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Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers

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Abstract AFLP markers were successfully employed to detect diversity and genetic differentiation among Indian and Kenyan populations of tea (*Camellia sinensis* (L.) O. Kuntze). Shannon's index of diversity was used to partition the total phenotypic variation into between and within population components. On average, most of the diversity was detected within populations, with 79% of the variation being within and 21% being between populations of Indian and Kenyan tea. A dendrogram constructed on the basis of band sharing distinctly separated the three populations of tea into China type (*sinensis*), Assam type (*assamica*) and Cambod type (*assamica* ssp. *lasiocalyx*) in a manner consistent with the present taxonomy of tea, the known pedigree of some of the genotypes and their geographical origin. Principal coordinate (PCO) analysis grouped Assam genotypes both from India and Kenya supporting the suggestion that the Kenyan clones have been derived from collections made in this region. The China types were more dispersed on the PCO plot which is a reflection of wider genetic variation. As would be expected, clones collected from the same region exhibited less overall genetic variation. AFLP analysis discriminated all of the tested genotypes from India and Kenya, even those which cannot be distinguished on the basis of morphological and phenotypic traits.

Key words AFLPs · Diversity · Genetic differentiation · *Camellia sinensis*

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Introduction

South-East Asia is the original home of tea (*Camellia sinensis* (L.) O. Kuntze), and China was the first country to use tea as a beverage, which is now popular throughout the world. Tea is a source of revenue for almost all of the producing countries, including India, and contributes significantly to the local economy. The collection of tea germplasm in India began with the discovery of Assam tea in 1823 by Robert Bruce (Singh 1979). Collections were subsequently made from Burma, China, Cambodia, Vietnam and Japan. These collections resulted in the development of superior planting materials in India and, subsequently, in many other tea-growing countries through the donation of elite germplasm selections. Thus, most of the world tea acreage has received its basic planting material directly or indirectly from the enhanced tea germplasm of India (Ukers 1935). Although the China type was introduced into India earlier than the discovery of the Assam type, it is mainly the Assam type which has been transferred to other countries (Singh 1979).

According to botanical classification (Barua 1965) there are three kinds of cultivated tea indigenous to the geographical regions of South-East Asia: China, Assam and Indo-China. The first two are considered to be distinct species, *Camellia sinensis* L. and *C. assamica* Masters, while the third, which is known as the 'Southern' (or Cambod) form, is regarded as a sub-species of the Assam type and was named *Camellia assamica* subsp. *lasiocalyx* (Planch MS) by Wight (1962). The 'China types' are characterised by a bush with small leaves resistant to cold, while 'Assam types' are tall trees with large leaves and less resistant to cold (Sealy 1958). The 'Cambod type' (Kingdom-Ward 1950; Roberts et al. 1958) has been treated as intermediate between the China and Assam types. Generally, all teas are classified under the name *C. sinensis* (L.) O. Kuntze irrespective of taxonomic variation (Sealy 1937, 1958; Barua 1965; Visser 1969).

Tea is a highly heterogeneous outbreeder, which results in a cline extending from extreme 'China type' plants to

those of distinct Assam origin (Wight 1959). The hybridisation has been so extreme that it is often debated if archetypal *C. sinensis*, *C. assamica* or *C. assamica* ssp. *lasiocalyx* still exist (Visser 1969). There is also doubt as to whether or not existing tea populations have resulted from free hybridisation between the three main taxa or if other *Camellia* species are also involved (Wood and Barua 1958; Cannell et al. 1977). The numerous hybrids that apparently have resulted are still generally referred to as Assam, Cambod or China types depending on their morphological proximity to the main taxa (Banerjee 1992).

The success of any breeding or genetic conservation programme is dependent on an understanding of the amount and distribution of the genetic variation present in the genepool. Traditionally, a combination of morphological and agronomic traits has been used to measure genetic diversity. However, tea is a heterogenous plant with many overlapping morphological, biochemical and physiological attributes (Purseglove 1968; Wickremasinghe 1979; Banerjee 1988b). In addition, since most of the vegetative characteristics are influenced by environmental factors and show a continuous variation and a high degree of plasticity, it has proved difficult to identify discrete taxonomic groups (Wickramaratne 1981). Furthermore, those which have been identified may not reflect true genetic similarities. In an attempt to overcome these problems biochemical and molecular techniques have been used to monitor genetic variability (Wachira et al. 1994). Isozymes have been used extensively to characterise plant genetic resources (Jana and Pietzrak 1988; Chengyin et al. 1992; Jelinski and Cheliak 1992), but they are limited by the relatively low levels of polymorphism detectable. Restriction fragment length polymorphisms (RFLPs) overcome this problem and have been used to investigate genetic diversity in cultivated plants and their wild relatives (Tanksley et al. 1989; Miller and Tanksley 1990; Wang et al. 1992). However, the RFLP assay requires large quantities of relatively pure DNA, and the frequent use of radioisotopes in the detection method makes it technically demanding, laborious and costly to characterise large numbers of samples. The polymerase chain reaction (PCR)-based randomly amplified polymorphic DNA (RAPD) assay (Williams et al. 1990; Welsh and McClelland 1990; Hu and Quiros 1992; Wilde et al. 1992; Chalmers et al. 1992; Russell et al. 1993; Dawson et al. 1995; Wachira et al. 1995) overcame many of the technical limitations of RFLPs but has proved sensitive to experimental conditions and as a result questions have been raised about their reproducibility. Recently a novel PCR-based assay for plant DNA fingerprinting, amplified fragment length polymorphism (AFLPs), has been developed which reveals significant levels of DNA polymorphism (Vos et al. 1995). Initial reports (Meksem et al. 1995; Becker et al. 1995) indicate that AFLP is a reliable and robust genetic molecular marker assay. The number of polymorphisms detected per reaction is much higher than revealed by RFLPs or RAPDs because of the number of loci sampled in a single assay. AFLPs offer an opportunity to perform detailed genetic studies in a large number of organisms and species. In the investigation reported here we have applied AFLPs to study genetic variation between and within populations of tea.

Materials and methods

Plant material

The materials for the present study consists of 32 genotypes collected from different parts of India and Kenya (Table 1). The leaf samples for 15 genotypes (from serial numbers 1–15) were collected from the Tea Experiment Station, Palampur, Kangra, India, and 17 genotypes (from serial numbers 16–32) were from the Tea Research Foundation, Kericho, Kenya.

DNA extraction

DNA was extracted from freeze-dried leaves using a modification of the method described by Gawel and Jarret (1991). Freeze-dried leaf material (2 g) was ground to a fine powder in liquid nitrogen using a pestle and mortar with the addition of 400 mg of insoluble polyclar AT. To this was added 100 ml of pre-heated (65°C) extraction buffer (2% CTAB, 100 m*M* TRIS-HCl, pH 8, 1.4 *M* NaCl, 20 m*M* EDTA, 0.1% DTT). The samples were then incubated for 30 min at 65°C with constant shaking, extracted with 75 ml of chloroform:isoamyl alcohol (24:1), centrifuged (Sorvall RC5C) at 8 K for 5 min and the resultant supernatant collected by filtration through several layers of muslin. The aqueous phase was mixed with an equal volume of ice-cold propan-2-ol and left at room temperature for 10 min to precipitate DNA. Following centrifugation at 5,000 rpm for 10 min, the supernatant was discarded and the DNA pellet drained and vacuum-dried. The dried DNA pellet was resuspended in 2 ml of TE buffer (10 m*M* TRIS-HCl, pH 7.5, 1 m*M* EDTA). After the addition of 20 µl RNase A (10 mg/ml), the samples were incubated for 15 min at 65°C and then stored at 4°C. Insoluble material was removed by centrifugation in an Eppendorf microfuge for 5 min at 14 K and the supernatant removed into clean tubes. The DNA was then reprecipitated in 2 volumes of ethanol (ice-cold), recovered by centrifugation, vacuum-dried and resuspended in TE buffer. The final sample was stored at 4°C.

AFLP

Digestion of DNA

The AFLP procedure was performed following the protocol of Keygene N.V. (Zabeau and Vos 1993) with minor modifications. DNA (2.5 mg) was digested at 37°C for 1 h using 12.5 U *Eco*RI, 12.5 U *Mse*I and 10 ml 5×restriction-ligation buffer (50 m*M* TRIS-acetate, 50 m*M* magnesium acetate, 250 m*M* potassium acetate, 25 m*M* DTT, and 0.25 µg/µl BSA) in a final volume of 50 µl, 10 µl of digested DNA was run on a 1.5% agarose gel to check for complete digestion.

Adaptor ligation

Adaptor ligation was achieved by adding 5 pmol *Eco*RI-adaptor, 50 - pmol *Mse*I adaptor, 0.96 µl 10 m*M* ATP, 1 U T4-DNA ligase, 0.8 ml of 10×restriction-ligation buffer and sterile H_2O to the double-digested DNA sample (60 µl final volume) and incubating for 3 h at 37°C. All primer adaptor sequences used are given in Table 2.

Pre-amplification

Pre-amplification of prepared template was performed with primers complementary to the core of the adaptor sequences (Table 2). Thus, 2 µl of DNA (digested and ligated) was mixed with 75 ng primer

Table 1 Tea genotypes studied and their country of origin

Accession	Type	Source of material	Variety type
1. Dhoedham	Seedjat	Dhoedham Tea Estate, Assam, India	Assam
2. T-253	Variety (selection)	Tea Research Association, Nagrakata Substation, West Bengal, India	China
3. TV9	Variety (selection)	Tocklai Experimental Station, Jorhat, Asam, India	Assam
4. Sundaram	Variety	UPASI Research Station, Nirardam PO Valparai, Coimbatore, India	Assam
5. P-312	Variety (selection)	Tea Research Association, Nagrakata Substation, West Bengal, India	China
6. Samsingh	Seedjat	Sam Sing Tea Estate, Mettallai PO Dooras, West Bengal, India	China
7. Kangra Asha (KVK-1)	Variety (selection)	Tea Experiment Station, Palampur, Kangra, India	China
8. Kangra Jwala (KVK-3)	Variety (selection)	Tea Experiment Station, Palampur, Kangra, India	Assam
9. M-6	Selection	Mansimble Tea Estate, Palampur, Kangra, India	China
10. M-18	Selection	Mansimble Tea Estate, Palampur, Kangra, India	China
11. M-51	Selection	Mansimble Tea Estate, Palampur, Kangra, India	China
12. M-93	Selection	Mansimble Tea Estate, Palampur, Kangra, India	China
13. C-33	Selection	Chambi Tea Estate, Palampur, Kangra, India	China
14. C-37	Selection	Chambi Tea Estate, Palampur, Kangra, India	China
15. C-124	Selection	Chambi Tea Estate, Palampur, Kangra, India	China
16. 382/1	Variety (selection)	TRFK, Kericho, Kenya	Assam
17. D99/10	Variety (selection)	TRFK, Kericho, Kenya	Assam
18. 301/2	Variety (introduction from Reunion)	TRFK, Kericho, Kenya	Cambod
19. 301/3	Variety (introduction from Reunion)	TRFK, Kericho, Kenya	Cambod
20. 6/8	Variety (selection)	TRFK, Kericho, Kenya	Assam
21. 7/9	Variety (selection)	TRFK, Kericho, Kenya	China
22. 56/89	Variety (selection)	TRFK, Kericho, Kenya	China
23. 4/28	Variety (selection)	TRFK, Kericho, Kenya	China
24. 14/1	Variety (selection)	TRFK, Kericho, Kenya	China
25. K/Purple	Variety (selection)	TRFK, Kericho, Kenya	China
26. SFS 150	Variety (introduction from Malawi)	TRFK, Kericho, Kenya	Assam
27. 430/43	Variety (selection)	TRFK, Kericho, Kenya	Assam
28. BB7	Variety (selection)	TRFK, Kericho, Kenya	Assam
29. BB21	Variety (selection)	TRFK, Kericho, Kenya	Assam
30. BB35	Variety (selection)	TRFK, Kericho, Kenya	Assam
31. TN 14/3	Variety (selection)	TRFK, Kericho, Kenya	China
32. 471/15	Variety (selection)	TRFK, Kericho, Kenya	Assam

Table 2 Sequences of primers and adaptors used

*Eco*RI adaptor		5′-CTCGTAGACTGCGTACC-3′ 3′-CTGACGCATGGTTAA-5′
*Mse*I adaptor		5′-GACGATGAGTCCTGAG-3′ 3′-TACTCAGGACTCAT-5′
*Eco*RI+0 primers	EOO:	5′-GACTGCGTACCAATTC-3′
*Eco*RI+1 primer	EO1:	5′-GACTGCGTACCAATTCA-3′
*Eco*RI+3 primers	E33:	5′-GACTGCGTACCAATTCAAG-3′
	E34:	5′-GACTGCGTACCAATTCAAT-3′
	E37:	5′-GACTGCGTACCAATTCACG-3′
	E38:	5′-GACTGCGTACCAATTCACT-3′
	E42:	5′-GACTGCGTACCAATTCAGT-3′
	E44:	5′-GACTGCGTACCAATTCATC-3′
	E46:	5′-GACTGCGTACCAATTCATT-3′
*Mse*I+0 primer	MOO:	5′-GATGAGTCCTGAGTAA-3′
*Mse*I+1 primer	MO1:	5′-GATGAGTCCTGAGTAAA-3′
*Mse*I+3 primers	M32:	5′-GATGAGTCCTGAGTAAAAC-3′
	M39:	5′-GATGAGTCCTGAGTAAAGA-3′
	M42:	5′-GATGAGTCCTGAGTAAAGT-3′
	M47:	5′-GATGAGTCCTGAGTAACAA-3′
	M55:	5′-GATGAGTCCTGAGTAACGA-3′

EO1, 75 ng primer MO1, 1 U *Taq* DNA polymerase (Perkin Elmer, USA), 2.5 µl of 10×PCR buffer (Perkin Elmer, USA) and 0.2 m*M* dNTPs, in a final volume of 24 µl. The PCR reaction was performed in a Perkin Elmer-9600 thermal cycler (Perkin Elmer, USA) using the following temperature profile: 30 cycles of 30 s at 94°C, 30 s at 60°C, 60 s at 72°C. After pre-amplification 55 µl TO.1E buffer (10 m*M* TRIS, pH 8.0, 0.1 m*M* EDTA) was added to each sample and 15 µl of this pre-amplified DNA was checked on 1% agarose gel where a smear (0.2 kb) was visible.

End labelling

Only *Eco*RI compatible primers were labelled. Sufficient primer was prepared for 35 selective amplifications by combining 3.5 µl δ-[^{33}P] ATP (1.11×10^{14} Bq/mmol), 3.5 µl of 10×T4 buffer (250 m*M* TRIS-HCl, pH 7.5, 100 m*M* MgCl2, 50 m*M* DTT, 5 m*M* spermidine), 4.7 µl E (plus three selective nucleotides) primer (50 ng/µl stock), 0.9 µl T4 kinase (10 units/ml) and 22.4 µl H2O (final volume 35 µl). Samples were incubated at 37°C for 30 min then heated to 70°C for 10 min to stop the reaction.

Selective PCR amplifications

Selective restriction fragment amplification was performed with a [^{33}P]-labelled *Eco*RI + 3 primer and an unlabelled *Mse*I + 3 primer

(Table 2). Each 20 µl PCR reaction consisted of 3 µl pre-amplified DNA, 50 ng labelled *Eco*RI+3 primer, 50 ng unlabelled *Eco*RI+3 primer, 50 ng unlabelled *Mse*I+3 primer, 2 m*M* dNTP, 0.1 µl *Taq* DNA polymerase (10 units/µl) and 2 µl PCR buffer (Perkin Elmer, USA). The following cycle profile ensured optimal primer selectivity: 1 cycle of 30 s at 94°C, 30 s at 65°C, 60 s at 72°C followed by 11 cycles of 0.7°C lower annealing temperature each cycle and 23 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C.

Gel electrophoresis

The PCR product was mixed with 20 µl formamide loading buffer (98% formamide, 10 m*M* EDTA, 0.005% each of xylene cyanol FF and bromophenol blue) and denatured by incubation for 5 min at 90°C. The 6% polyacrylamide gels were prepared by mixing 65 ml 6% Easigel (Scotlab) and 450 µl 10% ammonium persulphate, degassing and adding 20 µl TEMED. The gels were poured at least 4 h before use and pre-run at 80 W for 30 min. Samples of 5 µl were loaded per track and gels were run for 1.45 h at 80 W, transferred to 3 MM chromatography paper and dried on a gel drier for 2 h at 80°C. Gels were exposed to X-ray film for about 4–7 days.

Data analysis

Bands were scored as present or absent on autorads. The degree of polymorphism was quantified using Shannon's index of phenotypic diversity:

$$H_o = -\sum \pi_i \ln \pi_i$$

where pi is the frequency of phenotype i (King and Schaal 1989). H_o can be calculated and compared for different populations. Let:

$$H_{pop} = \tfrac{1}{n} \sum H_o$$

be the average diversity over the n different populations, and let:

$$H_{sp} = -\sum \pi \ln \pi$$

be the diversity calculated from the phenotypic frequencies p in all the populations considered together. Then the proportion of diversity present within populations, H_{pop}/H_{sp}, can be compared with that between populations, $(H_{sp}-H_{pop})/H_{sp}$.

Estimates of similarity between genotypes were based on the probability that an amplified fragment from one accession will also be present in another (Nei and Li 1979):

$$D_{AB} = 2 \times \text{Number of shared fragments} \div (\text{number of fragments}_A + \text{number of fragments}_B)$$

Principal coordinate analysis and single linkage cluster analysis (Kempton and McNicol 1990) were performed with the GENSTAT 5 (1987) statistical package.

Results and discussion

AFLP fingerprinting of 32 genotypes of tea with five primer combinations (E33/M39, E34/M39, E37/M32, E42/M39 and E46/M32; sequences in Table 2) revealed a total number of 73 unambiguous polymorphic amplified DNA fragments. On average, 15 polymorphic loci were scored per primer combination. Figure 1 shows fingerprints of the accessions studied after PCR with primer combination E37/M32 ($E_{acg}M_{aac}$). The size of the polymorphic amplified fragments that were scored ranged from 106–218 bp.

The phenotypic frequencies detected with the five primer combinations were calculated and used in estimating diversity (H_o) within population types. The Cambod population exhibited the lowest within-population variability, but its population size was the smallest which affects H_o. Both China and Assam populations exhibited high within-population variability. When all the genotypes from India and Kenya were grouped both populations showed high variability within populations (Table 3). Shannon's index of phenotypic diversity was then used to partition the diversity into within and between population components. An assessment of the proportion of diversity present within populations, H_{pop}/H_{sp}, compared with that between populations, $(H_{sp}-H_{pop})/H_{sp}$, indicates that on average, 79% is distributed within and 21% between populations of Indian and Kenyan tea (Table 4). When subdividing on the

Table 3 Estimates of genetic diversity (H_o) within populations of tea

Primer combination	Indian tea	Kenyan tea	Assam type	China type	Cambod type
1. E33/M39 (E_{aag} M_{aga})	2.62	2.60	2.36	2.75	0.69
2. E34/M39 (E_{aat} M_{aga})	2.71	2.83	2.56	2.83	0.69
3. E37/M32 (E_{acg} M_{aac})	2.71	2.83	2.56	2.83	0.69
4. E42/M39 (E_{agt} M_{aga})	2.71	2.83	2.56	2.83	0.69
5. E46/M32 (E_{att} M_{aac})	2.71	2.75	2.45	2.83	0.69
$\bar{x}$	2.69	2.77	2.50	2.81	0.69

Table 4 Partitioning of the genetic diversity into within and between populations of Indian and Kenyan tea for five primer combinations

Primer combination	H_{sp}	H_{pop}	H_{pop}/H_{sp}	$(H_{sp}-H_{pop})/H_{sp}$
1. E33/M39 (E_{aag} M_{aga})	3.30	2.61	0.79	0.21
2. E34/M39 (E_{aat} M_{aga})	3.46	2.77	0.80	0.20
3. E37/M32 (E_{acg} M_{aac})	3.46	2.77	0.80	0.20
4. E42/M39 (E_{agt} M_{aga})	3.46	2.77	0.80	0.20
5. E46/M32 (E_{att} M_{aac})	3.53	2.73	0.77	0.23
$\bar{x}$	3.44	2.73	0.79	0.21

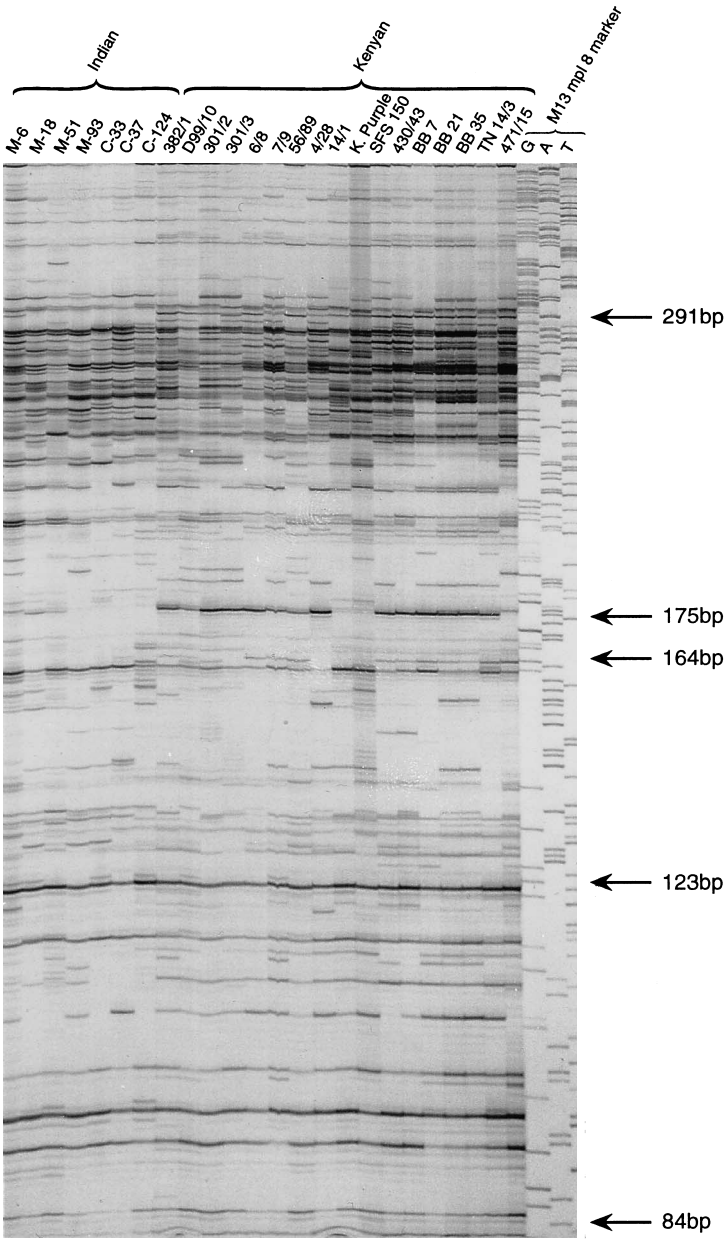

Fig. 1 A portion of AFLP fingerprint patterns in tea (*Camellia sinensis*) using primer combination E37/M32 ($E_{acg}M_{aac}$)

basis of type, 58% is within and 42% is between populations (Table 5) of China, Assam and Cambod types. These findings are in agreement with the observation that outbreeding woody plants retain considerable variability and that most variation is exhibited within populations (Hamrick 1990). Estimates of genetic diversity within populations showed that the China-type teas are the most variable. Importantly, estimates of diversity within Kenyan tea populations, which are considered to be based on the progeny from crosses undertaken in one region (Assam), were similar to those observed for the Indian population. This supports the observation of Wachira et al. (1995) who, using RAPD markers in Kenyan tea, observed a large amount of genetic diversity. The similarity matrix (Table 6) based on the proportion of shared fragments was used to show the relationships between and within Indian and Kenya genotypes of tea. Within Indian populations, the similarity ranged from 0.41 between T-253 and M-51 to 0.85 between M-6 and M-93, whereas within Kenyan populations it ranged from 0.41 between 6/8 and 301/2 to 0.96 between BB35 and BB21 (Table 6). Between Indian and Kenyan genotypes the similarity ranged from 0.35 between 14/1 and M-51, 471/15 and M-51, and 6/8 and C-124 to 0.87 between BB35 and Samsingh.

The dendrogram constructed on the basis of shared fragments divides the 32 genotypes into three types, Assam, China and Cambod (Fig. 2), which is generally consistent with existing knowledge on the systematics of tea and the

Table 5 Partitioning of the genetic diversity into within and between populations of Assam; China- and Cambod-type for five primer combinations

Primer combination	H_{sp}	H_{pop}	H_{pop}/H_{sp}	$(H_{sp}-H_{pop})/H_{sp}$
1. E33/M39 (E_{aag} M_{aga})	3.30	1.93	0.58	0.42
2. E34/M39 (E_{aat} M_{aga})	3.46	2.03	0.59	0.41
3. E37/M32 (E_{acg} M_{aac})	3.46	2.03	0.59	0.41
4. E42/M39 (E_{agt} M_{aga})	3.46	2.03	0.59	0.41
5. E46/M32 (E_{att} M_{aac})	3.53	1.99	0.56	0.44
$\bar{x}$	3.44	2.00	0.58	0.42

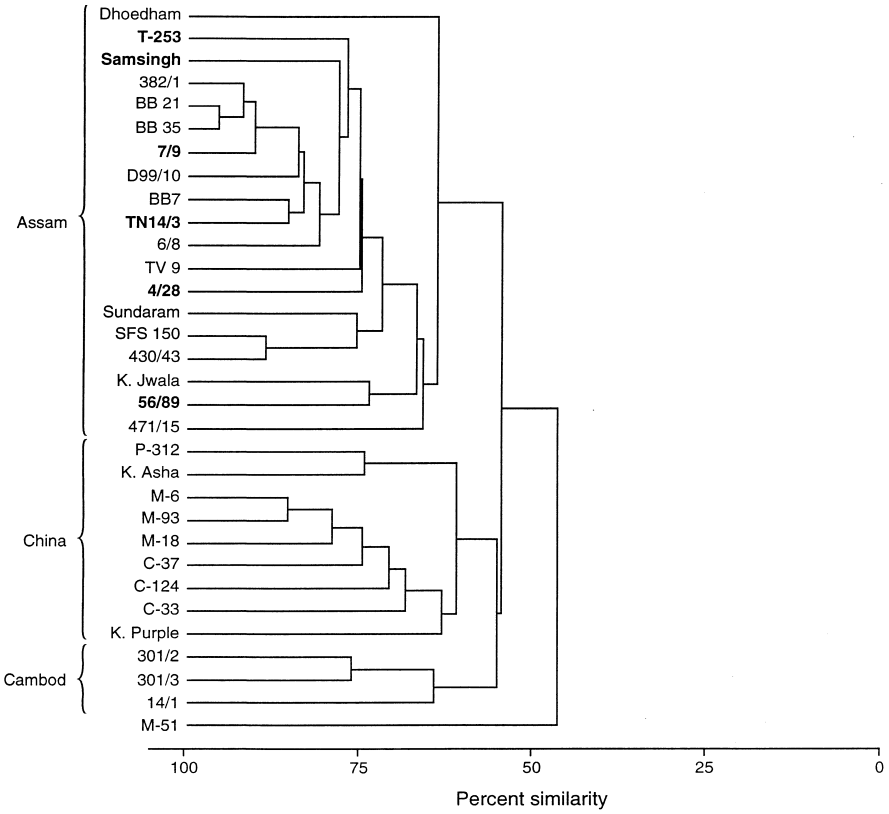

Fig. 2 Dendrogram of tea (*Camellia sinensis*) genotypes derived by average linkage cluster analysis. The *highlighted* genotypes have been classified as China type using morphological traits

genealogical data of some clones and hybrids. However, T-253 and Samsingh among the Indian material and in 7/9, TN 14/3, 4/28 and 56/89 the Kenyan material did not conform to their taxon affinity. Their classification however, has been based on morphological markers, which are subject to substantial environmental changes. With respect to the Kenyan material this exception has been reported previously by Wachira et al. (1995) based on RAPD analysis. It is possible that these genotypes are hybrids with morphological characters that predispose them to being identified as China tea.

Nine China genotypes, namely P-312, Kangra Asha, M-6, M-93, M-18, C-37, C-124, C-33 and K. Purple, form a group distinct from the Assam types which is consistent with their morphological classification. The three Cambod genotypes 301/2, 301/3 and 14/1 form a separate group between the China and Assam types. Wachira et al. (1995) also reported 301/2 and 301/3 in the Cambod group but reported 14/1 as a China type in contrast to the present finding. The reason for this is at present unclear.

The first two principal coordinates accounted for 33% of the variation and effectively differentiate between the variety type and their geographical distribution (Fig. 3).

From the principal coordinate (PCO) plot, the Assam, China and Cambod types are clearly demarcated. In addition, the China-type accessions M-6, M-18, M-51 and M-93 and C-33, C-37 and C-124, which were from the same estate but from different areas, form sub-groups within the China-type group showing clear geographical sub-differentiation. 'Kangra Asha' and 'Kangra Jwala', which were selections from the Tea Experiment Stations, were released as varieties to Kangra Valley planters. Both of these varieties differ extensively for AFLPs (similarity=0.55) and are not grouped in a same group. 'Kangra Jwala' resembles the Assam type varieties. During the early days of estate cultivation material from Assam was introduced to Kan-

Table 6 Similarity matrix based on Nei's estimates of similarity; genotypes are numbered as in Table 1

1																																
2	0.55																															
3	0.57	0.76																														
4	0.66	0.64	0.67																													
5	0.46	0.54	0.66	0.63																												
6	0.62	0.75	0.67	0.70	0.64																											
7	0.56	0.48	0.57	0.61	0.75	0.46																										
8	0.64	0.65	0.70	0.74	0.63	0.72	0.55																									
9	0.68	0.57	0.54	0.70	0.52	0.61	0.68	0.59																								
10	0.58	0.58	0.58	0.72	0.58	0.53	0.67	0.59	0.82																							
11	0.44	0.41	0.43	0.55	0.55	0.44	0.59	0.44	0.47	0.58																						
12	0.61	0.49	0.58	0.65	0.50	0.50	0.73	0.56	0.85	0.76	0.52																					
13	0.47	0.53	0.53	0.53	0.60	0.58	0.67	0.52	0.70	0.71	0.60	0.76																				
14	0.53	0.55	0.51	0.61	0.51	0.44	0.61	0.48	0.71	0.79	0.58	0.74	0.62																			
15	0.43	0.43	0.55	0.65	0.54	0.39	0.69	0.51	0.71	0.71	0.49	0.76	0.65	0.65																		
16	0.60	0.80	0.78	0.74	0.57	0.76	0.48	0.68	0.57	0.53	0.45	0.50	0.46	0.44	0.40																	
17	0.74	0.72	0.75	0.73	0.51	0.79	0.45	0.65	0.64	0.52	0.43	0.53	0.44	0.47	0.36	0.80																
18	0.46	0.44	0.59	0.43	0.50	0.41	0.43	0.50	0.56	0.41	0.30	0.53	0.44	0.48	0.47	0.51	0.50															
19	0.54	0.52	0.65	0.62	0.47	0.52	0.54	0.61	0.68	0.58	0.44	0.69	0.52	0.53	0.64	0.61	0.54	0.76														
20	0.61	0.72	0.72	0.74	0.61	0.76	0.40	0.62	0.52	0.50	0.36	0.42	0.42	0.46	0.35	0.82	0.83	0.41	0.44													
21	0.69	0.79	0.75	0.72	0.52	0.76	0.39	0.69	0.54	0.57	0.50	0.49	0.46	0.49	0.41	0.87	0.85	0.47	0.58	0.80												
22	0.61	0.70	0.63	0.69	0.55	0.74	0.59	0.74	0.65	0.55	0.36	0.63	0.52	0.53	0.56	0.69	0.61	0.43	0.64	0.63	0.62											
23	0.60	0.69	0.72	0.71	0.66	0.68	0.56	0.69	0.59	0.62	0.42	0.59	0.58	0.52	0.54	0.76	0.72	0.52	0.64	0.78	0.77	0.62										
24	0.37	0.51	0.64	0.58	0.63	0.52	0.58	0.59	0.64	0.62	0.35	0.65	0.58	0.56	0.58	0.58	0.54	0.62	0.67	0.54	0.53	0.53	0.68									
25	0.50	0.59	0.58	0.67	0.61	0.51	0.63	0.59	0.64	0.63	0.54	0.64	0.57	0.64	0.67	0.65	0.59	0.45	0.48	0.61	0.63	0.54	0.59	0.62								
26	0.70	0.66	0.70	0.74	0.58	0.71	0.52	0.66	0.75	0.70	0.43	0.64	0.60	0.54	0.48	0.75	0.72	0.50	0.55	0.72	0.68	0.63	0.72	0.59	0.62							
27	0.67	0.67	0.68	0.78	0.59	0.70	0.55	0.58	0.73	0.67	0.45	0.66	0.59	0.59	0.47	0.75	0.76	0.46	0.56	0.81	0.68	0.68	0.73	0.61	0.63	0.89						
28	0.61	0.80	0.79	0.69	0.61	0.78	0.48	0.70	0.63	0.57	0.41	0.52	0.50	0.51	0.51	0.84	0.79	0.49	0.58	0.75	0.82	0.64	0.73	0.63	0.64	0.73	0.71					
29	0.66	0.77	0.75	0.74	0.59	0.83	0.45	0.71	0.54	0.54	0.49	0.47	0.42	0.42	0.39	0.92	0.85	0.42	0.56	0.82	0.93	0.66	0.79	0.56	0.63	0.68	0.70	0.87				
30	0.73	0.87	0.79	0.80	0.60	0.87	0.47	0.71	0.67	0.63	0.54	0.57	0.52	0.52	0.38	0.92	0.88	0.44	0.63	0.83	0.91	0.71	0.79	0.58	0.68	0.79	0.81	0.88	0.96			
31	0.70	0.74	0.77	0.73	0.61	0.72	0.49	0.70	0.65	0.65	0.46	0.53	0.52	0.54	0.52	0.81	0.85	0.49	0.57	0.82	0.84	0.65	0.82	0.63	0.65	0.75	0.76	0.86	0.80	0.85		
32	0.62	0.60	0.65	0.67	0.53	0.70	0.52	0.62	0.63	0.56	0.35	0.59	0.54	0.60	0.49	0.63	0.69	0.52	0.55	0.68	0.61	0.72	0.57	0.55	0.58	0.72	0.76	0.68	0.61	0.69	0.64	
	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

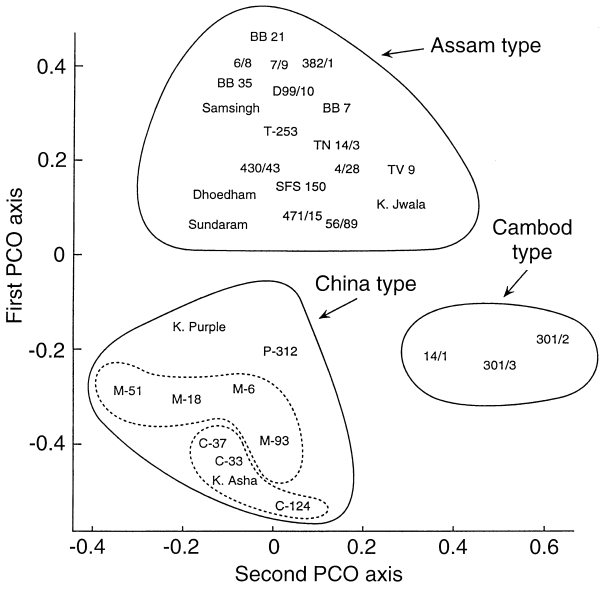

Fig. 3 Principal coordinate plot of 32 genotypes of tea (*Camellia sinensis*). The two principal components accounted for 32.56% of the total variation

gra Valley and planted randomly within the existing China type plants. 'Kangra Jwala' may therefore represent an introduced Assam type which was originally planted alongside the China types and later identified as a local 'Kangra Valley' selection. In contrast, 'Kangra Asha' resembles other local selections from that area and is considered one of the original China types. Both of the Cambod accessions 301/2 and 301/3 are selections from Reunion.

The Assam clones both from India and Kenya clustered closely in the PCO plot shown. This may offer credence to the belief that Kenyan teas originated from India and thus show common ancestry. The China types were more dispersed on the PCO plot, which is a reflection of wider genetic variation. However, the material collected from same geographic region (M and C series) exhibited higher levels of similarities, presumably because of selections being made on the same natural populations. These observations generally concur with results obtained using RAPD markers (Wachira et al. 1995) and RFLP markers (D. Chamberlain and P. Jack, PBI, Cambridge, England, personal communication). In conclusion, AFLP appears to represent an additional molecular polymorphism assay which can be applied to the analysis of genetic diversity and population genetics in tea and represents an important addition to the battery of PCR-based approaches that can be effectively employed in plant germplasm characterisation and analysis.

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