern, by means of manipulating its' genetic constitution by selection and/or by hybridization (the main two tools of classical breeding) or by creating transgenic plants (modern times). Identification of sources of genetic variability is one of the major factors involved in any plant-breeding programme (Banerjee, 1992). Accurate identification of parents based on true genetic differences is a more appropriate approach for breeding of crops, especially of perennial nature such as tea (*Camellia sinensis* L). Therefore, identification of genetically diverse tea cultivars is of paramount importance for prioritization of parents for generating heterosis by hybridization.

Over 55% of the replanted teas in Sri Lanka are clonal and most of the growers have used a limited number of tea cultivars in their plantations. Many of such cultivars are selections made from open pollinated (O.P.) progenies of a single genetic stock, ASM 4/10. Reliance on a limited number of selected cultivars threatens diversity and seriously narrow down the genetic diversity available for commercial use. In addition, it can cause detrimental effect in the aftermath of a natural disaster as drought, pest attack etc. To ensure sustainable tea production, scientifically based conservation of tea cultivars should be given high priority. In order to conserve valuable genetic material of both the cultivated gene pool and the diversity available within the natural tea population in Sri Lanka, an accurate assessment of the level of genetic variation is a prerequisite. This will enable the tea breeder to establish core collections that are representative of the total available genetic variation with minimum duplications in germplasm collections.

Furthermore, exploitation and characterization of available genetic variability of tea is an essential, important and fundamental step towards strengthening the breeding programmes in addition to the effective conservation and utilization of tea germplasms in Sri Lanka. Presently, the characterization of tea cultivars available in Sri Lanka is restricted to morphological descriptors or phenotypic characters which show a continuous variation and a high degree of plasticity. Therefore, comparison of tea cultivars by morphology alone could often lead to inaccurate conclusions and is not sufficient to identify diverse cutivars to be used in breeding programmes. Up to date, there are no published reports on systematic assessment of genetic variability in tea in Sri Lanka except for the comparison of tea cultivars by morphology and isozyme technique (Gunasekare *et al.* 2001; Liyanage *et al.*, 1999; Wickramaratne, 1981). These morphology and isozyme based studies too failed to reveal much information on tea cultivars due to their limitations in detection of sufficient number of polymorphic markers.

With the rapid development of molecular biology, plant breeders have been provided with a number of new powerful tools to allow crop improvement to proceed at a very rapid and higher scale. In view of the ease of using DNA based characterization systems, a study was conducted to screen some of the tea cultivars using Random Amplified Polymorphic DNA Marker (RAPD) technique to develop a molecular-marker based criterion for characterisation of tea germplasm of Sri Lanka.

MATERIALS AND METHODS

Plant materials

The plants assayed were a sample of germplasm of tea, comprising 22 'TRI cultivars', 14 'Estate selections' and three phenotypically distinct cultivars, namely 'Yabukita', 'China' and 'ASM 4/10', conserved at *ex-situ* gene bank at the Tea Research Institute (TRI) at Talawakelle, Sri Lanka (Table 1).

Table 1: Tea accessions used for RAPD analysis their parentage/pedigree

Code No:	Accession	Parentage/ Pedigree
1	TRI 2016	Seedling selection from St. Coombs estate
2	TRI 2023	ASM 4/10 O.P. introduced from Tocklai
3	TRI 2023 4X	Introduced tetraploid of TRI 2023
4	TRI 2025	ASM 4/10 O.P. introduced from Tocklai
5	TRI 2026	ASM 4/10 O.P. introduced from Tocklai
6	TRI 2027	ASM 4/10 O.P. introduced from Tocklai
7	TRI 2043	Shan Bansang 777
8	TRI 3013	TRI 2024 O.P.
9	TRI 3016	DT95 X ASM 4/10
10	TRI 3018	TRI 2024 X DT1
11	TRI 3020	TRI 2025 O.P.
12	TRI 3022	TRI 2026 X 2023
13	TRI 3025	TRI 2025 O.P.
14	TRI 3069	Induced tetraploid of TRI 2025
15	TRI 3072	TRI 2025 O.P.
16	TRI 4006	TRI 2023 X TRI 2026
17	TRI 4052	CY9 X ASM 4/10
18	TRI 4061	TRI 2020 X TRI 2023
19	TRI 4071	N2 X TRI 2024
20	TRI 4079	N2 SP
21	TRI 62/9	ASM 4/10 O.P. introduced from Tocklai
22	TRI 777	Shan Cho-Long No. 777
23	ASM 4/10	Clone of a bush from stock No124, Tocklai
24	CY 9	Seedling selection from Tangakelle estate
25	DN	Seedling selection from Dayagama estate

26	DT 1	Seedling selection from Drayton estate
27	DT 95	Seedling selection from Drayton estate
28	DG 39	Seedling selection from Balangoda estate
29	HS10A	Seedling selection from Heathersett estate
30	KEN 16/3	Seedling selection from Kenilworth estate
31	MT 18	Seedling selection from Balangoda estate
32	N2	Seedling selection from Norwood estate
33	NAY 3	Seedling selection from Nayabedde estate
34	PK 2	Seedling selection from Park estate
35	S 106	Seedling selection from Sirikandura estate
36	TK 48	Seedling selection from Talankande estate
37	UH 9/3	Seedling selection from Uva Highlands est.
38	China	Introduction from China
39	Yabukita	Introduction from Japan

SP – Self pollinated O.P. – Open pollinated

Isolation of DNA

DNA was extracted from fresh tender leaves (two leaves and a bud) using a modified CTAB method (Doyle and Doyle, 1990 and Weising and Kahl, 1997).

DNA amplification

Twenty random primers were selected from a stock of 100, 10-mer OPERON primers (Operon Technologies Inc., Alamada, California, USA.), after screening DNA of three tea cultivars ('TRI 2023', 'TRI 2026' and 'TRI 2043') for amplification consistency, for the RAPD assay. The amplification reaction was carried out as described by Williams *et al.* (1990). Amplified DNA products were separated on 1.5% agarose/ethidium bromide gels and visualized under UV by Photo Print Gel Documentation System (Vilber Lourmat, France).

RAPD data analysis

Amplified DNA fragments were scored in all cultivars as 1 if present and 0 if absent and assembled in a data matrix. Nei and Li's (1979) similarity coefficient was employed to generate a genetic distance matrix. Cluster analysis (Unweighted Pair Group Method with Arithmetic Means – UPGMA) based on Nei and Li's (1979) genetic similarity coefficient was used to construct a dendrogram with the help of 'RAPDISTANCE' software developed by J. Armstrong, Australian National University, Canberra.

RESULTS

Genetic diversity of tea cultivars

The Distance matrix, based on Nei and Li's estimate is presented in Table 2. The average genetic distances within and between 'TRI cultivars' and 'Estate selections' were estimated (Table 3) and a comparison of genetic distances between most diverse cultivars against 'TRI cultivars' and 'Estate selections' (Table 4) was carried out. The near similarity of average distances within and between 'TRI cultivars' and 'Estate selections' clearly indicates a close genetic association of all the material. The morphologically distinctive cultivars, 'China' and 'Yabukita' however, varied considerably from the rest. The 'TRI cultivars', 'TRI 2016' and 'TRI 4006' also varied considerably compared to rest of the 'TRI cultivars', 'Estate selections' and 'Yabukita' and 'China'.

Table 2. The matrix showing distance values of 39 tea cultivars determined and estimated by Nei and Li's coefficient Names of 39 tea cultivars are given in the box.

1.TRI 2016 2.TRI 2023 3.TRI 2023 4X 4. TRI 2025 5.TRI 2026 6.TRI 2027 7.TRI 2043 8.TRI 3013 9.TRI 3016 10. TRI 3018 11.TRI 3020 12.TRI 3022 13.TRI 3025 14.TRI 3069 15.TRI 3072 16.TRI 4006 17.TRI 4052 18.TRI 4061 19.TRI 4071 20.TRI 4079 21.TRI 62/9 22.TRI 777 23.ASM 4/10 24.CY 9 25.DN 26.DT 1 27.DT 95 28.DG 39 29.HS10A 30.KEN 16/3 31.MT 18 32.N2 33.NAY 3 34.PK 2 35.S 106 36.TK 48 37.UH 9/3 38.China 39.Yabukita

.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1	0.0																																					
2	0.42																																					
3	0.35	0.18																																				
4	0.43	0.29	0.26																																			
5	0.37	0.29	0.26	0.26																																		
6	0.45	0.36	0.31	0.30	0.29																																	
7	0.41	0.42	0.33	0.42	0.41	0.41																																
8	0.37	0.34	0.27	0.32	0.33	0.30	0.41																															
9	0.38	0.31	0.32	0.32	0.35	0.32	0.38	0.27																														
10	0.38	0.32	0.32	0.33	0.34	0.33	0.40	0.31	0.26																													
11	0.39	0.36	0.31	0.31	0.33	0.33	0.38	0.34	0.36	0.32																												
12	0.40	0.30	0.24	0.29	0.27	0.34	0.38	0.31	0.32	0.31	0.28																											
13	0.41	0.34	0.26	0.27	0.29	0.34	0.36	0.37	0.34	0.31	0.29	0.27																										
14	0.41	0.36	0.27	0.19	0.28	0.28	0.37	0.31	0.26	0.29	0.26	0.34	0.25																									
15	0.39	0.42	0.31	0.32	0.33	0.33	0.27	0.34	0.35	0.36	0.34	0.37	0.33	0.27																								
16	0.48	0.43	0.40	0.38	0.32	0.38	0.50	0.48	0.44	0.39	0.46	0.42	0.39	0.43	0.44																							
17	0.39	0.37	0.33	0.31	0.33	0.35	0.39	0.30	0.32	0.31	0.36	0.37	0.35	0.29	0.27	0.46																						
18	0.43	0.31	0.29	0.36	0.38	0.39	0.44	0.36	0.38	0.33	0.39	0.34	0.35	0.37	0.37	0.41	0.37																					
19	0.38	0.34	0.28	0.32	0.31	0.38	0.39	0.30	0.33	0.29	0.35	0.32	0.38	0.28	0.30	0.41	0.31	0.32																				
20	0.34	0.34	0.31	0.31	0.33	0.32	0.36	0.30	0.30	0.29	0.30	0.31	0.37	0.28	0.28	0.44	0.29	0.38	0.26																			
21	0.48	0.41	0.44	0.36	0.46	0.42	0.44	0.44	0.35	0.41	0.42	0.44	0.42	0.38	0.43	0.48	0.42	0.44	0.44	0.36																		
22	0.38	0.26	0.25	0.30	0.30	0.25	0.36	0.28	0.31	0.30	0.31	0.33	0.32	0.27	0.30	0.43	0.29	0.31	0.31	0.27	0.33																	
23	0.44	0.33	0.28	0.35	0.34	0.35	0.41	0.32	0.33	0.36	0.39	0.35	0.38	0.34	0.35	0.44	0.34	0.39	0.32	0.30	0.43	0.24																
24	0.44	0.35	0.34	0.36	0.37	0.34	0.37	0.40	0.38	0.38	0.34	0.39	0.40	0.36	0.38	0.45	0.34	0.38	0.35	0.36	0.39	0.34	0.40															
25	0.43	0.42	0.36	0.44	0.35	0.42	0.39	0.43	0.42	0.43	0.34	0.41	0.40	0.38	0.39	0.47	0.38	0.41	0.36	0.40	0.43	0.35	0.42	0.35														
26	0.48	0.40	0.36	0.43	0.44	0.36	0.41	0.37	0.34	0.35	0.31	0.37	0.38	0.39	0.40	0.48	0.41	0.36	0.34	0.39	0.46	0.36	0.46	0.31	0.40													
27	0.46	0.41	0.34	0.44	0.37	0.39	0.36	0.34	0.29	0.32	0.36	0.35	0.41	0.36	0.37	0.50	0.38	0.41	0.34	0.40	0.45	0.32	0.38	0.33	0.38	0.31												
28	0.49	0.40	0.37	0.42	0.38	0.36	0.38	0.40	0.39	0.38	0.40	0.41	0.43	0.33	0.38	0.48	0.39	0.37	0.35	0.35	0.34	0.32	0.40	0.33	0.38	0.37	0.33											
29	0.42	0.36	0.36	0.41	0.32	0.41	0.40	0.37	0.37	0.33	0.35	0.33	0.41	0.34	0.37	0.50	0.37	0.38	0.33	0.35	0.40	0.29	0.36	0.37	0.37	0.38	0.28	0.35										
30	0.45	0.42	0.29	0.40	0.35	0.31	0.35	0.32	0.36	0.38	0.31	0.35	0.36	0.30	0.30	0.42	0.37	0.35	0.31	0.36	0.43	0.30	0.35	0.31	0.28	0.29	0.30	0.32	0.35									
31	0.41	0.33	0.29	0.33	0.32	0.33	0.35	0.35	0.34	0.33	0.30	0.36	0.35	0.27	0.34	0.45	0.35	0.35	0.30	0.32	0.32	0.26	0.38	0.25	0.30	0.31	0.27	0.17	0.31	0.26								
32	0.42	0.41	0.34	0.36	0.34	0.35	0.39	0.36	0.35	0.36	0.31	0.35	0.36	0.30	0.35	0.43	0.37	0.37	0.29	0.34	0.37	0.33	0.43	0.31	0.33	0.29	0.34	0.28	0.29	0.26	0.19							
33	0.43	0.36	0.35	0.38	0.39	0.36	0.32	0.35	0.42	0.34	0.37	0.40	0.40	0.36	0.34	0.49	0.38	0.49	0.33	0.31	0.31	0.35	0.43	0.33	0.48	0.35	0.33	0.33	0.34	0.31	0.34	0.3						
34	0.46	0.41	0.37	0.40	0.43	0.42	0.37	0.40	0.39	0.37	0.38	0.35	0.42	0.40	0.38	0.50	0.39	0.44	0.39	0.39	0.39	0.36	0.43	0.33	0.39	0.37	0.30	0.37	0.34	0.30	0.30	0.32	0.31					
35	0.42	0.42	0.33	0.40	0.33	0.35	0.38	0.35	0.35	0.37	0.32	0.35	0.38	0.34	0.30	0.42	0.35	0.40	0.32	0.34	0.41	0.32	0.38	0.34	0.32	0.31	0.31	0.34	0.25	0.27	0.31	0.28	0.33	0.29				
36	0.51	0.42	0.41	0.45	0.44	0.35	0.42	0.42	0.38	0.39	0.36	0.46	0.42	0.39	0.42	0.45	0.42	0.40	0.41	0.41	0.41	0.34	0.43	0.30	0.36	0.28	0.36	0.31	0.40	0.29	0.29	0.35	0.32	0.33	0.28			
37	0.45	0.39	0.39	0.43	0.41	0.41	0.36	0.43	0.35	0.43	0.42	0.41	0.46	0.39	0.42	0.50	0.41	0.38	0.41	0.35	0.35	0.34	0.41	0.39	0.41	0.37	0.39	0.33	0.33	0.33	0.34	0.38	0.34	0.38	0.34	0.35		
38	0.45	0.47	0.49	0.52	0.46	0.47	0.43	0.42	0.41	0.43	0.46	0.50	0.50	0.46	0.48	0.56	0.44	0.52	0.47	0.42	0.46	0.43	0.40	0.42	0.42	0.48	0.47	0.45	0.42	0.46	0.46	0.44	0.44	0.43	0.44	0.45	0.41	
39	0.46	0.44	0.44	0.46	0.45	0.47	0.47	0.38	0.43	0.45	0.46	0.49	0.47	0.40	0.45	0.58	0.38	0.45	0.39	0.39	0.45	0.36	0.42	0.44	0.39	0.43	0.43	0.37	0.48	0.38	0.35	0.41	0.45	0.45	0.45	0.43	0.46	0.39

Table 3: Average genetic distances between and within 39 tea cultivars

Source of diversity	Avg. genetic distance	Range of the genetic distance
Within all the 39 cultivars	0.37	0.17 – 0.58
Within all the 'TRI cultivars' and 'Estate selections'	0.34	0.17 – 0.50
Within 'TRI cultivars'	0.35	0.18 – 0.50
Within 'Estate selections'	0.33	0.17 – 0.50
Between 'TR cultivars ' and 'Estate selections'	0.34	0.17 – 0.50

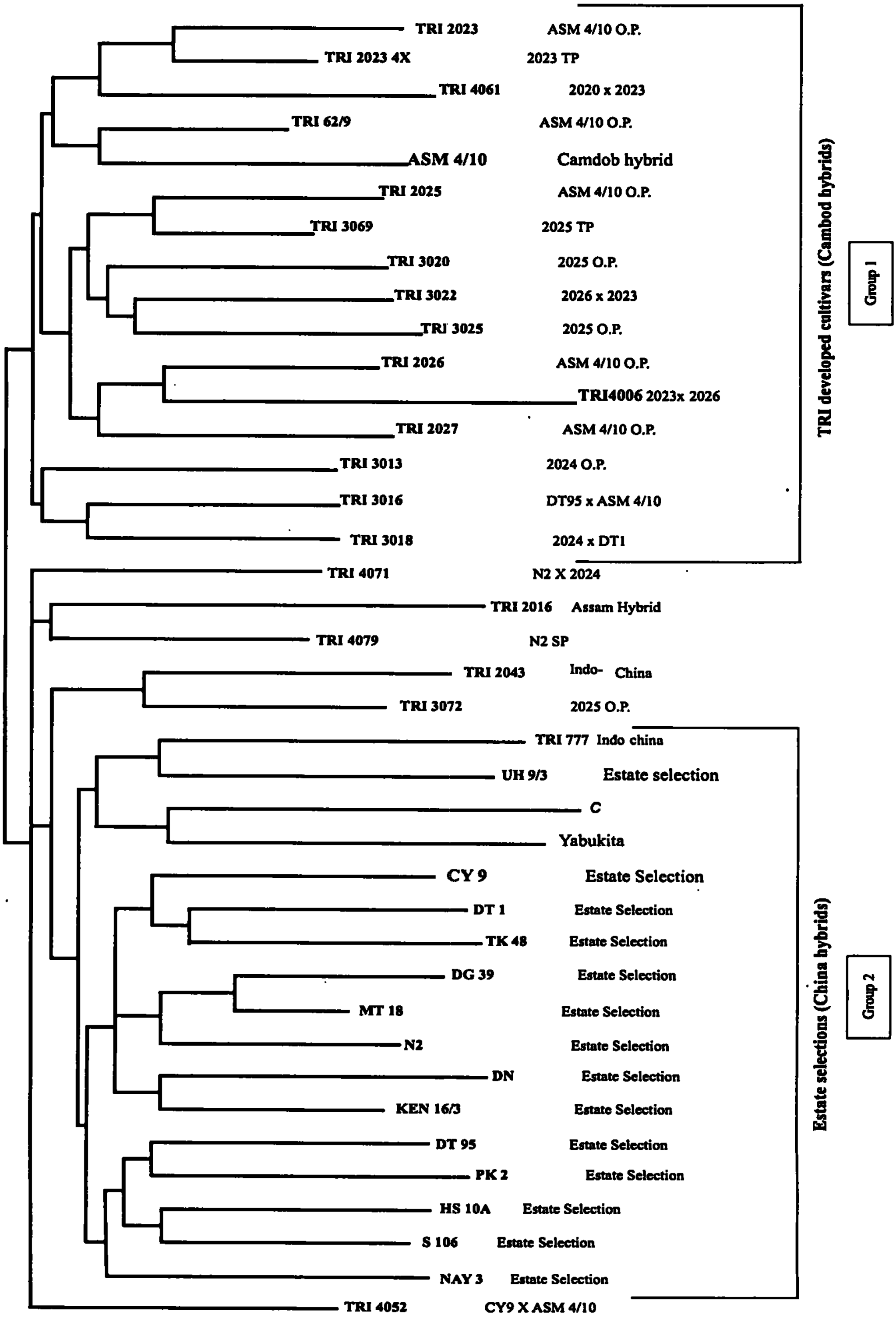
Table 4: Comparison of average genetic distance of the most diverse tea cultivars

Cultivar	Average genetic distance						
	Overall	TRI cultivars	Estate selections	TRI 777	TRI 2016	TRI 4006	China
TRI 777	0.39	0.42	0.39	-			
TRI 2016	0.42	0.40	0.45	0.48	-		
TRI 4006	0.45	0.43	0.47	0.48	0.48	-	
China0.44	0.48	0.44	0.46	0.45	0.56	-	
Yabukita	0.43	0.46	0.45	0.45	0.46	0.58	0.39

Genetic relationship between tea cultivars

A total of 223 polymorphic bands were used for the cluster analysis to construct the dendrogram. The dendrogram readily separated the 39 cultivars into two main clusters (Figure 1) with first cluster comprising only of 'TRI cultivars' (15 out of 22) together with phenotypically distinctive cultivar, ASM 4/10. The second cluster consisted of all of the 'Estate selections', 7 'TRI cultivars' and the two phenotypically distinct cultivars, 'China' and 'Yabukita'. It is interesting to note that all 'Estate selections' (except UH 9/3) formed a sub cluster within the second main cluster and this sub cluster is directly attached to the sub cluster formed by cultivars 'TRI 777', UH 9/3, 'China' and 'Yabukita' indicating a close genetic relationship between these cultivars. 'TRI 777' showed closer relationship to estate selections (0.39) than to TRI developed cultivars (0.42). Furthermore, although 'TRI 777' clustered with 'China' and 'Yabukita', it was considerably diverse from both distinct cultivars, 'Yabukita' (0.45) and 'China' (0.46).

Fig. 1: Dendrogram showing the genetic relatedness of 39 tea cultivars determined by cluster pair-wise genetic distances estimated by Nei and Li's coefficient and the NJTREE option of the RAPDISTANCE software package. 223 polymorphic RAPD fragments were used to construct this dendrogram



DISCUSSION

The RAPD based characterization clearly subdivided the cultivars tested into two main groups, one mainly comprising of "TRI cultivars" (15 'TRI cultivars' and distinct phenotype ASM 4/10) and the other predominantly with 'Estate selections' and 7 'TRI cultivars' and two phenotypically distinct cultivars 'China' and 'Yabukita'. The most popular 'TRI cultivars' of '2020 series' ('TRI 2021' up to 'TRI 2027') have been evolved from the open pollinated seeds from seed bearer, 'ASM 4/10', a 'Cambod' hybrid from India (Richards, 1965). Hence, grouping of 15 of the 22 TRI cultivars with 'ASM 4/10' clearly reflects a common origin of these cultivars. Subsequently the breeding programs at TRI, involving '2020 series', 'ASM 4/10' and some 'estate selections', resulted in development of '3000 series' and '4000 series' cultivars. In group I, sub-clustering of 'ASM 4/10' with cultivars of '2020 series', '3000 series' and '4000 series' confirms the common ancestry of all 'TRI cultivars'. Clustering of 'TRI 62/9' along with 'ASM 4/10' confirms the origin of 'TRI 62/9' which is a seedling selection made from the same seed source, 'ASM 4/10' later (Richards, 1965). In general, the group 1 cluster comprising of 'TRI cultivars' of the three TRI series, confirms the close genetic relationship of 'TRI cultivars', reflecting negatively on chances of achieving a further conspicuous improvement by making crosses within 'TRI cultivars' other than breeding for known inherited economic traits governed by major genes.

The second main group, which predominantly comprises of all 'Estate selections' assayed, indicates a close genetic relatedness of these selections made from elite bushes from naturally pollinated populations in tea estates in various locations of the country. The isozyme relationships too confirmed a similar separation of 'Estate selections' (Liyanage *et al.*, 1999). It is evident that materials received by Sri Lanka in the early days have originated from a more uniform seed stocks developed in India (Singh, 1979; Tubbs, 1939). Therefore, this pattern of grouping revealed the fact that prioritization of crosses between 'Estate selections' towards improvement by testing combining ability is not effective.

Cultivars 'TRI 4071' and 'TRI 4052' that are close to 'ASM 4/10' by their pedigree also grouped in cluster II with the 'Estate selections' because of the involvement of 'Estate selections', N2 and CY 9 respectively as one of their parents. Similarly, 'TRI 3072', which clustered with 'Estate selections', is an open pollinated progeny of 'TRI 2025' and it can be assumed that the male parent of this progeny may be related to some other cultivar of tea, which is not closer to 'ASM 4/10'. Therefore, use of these three cultivars as one of the parents with an 'Estate selection' as the other parent in heterosis breeding may be less effective.

In our study, cultivars 'TRI 2016', 'TRI 4006', 'China' and 'Yabukita' have been identified as the most genetically distinct cultivars. The reason for high genetic distinctiveness of 'TRI 2016' may be partly due to the contribution of genes from populations of different origin. The reason for high level of genetic difference of 'TRI 4006', which is a promising cultivar accepted by estates as well as small-holder sector is unclear. Cultivars like 'China' and 'Yabukita' have distinct morphological features, which are not present in other cultivars used in this study. Further, 'Yabukita' and 'China' have different geographical origins, Japan and China respectively, obviously resulting in high genetic differentiation. Kingdon-Ward, (1950) reported that the most divergent race, in its appearance is the 'China' type. High genetic distance detected in cultivar 'TRI 777' against other 'TRI cultivars' may partly be due to its different origin, which is Indo-china or due to the source of the seed material. Generally, use of these cultivars as progenitors in breeding programmes for generating heterosis could be very effective.

The information generated from this study, is of immense value in directing tea breeding programmes for the utilization of tea genetic resources effectively and determining progenitors in conventional breeding program. Further, the study showed that the genetic base of tea in Sri Lanka is low and therefore enrichment of local tea germplasm with exotic germplasm, is a more likely alternative for further genetic improvement of tea to meet the changing needs of the industry

ACKNOWLEDGMENT

This work was supported by SIDA/ SAREC grant for capacity building in Biotechnology awarded to Prof. E H Karunanayake and the grant from the Council for Agricultural Research Policy (CARP), Sri Lanka which constitutes a part of the post graduate programme of Mr K M Mewan.

REFERENCES

Banerjee B 1992 Selection and breeding of tea. In Tea Cultivation to Consumption. Ed. K C Willson and M N Clifford. pp53-86, Chapman and Hall, London.

Doyle J J and Doyle J L 1990 Isolation of plant DNA from fresh tissue. Focus 12, 13-15.

Gunasekare M T K, Kottawaarachchige J D, Mudalige K and Pieris T U S 2001 Morphological diversity of Camellia sinensis L. (Tea) genotypes in Sri Lanka. Proc. Sri Lanka Association for the Advancement of Science, Part 01 (abstracts) 57th annual session, Moratuwa, Sri Lanka. 83.

Kingdon-Ward F 1950 Does wild tea exist? Nature. 4191, 297-299.

Liyanage A C, Fernando W M U and Pathirana K P S K 1999 Determination of genetic diversity of tea cultivars in Sri Lanka using isozyme polymorphisms. Report on FAO IAEA seminar, 1999, Makati City, The Philippines. pp 85-87.

Nei M and Li W H 1979 Mathematical model for study genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 74, 5267-5273.

Richards A V 1965 The origin of the popular TRI cultivars. The Tea Quarterly of Ceylon. 36, 183-187.

Sealy J 1958 A version of the genus Camellia. J. Royal Horti. Soc. London. 62, 934 - 940.

Singh I D 1979 Indian tea germplasm and its contribution to the world's tea industry. Two and a Bud. 26, 23-26.

Tubbs F R 1939 The improvement of planting material. Tea Quarterly. 12, 38-43.

Weising K and Kahl R G 1997 Hybridization based microsatellite finger printing of plants and fungi. In DNA Markers; Protocols, Applications and Overviews. Eds. G Caetano-Anolles and P M Gresshoff Pp. 27-53, John Wiley & Sons, UK.

Wickramaratne M R 1981 Variation in some leaf characteristics in tea (Camellia sinensis) and their use in the identification of tea cultivars. The Tea Quarterly of Ceylon. 50, 183-189.

Williams J G K, Kubelik A R, Livak K, Rafalski J A and Tingey S V 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research. 18, 6531-6535.