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Biochemical differentiation in *Camellia sinensis* and its wild relatives as revealed by isozyme and catechin patterns

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Abstract

The variation in three NADP-linked dehydrogenase enzymes; glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and shikimate dehydrogenase as well as alpha and beta esterases was determined in 24 cultivars of *Camellia sinensis* and 2 other species of *Camellia*; *C. japonica* and *C. irrawadiensis*, using specific activity staining. The isozyme profiles partitioned the cultivars according to their phylogenetic origins; (China, Assam, Cambodia and Japan). At all the loci studied, tea cultivars from China expressed the highest number of alleles followed by the Assam/Cambodia cultivars while the Japanese cultivars expressed the least. *Camellia irrawadiensis* and *C. japonica* showed unique isozyme profiles. The F_1 progeny from an interspecific cross between *C. sinensis* and *C. japonica* displayed the normal Mendelian allelic segregation, while progeny from a *C. sinensis* and *C. irrawadiensis* cross displayed 'distorted' segregation for some alleles. Analysis of the catechin expression patterns using HPLC, also showed that Chinese teas expressed the highest number of prominent catechins while Japanese tea expressed the least. These results show that the catechin biosynthetic pathway is most diverse in China and least in Japan tea. Since the quality and pharmacological importance of tea is mainly derived from catechins and catechin precursors like the aromatic amino acids, these results have important implications in breeding strategies especially in connection with tea germplasm enrichment and quality.

Keywords: *Camellia*; Theaceae; Tea; Isozymes; Catechins; Biochemical differentiation

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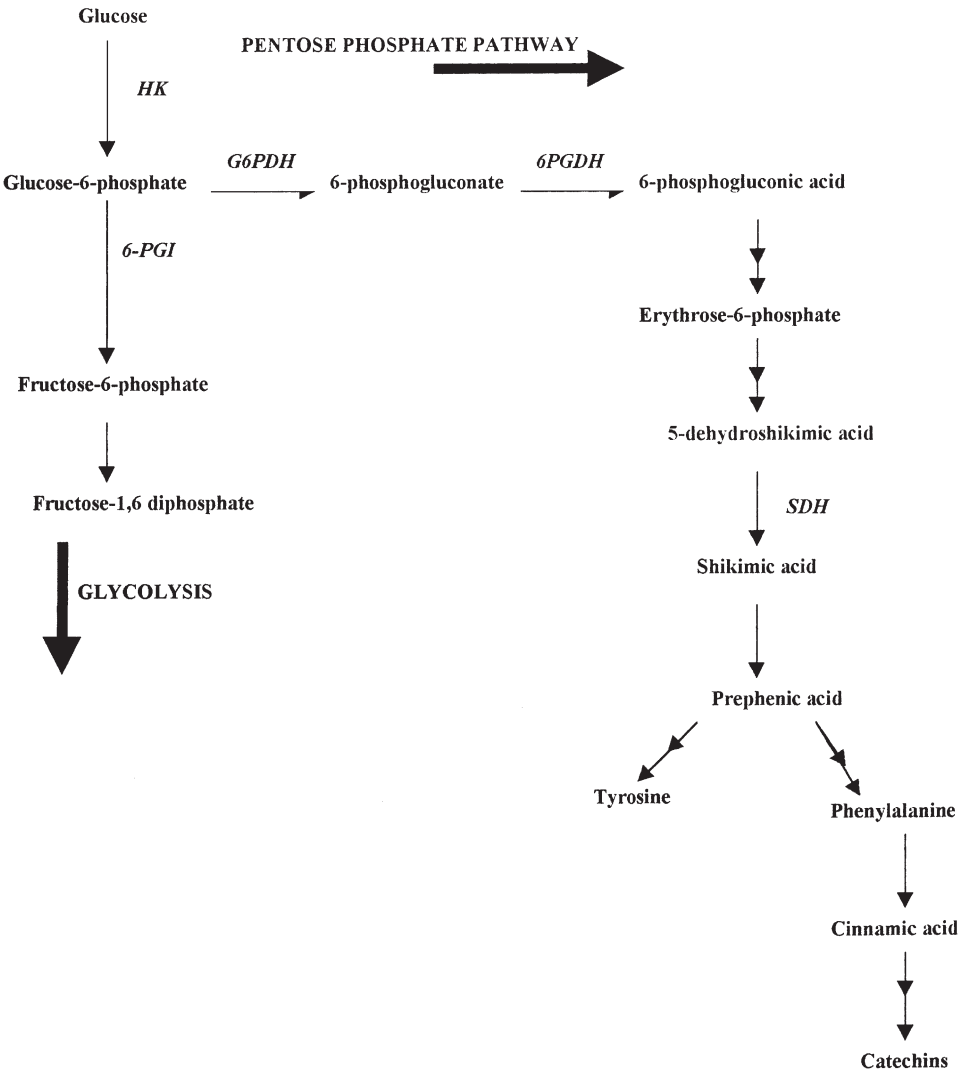
1. Introduction

The main constituents of the green tea leaf are the polyphenols, which comprise the four major catechins; epicatechin, (EC); epicatechin gallate, (ECG); epigallocatechin, (EGC) and epigallocatechin gallate (EGCG). The oxidation products of these catechins, the aromatic amino acids and caffeine are important in tea quality (Roberts and Smith, 1963; Takino et al., 1964). The catechins are also important pharmacologically due to their anticancer, antihypertension, antivascular disorders and anti-inflammatory properties (Adrian and Bolwell, 2000). Their general mechanism of action is thought to be through scavenging for endogenously formed free radicals and by inhibiting enzymes like cytochrome P450 which have potential to activate precarcinogens into carcinogens (Stoner and Mukhtar, 1995; Thomas, 1995; Salah et al., 1995).

The above polyphenols are formed through intermediary glucose metabolism comprising the pentose pathway, the shikimate pathway and the flavonoid pathway (Scheme 1). Some of the key enzymes catalyzing the biosynthesis of polyphenols include glucose-6-phosphate dehydrogenase (G6PDH) and shikimate dehydrogenase (SDH). G6PDH catalyses the first committed and rate limiting step of the pentose pathway (Copeland and Turner, 1987, Von Schaewen et al., 1995), while SDH catalyses the conversion of dehydroshikimate to shikimic acid, an essential precursor in the formation of catechins, caffeine and aromatic amino acids (Schnarrenberger et al., 1995). Up to 60% of the dry weight of some plant tissues can consist of metabolites derived from the shikimate pathway and are dependent on the flux from the pentose pathway (Schnarrenberger et al., 1995). It has been observed that during microbial attack the pentose pathway flux in plants increases in tandem with the shikimate pathway to provide sufficient secondary metabolites important in defence against microbes (Herrmann, 1995).

In some instances, it has been observed that variation at a single enzyme locus in a pathway can have a major impact on physiological variation and components of fitness. For example, variation in 6-phosphogluconate dehydrogenese (6PGDH) in rye grass has been shown to be related to the biomass accumulation of the grass (Rainey et al., 1987). In strawberry, SDH isozyme variation has been shown to have a close linkage with fruit colour (Williamson et al., 1995). Studies on relationships between variation at specific isozyme loci and the concentrations of the secondary metabolites have, however, not been carried out in most plant species. Studies on *C. sinensis* have shown that the tannin content, which is a measure of total catechin content, can be used in the determination of genetic variability in tea (Takeda, 1994). However, studies on the catechin biosynthetic enzymes and the expression of the individual catechins have not been carried out. In this study, we report on variability in some isozymes and catechin profiles in *C. sinensis* and two wild relatives; *C. japonica* and *C. irrawadiensis*.



Scheme 1. Flow of carbon compounds in *Camellia* species.

2. Materials and methods

2.1. Isozyme extraction and electrophoresis

Leaf material comprising two leaves and a terminal bud, was sampled from 24 clonal cultivars of *C. sinensis*, one cultivar of *C. irrawadiensis* (91/1) and *C. japonica* at the Tea Research Foundation of Kenya *ex- situ* field genebank (Table 1). The isozymes were extracted using a modified Tris–HCl extraction buffer (Soltis et al., 1983); one gram of the fresh leaf was crushed in a pre-chilled mortar to form a fine

Table 1
Passport information for *Camellia* cultivars used in the study

Cultivar	Source	Variety type	Specimen[a] Voucher No.
1. *C. sinensis* cv. 301/1	Re-Union	Cambod	EA. TRF01
2. *C. sinensis* cv. 301/2	″	″	EA. TRF02
3. *C. sinensis* cv. 301/3	″	″	EA. TRF03
4. *C. sinensis* cv. 301/4	″	″	EA. TRF04
5. *C. sinensis* cv. 301/5	″	″	EA. TRF05
6. *C. sinensis* cv. 301/6	″	″	EA. TRF06
7. *C. sinensis* cv. 6/8	Tea Research Foundation, Kericho, Kenya	Assam	EA. TRF07
8. *C. sinensis* cv. TN14-3	Eastern Produce, Nandi, Kenya	″	EA. TRF08
9. *C. sinensis* cv. 31/8	Tea Research Foundation, Kericho, Kenya	″	EA. TRF09
10. *C. sinensis* cv. S15/10	African Highlands, Kericho, Kenya	″	EA. TRF10
11. *C. sinensis* cv. KAG28	KTDA, Kirinyaga, Kenya	″	EA. TRF11
12. *C. sinensis* cv.BB7	Brooke Bond, Kericho, Kenya	″	EA. TRF12
13. *C. sinensis* cv. BB35	″	″	EA. TRF13
14. *C. sinensis* cv.KAG4	KTDA, Kirinyaga, Kenya	″	EA. TRF14
15. *C. sinensis* cv. SC12/28	African Highlands, Kericho, Kenya	″	EA. TRF15
16. *C. sinensis* cv. Ejulu	George Williamson, Sotik, Kenya	China	EA. TRF16
17. *C. sinensis* cv. K/purple	Molo South, Kenya	″	EA. TRF17
18. *C. sinensis* cv. 90/1	Tocklai, India	″	EA. TRF18
19. *C. sinensis* cv. Dwarf china	Tea Research Foundation, Kericho, Kenya	″	EA. TRF19
20. *C. irrawadiensis* cv. 91/1	Tocklai, India	‘Wild tea’	EA. TRF20
21. *C. japonica* cv.	NIVOT, Makurazaki, Japan	″	EA. TRF21
22. *C. sinensis* cv. 378/1	Tea Research Foundation, Kericho, Kenya.	Assam Polyploid (3x)	EA. TRF22
23. *C. sinensis* cv. 311/287	″	(4x)	EA. TRF23
24. *C. sinensis* cv 371/1	″	(3x)	EA. TRF24
25. *C. sinensis* cv. Yabukita	NIVOT, Makurazaki, Japan	Japan	EA. TRF25
26. *C. sinensis* cv. Yutakamidori	″	″	EA. TRF26

[a] Specimens are deposited at the East African Herbarium, Nairobi, Kenya.

paste before adding 10 volumes of a modified Tris-HCl extraction buffer with the following composition: 0.1M Tris-HCl pH 7.8, 0.001M EDTA, 0.01M KCl and 0.1M ascorbic acid. The mixtures were centrifuged (Microfuge, Hermle, Model Z-252M; 1000×g) for 2 min. at 25 °C and the resulting supernatants diluted ×10 in 5% sucrose. Five microlitres of the diluted supernatant was loaded onto a 7.6% starch gel and electrophoresed (150 V, 4 °C, 3 hr) on a horizontal gel apparatus (Pharmacia LKB, Multiphor II) linked to a temperature regulator (Pharmacia LKB, Multitemp II). After electrophoresis, the gels were stained for glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), 6-phosphogluconate dehydrogenase (6PGDH; EC 1.1.1.44), shikimate dehydrogenase (SDH; EC 1.1.1.25) and the alpha and beta esterases (EST; EC 3.1.1) according to Soltis et al. (1983).

2.2. Data analysis

Isozymes were designated sequentially from the anode according to conventional nomenclature. The twenty-six cultivars were scored for the two loci coding for the NADP-linked dehydrogenases. The presence of an allele was scored as 1 and the absence as 0. A total of eighteen alleles were scored for each cultivar and the data subjected to analysis by the POPGENE Version 1.31 computer software program (Yeh and Yang, 1999). The level of genetic similarity was determined based on Nei' s (1978) measure of genetic identity while a phylogenetic dendrogram was generated based on Nei's (1978) genetic distance.

2.3. Isozyme segregation

Four progeny from a *C. sinensis* cultivar Ejulu (♀) × *C. japonica* (♂) cross and 6 progeny from *C. sinensis* cultivar BB 35 (♀) × *C. irrawadiensis* (♂) cross were stained for 6PGDH and SDH as previously described to assess allele segregation. Due to the small number of progeny, the segregation data were not subjected to statistical analysis.

2.4. Catechin extraction and analysis

The catechins were extracted from fresh green leaves using an acetonitrile: H_2O (1:1) solvent system and analyzed on HPLC (Cecil, Model 1000 series) according to Obanda et al. (1999); under the following running conditions: Column: Capcell Pack C18UG 120S-5, Solvent A: 0.1% phosphoric acid in acetonitrile, Solvent B: 0.1% phosphoric acid in water, flow rate: 1.0 ml/min; injection vol. 20 μl, column temp. 40 °C; detection 230 nm.

The running program used (A:B) was: 0–5 min, 10:90; 5–25 min, 25:75; 25–26 min, 60:40; 26–35 min, 10:90; 35–45 min, 10:90. The % B used in each case was (100%–% of A). The following standards purchased from Sigma-Aldrich (U.K.), were used in the calibration: (+) catechin (+C), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG) and caffeine. The concentrations of the mixed standards used in the calibration ranged between 2.5–

10 μg/ml. In order to ascertain the elution sequence of the individual catechins or caffeine, mixed standards were spiked with the pure compounds before injection into the column.

3. Results and discussion

3.1. Isozyme activity and resolution

The isozyme activities and resolution decreased with increasing concentration of the following buffer additives; polyvinylpolypyrrolidone (PVPP), bovine serum albumin (BSA) and polyvinylpyrrolidone (PVP) to the simple Tris-HCl extraction buffer (0.1 M Tris-HCl pH 7.8, 0.001 M EDTA, 0.01 M KCl, 0.1 M ascorbic acid). The activities of the enzymes from young leaves were higher than those from old leaves (Fig. 1). This was in agreement with the observation of Sanderson (1966). However, the enzyme activities in the shikimate pathway enzymes in *C. sinensis* are not related to the total amounts of catechins (Saijo and Takeo, 1979; Magoma et al., 2000). This may be attributed to the metabolic branching at prephenic acid which may direct synthesis of more tyrosine and tyrosine derivatives than the formation of phenylalanine, the primary precursor of catechins (see Scheme 1).

The electrophoretic analysis of enzymes from plants containing high levels of

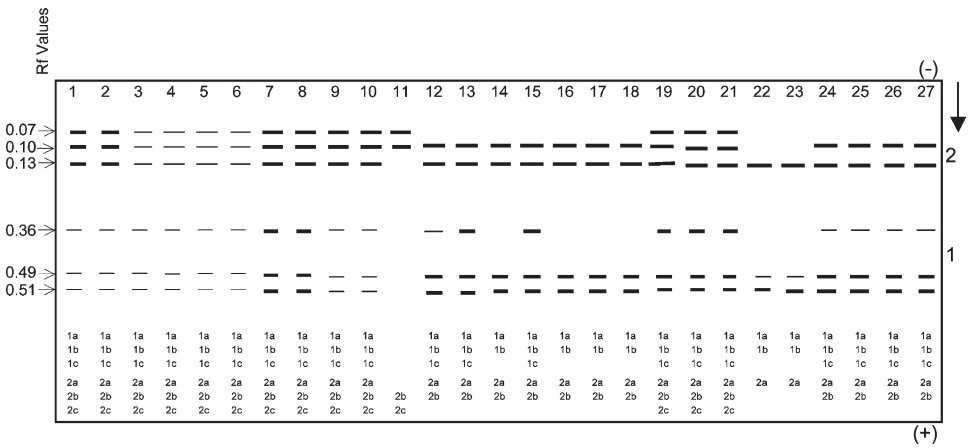


Fig. 1. Schematic illustration of SDH activity profiles in some *Camellia* cultivars and effect of buffer additives on activity: Lanes 1–10: Effect of buffer additives and leaf age on SDH activity. Lanes 1 & 2 — 2% PVPP & 0.5% PVP, lanes 3 & 4 — 4% PVPP & 1% PVP, lanes 5 & 6 — 8% PVPP & 2% PVP, lanes 7 & 8 — young leaf, lanes 9 & 10 — old leaf. Lanes 11–27 are different cultivars as follows; lane 11, *C. irrawadiensis*, lanes 12–15 (Assam tea), cultivars S15/10, 31/8, TN14-3, 6/8. Lanes 16–18 (Cambod tea), cultivars 301/3, 301/4, and 301/2. Lanes 19–21 (China tea), cultivars 90/1, Ejulu, K-purple. Lanes 22 & 23 (Japan tea), cultivars Yabukita,Yutakamidori. Lanes 24–27 (Polyploid tea), cultivars 371/1 (3×), 311/287 (4×), 371/8 (3×), 378/1 (3×). The arrow on the right indicates the direction of migration of the isozymes. The anodal locus is designated 1 while the cathodal one is designated 2. The Rf values are shown on the left margin.

polyphenols and tannins is difficult since these phenolic compounds are oxidized by phenol oxidases to quinones which inactivate the enzymes by reacting with the amino (NH_2) and sulfhydryl (SH) moieties of the enzymes (Loomis, 1974; Wendel, 1980). In order to minimize these effects, the inclusion of polyphenol adsorbents like PVPP, PVP and serum albumin in the extraction buffer usually yield isozymes with high activity (Soltis et al., 1983). The fact that these buffer additives decreased isozyme resolution and activity in our experiments shows that the ascorbic acid used in the extraction buffer was sufficient to inhibit polyphenol oxidase since there was no browning of the extracts.

3.2. Interpretation of isozyme patterns

The activity profiles for G6PDH, 6PGDH, SDH and α and β EST in the tea cultivars showed that they were all expressed at 2 loci and the phenotypes at each locus migrated very closely. In *C. irrawadiensis* and *C. japonica* the enzymes were, however, expressed in only one locus (Figs. 1 and 2). All the enzymes used in this study except SDH have been reported to exist as dimers (Schnarrenberger et al., 1973; Weeden and Marx, 1984; Tanksley and Kuehn, 1985; Murphy et al., 1990). The three-banded isozyme profile in cultivars expressing the highest number of alleles/locus in our results fits a model where products from the same allele preferentially combine to form active homodimers. Although SDH is monomeric in many plant species (Weeden and Weeden, 1989; Carre et al., 1993), it also exhibited a three-banded profile at both loci (Fig. 1). A possible explanation for these phenotypes is the duplication of loci with each having up to three alleles, all of which express an active polypeptide.

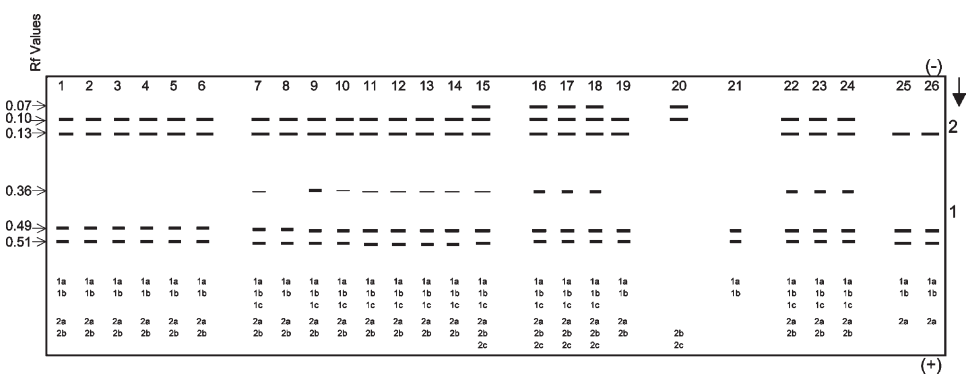


Fig. 2. A schematic representation of 6PGDH profiles in some *Camellia* cultivars: Lanes 1–6 (Cambod tea), cultivars 301/1, 301/2, 301/3, 301/4, 301/5, 301/6. Lanes 7–15 (Assam tea), cultivars 6/8, TN14-3, 31/8, S15/10, KAG28, BB7, BB35, KAG4, SC12/28. Lanes 16–19 (China tea), cultivars Ejulu, K/purple, 90/1, Dwarf. Lane 20 *C. irrawadiensis*; Lane 21 *C. japonica.* Lanes 22–24. (Polyploid tea), cultivars 378/1 (3×), 311/287 (4×), 371/1 (3×). Lanes 25 & 26 (Japan cultivars), cultivars Yabukita, Yutakamidori. The arrow on the right indicates the direction of migration of the isozymes. The anodal locus is designated 1 and the cathodal one is designated 2. The Rf values are shown on the left margin.

3.3. Isozyme linkage

All the five enzymes studied were expressed at two loci and the isozyme pattern exhibited by each cultivar was the same for all the enzyme systems. This expression suggests that the genes coding for these enzymes could be in the same linkage group. Enzyme linkage groups exist in many plant species; Navot and Zamir (1986) showed that esterase and shikimate dehydrogenase are linked in C*itrullus* and C*ucurbita*. Mutschler et al. (1987) also reported an EST-6PGI-6PGDH linkage group in tomato. A linkage group for G6PDH, 6PGDH, MDH, SDH, GOT and IDH has also been reported in strawberry (Granger, 1996).

3.4. Polyploidy in C. sinensis

Tea forms stable polyploids and a number of them have been identified in seed populations while others have arisen through artificial crossing of known diploids (Wachira and Kiplangat, 1991). The tea polyploids of Assam origin; 378/1 (3×), 371/1 (3×) and 311/287 (4×), showed activity profiles similar to the typical diploid Assam teas (Figs. 1 and 2). This expression suggests that the type of polyploidy exhibited by these teas is autoploidy, in which the inheritance of the isozymes is expected to be disomic. These cultivars have also shown a tendency to form multivalent associations during meiosis (Wachira, 1997), suggesting that they could also have arisen from fertilization involving unreduced gametes.

3.5. Allele frequencies and genetic differentiation

The allele frequencies of the different varieties used in the study and a summary of the genic variation statistics for all the loci are shown in Tables 2 and 3, respectively. The China cultivars expressed the most alleles; that is *a*, *b* and *c* at both locus 1 and 2 in almost equal proportions. The Assam cultivars expressed mainly alleles *a* and *b* at locus 2 and alleles *a*, *b*, *c* at locus 1 with allele *c* showing little stain intensity compared to the other two alleles. The Cambod cultivars expressed alleles *a* and *b* at both loci while the Japanese clones expressed alleles *a* and *b* at locus 1 and only allele *a* at locus 2. The decline of alleles from China to Japanese tea may be due to the domestication process of *Camellia sinensis*. Decline in allele frequencies with domestication has also been observed in insects such as *Aedes aegypti* (Mukiama, 1980).

3.6. Genetic diversity and phylogenetic affinities among Camellia varieties

Genetic analysis of the isozyme data using the POPGENE software revealed a mean total diversity of 0.333, while the diversity within the variety groups was 0.035 and genetic differentiation of 0.895 (Table 4). These results show that almost 90% of the total genetic diversity was due to differences among the variety groups. The level of total genetic diversity is higher than the average mean values for long-lived woody perennials (0.177), gymnosperms (0.169) and long-lived woody widespread

Table 2
Allele frequencies for the different taxa of *C.sinensis* and its wild relatives

Locus/Allele		Assam $n = 9$	China $n = 4$	Cambod $n = 6$	Japan $n = 2$	*C. irrawa*[a]. $n = 1$	*C. jap*[b] $n = 1$	Polyploid $n = 3$
SDH1	a	0.333	0.364	0.5	0.5	0	0.5	0.333
	b	0.333	0.364	0.5	0.5	0	0.5	0.333
	c	0.333	0.272	0	0	0	0	0.333
SDH2	a	0.474	0.364	0.5	1	0	0	0.5
	b	0.474	0.364	0.5	0	0.5	0	0.5
	c	0.052	0.272	0	0	0.5	0	0
G6PDH1	a	0.346	0.364	0.5	0.5	0	0.5	0.333
	b	0.346	0.364	0.5	0.5	0	0.5	0.333
	c	0.308	0.272	0	0	0	0	0.333
G6PDH2	a	0.474	0.333	0.5	1	0	0	0.5
	b	0.474	0.333	0.5	0	0.5	0	0.5
	c	0.052	0.333	0	0	0.5	0	0
6PGDH1	a	0.346	0.364	0.5	0.5	0	0.5	0.333
	b	0.346	0.364	0.5	0.5	0	0.5	0.333
	c	0.308	0.272	0	0	0	0	0.333
6PGDH2	a	0.474	0.333	0.5	1	0	0	0.5
	b	0.474	0.333	0.5	0	0.5	0	0.5
	c	0.052	0.333	0	0	0.5	0	0

[a] *C. irrawadiensis.*
[b] *C. japonica.*

species (0.257) (Hamrick et al., 1992). However, the level of diversity observed in our studies is lower than in *Eurya japonica* (0.46), which also belongs to the same family as *Camellia sinensis*. The observed mean number of alleles and the effective number of alleles are also lower than those reported for *E. japonica* (*na* = 3.79; *ne* = 2.09, respectively) (Chung and Kang, 1994). The lower level of diversity in this study could be attributed to the small number of cultivars and enzyme systems assayed. Increasing both the isozyme loci and cultivars from the individual groups may reveal a higher level of diversity.

Genetic identity was highest between Assam and polyploid tea (0.985) and lowest between *C. irrawadiensis* and polyploid tea (Table 5). Analysis of the phylogenic affiliations of the cultivars based on Nei's (1978) genetic distance yielded three major cluster groups (Fig. 4). The first group comprised of Cambod, China, Assam and polyploid tea cultivars; the second comprised of *C. japonica* and the Japanese cultivars while *C. irrawadiensis* was most isolated. The tight clustering of polyploid and Assam tea is expected since the polyploids are of Assam variety type. The first cluster group also shows that the Assam cultivars could have evolved from China tea while the Cambod tea could have evolved differently. This finding is in agreement with the history of tea cultivation. The origin of tea is thought to be in western China and speciation is thought to have taken place during migration to the south west (Assam), south (Cambodia) and the east (China /Taiwan/Japan) (Yamaguchi et

Table 3
Summary statistics of genic variation for all loci in *C. sinensis* and its wild relatives

Locus/Allele	*na*	*ne*	*H*	*I*
SDH1 a	2	1.08	0.074	0.163
SDH1 b	2	1.08	0.074	0.163
SDH1 c	2	1.95	0.49	0.68
SDH2 a	2	1.17	0.14	0.271
SDH2 b	2	1.26	0.2	0.358
SDH2 c	2	1.31	0.23	0.396
G6PDH1 a	2	1.08	0.074	0.163
G6PDH1 b	2	1.08	0.074	0.163
G6PDH1 c	2	1.95	0.49	0.68
G6PDH2 a	2	1.17	0.14	0.271
G6PDH2 b	2	1.26	0.2	0.358
G6PDH2 c	2	1.31	0.23	0.396
6PGDH1 a	2	1.08	0.074	0.163
6PGDH1 b	2	1.08	0.074	0.163
6PGDH1 c	2	1.95	0.49	0.68
6PGDH2 a	2	1.17	0.14	0.271
6PGDH2 b	2	1.26	0.2	0.43
6PGDH2 c	2	1.26	0.23	0.396
Mean	1.947	1.296	0.195	0.325
St. Dev.	0.229	0.309	0.149	0.195

na = Observed number of alleles, *ne* = Effective number of alleles, *H* = Nei's 1973 gene diversity, *I* = Shannon's information index, St. Dev.= Standard deviation.

al., 1999). The clustering of Japanese tea with *C. japonica* could be attributed to the highly outcrossing nature of *Camellia's*, resulting in the co-evolution of the Japan tea with *C. japonica.*

3.7. Segregation of alleles

The F_1 progeny from *C. sinensis* cultivar Ejulu and *C. japonica* displayed 1:1 Mendelian inheritance for allele *1c*. The other alleles were not inherited in a Mendelian fashion (Fig. 3(a)). The F_1 progeny of the cross between *C. irrawadiensis* and *C. sinensis* cultivar BB35 displayed a 1:2 non-Mendelian segregation ratio for allele *1c*. The allele *2c* in *C. irrawadiensis* was inherited in a distorted manner since it was not present in the F_1 progeny (Fig. 3(b)). The sample sizes of the segregating populations were, however, too small to qualify whether the above segregation ratios were distorted.

3.8. Expression of catechins and isozymes and their taxonomic significance

The elution of the individual catechins and caffeine from the HPLC column occurred in the following order: EGC, +C, caffeine, EC, EGCG and ECG. The China cultivars, Ejulu and 90/1 expressed the highest number of catechin peaks, the Cam-

Table 4
Genetic diversity analyses for *C. sinensis* and its wild relatives

Locus/Allele	H_t	H_s	G_{st}
SDH1 a	0.245	0	1
SDH1 b	0.245	0	1
SDH1 c	0.427	0.135	0.684
SDH2 a	0.408	0	1
SDH2 b	0.408	0	0.749
SDH2 c	0.346	0.087	1
G6PDH1 a	0.245	0	1
G6PDH1 b	0.245	0	1
G6PDH1 c	0.427	0.135	0.684
G6PDH2 a	0.408	0	1
G6PDH2 b	0.408	0	0.749
G6PDH2 c	0.346	0.087	1
6PGDH1 a	0.245	0	1
6PGDH1 b	0.245	0	1
6PGDH1 c	0.427	0.135	0.684
6PGDH2 a	0.408	0	1
6PGDH2 b	0.408	0	0.749
6PGDH2 c	0.346	0.087	1
Mean	0.333	0.035	0.895
St. Dev.	0.132	0.003	

H_t=Total gene diversity, H_s=Diversity within groups, G_{st}=Genetic differentiation.

Table 5
Genetic identity matrix of the test germplasm

	China	Cambod	Japan	*C. irrawa*[a]	*C. jap*[b].	Polyploid tea
Assam	0.976	0.93	0.764	0.231	0.598	0.985
China	–	0.93	0.756	0.349	0.581	0.93
Cambod		–	0.842	0.316	0.684	0.842
Japan			–	0.263	0.842	0.684
C. irrawa[a]				–	0.421	0.158
C. jap[b]					–	0.526
Polyploid tea						–

[a] *C. irrawadiensis.*
[b] *C. japonica.*

bod and Assam cultivars had intermediate number of peaks while a Japanese cultivar, Yabukita, expressed the least number of catechins (Table 6). The catechin peaks in this cultivar were less pronounced compared to the other cultivars used in this study. Furthermore, the expression of + C was particularly diminished. These results corroborate those of Takeda (1994) and Obanda, (1997) who showed that the Japanese tea

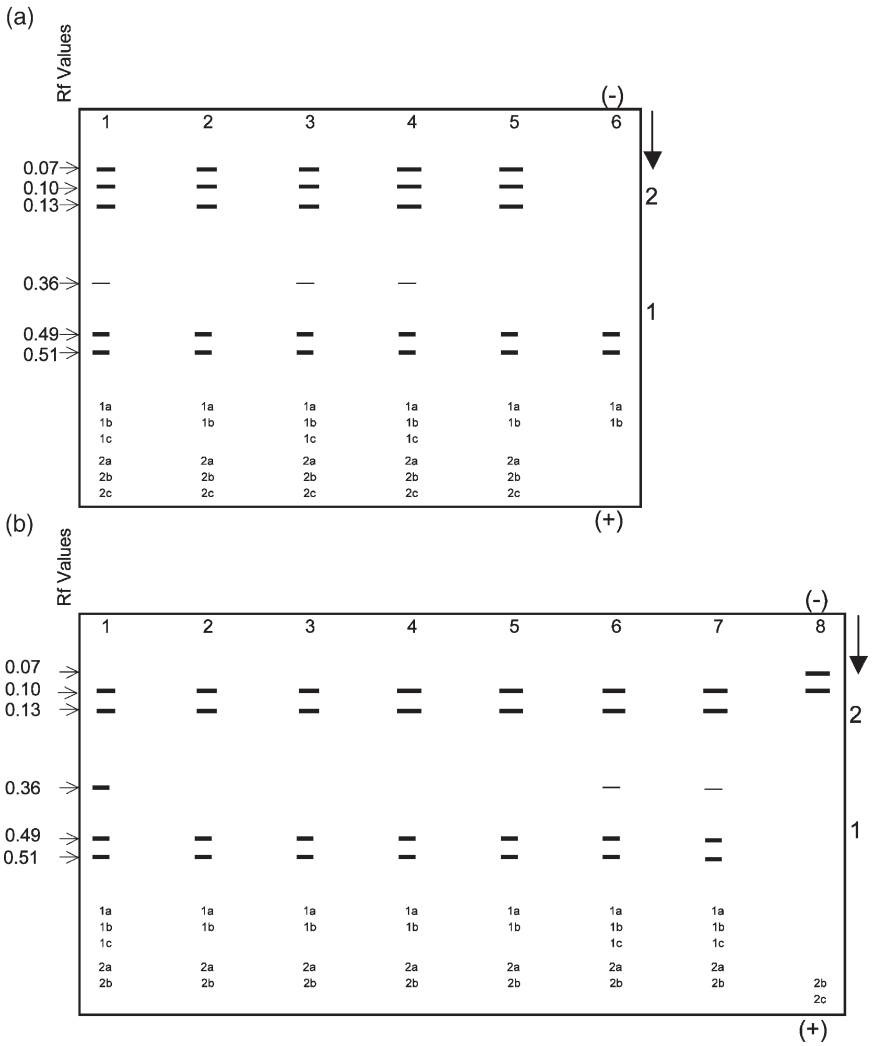


Fig. 3. Schematic illustration of segregation of alleles in progeny from 'wild tea' and *Camellia sinensis* crosses. (a): Segregation of alleles at 6PGDH loci in *C. sinensis* cultivar Ejulu x *C. japonica* progeny. Lane 1. Ejulu, Lanes 2–5 F_1 progeny, Lane 6 *C. japonica*. (b): Segregation of alleles at SDH loci in BB35 x *C. irrawadiensis* progeny. Lane 1 BB35, Lanes 2–7 F_1 progeny, Lane 8 *C. irrawadiensis.*

generally has low tannin contents compared to China, Assam tea and Kenyan cultivars of Assam origin.

The EC and ECG peaks were more prominent in the China and Cambod cultivars and diminished in the Assam and Japan cultivars, indicating that the expression of the individual catechins is variety dependent. Indeed recent studies have shown that the ratio of the dihydroxylated: trihydroxylated catechins (EC+ECG/EGC+EGCG) is high for the China and Cambod cultivars and low for the Assam and polyploid

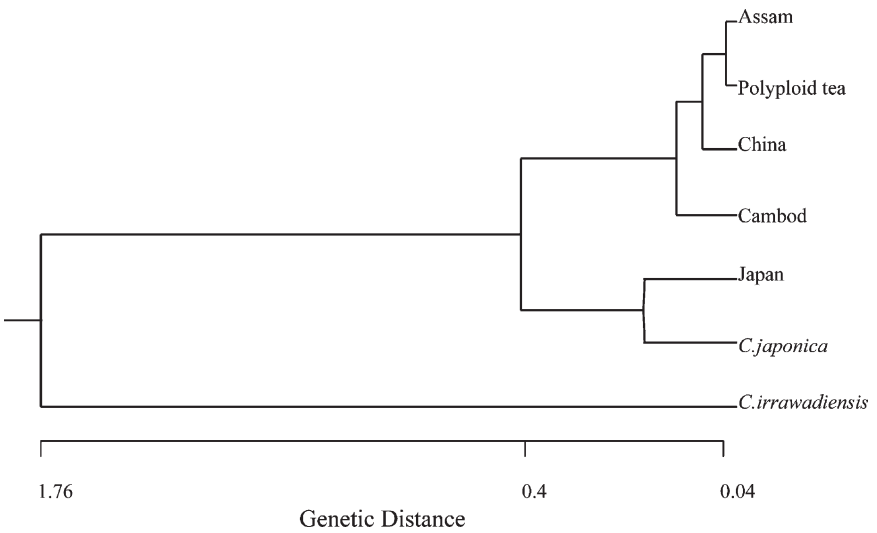


Fig. 4. A phylogenetic dendrogram for different taxa of tea and its related species based on Nei's genetic distance.

Table 6
The presence and relative abundance of catechins and caffeine in some *C. sinensis* varieties and *C. irrawadiensis*

Compound	China		Cambod	Assam	Japan	Wild tea
	cv. Ejulu	cv. 90/1	cv. 301/2	cv. 6/8	cv. Yabukita	*C. irrawadiensis*
1. Unidentified[a]	+++	+++	+	+	–	+
2. EGC	++	+++	++	+++	++	+
3. +C	+++	+++	+	–	–	–
4. Caffeine	++	++	+++	++	+	+
5. EC	++	+++	+++	++	+	+
6. EGCG	+++	+++	++	+++	+	+++
7. ECG	+++	+++	+++	++	+	+

+++ = High concentration, ++ = Medium concentration, + = Low concentration, − =absent.
[a] Several unidentified peaks probably other unidentified catechins, their isomers or flavonol glycosides.

teas and is therefore important in the biochemical differentiation of tea (Magoma et al., 2000).

In this study, the trend in the decline of the number of catechin peaks from Chinery to Japanese cultivars (Table 6) is also reflected in the decline of the number of isozyme alleles (Figs. 1 and 2). These results show that cultivars from China have a more diverse catechin biosynthetic pathway compared to tea from other origins. This decline in the diversity of the catechin pathway is not unexpected since China

is believed to be the centre of origin of tea. The speciation process which occurred during dispersal of tea from China to Assam and Japan, could have led to this loss of diversity (Yamaguchi et al., 1999). *C. irrawadiensis* which expressed one major catechin peak (Table 6) also expressed a unique isozyme allele (*c*) at locus 2 (Figs. 1 and 2). This finding also seems to suggest a correlation between the number of catechin peaks and expression of the isozyme alleles.

The results of this study enabled distinction of the two purple/ brick-red pigmented cultivars, *C. irrawadiensis* and cultivar K/purple; which have previously been shown to be genetically very close using RAPDs (Wachira et al., 1997). Apart from having only alleles *b* and *c* at the cathodal locus for all the isozymes studied, *C. irrawadiensis*, displayed the least number of major catechin peaks and particularly diminished levels of (+) catechin and caffeine (Table 6). This shows that although the isozymes and catechins used in this study may have limited polymorphism, they could supplement results from other classification techniques and in this case they would be very useful in studying interspecific gene introgression within the genus *Camellia*.

3.9. Catechin composition and tea quality

The catechins and caffeine composition of the green leaf has been shown to be a potential indicator of black tea quality (Takino et al., 1964; Obanda et al., 1997). Indeed, cultivar Ejulu which had the highest number of catechin peaks is a very high black tea quality cultivar (Obanda et al., 1997) while *C. irrawadiensis* with the least number of catechin peaks lacks the quality acceptable as tea (Bezbaruah, 1987). This finding may suggest that the number, diversity and relative amounts of catechins may be important in the determination of the quality of a cultivar. However, it is important to note that the brick-red pigmented clone 90/1, which also has a high number of catechin peaks (Table 6) is considered to be of medium quality rather than of high quality as expected from its number of catechin peaks. This study, therefore, shows that although the catechin diversity of a clone is important, it may not be the only single determinant of the quality of black tea. Further study on this is required.

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