*Genetic Resources and Crop Evolution* **47:** 107–114, 2000.



# The use of catechins as biochemical markers in diversity studies of tea (*Camellia sinensis*)

G.N. Magoma[1], F.N. Wachira*, M. Obanda, M. Imbuga[1] & S.G. Agong[2]
*Department of Botany, Tea Research Foundation of Kenya, P.O. Box 820, Kericho, Kenya; E-mail: tearfk@insightkenya.com. Present address: [1]Department of Biochemistry, [2]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box 62000, Nairobi, Kenya (*Author for correspondence)*

Received 14 September 1998; accepted in revised form 29 March 1999

*Key words: Camellia sinensis*, catechins, genetic differentiation

**Abstract**

The concentrations of catechins in a Kenyan tea germplasm collection of 102 accessions were determined by HPLC. Total green leaf catechin concentrations and the ratio of dihydroxylated to trihydroxylated catechins were used to establish genetic differentiation in the germplasm. Upon multivariate analysis, accumulation of the various catechins separated the tea clones into 3 major and 5 minor groups according to their phylogenetic origins. The Cambod teas had the highest ratio (7:10) followed by China teas (3:5) while Assam teas had the lowest ratio (1:4). This biochemical differentiation indicates that there is potential for broadening the genetic base of the mainly Assam teas in Kenya (90%) with the putative China and Cambod teas.

## Introduction

Tea is one of the most widely consumed soft beverages in the world. The main classes of cultivated tea consist of *C. sinensis* (L.) O. Kuntze and *C. assamica* (Masters) Wight with the intermediate *C. assamica* ssp *lasiocalyx* (Planchon ex Watt) Wight (i.e. China, Assam and Cambod varieties respectively) (Banerjee, 1992). However, tea is highly heterogenous and all the above taxa freely interbreed, resulting in a cline extending from extreme China types to those of Assam origin (Wight, 1959). Hybridization has been so extreme that it is often debated if archetypal *C. sinensis, C. assamica* or *C. assamic* ssp *lasiocalyx* still exist (Visser, 1969). Cultivated tea is largely based on high yielding clones from vegetatively propagated materials. This widespread cultivation of clonal tea can diminish genetic diversity if care is not taken to use clones of disparate origin. A few genebanks for tea germplasm conservation have been established, though most have not been rationalized and were not based on optimized collection strategies. Since the conservation of germplasm resources is critical for future sustainability of the tea industry, there is need to rationalize the collections and especially the one at the Tea Research Foundation of Kenya which is one of the few held anywhere in the world to ascertain the prevailing diversity. Previous studies using RAPD and AFLP markers on representative samples from Kenya and India (which is believed to be the primary centre of dispersal) show that Kenyan tea has close similarity with the Indian germplasm (Wachira et al., 1995; Paul et al., 1997). However, deliberate selection for diverse clones with high quality has been lacking.

The selection for high quality tea requires precise information on the diversity available and also a careful study of the biochemicals which contribute towards the quality. The important biochemicals in determining tea quality include the green leaf tea catechins, their oxidation products and caffeine. The major catechins in green leaf consist of (−) epicatechin (EC), (−) epigallocatechin (EGC), (−) epigallocatechin gallate (EGCG) and epicatechin gallate (ECG). The oxidation products of these catechins, the theaflavins and thearubigins are responsible for most of the plain black tea quality attributes (Robertson & Smith, 1963;

Takino et al., 1964; Brown et al., 1966; Robertson, 1992).

Total catechin content could be used to indicate the quality potential of tea, with high content being related to high quality (Obanda & Owuor, 1997). Other studies have also shown that tannins content, which is a measure of total catechins content, can be used in the determination of genetic diversity in tea (Takeda, 1994). However, these methods are not fully reliable as they do not take into account the individual catechins present. The individual proportions of the catechins could be important in the determination of tea quality and genetic diversity (Owuor & McDowell, 1994). There is need therefore to study the relative expression of the individual tea catechins.

The formation of dihydroquercetin and dihydromyricetin, which are the precursors of dihydroxylated catechins (EC & ECG) and trihydroxylated catechins (EGC & EGCG), respectively, is under genetic control (Gerats & Martin, 1992). Indeed, the dihydroxylated and trihydroxylated catechins arise from different branches of an initial common pathway depending on the proportionate activity of the flavonoid 3′ hydroxylase to flavonoid 3′5′ hydroxylase (Scheme 1; Gerats & Martin, 1992).

In grapes (*vitis vinifera*), in nearly all the varieties the ratio between the quantities of the dihydroxylated and trihydroxylated anthocyanin is of the order 1:4, and in muscats it is about 1:2, this is a constant pigment character which is independent of growing conditions (Harbone, 1967). In *Petunia hybrida* (L.), different anthocyanins give rise to different colours according to the degree of hydroxylation of the B-ring; flower colour shifts from orange to red to purple/blue when the anthocyanin present is pelargonidin, cyanidin or delphinidin, respectively. Since the pathway leading to the formation of tea catechins is similar to that in anthocyanin formation in *P. hybrida* (L.) and *V. vinifera* (L.), the dihydroxylated and trihydroxylated catechin ratios could be used to study genetic variation in tea with the aim to elucidate questions of diversity. We report here for the first time the utilisation of dihydroxylated: trihydroxylated catechin ratios and total catechins in the determination of the genetic differentiation of the Kenyan tea germplasm.

## Materials and methods

### *Sample collection*

Fresh plant material (two leaves and a bud) were collected three times over a period of eight months from randomly selected plants in 102 clonal plots from the Kenyan ex-situ germplasm collection. The collection was established during 1983–1997 at the TRFK station at an altitude of 2180 m.a.m.s.l. The collection currently holds 102 accessions (Table 1), some of which are semi-wild. Each accession is represented by a plot of 100 plants. The characters assessed were the accumulation of the major tea catechins: EC, ECG, EGC and EGCG.

### *Catechin analysis on HPLC*

The catechins were extracted and analyzed on HPLC (Cecil, Model 1000 series) according to the method of Obanda & Owour (1997) under the following running conditions:

Column: Capwell Pack C18UG 120 5-5
Solvent B: 0.1% phosphoric acid in acetonitrile
Solvent C: 0.1% phosphoric acid in water
Flow rate: 1.0 ml/min; Injection vol. 20 ml, column temperature 40 °C; Detection 270 nm.

The results were recorded and analyzed using a JCL 600 data system and the individual catechin concentrations calculated using the following formula:

Y=[(aX+K). Extraction vol.×dil. volume]/Dry Matter (DM)

where:

Y is the catechin concentration, a is the gradient, X is the peak area and K is the constant for each catechin.

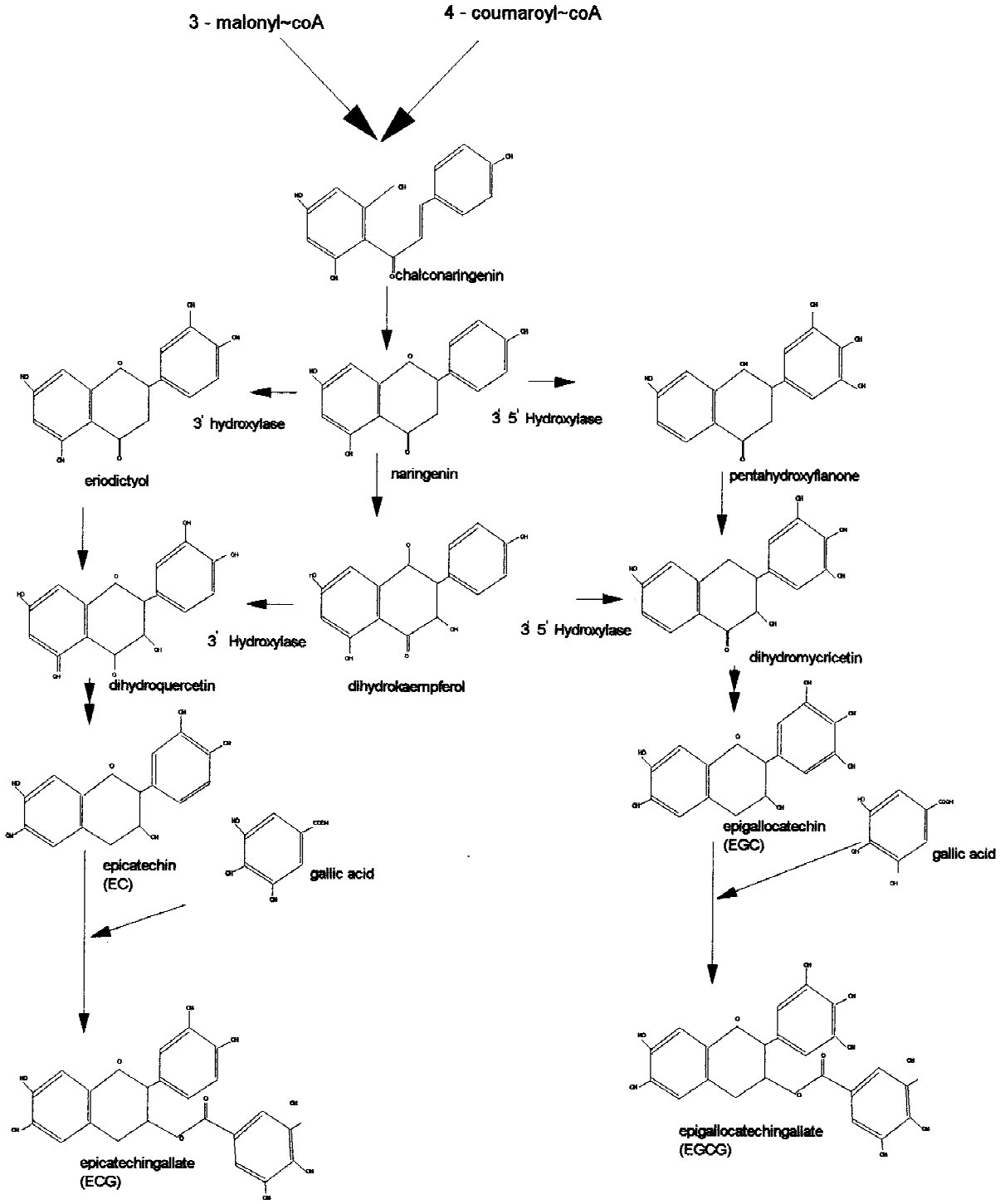
The concentrations of EGC, EGCG, EC and ECG were determined in three replicates for all the clones.

## Data analysis

Data was analysed by the GENSTAT version 5 computer software program based on Principal Component Analysis (PCA) and hierarchical cluster analysis (Genstat 5 committee, 1989).

## Results

The order of elution of the catechins from the HPLC column was as follows: EGC, EC, EGCG and ECG. Principal Component Analysis based on the factorial co-ordinates on the first two axes produced a hierarchical ascendant classification which partitioned the clones into several groups. The first principal component, which is the weighted average of the various catechin concentrations and the dihydroxylated




*Scheme 1.* Biosynthesis of catechins; modified from Gerats and Martin (1992).

to trihydroxylated catechin ratios, accounted for 45% of the total variability of which the ratios were the most important contributor with a vector loading of 0.55. The second principal component, which accounted for 32% of the total variability, constrasted dihydroxylated catechins and the total catechins contents with the weighted average. The PCA allowed identification of 3 main groups and 5 sub-groups (Figure 1).

The identification of the groups was achieved by ascertaining the location of the putative Cambod, Assam and China clones, the sub-groups were identified by judging whether there was sub-clustering within the main groups. Group 1, which represents 90% of the clones, comprises Assam tea. This group had 3 subgroups; subgroup (1a) which was an interface between Assam and Cambod tea had a catechin ratio of 1:2; subgroup (1b) with the catechin ratio of 1:4 and the highest total catechins comprised the polyploid teas, while subgroup (1c) which comprised the typical Assam tea had a catechin ratio of 1:4. Group 2 comprised the Cambod clones and it had a catechin ratio of 7:10. Group 3 which comprised China tea had 2 subgroups: subgroup (3a) with a ratio of 1:2 represented the wild species of *Camellia*; cultivars K. purple and 91/1 (*Camellia irrawadiensis* P.K. Barua), and mate tea (*Ilex paraguariensis* St. Hil.); subgroup (3b) with the ratio of 2:3 and the lowest total catechins comprised clones Ejulu and 90/1. Summary statistics of the various groups as defined by the PCA showed that the catechin ratios had the highest resolving power compared to the other variables, allowing clear identification between the three major groups of tea. Both the total catechin and the trihydroxylated catechin concentrations only allowed identification of China tea from the other groups of tea whereas the dihydroxylated catechins did not give any clear group identity (Table 2).

## Discussion

A clear structure was observed within *C. sinensis* through the identification of 3 major groups: the Assam, Cambod and China teas. China teas have low total catechins compared to Assam teas (Table 2). These results generally conform with those of Takeda (1994) where the China teas had low tannins content compared to Assam teas. Specifically, our results also conform with those of Paul et al. (1997) and Wachira et al. (1995) where the AFLP and RAPD markers respectively tightly clustered the two Brooke bond clones BBKL21 and BBLK 35, and the Cambod clones 301/1, 301/3, 301/4 and 301/5. A further corroboration of our results with AFLP markers is the classification of clones 7/9, 56/89 and TN14/3 under the the Assam group, although morphological markers depict them as China type (Table 1). Another interesting observation of the resolving power of the catechin profiles compared to morphological markers is the classification of clone 303/577 which is an offspring of clone 6/8, a putative Assam clone. The catechin profiles place it under the Assam group as expected but morphological markers place it under the China group (Table 1). Our results also conform with those of Wachira et al. (1997) where RAPD markers also tightly clustered the two brick-red pigmented clones K/purple and 91/1. However, a Cambod clone 301/2 clustered with the Assam tea sub-group which has a catechin ratio of 1:2. This differed with the results of Paul et al. (1997) where clone 301/2 clustered with clone 301/3 in the Cambod group, implying that most of the Assam teas which clustered with 301/2 may actually be hybrids of the Cambod and Assam teas. Catechin ratio hierarchical ascendance classification showed that even if this clone is classified under Assam sub-group 1(a), it is also quite similar to the other putative Cambod clones (Table 3). Hybridization between different tea taxa is a very common phenomenon which sometimes makes it difficult to assign a clone to a particular varietal taxa (Visser, 1969). Tea clones selected from the same sites clustered together: e.g. KAG 4, KAG 27 and KAG 28 from Kagochi Farm in the East of the great Rift Valley; the African Highland Produce clones: PMC45, PMC46 and PMC61, and the Brooke Bond clones BBLK21 and BBLK 35. The results presented here using catechins accumulation differentials of the tea clones can form a basis for further elucidation of the biochemical structure of *C. sinensis*, especially studies on the enzymes involved in the biosynthesis of the catechins and other important metabolites. The highest proportion (90%) of clones grown in Kenya are of Assam origin. All the other groups combined comprise about 10% of the total number of clonal tea cultivated in Kenya. Interestingly, group 3 includes clone Ejulu which has superior tea quality characteristics. The total catechin concentration in this group was the lowest but the ratio of the dihydroxylated:trihydroxylated catechins was quite high, (2:3). The clones in group 2 with a high ratio (7:10) have also been reported to have good tea quality, comparable to the standard high quality clone

*Table 1.* Tea genotypes and their origins

| Clone | Source of material | Variety type | Grp in PCA | Clone | Source of material | Variety type | Grp in PCA |
|---|---|---|---|---|---|---|---|
| 1. 12/12 | TRFK, Kericho, Kenya | Assam | 1c | 52. 7/14 | TRFK, Kericho, Kenya | Assam | 1c |
| 2. H81/22 | EAPA, Nandi, Kenya | China | 1c | 53. BB207 | BB, Kericho, Kenya | China | 1c |
| 3. 12/19 | TRFK, Kericho Kenya | Assam | 1c | 54. 303/216 | Rwebitaba, Uganda | Assam | 1c |
| 4. 38/3 | GW, Kericho, Kenya | Assam | 1c | 55. 303/259 | Rwebitaba, Uganda | Assam | 1c |
| 5. **S8/38** | **AHP, Kericho, Kenya** | **Assam** | **2** | 56. 54/40 | TRFK, Kericho, Kenya | Assam | 1c |
| 6. D99/10 | EAPA, Nandi, Kenya | Assam | 1c | 57. 12/28 | TRFK, Kericho, Kenya | Assam | 1c |
| 7. **7/9** | **TRFK, Kericho, Kenya** | **China** | **1c** | 58. 303/178 | Rwebitaba, Uganda | Assam | 1c |
| 8. **TN14/3** | **EAPA, Nandi, Kenya** | **China** | **1c** | 59. Ejulu | GW, Kericho, Kenya | China | (3b) |
| 9. **C12** | **EAPA, Nandi, Kenya** | **China** | **1c** | 60. F5/222 | AHP, Kericho, Kenya | Assam | 1c |
| 10. 182/40 | EAPA, Nandi, Kenya | Assam | 1c | 61. **303/577** | **Rwebitaba, Uganda** | **China** | **1c** |
| 11. 31/8 | TRFK, Kericho, Kenya | Assam | 1b | 62. 12/2 | TRFK, Kericho, Kenya | Assam | 1c |
| 12. 6/8 | TRFK, Kericho, Kenya | Assam | 1c | 63. 91/1 | Toklai, India | China | 3a |
| 13. S15/10 | AHP, Kericho, Kenya | Assam | 1c | 64. 301/6 | Re-Union | Cambod | 2 |
| 14. 89/5 | Siret, Nandi, Kenya | Assam | 1c | 65. 301/4 | Re-Union | Cambod | 2 |
| 15. 7/3 | TRFK, Kericho, Kenya | Assam | 1c | 66. 301/5 | Re-Union | Cambod | 2 |
| 16. **Changoi** | **GW, Kericho, Kenya** | **China** | **1c** | 67. 90/1 | Toklai, India | China | 3b |
| 17. **108/82** | **Rwebitaba, Uganda** | **China** | **1b** | 68. Mate | France | China | 3a |
| 18. 100/5 | Rwebitaba, Uganda | Assam | 1c | 69. 301/1 | Re-Union | Cambod | 2 |
| 19.11/26 | TRFK, , Kericho, Kenya | Assam | 1c | 70. **301/2** | **Re-Union** | **Cambod** | **1a** |
| 20. 11/52 | TRFK, , Kericho, Kenya | Assam | 1c | 71. 5/3 | Sotik, Kenya | Assam | 1c |
| 21. PMC 2 | AHP, Kericho, Kenya | Assam | 1c | 72. 301/3 | Re-Union | Cambod | 2 |
| 22. 11/4 | TRFK, Kericho, Kenya | Assam | 1c | 73. 57/15 | TRFK, Kericho, Kenya | Assam | 1c |
| 23. 89/2 | Siret, Nandi, Kenya | Assam | 1c | 74. 31/27 | Ambangulu, Tanzania | Assam | 1c |
| 24. B8 | KTDA, Kerinyaga, Kenya | Assam | 1c | 75. 2xi/4 | TRFK, Kericho, Kenya | Assam | 1c |
| 25. PMC61 | AHP, Kericho, Kenya | Assam | 1c | 76. G28U864 | AHP, Kericho, Kenya | Assam | 1c |
| 26. PMC46 | AHP, Kericho, Kenya | Assam | 1c | 77. 303/999 | Rwebitaba, Uganda | Assam | 1c |
| 27. KAG27 | KTDA, Kerinyaga, Kenya | Assam | 1c | 78. 303/231 | Rwebitaba, Uganda | Assam | 1c |
| 28. KAG4 | KTDA, Kerinyaga, Kenya | Assam | 1c | 79. 31/29 | Ambangulu, Tanzania | Assam | 1c |
| 29. 89/1 | Siret, Nandi, Kenya | Assam | 1c | 80. 31/30 | Ambangulu, Tanzania | Assam | 1c |
| 30. 89/3 | Siret, Nandi, Kenya | Assam | 1c | 81. 247/26 | TRFK, Kericho, Kenya | Assam | 1c |
| 31. B1 | KTDA, Kerinyaga, Kenya | Assam | 1c | 82. 311/287 | Rwebitaba, Uganda | Assam | 1b |
| 32. S1/99 | AHP, Kericho, Kenya | Assam | 1c | 83. 55/56 | TRFK, Kericho, Kenya | Assam | 1c |
| 33. 38/8 | GW, Kericho, Kenya | Assam | 1c | 84. 303/1199 | Rwebitaba, Uganda | Assam | 1c |
| 34. BB2 | BB, Kericho, Kenya | Assam | 1c | 85. 31/37 | Ambangulu, Tanzania | Assam | 1c |
| 35. M/1/1/10 | KTDA, Meru, Kenya | Assam | 1c | 86.55/55 | TRFK, Kericho, Kenya | Assam | 1c |
| 36. S7/268 | AHP, Kericho, Kenya | Assam | 1c | 87. 347/314 | TRFK, Kericho, Kenya | Assam | 1b |
| 37. MN11/96 | AHP, Kericho, Kenya | Assam | 1a | 88. 371/1 | AHP, Kericho, Kenya | Assam | 1b |
| 38. B5/63 | AHP, Kericho, Kenya | Assam | 1c | 89. 337/138 | TRFK, Kericho, Kenya | Assam | 1c |
| 39. F7/346 | AHP, Kericho, Kenya | Assam | 1c | 90. 303/179 | AHP, Kericho, Kenya | Assam | 1c |
| 40. M2/10 | AHP, Kericho, Kenya | China | 1c | 91. 347/573 | TRFK, Kericho, Kenya | Assam | 1c |
| 41. M3 | Sotik, Kericho, Kenya | Assam | 1c | 92. 31/28 | Ambangulu, Tanzania | Assam | 1c |
| 42. BB21 | BB, Kericho, Kenya | Assam | 1c | 93. 338/13 | TRFK, Kericho, Kenya | Assam | 1c |
| 43. BBLK/5 | BB, Kericho, Kenya | Assam | 1c | 94. 31/11 | Ambangulu, Tanzania | Assam | 1c |
| 44. SFS150 | TRFCA, Malawi | Assam | 1c | 95. 303/35 | Rwebitaba, Uganda | Assam | 1c |
| 45. 38/1 | GW, Kericho, Kenya | Assam | 1c | 96. 347/336 | TRFK, Kericho, Kenya | Assam | 1c |
| 46. K.Purp | Molo, Nakuru, Kenya | China | 3a | 97. 337/3 | TRFK, Kericho, Kenya | Assam | 1b |
| 47. BB35 | BB, Kericho, Kenya | Assam | 1a | 98. 303/152 | Rwebitaba, Uganda | Assam | 1c |
| 48. 56/89 | TRFK, Kericho, Kenya | China | 1c | 99. 89/4 | Siret, Nandi, Kenya | Assam | 1c |
| 49. BB152 | BB, Kericho, Kenya | Assam | 1c | 100. BB7 | BB, Kericho, Kenya | Assam | 1c |
| 50. M1 | Sotik, Kericho, Kenya | Assam | 1c | 101. 378/1 | BB, Kericho, Kenya | Assam | 1b |
| 51. KAG8 | KTDA, Kerinyaga, Kenya | Assam | 1c | 102. PMC45 | AHP, Kericho, Kenya | Assam | 2 |

Acronyms for source of material: AHP–African Highland Produce, BB–Brooke Bond, EAPA–East African Produce African LTD, GW–George Williamson, KTDA–Kenya Tea Development Authority, TRFCA–Tea Research Foudation of Central Africa, TRFK–Tea Research Foundation of Kenya. Highlighted rows show cases where morphological features and catechin profiles give incongruent classifications.

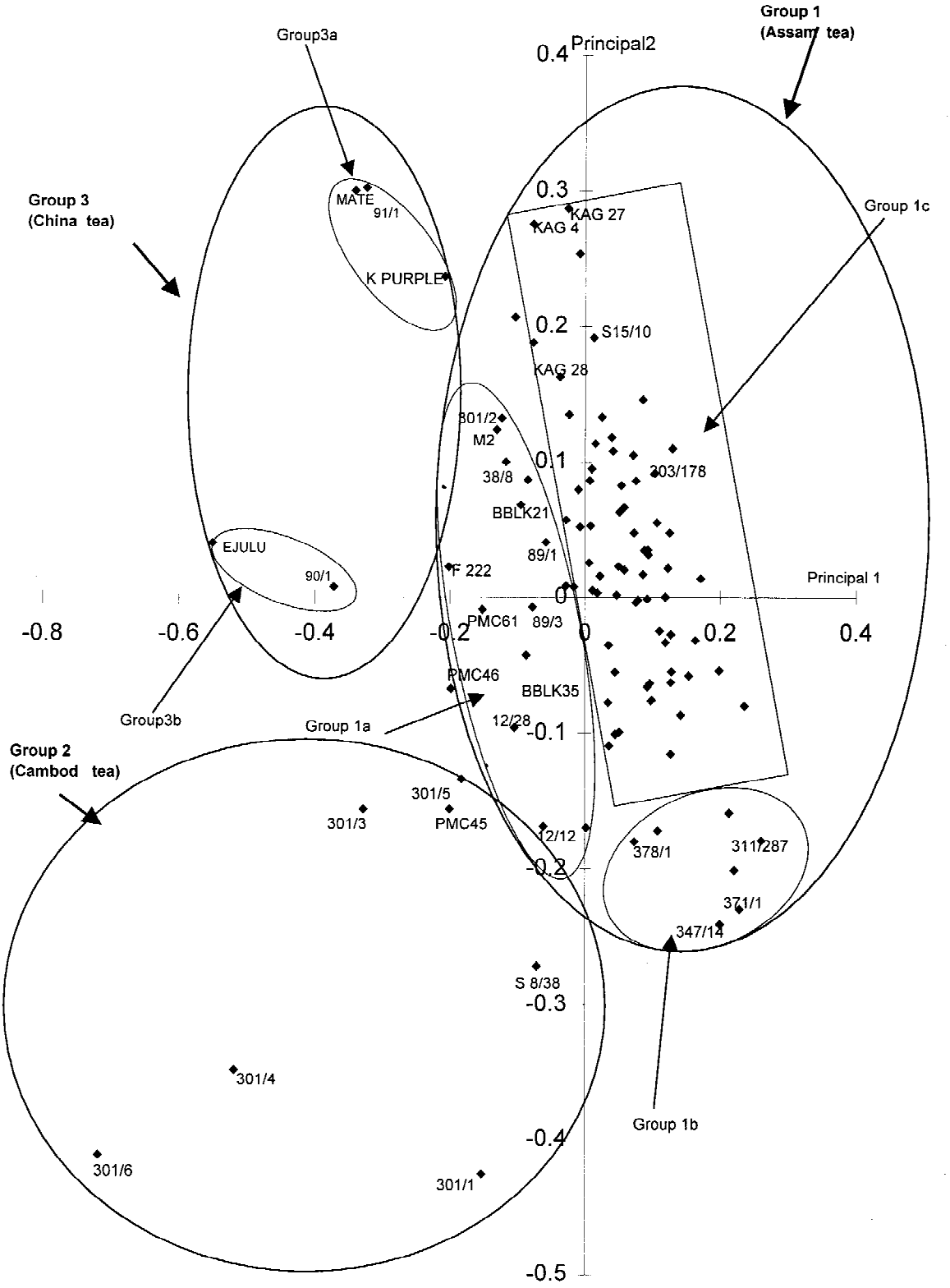



*Figure 1.* Principal component analysis of Kenyan tea clones based on total catechins and catechin ratios.

*Table 2.* Summary statistics for four measures of catechins in 102 clones of *C. sinensis* from Kenya. Groups as defined in the PCA plot

| Variable | Group as defined in PCA plot | No. of genotypes | Mean total catechins $\mu$Mol/$\mu$g DM | Standard error | Min. | Max. |
|---|---|---|---|---|---|---|
| 1.Total catechin | (1a) | 14 | 6.60 | 1.52 | 5.75 | 8.11 |
| | (1b) | 7 | 8.90 | 1.65 | 8.33 | 9.38 |
| | (1c) | 69 | 6.90 | 1.46 | 5.15 | 8.78 |
| | 2 | 7 | 6.90 | 2.06 | 6.29 | 8.49 |
| | 3(a) | | 4.86 | 1.47 | 4.06 | 5.43 |
| | 3(b) | 2 | 3.91 | 1.12 | 3.23 | 4.59 |
| 2. Catechin ratios | 1(a) | 14 | 0.40 | 0.08 | 0.32 | 0.49 |
| | (1b) | 7 | 0.24 | 0.05 | 0.20 | 0.30 |
| | (1c) | 69 | 0.24 | 0.06 | 0.17 | 0.34 |
| | 2 | 7 | 0.73 | 0.36 | 0.48 | 1.25 |
| | 3(a) | 3 | 0.47 | 0.29 | 0.37 | 0.63 |
| | 3(b) | 2 | 0.70 | 0.26 | 0.52 | 0.89 |
| 3. EGC+EGCG | (1a) | 14 | 4.72 | 0.72 | 3.94 | 5.85 |
| | (1b) | 7 | 7.22 | 1.06 | 6.41 | 7.67 |
| | (1c) | 69 | 5.60 | 0.77 | 3.96 | 7.23 |
| | 2 | 7 | 4.85 | 0.73 | 2.79 | 5.50 |
| | 3(a) | 3 | 3.28 | 0.58 | 3.07 | 3.41 |
| | 3(b) | 2 | 2.27 | 0.38 | 2.11 | 2.50 |
| 4. EC+ECG | (1a) | 14 | 1.85 | 0.26 | 1.37 | 2.43 |
| | (1b) | 7 | 1.73 | 0.34 | 1.54 | 1.93 |
| | (1c) | 69 | 1.35 | 0.27 | 0.83 | 1.74 |
| | 2 | 7 | 2.88 | 0.46 | 2.30 | 3.50 |
| | 3(a) | 3 | 1.58 | 1.10 | 1.38 | 2.10 |
| | 3(b) | 2 | 1.64 | 0.78 | 1.12 | 2.16 |

*Table 3.* Dissimilarity coefficient matrix of some selected clones based on the catechin ratio

| Clone | 301/1 | 301/2 | 301/3 | 301/4 | 301/5 | 301/6 | 12/12* | PMC61* | PMC46* | 89/1* |
|---|---|---|---|---|---|---|---|---|---|---|
| 301/2 | 0.006 | 0.000 | 0.292 | 0.226 | 0.000 | 0.596 | 0.027 | 0.005 | 0.003 | 0.001 |

Assam clones which clustered with clone 301/2.

6/8. Therefore, a high dihydroxylated:trihydroxylated catechin ratio could be used along with other characteristics like caffeine content as a marker for superior quality in the tea breeding programmes.

These results showed that differences in the catechin ratios can unequivocably identify clones as belonging to different origins, even if the clones have similar catechin contents. For instance, clones 303/178 and 301/6 have similar amounts of catechins, but their catechin ratios gave a distinct difference which aligned them with the Assam and Cambod groups, respectively. These results clearly illustrate that this technique of identification is more discriminative compared to the use of the total catechins. The utilization of the catechin ratios in the determination of genetic diversity could prove to be a novel and handy technique in the future in establishing affinities of hybrids to the major taxonomic categories and identification of genotypes for future utilization in tea breeding programs. The main advantage of this method of diversity detection is that it is robust, cheap and has high throughput. Another advantage with this

technique is that, should it be developed as one of the markers of tea quality, it can be used to monitor the type of tea coming from the production line. This technique would be a useful complement to new molecular biology techniques like Restriction Fragment Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Random Amplified Polymorphic DNA (RAPD) where these are difficult to adopt because of the high cost of appropriate equipments and consumables and lack of enough qualified personnel.

## Acknowledgements

We would like to thank the Germany Academic Exchange Services (DAAD) for funding this project and the staff of the Chemistry Department at TRFK for technical assistance in catechin separation and identification. This paper is published with the permission of the Director, Tea Research Foundation of Kenya.

## References

Banerjee, B., 1992. Botanical classification of tea. In: Willson, K.C. & M.N. Clifford (Eds) Tea Cultivation to Consumption, p. 39. Chapman and Hall, London.

Brown, A.G., C.P. Falsaw, E. Halsam, A. Holmes, & W.D. Ollis, 1966. The constitution of theaflavins. Tetrahedron Lett. 11: 1193–1204.

Harbone, J.B., 1967. Plant phenolics. In: Comparative Biochemistry of Flavonoids, pp. 226–227. Academic Press, London.

Obanda, M. & P. Owuor, 1997. Flavanols composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas. J. Sci. Food Agric. 74: 209–215.

Owuor, P.O. & I.J. McDowell, 1994. Changes in the theaflavin composition and astringency during black tea fermentation. Food Chem. 51: 251–254.

Genstat 5 committee, 1989. Principal component analysis and hierarchical ascendance classification. In: Genstat Reference Manual, pp. 471–508. Clarendon Press, Oxford.

Gerats, A.M. & C. Martin, 1992. Flavanoid Synthesis in *Petunia hybrida*; Genetics and Molecular Biology of flower colour. In: H.A. Stafford & R.K. Ibrahim (Eds) Phenolic Metabolism in Plants, pp. 167–175. Plenum Press, New York, NY.

Paul, S., F.N. Wachira & R. Waugh, 1997. Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers. Theor. Appl. Genet. 94: 255–263.

Robertson, A., 1992. The Chemistry and Biochemistry of Black Tea Production, the Non-Volatiles. In: K.C. Willson & M.N. Clifford (Eds) pp. 603–647. Chapman and Hall, London.

Roberts, E.A.H. & R.F. Smith, 1963. Phenolic substances of manufactured tea spectrophotometric evaluation of tea liquors. J. Sci. Food Agri. 14: 489–700.

Takeda, Y., 1994. Differences in caffeine and tannin contents between tea cultivars and application to tea breeding. Jap. Agric. Res. Quart. 28: 117–123.

Takino, Y., H. Imagawa, H. Harikawa & A. Tanaka, 1964. Studies of the mechanism of the oxidation of tea catechins part III. Formation of a reddish orange pigment and its spectral relationship to some benzotropolone derivatives. Agric. Biol. Chem. 28: 64–71.

Visser, T., 1969. Tea, *Camellia sinensis* (L.) O. Kuntze. In: Ferwarda, F.P. & F. Wit (Eds) Outlines of perrenial crop breeding in tropics, pp. 459–493. H. Veenman & Zonen, Wageningen.

Wachira, F.N., R. Waugh, C.A. Hackett & W. Powel, 1995. Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. Genome 38: 201–210.

Wachira, F.N., W. Powell & R. Waugh, 1997. Assessment of genetic diversity among *Camellia sinensis* L. (cultivated tea) and its wild relatives based on randomly amplified polymorphic DNA and organelle specific STS. Heredity 78: 603–611.

Wight, W., 1959. Nomenclature and classification of the tea plant. Nature 183: 1726–1728.