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Genetic diversity of UPASI tea clones (*Camellia sinensis* (L.) O. Kuntze) on the basis of total catechins and their fractions

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Abstract

Tea leaf catechins and the ratio of dihydroxylated to trihydroxylated catechin fractions were analysed to identify the genetic diversity of 26 UPASI released tea clones. Principal component analysis (PCA) based on regression factor separated tea clones into five groups according to their jats (Jats are region based rays for e.g., Assam, China and Cambod origin) as well as their quality constituents (such as total polyphenols, total catechins, amino acids in the green leaves and liquor characteristics of black tea), particularly the catechins. Group 1 represented medium quality (quality of the final produce) clones, such as UPASI-10, UPASI-12 and UPASI-15 and drought tolerant clones like UPASI-1, UPASI-2, UPASI-9 and UPASI-10. Group 2 contained purely ''China'' cultivars while group 3 possessed high quality tea cultivars. ''Assam'' (group 5) teas had the lowest ratio of dihydroxylated to trihydroxylated catechin fractions (1:4) than the ''Chinery'' (group 2) teas (1:5). This biochemical differentiation indicated that there is a vast genetic diversity in UPASI released tea clones in terms of catechin fractions, even though the majority of them were selected from one tea estate located in the Nilgiris.

Keywords: Genetic diversity; Total catechins and catechin fractions

1. Introduction

Present day tea cultivation (particularly, infilling, inter planting, inter row planting and replanting) is largely based on clones from vegetatively propagated materials. This widespread planting of clonal tea would diminish the genetic diversity, if attention is not given to utilise clones of disparate origin. Earlier studies using randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers studies showed that Kenyan teas showed close similarity with Indian germplasm (Wachira et al., 1995; Paul et al., 1997). Nonetheless, a premediated selection among the germplasm with high quality has been lacking. Selection for good quality tea plants needs a close study on the biochemical constituents, which donate towards the liquor characteristics. Important biochemicals that settle on tea quality are the green tea catechins and their oxidation products. Catechins in the green leaf primarily composed of (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG) and (−)-epicatechin gallate (ECG).

Oxidation products such as theaflavins and thearubigins are responsible for most of the black tea quality characteristics (Robertson, 1992). Higher amount of total catechin content could be used to correlated with high quality potential of final product (Obanda and

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Owuor, 1997). Conversely, these methods are not fully steadfast as they do not considered the individual catechins. Fractions of catechins could be important in the determination of tea quality and genetic bioconstituent (Owuor and McDowell, 1994). Thus, it becomes essential to study the relative expression of the individual catechin fractions. Formation of dihydroquercetin and dihydromyricetin, which are the precursors of dihydroxylated catechins (EC and ECG) and trihydroxylated catechins (EGC and EGCG), respectively, where under genetic control (Gerats and Martin, 1992). Di- and trihydroxylated catechin ratios could be used to study genetic variation in tea. In this context, an attempt has been made to analyse the di- and tri-hydroxylated catechin ratio and total catechins of UPASI tea clones for their genetic and geographical diversity.

2. Materials and methods

Tea shoots comprising two leaves and a bud were harvested four times in an year coinciding with two lean and peak periods. Tea plants are grown at UPASI Experimental Farm, which is located at an altitude of 1150 m above MSL. Plants with same age and uniformly pruned at 26″ above ground level were selected to avoid variations. Generic and geographic data on different type of UPASI clones are presented in Table 1. Basically recommended UPASI clones comprised of ''Assam'', ''China'' and ''Cambod'' hybrids. These were catagorised on the basis of phenological characteristics (Mohanan and Sharma, 1981; Venkataramani and Sharma, 1975). Recent biotechnological tools like RAPD and-AFLP techniques are used to group the plants under different classes on the basis of their genetic and geographical origin (Balasaravanan et al., 2003).

Tea shoots collected were used determine the total catechin content using spectrometer (Model: Genesys 10UV) adopting the method reported by Swain and Hillis (1959). Catechin fractions were quantified using HPLC (Model HP 1100 series) by ISO method (14502-2, 1999). Tea clone, UPASI-23 was not subjected to biochemical estimations, since they were grown under high elevation area, The Nilgiris. Based on the data obtained, values were subjected to linear regretion analysis and principal component analysis using the Special Purpose Software for Statistics (SPSS, Ver. 7.0) for clustering the tea clones. For PCA analysis total catechins of individual cultivar and their fractions were considered. Summary statistics of the various group as defined by the PCA expressed that the catechin ratios had the highest resolving power compared to the other variables, allow-

Table 1
Origin and descriptive characters of UPASI tea clones

Clone	Accession	Source of the material	Variety	Reference
UPASI-1	B/4/141	Brooklands Estate, The Nilgiris	Assam	Mohanan and Sharma (1981)[a]
UPASI-2	B/4/142	Brooklands Estate, The Nilgiris	Assam	Mohanan and Sharma (1981)[a]
UPASI-3	B/5/63	Brooklands Estate, The Nilgiris	Assam	Mohanan and Sharma (1981)[a]
UPASI-4	B/6/1O	Brooklands Estate, The Nilgiris	Assam	Balasaravanan et al. (2003)[b]
UPASI-5	B/6/21	Brooklands Estate, The Nilgiris	Cambod	Balasaravanan et al. (2003)[b]
UPASI-6	B/6/24	Brooklands Estate, The Nilgiris	Assam	Balasaravanan et al. (2003)[b]
UPASI-7	B/6/34	Brooklands Estate, The Nilgiris	Assam	Balasaravanan et al. (2003)[b]
UPASI-8	B/6/36	Brooklands Estate, The Nilgiris	Cambod	Balasaravanan et al. (2003)[b]
UPASI-9	B/6/61	Brooklands Estate, The Nilgiris	China	Mohanan and Sharma (1981)[a]
UPASI-10	B/6/62	Brooklands Estate, The Nilgiris	China	Mohanan and Sharma (1981)[a]
UPASI-11	B/6/127	Brooklands Estate, The Nilgiris	Assam	Balasaravanan et al. (2003)[b]
UPASI-12	B/6/129	Brooklands Estate, The Nilgiris	Cambod	Balasaravanan et al. (2003)[b]
UPASI-13	B/6/137	Brooklands Estate, The Nilgiris	Assam	Balasaravanan et al. (2003)[b]
UPASI-14	S/6/99	Singara Estate, The Nilgiris	Cambod	Balasaravanan et al. (2003)[b]
UPASI-15	SP/4/5	Springfield Estate, The Nilgiris	China	Mohanan and Sharma (1981)[a]
UPASI-16	B/6/182	Brooklands Estate, The Nilgiris	China	Mohanan and Sharma (1981)[a]
UPASI-17	B/6/203	Brooklands Estate, The Nilgiris	Cambod	Mohanan and Sharma (1981)[a]
UPASI-18	B/6/57	Brooklands Estate, The Nilgiris	Cambod	Balasaravanan et al. (2003)[b]
UPASI-19	SP/4/6	Springfield Estate, The Nilgiris	Cambod	Balasaravanan et al. (2003)[b]
UPASI-20	B/7/372	Brooklands Estate, The Nilgiris	China	Balasaravanan et al. (2003)[b]
UPASI-21	B/4/198	Brooklands Estate, The Nilgiris	Assam	Mohanan and Sharma (1981)[a]
UPASI-22	B/6/29'	Brooklands Estate, The Nilgiris	Assam	Balasaravanan et al. (2003)[b]
UPASI-23	B/5/148	Brooklands Estate, The Nilgiris	Assam	Balasaravanan et al. (2003)[b]
UPASI-24	B/5/149	Brooklands Estate, The Nilgiris	Cambod	Balasaravanan et al. (2003)[b]
UPASI-25	K/19/16	UPASI TRF, Anamallais	Cambod	Balasaravanan et al. (2003)[b]
UPASI-26	DVS/3A/39	Devarshola Estate, Nilgiri-Wynaad	Assam	Balasaravanan et al. (2003)[b]
UPASI-27	A/58	Anaimudi Estate, Anamallais	Assam	Balasaravanan et al. (2003)[b]

There may be certain variations between morphological, systematic analysis and AFLP analysis.
[a] Based on morphology and systematics.
[b] Confirmed based on AFLP analysis.

ing clear identification between the various types of tea. Both total catechins and trihydroxylated catechin concentrations only allowed identification of quality clones from existing germplasm.

3. Results and discussion

Though, UPASI tea clones (UPASI-1 to UPASI-27) released for cultivation/commercial exploitation they are belonging to one geographical origin within south India. Most of them were identified from Brooklands Tea Estates, The Nilgiris. These clones contained variable quantum of total catechins (Table 2). Variations are not only observed in their phenological but also in genetic characteristics (Balasaravanan et al., 2003). UPASI clones exhibited wide variation in their phenotypic characteristics. Among the clones, UPASI-17 (''Cambod'') contained higher quantum of catechin content followed by UPASI-3 (''Assam'').

(−)-Epigallocatechin (EGC) was eluted first followed by (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG) and (−)-epicatechin gallate (ECG). Their quantum and their ratios were also varied with different tea clones. (Table 3). Although there was a marginal variation in the amount of catechin fractions, total catechin contents seems to be very high in UPASI-17 followed by UPASI-3. While UPASI-26 registered lower values of total catechins and their fractions. It may be noted that the values of total catechins and their fractions are not tallying with each other. This may be due to the variation in precision of spectrophotometer method and HPLC method followed in the present study. Results obtained in this study confirmed the earlier studies on biochemical and metabolic functions of tea clones (Ranganath and Marimuthu, 1992; Raj Kumar et al., 1993).

Table 2
Individual catechin fractions in UPASI clones

Clones	Relative distribution of catechin fractions[a]				
	EGC (%)	EC (%)	EGCG (%)	ECG (%)	Total catechins (%)[b]
UPASI 1	2.52	1.35	11.82	1.18	16.87
UPASI 2	1.68	1.45	12.53	1.05	16.71
UPASI 3	2.28	1.74	13.86	1.86	19.74
UPASI 4	2.02	1.43	11.66	1.31	16.42
UPASI 5	1.83	1.50	10.29	1.08	14.70
UPASI 6	2.73	1.73	10.81	1.16	16.44
UPASI 7	2.08	1.32	10.46	1.13	15.00
UPASI 8	2.12	1.52	11.52	1.29	16.45
UPASI 9	1.97	1.29	12.28	1.34	16.88
UPASI 10	1.51	1.41	12.95	1.26	17.12
UPASI 11	1.85	1.34	11.08	1.06	15.32
UPASI 12	1.45	1.34	13.62	1.52	17.93
UPASI 13	1.91	1.61	10.55	1.08	15.14
UPASI 14	1.55	1.54	12.29	1.29	16.67
UPASI 15	2.06	1.44	13.26	1.14	17.91
UPASI 16	1.27	1.21	10.54	1.41	14.44
UPASI 17	2.73	2.04	13.85	1.77	20.39
UPASI 18	2.26	1.75	10.84	1.14	16.00
UPASI 19	1.64	1.43	12.99	2.09	18.14
UPASI 20	1.63	1.38	12.86	1.70	17.57
UPASI 21	1.56	1.35	12.14	1.97	17.03
UPASI 22	1.66	1.54	11.03	1.54	15.78
UPASI 24	2.04	1.87	12.43	1.67	18.01
UPASI 25	2.03	1.33	10.62	0.88	14.86
UPASI 26	1.91	1.50	9.51	0.91	13.83
UPASI 27	1.54	2.17	12.60	1.78	18.09

Values are mean of four seasons and triplicate samples.
[a] Catechin fraction determined using HPLC with oven dried tea sample extract.
[b] Total catechins were determined by adopting spectrophotometer method with fresh tea shoots.

Statistic summary of UPASI clones revealed that there were five distinct groups segregated on the basis of catechins and their fractions (Table 3). When considering the total catechins alone, six genotypes exhibited high quality category while seven prototypes come under poor category. Two clones were grouped into moderate category while three others were good quality clones. Remaining two clones are premium clones (Table 3). Similar trend was observed with the individual catechin fractions and their combinations as well (Tables 2 and 3). Pooled data of total catechins and their fractions showed distinct difference among the groups. (Table 4). Group II and III had a prominent variation between the cluster centroids followed by cluster III and IV, group II and V and finally group I and III. Cluster IV and V showed very narrow relationship (Fig. 1). Principal component regression plot also confirmed the analysis (Fig. 1). First component of principal component analysis accounted for 60.3% variability while second component accounted for 39.7% of the total variability. Distance between the clusters II and III was very high followed by group IV and III. Group IV and V had very close relationship. Except one or two clones like UPASI-26 (Devarshola), etc. grouped into other hierarchial cluster, confirming the free natural breeding.

Though the tea clones are segregated as ''Assam'', ''China'' and ''Cambod'' hybrids, as for as the catechin fractions, none of them segregated individual groups. This may be due to the prolonged free hybridisation that occurred over the ages, which in turn responsible for significant variations in biochemical and physiological/metabolic functions. For example, cluster I contained UPASI-1, UPASI-2, UPASI-9, UPASI-10 and UPASI-15. Among the clones, UPASI-9, UPASI-10 and UPASI-15 had been recognised as ''China'' cultivar while UPASI-2 considered as ''Assam'' type (Mohanan and Sharma, 1981). In cluster III, both broad-leaved ''Assam'' (UPASI-3) and ''Cambod'' (UPASI-17) were segregated together. Here again, both the clones are recognised as moderate to good quality clones. Cluster analysis did not show any distinct difference on the basis

Table 3
Summary statistics of four measures of catechins in UPASI tea clones

Variables	Number of groups	Number of genotypes	Percent catechins (mean)	Standard deviation	Min	Max
Total catechin	1[a]	6	17.23	0.53	16.70	17.90
	2	7	14.74	0.51	13.80	15.30
	3	2	20.05	0.49	19.70	20.40
	4	6	16.25	0.28	15.80	16.50
	5	5	17.76	0.47	17.00	18.10
Number of genotypes			26			
Catechin ratios	1	6	0.18	0.01	0.17	0.19
	2	7	0.21	0.02	0.17	0.24
	3	2	0.23	0.01	0.22	0.23
	4	6	0.21	0.02	0.20	0.24
	5	5	0.24	0.02	0.21	0.28
Number of genotypes			26			
EGC + EGCG	1	6	14.62	0.47	14.20	15.30
	2	7	12.26	0.52	11.40	12.90
	3	2	16.35	0.35	16.10	16.60
	4	6	13.40	0.42	12.70	13.80
	5	5	14.28	0.38	13.70	14.60
Number of genotypes			26			
EC + ECG	1	6	2.63	0.13	2.50	2.86
	2	7	2.48	0.16	2.21	2.68
	3	2	3.70	0.16	3.59	3.81
	4	6	2.87	0.12	2.73	3.08
	5	5	3.48	0.32	3.08	3.95
Number of genotypes			26			

[a] Group 1 contained Assam and China cultivars, group 2 pure China, group 3 Assam and Cambod cultivars, group 4, Assam and China and group 5 pure Assam cultivars.

Table 4
Distances between final cluster centroids

Cluster number	I	II	III	IV	V
I	0.000	2.554	3.051	1.297	1.535
II		0.000	5.338	1.565	3.128
III			0.000	3.78	2.315
IV				0.000	1.154
V					0.000

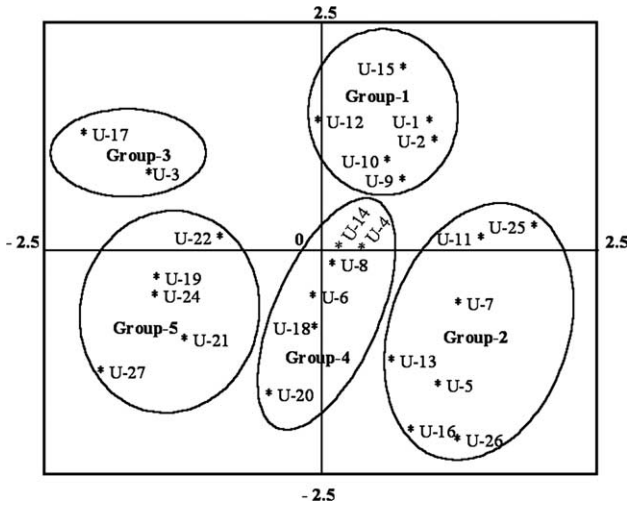

Fig. 1. Principal component analysis based on regression factor score extraction.

of geographical origin like Indo-China and Cambodiansis. But the present study clearly indicated that variation in the classification is due to free natural hybridisation resulted which in turn segregated five intermittent groups.

Group 1 is a mixture of ''Assam'' and ''China'' cultivars, represented medium quality clones such as UPASI-10, UPASI-12 and UPASI-15 and drought tolerant clones such as UPASI-1, UPASI-2, UPASI-9, UPASI-10 and UPASI 15. It is imperative to note that UPASI-2, UPASI-10 and UPASI-15 of group 1 are blister tolerant (Agnihothrudu and Chandramouli, 1990). Group 2 contained purely ''Chinery'' jats such as UPASI-5, UPASI-7, UPASI-11, UPASI-13, UPASI-16, UPASI-25 and UPASI-26. UPASI-5 and UPASI-13 are average quality clones. Though the group 3 possessed high quality cultivars (UPASI-3 and UPASI-17) they were known for their susceptibility to drought because of their phenological character (broad leafs with more number of stomata).

Group 4 contained both ''Assam'' and ''China'' cultivars (UPASI-4, UPASI-6, UPASI-8, UPASI-14 and UPASI-18) besides the ''Combod'' cultivar, UPASI-20 (based on the morphological characterisation). Group 5 comprised only ''Assam'' type such as UPASI-19, UPASI-21, UPASI-22, UPASI-24 and UPASI-27. Dihydr-

oxylated catechins did not give any clear group identity (Table 3). Group 3 which contained high quality cultivars had a catechin ratio of 1:4 while group 1 which contained medium quality clones registered a catechin ratio of 1:6. ''Assam'' type (group 5) had a catechin ratio of 1:4 while the ''China'' jats (group 2) possessed a catechin ratio of 1:5. Intermediate between these two groups (group 4) registered ratio of 1:5.

Very distinct variation was observed within *Camellia* spp. and they were grouped in to three categories like ''Assam'', ''Cambod'' and ''China''. ''Chinery'' types had low total catechins when compared to ''Assam'' jats. Results obtained in the present study confirmed the earlier report of Takeda (1994). Results derived from this study also coincide with the results of Mohanan and Sharma (1981) where the leaf and floral characteristics were used as markers in the identification of jats. Nevertheless, certain groups contained all the three types in turn make difficult to assign into a particular cultivar (Visser, 1969). Results presented here using catechins content of UPASI clones can form a basis for further exploitation of identification of potential/premium quality mother bushes from the existing tea germplasm. In this study, high values of dihydroxylated: trihydroxylated catechin ratio used along with other characteristics like caffeine content as a marker to identify the superior quality clone.

Clones, UPASI-2 and UPASI-6 showed identical values of catechins; but their catechin ratios resulted in to a distinct difference which separated them in to ''Assam'' and ''China'', respectively. Utilisation of the catechin ratios (dihydroxylated to trihydroxylated catechins) in determination of genetic diversity could prove to be a novel and handy technique in future in establishing affinities of hybrids to the major taxonomic categories. Advantage with this technique is that it can be used as a marker for high quality tea varieties. This technique would be a useful complement to new molecular biology techniques like RFLP, AFLP and RAPD where these are difficult to adopt because of laborious and higher cost involved in these processes.

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