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Biochemical Characteristics of Tea (*Camellia* L. spp.) Germplasm Accessions in Sri Lanka: Correlation between Black Tea Quality Parameters and Organoleptic Evaluation

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ABSTRACT: Biochemical composition of tea leaf (*Camellia* L. spp.) is dependent on climatic conditions and the genetic makeup of the plant. Many attempts have been focused to identify various biochemical compounds responsible for quality. Although, currently about 500 germplasm accessions are being maintained in the Tea Research Institute of Sri Lanka, these accessions are not adequately evaluated for biochemical components present in processed black tea. This is the first attempt to quantify various black tea biochemical compounds *viz.*, theaflavins, thearubigins, polyphenols, amino acids, total catechins and caffeine content in a range of germplasm accessions and to correlate with organoleptic parameters such as infused leaf colour, liquor colour, strength, liquor quality and total quality scores using correlation analysis. A strong positive correlation ($P \leq 0.001$) of theaflavins with brightness, total catechins, infused leaf colour, liquor quality and total quality scores was observed. Principal Component Analysis (PCA) based on biochemical parameters and tasters' evaluation separated thirty five tea accessions into four groups. These groups also depicted a relationship with their origin and ancestry. First component of PC analysis accounted for 43.0% variability while second component accounted for 13.3% of the total variability. The significant variation of biochemical compounds detected in the present study indicates the wider diversity of tea germplasm in terms of its biochemical components present in black tea in Sri Lanka. Results could be used to facilitate establishing inherent biochemical constituents that reflect quality of black tea that provides early selection criteria to speed up the tea breeding programme.

KEYWORDS: *Biochemical compounds; Black tea; Quality; Organoleptic evaluation; Germplasm*

Introduction

Tea (*Camellia* L. spp.) is one of the most popular beverages in the world and it is the most consumed beverage next to water. The major tea-producing countries are China, India, Sri Lanka, Kenya and Japan and is also grown in Indonesia, Vietnam, Argentina, Georgia, and other countries.[1] Black, Green and Oolong teas are all produced from the hybrids with an affinity to three key taxa of the genus *Camellia* L. Black, green and Oolong tea differ in their appearance, taste, chemical content as well as flavour due to the difference in the fermentation process involved in making three main tea types.

Black tea quality is a complex phenomenon and depends mainly on the chemical components and colour attached to the tea infusions. As such, tea prices vary greatly depending on the quality which has traditionally been assessed by a tea taster who has developed a language of his own, the organoleptic evaluation, to describe various quality attributes of a tea infusion.[2]

The chemical components of tea leaf include polyphenols (catechins/flavonoids), alkaloids (caffeine, theobromine, theophylline), volatile oils, polysaccharides, amino acids, lipids and vitamins. During black tea processing, catechins (flavan-3-ol) in tea shoots undergo oxidative changes to form theaflavins (TFs) and thearubigins (TRs), which are largely responsible for brightness, strength and colour of black tea.[3] Free amino acids in tea leaves are important chemical constituents that remarkably influence the quality of tea and they are considered as the major contributors to the freshness of tea.[4] Caffeine represents the major methylxanthine, followed by theobromine in much smaller amounts. Caffeine is pharmacologically classified as a central nervous system stimulant and a diuretic.[5]

In some countries, biochemical parameters such as

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polyphenols, catechins, caffeine and others in fresh tea leaves have been used as discriminative markers to evaluate their germplasms. Besides, considerable attempts have been made to relate the organoleptic assessment of tea quality with chemical compounds for black tea, green tea and oolong.[2,6–10] However, most of the previous studies conducted by early scientists at the Tea Research Institute of Sri Lanka (TRISL) were focused only on individual biochemical constituents present in either fresh leaf or processed tea using limited number of accessions.[11] Therefore, a comprehensive study to quantify a range of biochemical compounds of black tea using a representative sample of accessions selected from the germplasm collection is vital to identify unique accessions which possess important biochemical compounds that exert potential health benefits and produce high quality black tea.

Materials and Methods

Source of Materials

Total 35 tea germplasm accessions representative of the hybrids of the three main taxa were selected from *ex situ* field gene bank at the Tea Research Institute of Sri Lanka, Talawakelle (latitude 60° 54'N, longitude 80° 42'E, 1394 m above mean sea level). Monthly average rainfall is about 227 mm in wet season (during September–December) and 72 mm in dry season (during January–April).

Processing of Black Tea

Tea shoots (two leaves and an active bud) (500 g in quantity) were harvested from each accession and black tea was produced in an environmental control manufacture system designed by Teacraft®. Shoots were withered in a withering cabinet for 14–18 hr at 20°C. Withered leaves were rolled for 15 min in a miniature roller and fed into a miniature spiral rotorvane. The macerated leaves (*dhool*) were sieved through a 2.5-mm mesh and placed in a cabinet for optimum fermentation (2.5–3 hr at 20°C). The inlet temperature of the miniature fluid bed dryer was set at 125°C and fermented *dhool* was fed to it. The drying step was terminated when the outlet temperature reached 98°C and ungraded black tea was obtained. The black tea production was repeated twice in wet season (during September–December 2011) and dry season (during January–April 2012) to ensure capturing seasonal variability.

Preparation of Standard Black Tea Infusion

Black tea (9 g) were infused in 375 ml boiling distilled water in a vacuum flask for 10 min using a mechanical shaker. The liquor was then filtered through a cotton wool plug and allowed to cool to room temperature.

Determination of Theaflavins (TF), Thearubigins (TR), Total Colour (TC) and Brightness (BT)

Contents of theaflavin, thearubigin, total colour and brightness of black tea samples were determined by colorimetric method.[12,13]

Theaflavin (g kg^{-1}) = [2.25× EC]×10
Thearubigin (g kg^{-1}) = [1.77×ED + (EA – EC) × 7.06]×10
Total liquor colour % = 6.25× (EA* + 2EB*)
Brightness % = 100× EC*/(EA* + 2EB*)
EA, EB, EC and ED: Spectrophotometer reading at 380 nm
EA*, EB*, EC* and ED*: Spectrophotometer reading at 460 nm

Determination of Total Polyphenols (TPP), Total Catechins (CAT), Free Amino Acids (AA) and Caffeine (CF)

The total polyphenol content in the black tea extract was determined based on the colorimetric method using Folin-Ciocalteu phenol reagent in alkaline medium and the absorbance was measured at 765 nm.[14] Total catechins in the tea extract were estimated using acidified vanillin reagent and the absorbance was measured at 500 nm.[15] Total free amino acid content in the tea extract was determined using Ninhydrin as previously described and the absorbance at 570 nm.[16] D-L-Alanine was used for calibration of the standard curve. Caffeine content in the tea extract was determined by using 50% lead acetate basic solution [$Pb(CH_3COO)_2 \cdot Pb(OH)_2$] by measuring the absorbance at 274 nm.[17] Caffeine was used for calibration of a standard curve.

Determination of dry matter content

Black tea (5 g) was weighed to the nearest 0.001 g, placed in aluminum dishes and heated in an oven at 103 ± 2°C for at least 18 hr to a constant weight. The percentage of dry matter (DM) in the samples was then calculated. All the chemicals used for this study were of analytical grade. UV-VIS spectrophotometer (CARY 510 Bio®) was used for all measurement.

Organoleptic Evaluation of Black Tea

A sample of black tea (2.5 g) was extracted with 150 ml of boiling water for 5 min and was subjected to organoleptic evaluation made by four professional tea tasters with scores based on infused leaf colour (IFL), liquor colour (LC), strength (STN) and liquor quality (LQ).

Statistical Analysis

Results were statistically analysed by analysis of variance (ANOVA) using Statistical Analysis Software (SAS Version 9.1). The differences between mean values of each biochemical parameter were compared by Duncan's Multiple Range Tests. Correlation analysis of biochemical parameters and organoleptic evaluation were carried out, and a principal component analysis was performed to determine the major contributory factors associated with grouping of accessions.

Results and Discussion

A highly significant variation in all biochemical parameters was observed among accessions studied. Black tea biochemical constituents (TF, TR, TR/TF ratio, TC, BT, TPP, CAT, AA and CF) of 35 tea germplasm accessions in wet and dry seasons are presented in Tables 1.a and 1.b and organoleptic evaluations in Table 1.c. Minimum and maximum values, means, standard deviation (SD) and standard error (SE) of biochemical parameters are given in Table 2.

Theaflavins content (TF)

A significant variation of theaflavins content in black tea was observed among the accessions studied (Table 1.a). Irrespective of the seasons, higher values of TF (above 12 g kg^{-1}) were observed in estate selections, a group of accessions that has ancestral affinity towards Cambod type tea (*C. sinensis* ssp. lasiocalyx) such as PK 2, DT 1, N 2 and NAY 3. On the other hand, TF content of known low quality cultivars (TRI 2023, TRI 2016, TRI 3072 and TRI 2043) indicated low values. Present study showed that the TF content in wet season ranges from 6.44 to 18.89 g kg^{-1} with a mean of 11.09 g kg^{-1} whereas

Table 1.a: Mean ±Standard Deviations of the biochemical parameters in different tea accessions

Accessions	TF (g kg^{-1})		TR (g kg^{-1})		TR:TF		TC (%)		BT (%)	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
ASM 4/10	7.67 ± 0.1^{klm}	10.14 ± 0.8^{ijk}	171.16 ± 1.9^{a}	113.85 ± 1.3^{k}	22.31 ± 0.6^{a}	11.27 ± 1.0^{e-i}	4.83 ± 0.1^{e-j}	3.70 ± 0.1^{k}	13.53 ± 1.2^{q}	25.74 ± 1.4^{e-k}
CY 9	9.91 ± 0.6^{hi}	12.63 ± 0.6^{ef}	157.78 ± 0.1^{b-d}	135.88 ± 0.2^{c-f}	15.95 ± 1.0^{e-h}	10.77 ± 0.5^{h-k}	4.75 ± 0.0^{g-k}	4.54 ± 0.1^{c-k}	19.21 ± 0.4^{k-n}	23.60 ± 1.3^{f-n}
DN	8.65 ± 0.6^{jk}	14.41 ± 0.8^{cd}	116.93 ± 2.3^{p-r}	138.22 ± 0.8^{cd}	13.55 ± 1.1^{hk}	9.61 ± 0.5^{j-n}	4.02 ± 0.0^{mno}	5.23 ± 0.0^{b}	20.22 ± 0.0^{ij}	27.67 ± 1.7^{c-k}
DT 1	13.19 ± 0.6^{cd}	13.30 ± 1.3^{de}	157.25 ± 6.0^{b-e}	139.37 ± 8.5^{bc}	11.92 ± 0.1^{k-o}	10.50 ± 0.4^{h-m}	5.37 ± 0.3^{ab}	5.02 ± 0.1^{b-d}	22.88 ± 0.7^{f-h}	26.60 ± 1.8^{d-j}
DT 95	7.68 ± 0.3^{k-n}	12.47 ± 0.4^{ef}	146.56 ± 0.3^{d-i}	130.58 ± 1.6^{e-j}	19.09 ± 0.6^{bc}	10.48 ± 0.3^{h-m}	4.70 ± 0.2^{h-l}	4.68 ± 0.3^{b-f}	14.89 ± 0.2^{pq}	27.71 ± 0.8^{c-i}
DUN 7	12.05 ± 0.2^{def}	11.91 ± 0.3^{efg}	159.63 ± 1.5^{abc}	127.08 ± 0.4^{j}	13.25 ± 0.1^{i-l}	10.67 ± 0.2^{h-k}	5.09 ± 0.1^{a-h}	3.73 ± 0.1^{k}	22.65 ± 0.7^{f-h}	30.06 ± 0.3^{c-f}
H 1/58	13.91 ± 0.8^{c}	11.67 ± 0.3^{f-i}	122.03 ± 4.7^{n-q}	92.48 ± 2.3^{q}	8.80 ± 0.8^{pq}	7.93 ± 0.4^{pq}	5.04 ± 0.1^{a-i}	4.20 ± 0.1^{h-j}	28.45 ± 1.9^{b-d}	27.53 ± 1.7^{c-k}
INTRI 6	13.12 ± 0.1^{cd}	12.40 ± 0.2^{ef}	146.34 ± 1.3^{d-i}	93.73 ± 0.4^{pq}	11.16 ± 0.0^{mno}	7.56 ± 0.1^{q}	5.48 ± 0.3^{a-d}	4.39 ± 0.1^{e-i}	23.13 ± 1.2^{fg}	28.18 ± 0.9^{c-j}
KEN 16/3	8.60 ± 0.5^{jk}	8.25 ± 0.4^{l}	127.77 ± 3.2^{m-p}	99.10 ± 0.5^{n-p}	14.88 ± 0.6^{d-i}	12.03 ± 0.6^{c-g}	4.69 ± 0.2^{h-l}	4.49 ± 0.1^{c-h}	17.96 ± 1.8^{l-n}	18.71 ± 0.6^{no}
N 2	15.63 ± 1.0^{b}	14.39 ± 0.4^{cd}	150.15 ± 7.0^{c-h}	139.64 ± 1.1^{bc}	9.67 ± 2.3^{opq}	9.71 ± 0.4^{j-n}	5.04 ± 0.1^{a-i}	4.46 ± 0.2^{c-i}	27.66 ± 2.2^{cd}	31.32 ± 2.4^{bc}
NAY 3	12.97 ± 1.6^{cd}	16.19 ± 1.3^{a}	149.69 ± 5.6^{c-h}	157.95 ± 2.8^{a}	11.60 ± 1.0^{l-o}	9.78 ± 0.6^{j-n}	5.33 ± 0.5^{a-f}	5.63 ± 0.2^{a}	23.45 ± 1.3^{fg}	28.27 ± 1.5^{c-i}
PK 2	15.78 ± 0.4^{b}	14.57 ± 0.5^{bcd}	162.50 ± 5.8^{ab}	107.70 ± 0.8^{lm}	10.30 ± 0.6^{n-p}	7.40 ± 0.2^{q}	5.44 ± 0.0^{a-e}	2.98 ± 0.0^{l}	28.33 ± 1.0^{bcd}	48.02 ± 3.7^{a}
PLLG 2	7.48 ± 0.2^{k-n}	5.70 ± 0.2^{n}	129.98 ± 1.7^{l-o}	96.49 ± 0.8^{pq}	17.38 ± 0.3^{de}	16.93 ± 0.6^{b}	3.82 ± 0.2^{no}	3.03 ± 0.1^{l}	18.10 ± 0.1^{k-n}	17.51 ± 0.8^{no}
S-106	16.50 ± 1.4^{b}	11.26 ± 0.8^{g-j}	146.28 ± 5.7^{d-i}	95.50 ± 0.1^{pq}	8.88 ± 0.4^{pq}	8.50 ± 0.6^{n-q}	5.83 ± 0.6^{a}	4.08 ± 0.1^{j}	29.11 ± 0.2^{bc}	26.94 ± 1.6^{d-j}
TC 10	7.99 ± 0.2^{klm}	8.18 ± 0.3^{l}	112.62 ± 2.1^{qr}	102.32 ± 2.0^{mno}	16.79 ± 0.4^{e-g}	11.54 ± 0.3^{d-k}	4.58 ± 0.0^{o}	4.23 ± 0.0^{j}	20.56 ± 1.4^{ij}	29.45 ± 1.9^{b-i}
TC 9	9.69 ± 0.2^{ij}	11.75 ± 0.1^{f-h}	162.58 ± 0.8^{ab}	135.56 ± 2.1^{c-g}	14.10 ± 0.0^{def}	12.53 ± 0.8^{c-i}	3.68 ± 0.1^{ij}	3.21 ± 0.0^{g-k}	19.95 ± 0.2^{i-k}	23.74 ± 1.1^{f-l}
TRI 2016	6.44 ± 0.2^{n}	9.82 ± 0.1^{jk}	133.04 ± 1.2^{h-o}	137.82 ± 0.2^{cd}	20.69 ± 0.6^{b}	14.03 ± 0.2^{cd}	4.06 ± 0.1^{mno}	5.08 ± 0.1^{bc}	13.40 ± 0.8^{q}	18.79 ± 1.4^{no}
TRI 2023	8.31 ± 0.2^{kl}	8.29 ± 0.0^{l}	119.45 ± 0.1^{o-r}	97.11 ± 1.3^{pq}	14.37 ± 0.3^{f-i}	11.71 ± 0.2^{c-h}	4.06 ± 0.1^{mno}	4.39 ± 0.0^{e-i}	19.41 ± 0.9^{j-m}	18.59 ± 0.4^{no}
TRI 2024	10.49 ± 0.2^{gh}	8.21 ± 0.3^{l}	147.79 ± 0.8^{d-i}	103.03 ± 0.7^{mn}	14.10 ± 0.3^{hi}	12.55 ± 0.5^{de}	5.30 ± 0.0^{a-g}	4.68 ± 0.0^{b-f}	18.33 ± 0.1^{klm}	17.64 ± 0.8^{no}
TRI 2025	12.20 ± 0.1^{de}	8.98 ± 0.5^{kl}	155.91 ± 0.8^{b-f}	129.57 ± 1.4^{ij}	12.79 ± 0.1^{j-n}	14.46 ± 0.9^{c}	4.42 ± 0.3^{j-m}	4.38 ± 0.7^{e-i}	26.20 ± 1.0^{de}	19.25 ± 4.0^{no}
TRI 2043	7.58 ± 0.3^{k-n}	5.95 ± 0.4^{n}	138.20 ± 1.1^{h-n}	133.30 ± 1.0^{d-h}	18.24 ± 0.5^{cd}	22.46 ± 1.8^{a}	4.35 ± 0.1^{klm}	4.17 ± 0.0^{ij}	15.50 ± 1.4^{opq}	13.30 ± 1.6^{p}
TRI 2142	13.31 ± 0.1^{cd}	14.82 ± 1.9^{abc}	155.07 ± 2.4^{b-f}	131.65 ± 5.3^{e-i}	11.65 ± 0.3^{lmn}	8.99 ± 1.5^{l-q}	4.99 ± 0.2^{b-j}	4.68 ± 0.1^{b-f}	25.04 ± 0.5^{ef}	27.56 ± 1.2^{c-k}
TRI 3013	10.45 ± 0.1^{gh}	9.95 ± 0.6^{ijk}	140.75 ± 0.5^{g-l}	113.95 ± 0.2^{k}	15.47 ± 0.1^{hk}	11.48 ± 0.6^{e-i}	4.79 ± 0.1^{f-k}	4.12 ± 0.1^{ij}	21.32 ± 0.7^{ghi}	24.04 ± 1.2^{e-l}
TRI 3019	7.07 ± 0.7^{lmn}	10.06 ± 0.2^{ijk}	140.60 ± 1.5^{g-l}	123.07 ± 3.6^{j}	20.01 ± 2.2^{b}	12.24 ± 0.6^{c-f}	4.28 ± 0.0^{lmn}	3.58 ± 0.1^{k}	15.78 ± 2.5^{n-q}	20.92 ± 0.8^{mn}
TRI 3072	6.76 ± 0.1^{mn}	7.62 ± 0.9^{l}	113.67 ± 2.2^{qr}	133.12 ± 1.4^{d-h}	16.81 ± 0.1^{def}	17.58 ± 1.8^{b}	3.73 ± 0.1^{o}	4.23 ± 0.0^{g-j}	17.68 ± 0.1^{lmno}	17.06 ± 2.4^{o}
TRI 3073	10.36 ± 0.6^{gh}	12.27 ± 0.6^{ef}	131.63 ± 4.0^{klmn}	122.07 ± 5.0^{j}	12.74 ± 1.1^{j-n}	9.95 ± 0.1^{j-n}	4.24 ± 0.0^{lmn}	4.43 ± 0.2^{d-i}	24.83 ± 2.6^{ef}	28.31 ± 0.3^{c-i}
TRI 4052	12.76 ± 0.3^{cd}	14.36 ± 0.1^{cd}	137.07 ± 1.7^{i-n}	134.05 ± 0.3^{c-g}	10.75 ± 0.4^{no}	9.34 ± 0.0^{k-p}	4.04 ± 0.2^{mno}	5.11 ± 0.1^{b}	28.12 ± 0.7^{cd}	30.98 ± 1.8^{b-d}
TRI 4067	11.34 ± 0.5^{efg}	10.33 ± 0.8^{h-l}	142.56 ± 4.1^{f-k}	144.16 ± 3.4^{b}	12.59 ± 0.9^{j-n}	13.99 ± 0.7^{cd}	4.40 ± 0.2^{k-n}	4.47 ± 0.3^{c-i}	24.13 ± 1.3^{ef}	23.00 ± 2.3^{lm}
TRI 4071	10.77 ± 0.5^{fgh}	12.75 ± 0.5^{ef}	128.06 ± 3.6^{m-p}	130.36 ± 2.0^{f-j}	11.92 ± 0.9^{k-n}	10.24 ± 0.5^{h-n}	3.82 ± 0.2^{no}	4.33 ± 0.1^{fhi}	27.51 ± 0.3^{cd}	30.91 ± 2.2^{b-d}
TRI 4078	11.14 ± 0.3^{e-h}	15.90 ± 0.3^{ab}	126.47 ± 0.5^{n-p}	129.98 ± 0.9^{h-j}	11.36 ± 0.4^{mno}	8.18 ± 0.1^{opq}	4.84 ± 0.1^{d-j}	5.03 ± 0.2^{b-d}	21.57 ± 0.3^{g-j}	33.95 ± 0.3^{b}
TRI 4079	15.36 ± 1.4^{b}	12.68 ± 0.2^{ef}	148.28 ± 1.9^{c-i}	133.36 ± 0.9^{d-h}	9.71 ± 1.0^{opq}	10.52 ± 0.3^{h-l}	4.96 ± 0.2^{c-k}	4.74 ± 0.0^{b-e}	30.63 ± 2.9^{b}	27.22 ± 1.0^{d-i}
TRI 62/5	7.14 ± 0.3^{lmn}	7.73 ± 0.4^{l}	108.76 ± 0.3^{r}	94.78 ± 1.2^{pq}	15.18 ± 0.7^{fgh}	12.29 ± 0.8^{cf}	3.78 ± 0.2^{o}	3.13 ± 0.1^{l}	17.19 ± 1.3^{m-p}	23.47 ± 1.3^{klm}
TRI 777	11.36 ± 0.3^{efg}	15.36 ± 0.1^{abc}	144.79 ± 1.8^{e-j}	137.46 ± 3.9^{cde}	12.75 ± 0.5^{j-m}	8.95 ± 0.3^{l-q}	5.63 ± 0.2^{abc}	5.18 ± 0.1^{b}	19.78 ± 0.5^{ijk}	30.74 ± 0.2^{bc}
VHMOR	15.74 ± 0.2^{b}	12.69 ± 0.2^{ef}	151.92 ± 0.6^{b-i}	112.03 ± 0.1^{kl}	9.65 ± 0.2^{opq}	8.83 ± 0.1^{m-q}	5.49 ± 0.0^{a-d}	4.16 ± 0.2^{hij}	28.57 ± 0.3^{bcd}	31.43 ± 0.1^{bc}
WT 26	18.89 ± 0.4^{a}	10.73 ± 0.4^{h-j}	156.22 ± 3.7^{b-e}	107.50 ± 1.1^{lm}	8.27 ± 0.0^{q}	10.03 ± 0.5^{i-n}	5.11 ± 0.2^{a-h}	3.88 ± 0.0^{k}	35.87 ± 1.1^{a}	27.34 ± 0.6^{d-i}

TF= theaflavins content, TR= thearubigin content , TR/TF= TR:TF ratio, TC= Total colour of liquor, BT =Brightness of liquor

Table 1.b: Mean ±Standard Deviations of the biochemical parameters in different tea accessions

Accessions	TPP (g kg^{-1})		CAT (g kg^{-1})		AA (g kg^{-1})		CF (g kg^{-1})	
	wet	Dry	Wet	dry	wet	dry	wet	dry
ASM 4/10	182.42 ± 2.2^{f-j}	181.75 ± 9.8^{f-j}	94.78 ± 1.7^{mn}	102.50 ± 7.8^{o}	16.12 ± 0.3^{cde}	11.05 ± 0.3^{jkl}	49.33 ± 0.5^{c-f}	44.95 ± 1.8^{g-m}
CY 9	161.37 ± 0.4^{n-q}	153.49 ± 2.7^{lm}	87.65 ± 2.3^{n}	106.44 ± 7.7^{no}	7.84 ± 0.1^{p}	9.49 ± 0.2^{mn}	35.71 ± 0.8^{lm}	49.21 ± 0.8^{c-i}
DN	173.95 ± 1.3^{h-n}	180.19 ± 3.7^{f-j}	106.48 ± 2.6^{jk}	115.03 ± 2.0^{k-n}	13.70 ± 0.1^{ghi}	14.39 ± 0.0^{def}	51.27 ± 1.7^{b-e}	48.11 ± 0.2^{d-j}
DT 1	179.68 ± 1.6^{f-k}	189.75 ± 3.6^{e-h}	130.33 ± 6.2^{cde}	134.19 ± 3.2^{def}	11.37 ± 1.2^{lmn}	12.36 ± 0.1^{hij}	47.87 ± 0.3^{c-g}	51.12 ± 2.5^{b-h}
DT 95	144.55 ± 3.1^{s}	181.05 ± 2.6^{f-j}	101.76 ± 3.6^{kl}	92.22 ± 6.9^{p}	13.56 ± 0.5^{ghi}	12.43 ± 0.6^{hij}	36.82 ± 0.2^{klm}	33.66 ± 0.1^{q}
DUN 7	207.71 ± 5.3^{cd}	247.93 ± 10.6^{a}	132.63 ± 1.3^{cd}	133.08 ± 1.6^{efg}	9.98 ± 0.3^{no}	12.29 ± 0.1^{hij}	33.43 ± 1.3^{m}	34.03 ± 7.7^{pq}
H 1/58	184.53 ± 1.6^{e-i}	210.23 ± 3.7^{bcd}	135.76 ± 2.8^{bc}	124.55 ± 0.7^{g-j}	10.67 ± 0.6^{mn}	14.52 ± 0.1^{de}	40.51 ± 0.1^{h-k}	45.98 ± 0.8^{f-l}
INTRI 6	181.17 ± 3.8^{f-k}	196.06 ± 1.4^{c-g}	126.44 ± 0.9^{de}	128.63 ± 2.9^{f-i}	18.20 ± 0.2^{b}	15.04 ± 1.1^{cde}	49.98 ± 5.1^{cde}	47.39 ± 1.7^{e-j}
KEN 16/3	168.29 ± 1.4^{k-o}	155.44 ± 1.8^{klm}	106.44 ± 1.6^{jk}	128.48 ± 1.7^{f-i}	13.27 ± 1.7^{hi}	15.41 ± 0.8^{bcd}	36.88 ± 0.8^{klm}	46.10 ± 5.2^{f-l}
N 2	158.86 ± 3.7^{o-r}	205.39 ± 5.9^{bcd}	113.57 ± 1.2^{g-j}	145.82 ± 1.8^{c}	14.88 ± 0.4^{efg}	10.57 ± 0.2^{klm}	51.43 ± 3.7^{b-e}	49.02 ± 2.1^{c-i}
NAY 3	227.52 ± 7.4^{b}	194.48 ± 7.1^{d-g}	119.47 ± 1.1^{fg}	142.68 ± 1.9^{cd}	11.90 ± 1.5^{j-m}	11.03 ± 0.3^{jkl}	45.00 ± 3.2^{fgh}	46.09 ± 0.7^{f-l}
PK 2	178.34 ± 2.0^{g-l}	246.72 ± 1.8^{a}	142.78 ± 2.3^{a}	173.89 ± 5.0^{a}	10.78 ± 0.5^{mn}	12.00 ± 0.9^{hij}	41.26 ± 0.2^{h-k}	43.58 ± 0.1^{i-n}
PLLG 2	189.53 ± 7.0^{efg}	205.35 ± 3.5^{bcd}	114.76 ± 1.3^{ghi}	121.46 ± 6.2^{i-l}	11.01 ± 0.4^{lmn}	12.03 ± 0.1^{hij}	42.45 ± 2.2^{hij}	45.48 ± 1.9^{g-m}
S-106	235.48 ± 6.3^{a}	221.46 ± 1.1^{b}	133.19 ± 6.6^{cd}	141.63 ± 2.4^{cd}	9.29 ± 0.0^{o}	12.61 ± 0.1^{ghi}	39.07 ± 1.1^{i-l}	50.48 ± 0.7^{b-h}
TC 10	171.27 ± 1.7^{i-o}	176.29 ± 7.7^{hij}	112.70 ± 2.1^{g-j}	126.14 ± 6.9^{f-i}	11.37 ± 0.9^{lmn}	14.11 ± 0.2^{def}	39.59 ± 0.4^{i-l}	45.34 ± 2.5^{g-m}
TC 9	174.78 ± 8.9^{h-m}	189.19 ± 1.3^{e-g}	124.21 ± 1.7^{ef}	122.87 ± 1.4^{h-k}	12.48 ± 0.2^{i-l}	10.37 ± 0.0^{klmn}	47.41 ± 1.0$^{d b}$	39.23 ± 1.5^{m-q}
TRI 2016	169.76 ± 4.8^{j-o}	180.83 ± 1.5^{f-j}	93.11 ± 1.2^{mn}	101.55 ± 5.4^{o}	14.50 ± 0.0^{fgh}	14.66 ± 0.0^{cde}	54.78 ± 1.6^{b}	56.76 ± 8.7^{b}
TRI 2023	218.67 ± 4.5^{bc}	215.14 ± 1.0^{bcd}	129.89 ± 2.2^{cde}	112.91 ± 5.7^{lmn}	11.81 ± 0.8^{klm}	16.43 ± 1.3^{b}	63.61 ± 4.8^{a}	63.26 ± 0.6^{a}
TRI 2024	191.19 ± 3.6^{efg}	170.33 ± 3.5^{ijk}	90.12 ± 0.9^{n}	104.03 ± 1.1^{o}	15.47 ± 0.5^{def}	16.02 ± 0.5^{bc}	51.42 ± 1.8^{b-e}	54.56 ± 3.5^{bcd}
TRI 2025	196.75 ± 7.1^{de}	221.73 ± 0.6^{b}	87.90 ± 7.3^{n}	122.63 ± 1.9^{h-k}	13.17 ± 0.9^{hij}	9.97 ± 0.1^{lmn}	52.60 ± 0.4^{bc}	64.26 ± 0.6^{a}
TRI 2043	162.86 ± 0.0^{m-q}	142.43 ± 1.3^{m}	98.48 ± 4.3^{lm}	82.27 ± 0.3^{q}	13.83 ± 0.5^{ghi}	19.96 ± 0.4^{a}	38.37 ± 0.5^{jkl}	46.48 ± 0.9^{f-k}
TRI 2142	192.55 ± 8.5^{ef}	196.38 ± 4.6^{c-g}	131.09 ± 1.7^{cde}	130.95 ± 1.2^{efgh}	11.32 ± 0.4^{lmn}	14.93 ± 1.6^{cde}	40.97 ± 3.2^{h-k}	41.57 ± 0.7^{j-o}
TRI 3013	150.91 ± 0.1^{qrs}	197.64 ± 9.0^{c-g}	113.38 ± 2.4^{g-j}	127.56 ± 3.9^{f-i}	13.88 ± 0.1^{ghi}	10.49 ± 0.9^{klmn}	43.73 ± 0.6^{ghi}	54.31 ± 3.8^{bcd}
TRI 3019	187.14 ± 9.7^{efgh}	195.23 ± 5.7^{c-g}	107.87 ± 0.7^{ijk}	117.21 ± 5.5^{j-m}	15.85 ± 1.0def	14.44 ± 0.4^{def}	47.80 ± 0.2^{c-g}	49.45 ± 1.1^{c-i}
TRI 3072	162.21 ± 4.5^{m-q}	173.55 ± 1.7^{hij}	90.41 ± 0.1^{n}	113.38 ± 3.6^{lmn}	26.68 ± 0.2^{a}	14.84 ± 0.2^{cde}	50.58 ± 1.8^{b-e}	52.36 ± 6.0^{b-f}
TRI 3073	195.66 ± 3.7^{e}	212.68 ± 2.5^{bc}	130.99 ± 2.8^{cde}	120.85 ± 3.5^{i-l}	17.35 ± 0.6^{bc}	14.20 ± 0.5^{def}	47.27 ± 1.9^{d-g}	55.04 ± 1.4^{bc}
TRI 4062	144.23 ± 0.0^{s}	171.56 ± 0.5^{ijk}	127.11 ± 6.2^{de}	124.84 ± 1.4^{g-j}	10.80 ± 1.0^{mn}	13.82 ± 0.2^{efg}	40.64 ± 0.1^{h-k}	53.15 ± 0.4^{b-e}
TRI 4067	154.66 ± 1.4^{pq}	165.24 ± 1.5^{jkl}	116.21 ± 5.0^{gh}	108.68 ± 0.6^{mno}	7.24 ± 0.9^{p}	11.85 ± 0.6^{h-k}	37.39 ± 1.8^{klm}	39.85 ± 0.7^{l-q}
TRI 4071	*147.00 ± 3.4^{rs}*	*187.47 ± 1.2^{f-i}*	*111.73 ± 8.4^{hij}*	*137.31 ± 1.4^{cde}*	*13.06 ± 0.2^{hijk}*	*8.60 ± 1.4^{o}*	*47.41 ± 1.4^{d-g}*	*43.71 ± 0.3^{i-n}*
TRI 4078	175.42 ± 5.5^{h-m}	197.42 ± 0.4^{c-g}	130.65 ± 1.3^{cde}	145.34 ± 5.2^{c}	14.02 ± 0.3^{gh}	10.50 ± 0.2^{klm}	48.58 ± 1.7^{c-f}	53.13 ± 3.8^{b-e}
TRI 4079	151.45 ± 2.5^{qrs}	206.69 ± 0.0^{bcd}	102.58 ± 3.5^{kl}	109.57 ± 3.9^{mno}	11.74 ± 0.6^{klm}	9.12 ± 0.0^{no}	49.19 ± 2.1^{c-f}	35.57 ± 0.2^{opq}
TRI 62/5	165.75 ± 5.6^{l-p}	196.69 ± 3.5^{c-g}	110.79 ± 1.6^{hij}	115.05 ± 3.9^{k-n}	10.74 ± 0.2^{mn}	14.67 ± 0.6^{cde}	51.63 ± 3.4^{b-e}	51.72 ± 2.0^{b-g}
TRI 777	172.90 ± 4.7^{i-n}	203.16 ± 4.3^{c-f}	91.96 ± 1.2^{mn}	114.88 ± 1.7-kn	16.65 ± 0.3^{cd}	13.06 ± 0.6^{fgh}	52.04 ± 0.6^{bcd}	51.47 ± 1.4^{b-h}
VHMOR	184.07 ± 1.8^{e-i}	174.65 ± 6.5^{hij}	141.96 ± 2.2^{ab}	141.53 ± 3.2^{cd}	11.23 ± 0.3^{lmn}	11.32 ± 0.4^{i-l}	47.08 ± 2.3^{efg}	37.60 ± 0.0^{n-q}
WT 26	180.90 ± 2.3^{f-k}	187.06 ± 3.4^{f-i}	91.24 ± 1.0^{mn}	153.88 ± 1.3^{b}	11.72 ± 0.3^{klm}	13.05 ± 0.1^{fgh}	48.65 ± 0.9^{c-f}	40.21 ± 3.0^{k-p}

TPP= polyphenol content, CAT= Total catechins content, AA= Free amino acid content, CF= Caffeine content
Within a raw followed by the same letters are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test

in dry season it varies from 5.70 to 16.19 g kg^{-1} with a mean of 11.36 g kg^{-1}. However, there was no significant difference in TF values between wet and dry seasons.

It was reported that TF content and its range in tea cultivars vary with the country in which those black teas were produced.[1] Favourable climatic conditions and the leaf standards (tea leaves exclusively consisting of two leaves and a bud) may be the reasons for presence of higher content of TFs, in the present study. It was generally recognized that the theaflavins play a prime role in determining the characteristic cup quality of black tea infusion described by tea tasters as "brightness" and "briskness". Several studies conducted to evaluate the possibility of relating black tea quality to TF content in Sri Lanka,[18] Kenya[19] and India[20] confirmed that TFs and their fractions could be used as a black tea quality parameter.

Thearubigins (TR) Content and TR/TF Ratio

The TR content observed in the present study was in the range of 108.36–171.16 g kg^{-1} with a mean of 141.11 g kg^{-1} and 92.48–157.95 g kg^{-1} with a mean of 121.54 g kg^{-1} in wet and dry seasons respectively (Table 2). TR content of most of cultivars in dry season was lower than wet season. The TRs are a heterogeneous group of orange brown, weakly acidic pigments formed by enzymatic oxidative transformation of flavanols during the fermentation process of black tea. TRs are responsible for much of the colour of tea infusions and major constituent of black teas accounting for 70–80% of the total polyphenols.[3] TR/TF ratio is another important factor in determining the quality of black tea. The TR/TF ratio should be 10–12 to get balanced liquor and taste.[12] According to the results, a large number of accessions processed in dry season recorded the ratio between 10 and 12 (Table 1.a).

Total Colour (TC) and Brightness (BT)

The spectrophotometric determination of total colour of 35 black tea infusions ranged beteen 3.68% and 5.83% and 2.98% and 5.63% in wet and dry seasons, respectively. The spectrophotometric determination of brightness of 35 black tea infusions ranged between 13.40% and 35.87% and 13.30% and 48.02% in wet and dry seasons, respectively (Table 2). Usually, the value for TC of Ceylon orthodox black teas was 4.17% and BT was 23.1%.[1] In most studies, BT of tea has been associated with total TFs. Conversely, the relationship between TRs and TFs has been inversely related in many instances and TRs reduced black tea brightness and increased its total colour. TFs had a positive and major role in spectrophotometrically measured BT whilst TRs had a negative role.[13]

Total Polyphenols (TPP) Content

The present study showed that the total polyphenol content in wet season ranges from 144.23 to 235.48 g kg^{-1} with a mean of 177.65 g kg^{-1} whereas in dry season from 142.43 to 246.72 g kg^{-1} with a mean of 192.02 g kg^{-1}. Higher values of TPP were observed in known high quality black tea producing accessions with ancestral affinity to Cambod type, such as DT 1, N 2, H 1/58, PK 2, TC 9 and S 106, whereas lower values were detected in known low quality accessions having Assam ancestry relations (ASM 4/10, TRI 2016, TRI 2024 and TRI 3072) in dry season.

The polyphenolics in general have shown several biological activities such as antioxidant, antimutagenic and/or anticarcinogenic as well as free-radical scavenging ability.[5]

Table 1.c: Mean ±Standard Deviations of the tasters' evaluated parameters in different tea accessions

Accessions	IFL		LC		STN		LQ		TQS	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
ASM 4/10	2.50 ± 0.58	3.25 ± 0.50	2.00 ± 0.82	3.00 ± 0.00	4.25 ± 0.50	4.50 ± 0.58	4.25 ± 1.71	6.50 ± 1.73	13.00 ± 2.45	17.25 ± 2.06
CY 9	5.00 ± 0.82	4.50 ± 1.29	3.50 ± 0.58	3.25 ± 0.50	3.50 ± 0.58	3.50 ± 0.58	6.00 ± 0.82	6.75 ± 0.50	18.00 ± 1.41	18.00 ± 1.41
DN	3.25 ± 0.50	3.25 ± 0.50	3.50 ± 0.58	3.25 ± 0.50	3.75 ± 0.50	4.00 ± 0.00	7.50 ± 0.58	7.00 ± 1.15	18.00 ± 0.82	17.50 ± 1.29
DT 1	5.94 ± 0.35	5.81 ± 0.63	2.69 ± 0.46	2.81 ± 0.77	5.66 ± 0.53	4.81 ± 0.55	7.78 ± 0.53	7.50 ± 1.37	21.53 ± 1.44	21.31 ± 2.01
DT 95	3.75 ± 0.50	4.25 ± 0.50	3.75 ± 0.50	3.00 ± 0.82	3.25 ± 0.50	5.00 ± 0.00	6.50 ± 1.00	7.50 ± 0.58	17.25 ± 1.50	19.75 ± 1.50
DUN 7	5.75 ± 0.50	6.25 ± 0.50	3.50 ± 0.58	2.50 ± 1.00	5.50 ± 0.58	5.00 ± 1.41	8.00 ± 0.82	7.25 ± 1.50	22.75 ± 2.06	21.00 ± 2.16
H 1/58	4.75 ± 0.50	4.50 ± 0.58	3.50 ± 0.58	3.00 ± 0.82	5.25 ± 0.96	4.75 ± 0.50	7.50 ± 0.58	8.00 ± 0.82	21.00 ± 0.00	20.25 ± 1.26
INTRI 6	3.75 ± 0.96	3.50 ± 1.29	2.50 ± 0.58	3.00 ± 1.41	4.75 ± 1.71	5.25 ± 1.26	7.00 ± 0.82	7.50 ± 1.29	18.00 ± 2.45	19.25 ± 1.89
KEN 16/3	3.75 ± 0.96	2.75 ± 0.96	3.50 ± 0.58	3.25 ± 0.96	4.50 ± 0.58	4.25 ± 0.50	5.50 ± 1.29	6.50 ± 0.58	17.25 ± 1.71	16.75 ± 0.96
N 2	4.25 ± 0.50	3.50 ± 0.58	3.00 ± 0.00	3.50 ± 0.58	4.25 ± 0.50	4.25 ± 1.26	7.00 ± 1.41	7.25 ± 0.50	18.50 ± 1.73	18.50 ± 2.08
NAY 3	5.25 ± 0.96	5.00 ± 0.82	3.00 ± 0.82	2.75 ± 0.96	5.75 ± 0.50	5.50 ± 0.58	6.00 ± 0.82	8.00 ± 0.82	20.00 ± 1.83	21.25 ± 1.26
PK 2	6.25 ± 0.50	3.75 ± 2.06	3.25 ± 0.50	2.25 ± 0.50	4.25 ± 0.50	3.50 ± 0.58	7.50 ± 1.73	6.00 ± 3.56	21.25 ± 2.22	15.50 ± 5.92
PLLG 2	2.75 ± 0.50	3.50 ± 0.58	2.50 ± 0.58	2.50 ± 0.58	5.75 ± 0.96	4.75 ± 0.50	6.00 ± 0.82	5.50 ± 0.58	17.00 ± 1.15	16.25 ± 0.96
S-106	5.25 ± 0.50	4.25 ± 0.50	3.50 ± 0.58	2.00 ± 0.82	5.25 ± 0.50	5.00 ± 0.82	7.75 ± 0.50	8.25 ± 0.96	21.75 ± 1.50	19.50 ± 1.29
TC 10	3.75 ± 0.50	4.00 ± 0.82	2.25 ± 0.50	3.75 ± 0.50	3.75 ± 0.50	3.75 ± 0.50	7.25 ± 0.96	5.00 ± 1.41	18.00 ± 1.41	16.50 ± 2.38
TC 9	5.25 ± 0.96	4.25 ± 0.96	3.50 ± 0.58	3.25 ± 0.50	5.00 ± 1.41	4.00 ± 0.00	9.00 ± 0.82	6.00 ± 0.82	22.75 ± 1.50	17.50 ± 1.91
TRI 2016	2.25 ± 0.50	3.00 ± 0.82	3.25 ± 0.96	3.25 ± 0.50	3.75 ± 0.50	3.75 ± 0.96	4.75 ± 0.96	4.25 ± 0.96	14.00 ± 2.16	14.25 ± 1.50
TRI 2023	3.50 ± 0.58	2.50 ± 1.00	3.50 ± 0.58	2.75 ± 1.26	4.25 ± 0.50	4.25 ± 0.50	5.25 ± 1.26	5.25 ± 0.96	16.50 ± 2.08	14.75 ± 1.71
TRI 2024	3.50 ± 0.58	2.75 ± 0.96	3.25 ± 0.96	2.75 ± 1.26	5.25 ± 0.50	4.25 ± 1.26	5.75 ± 0.50	5.75 ± 1.26	17.75 ± 1.50	15.50 ± 1.91
TRI 2025	2.75 ± 0.50	3.25 ± 0.96	2.50 ± 1.29	3.25 ± 0.96	4.50 ± 0.58	4.75 ± 0.50	5.75 ± 0.96	7.25 ± 0.50	15.50 ± 1.00	18.50 ± 1.29
TRI 2043	4.00 ± 0.82	3.75 ± 0.50	2.00 ± 0.82	2.25 ± 1.26	5.00 ± 0.82	4.25 ± 1.89	6.50 ± 1.29	4.75 ± 0.96	17.50 ± 1.91	15.00 ± 2.58
TRI 2142	4.50 ± 0.58	4.25 ± 0.96	3.00 ± 0.82	3.75 ± 0.50	4.50 ± 0.58	4.75 ± 0.50	4.00 ± 0.82	6.50 ± 0.58	16.00 ± 0.82	19.25 ± 0.50
TRI 3013	4.25 ± 0.50	4.25 ± 0.96	3.25 ± 0.50	3.50 ± 0.58	4.50 ± 0.58	4.75 ± 0.96	7.00 ± 1.41	7.50 ± 0.58	19.00 ± 2.16	20.00 ± 0.00
TRI 3019	2.50 ± 0.58	3.75 ± 1.26	2.75 ± 0.50	3.25 ± 0.5	4.00 ± 0.82	4.25 ± 0.50	4.25 ± 0.96	6.75 ± 0.50	13.50 ± 1.73	18.00 ± 0.82
TRI 3072	2.75 ± 0.50	4.75 ± 0.50	3.00 ± 0.82	2.00 ± 0.82	3.25 ± 0.50	4.75 ± 1.71	4.75 ± 0.96	6.00 ± 1.41	13.75 ± 0.50	17.50 ± 2.65
TRI 3073	3.50 ± 0.58	5.00 ± 0.82	3.25 ± 0.50	3.00 ± 0.82	3.75 ± 0.50	3.50 ± 0.58	6.75 ± 0.50	5.50 ± 1.73	17.25 ± 1.26	17.00 ± 3.56
TRI 4052	3.25 ± 0.50	3.25 ± 0.96	3.13 ± 0.53	2.25 ± 0.50	3.75 ± 0.71	4.50 ± 1.29	4.13 ± 0.75	6.00 ± 0.82	14.25 ± 1.50	16.00 ± 1.63
TRI 4067	4.25 ± 0.50	3.25 ± 0.96	2.75 ± 0.50	3.50 ± 0.58	4.00 ± 0.82	3.00 ± 0.00	4.00 ± 0.82	7.00 ± 0.82	15.00 ± 1.15	16.75 ± 1.50
TRI 4071	2.50 ± 0.35	3.50 ± 0.58	2.88 ± 0.88	3.25 ± 0.50	4.25 ± 0.50	4.00 ± 0.00	3.50 ± 1.06	6.50 ± 0.58	13.13 ± 2.30	17.25 ± 1.26
TRI 4078	3.75 ± 0.50	3.50 ± 0.58	3.00 ± 0.82	3.25 ± 0.50	4.75 ± 0.96	4.25 ± 0.50	6.75 ± 0.96	5.75 ± 1.26	18.25 ± 1.26	16.75 ± 2.06
TRI 4079	3.38 ± 0.18	3.75 ± 0.96	3.50 ± 0.50	3.25 ± 0.96	3.75 ± 0.50	4.75 ± 0.96	6.50 ± 0.71	7.25 ± 0.50	17.13 ± 0.88	19.00 ± 1.15
TRI 62/5	2.25 ± 0.50	2.75 ± 0.50	2.75 ± 0.96	3.25 ± 0.50	4.50 ± 0.58	4.25 ± 0.50	3.50 ± 1.73	4.50 ± 0.58	13.00 ± 2.16	14.75 ± 1.26
TRI 777	5.00 ± 1.41	4.75 ± 0.50	3.25 ± 0.96	2.25 ± 0.50	5.50 ± 0.58	5.75 ± 0.50	7.50 ± 0.58	7.50 ± 1.00	21.25 ± 1.71	20.25 ± 1.71
VHMOR	5.75 ± 0.50	5.50 ± 0.58	2.25 ± 0.50	3.25 ± 0.96	5.25 ± 0.50	4.75 ± 0.50	8.25 ± 0.50	7.00 ± 0.82	21.50 ± 1.00	20.50 ± 1.73
WT 26	5.25 ± 1.26	4.75 ± 0.96	3.50 ± 0.58	2.75 ± 0.96	4.50 ± 0.58	5.25 ± 0.96	7.75 ± 1.26	7.75 ± 1.89	21.00 ± 2.16	20.50 ± 2.65

IFL= infused leaf colour; LC= liquor colour; STN= Strength; LQ= liquor quality; TQS= total quality score.

Total Catechins Content (CAT)

Mean values of catechins in wet and dry seasons were 115.04 g kg^{-1} and 124.26 g kg^{-1}, respectively. Interestingly, the highest values of TPP (246.72 g kg^{-1}) and CAT (173.89 g kg^{-1}) were observed in PK 2 during dry season (Table 1.b). Higher amount of CAT was detected in DT 1, NAY 3, PK 2, WT 26, and TRI 4078 during dry season. These accessions classified as Cambod types based on floral morphology, although leaf morphology depicts them as China type to a certain extent (unpublished data). Generally, it is considered that the China type with small leaves have low amount of catechins compared to Cambod or Assam types.[21] Most of the accessions having high amount of catechins can be classified as Assam or Cambod hybrids rather than China type (*C. sinensis*, ssp. *sinensis*) based on the information available (data unpublished). Therefore, catechin content can be considered as a suitable and important biochemical marker for characterization of tea germplasm.

Moreover, it was found that the CAT of tea leaves increases with the time of exposure to sunlight;[22,23] It may be the reason for higher amount of CAT detected in most of the cultivars during dry season than wet season. Catechins, together with their oxidation products (TFs and TRs) are responsible for most of the sensory characteristics associated with black tea liquors.[24] Moreover, the proportions of catechins are important not only for tea quality, but also for the efficiency of cancer prevention.[25] Catechins present in tea being powerful antioxidants, may play an important role in the prevention of cancer by reducing damage.[5] Therefore, tea cultivars that posses high catechin levels or the processing condition that could enhance catechins levels would be worth exploring to enhance the health benefit potential of black tea.

Total Amino Acid (AA) Content

Total amino acid content ranged from 7.24 to 26.68 g kg^{-1} and 8.60 to 19.96 g kg^{-1} in wet and dry seasons, respectively (Table 2). Mean values of AA in wet and dry seasons were 13.07 g kg^{-1} and 12.90 g kg^{-1}. Previous studies show that the AA content in black teas varies from 9.31 to 15.58 g kg^{-1} in Sri Lankan tea and 18.1–50.4 g kg^{-1} in Chinese tea.[2,26] These differences could be due to geographical locations. The content of AA is considered to be one of the parameters that enhance black tea quality.[4] Among the amino acids, theanine (*N*-ethylglutamic acid) has been the centre of attraction for a long time as it occurs exclusively only in tea and accounts for 50% of the free amino acids in black tea.

Caffeine Content (CF)

In the 35 black tea samples tested, caffeine content ranged from 33.43 to 63.61 g kg^{-1} and 33.66 to 64.26 g kg^{-1} in wet and dry seasons, respectively (Table 2). In contrast, the level detected in Chinese accessions was in the range of 35.3–58.7 g kg^{-1} that was comparable with the levels recorded in the present study.[2] The results of the present study showed that the mean values of CF in 35 black tea

Table 2: Minimum and maximum value, mean, standard deviation (SD) and standard error (SE) of black tea quality parameters

Parameters	Wet season					Dry season				
	Min	Max	Mean	SD	SE	Min	Max	Mean	SD	SE
TF (g kg^{-1})	6.44	18.89	11.09	3.25	0.39	5.70	16.19	11.36	2.82	0.34
TR (g kg^{-1})	108.36	171.16	141.11	16.33	1.95	92.48	157.95	121.54	17.72	2.12
TR:TF	8.27	22.31	13.62	3.58	0.43	7.40	22.46	11.29	3.09	0.37
TC (%)	3.68	5.83	4.69	0.64	0.08	2.98	5.63	4.33	0.65	0.08
BT (%)	13.40	35.87	22.31	5.39	0.64	13.30	48.02	25.57	6.43	0.77
TPP (g kg^{-1})	144.23	235.48	177.65	21.84	2.61	142.43	246.72	192.02	23.39	2.79
CAT (g kg^{-1})	87.65	142.78	115.04	17.25	1.93	82.27	173.89	124.26	18.52	2.08
AA (g kg^{-1})	7.24	26.68	13.07	3.45	0.41	8.60	19.96	12.90	2.43	0.29
CF (g kg^{-1})	33.43	63.61	45.69	6.48	0.74	33.66	64.26	47.81	7.33	0.84

TF=theaflavins; TR=thearubigins; TC=total liquor colour,; BT=brightness of liquor; TPP=polyphenols; CAT=total catechins AA=amino acids; CF=caffeine;
SD=standard deviation; SE=standard error.

samples were 45.69 g kg^{-1} and 47.81 g kg^{-1} in dry and wet seasons, respectively. Earlier studies showed that CF content of black teas was affected by accessions, season, and stage of harvesting.[27] Among the accessions tested, most of the TRI 2000 and TRI 3000 series accessions (TRI 2023, TRI 2024, TRI 2025, TRI 3019, TRI 3072 and TRI 3073) which are having ancestral relationship with ASM 4/10 reported higher CF content (>50 g kg^{-1}) during dry season. Estate selections such as CY 9, DT 95, DUN 7, KEN 16/3, S 106 and TC 10 recorded low CF content (< 40 g kg^{-1}) during wet season. It is indicated that the screening of existing old seedling populations for caffeine content would be useful to find out low caffeine tea accessions.

Caffeine is an important compound in tea brew. The interaction of caffeine with catechins, which is well known, could lead to changes in the sensory properties of tea.[28] Due to pharmacological properties of CF which has a stimulating effect to the central nervous system, the demand for low-caffeine tea is increasing greatly from 2% of total tea consumption in 1980 to 15% in the early 21st century, especially for aged people, pregnant women, and children.[17] The decaffeination process using artificial techniques not only reduced caffeine but also reduced theaflavins, thearubigins, colour and brightness which are some important and unique characteristics of black tea. This affects the consumer acceptance for decaffeinated black tea and can reduce its market value.[29] Therefore, development of tea cultivars possessing low caffeine content through breeding and selection is more acceptable than making decaffeinated tea through artificial means. The lower caffeine containing cultivars could be used in engineering of caffeine free accession and this is being attempted to produce decaffeinated tea accessions by natural means.[30,31]

The ranges of important biochemical constituents such as thearubigins, total pollyphenols, total catechins and caffeine are significantly different between wet and dry seasons (Fig. 1). Therefore, results of the present study confirm that there was a seasonal fluctuation of black tea biochemical constituents.

Organoleptic Evaluation of Black Tea

In organoleptic evaluation, parameters such as infused leaf colour (IFL), liquor colour (LC), liquor strength (STN), liquor quality (LQ) and total quality scores (TQS) were assessed by professional tea tasters (Table 1.c). Higher values for total quality scores (TQS) were observed in most of the well-known high quality accessions such as DT 1, TRI 777, WT 26, DUN 7 and S 106

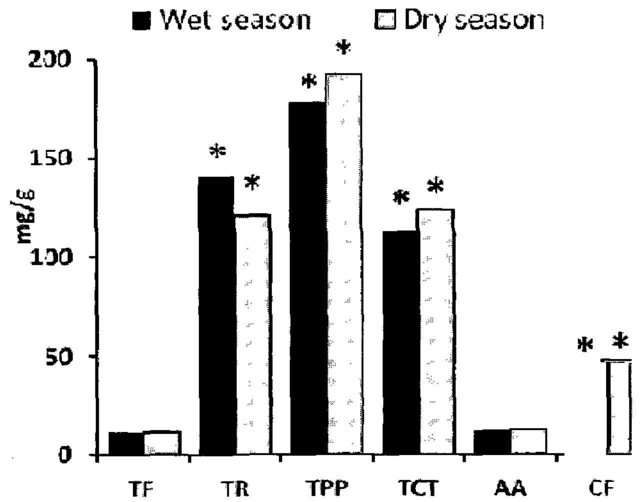

Fig. 1: Variation of black tea biochemical compounds in wet and dry seasons. *Significant difference at $P \leq 0.05$.

whereas, lower values were detected in known low quality accessions having Assam ancestry relations (TRI 2016, TRI 62/5 and TRI 2023), irrespective of the seasonal effect. In addition, two introductions (VHMOR and INTRI 6) also showed higher values for total quality scores (TQS), and those could be used in future breeding programs to exploit the potential of quality character.

Correlation Analysis

Correlation analysis of biochemical parameters and tea tasters' evaluation, are presented in Table 3. There were significant positive correlations of TF with BT, TCT, LQ and TQS exhibiting strong negative correlation (–0.89) with TR:TF. A strong positive correlation (0.75) was observed between TCT and BT. TPP has positive correlation with TCT. Thus, the positive correlation among TF, TPP and TCT has a direct impact on black tea quality. Quality of black tea is correlated to theaflavins, total polyphenol and total catechins content in black tea.[18–20] Results obtained in the present study confirmed that theaflavins play a major role to determine black tea quality.

There was a negative correlation of AA and CF with other quality parameters and the correlation was non-significant. Amino acids play an important role in the development of tea aroma during the black tea manufacturing process.[1] Theanine is considered to be a unique amino acid in nature because its occurrence appears to be limited to the *Camellia* genus, mostly the tea-producing plants. Because of its contribution to favourable flavour and health benefits, theanine has great potential for utilization as a food ingredient or as a dietary supplement.[32]

Table 3: Correlation coefficients of biochemical and organoleptic parameters

	TF	TR	TR:TF	TC	BT	TPP	TCT	AA	CF	IFL	LC	STN	LQ	TQS
TF	1.00													
TR	0.44	1.00												
TR:TF	-0.89**	-0.05	1.00											
TC	0.60**	0.36*	-0.48*	1.00										
BT	0.88**	0.17	-0.87**	0.31	1.00									
TPP	0.31	-0.05	-0.37*	0.07	0.32	1.00								
TCT	0.64**	-0.06	-0.73**	0.12	0.75**	0.53**	1.00							
AA	-0.43*	-0.29	0.40*	-0.06	-0.46*	-0.14	-0.34*	1.00						
CF	-0.20	-0.23	0.09	-0.001	-0.25	0.15	-0.16	0.39*	1.00					
IFL	0.59**	0.41*	-0.46*	0.41*	0.49*	0.31	0.50*	-0.29	-0.47*	1.00				
LC	0.19	0.04	-0.32*	0.06	0.13	-0.08	0.03	-0.34*	-0.08	-0.03	1.00			
STN	0.29	0.07	-0.26	0.36*	0.10	0.45*	0.25	-0.03	-0.04	0.44*	-0.34*	1.00		
LQ	0.60**	0.25	-0.55**	0.44*	0.47*	0.34*	0.38*	-0.25	-0.30	0.75**	0.12	0.50*	1.00	
TQS	0.63**	0.30	-0.57**	0.48*	0.48*	0.40*	0.46*	-0.31	-0.37*	0.89**	0.11	0.63**	0.94**	1.00

*Significant at $P<0.05$, **significant at $P<0.001$
Biochemical parameters (TF= theaflavins, TR= thearubigins, TC= total colour, BT= brightness, TPP= total polyphenols, TCT= total catechins, AA= total amino acids, CF= caffeine).
Organoleptic parameters (IFL = Infused leaf colour, LC= liquor colour, STN= liquor strength, LQ= liquor quality, TQS = Total quality score.

Therefore, further investigation on individual amino acid content and flavour profile of accessions would be more meaningful than total amino acid content.

A significant correlation between biochemical parameters (TF, TC, BR, TPP and TCT) and organoleptic parameters (IFL, LQ and TQS) was observed. There were strong positive correlations of IFL with STN (0.44), LQ (0.75) and TQS (0.89), respectively. Therefore, infused leaf colour could be considered as a good organoleptic quality parameter. In addition, a strong positive correlation (0.94) could be observed between LQ and TQS. Above correlations (such as biochemical constituents and organoleptic parameters) are in agreement with the results of previous studies to a greater extent.[2,6,7] Furthermore, quality of tea accessions was frequently assessed by professional tea taster's by sensory evaluation and present results revealed that it is worthwhile to incorporate biochemical parameters to derive more meaningful conclusions about black tea quality.

Average Linkage Cluster Analysis

According to the results of the principal component and cluster analysis, four distinct groups segregated on the basis of biochemical parameters and tasters' evaluation (Fig. 2). First component of PC analysis accounted for 43.0% variability while second component accounted for 13.3% of the total variability.

Out of the 35 accessions, 9 accessions grouped in cluster 1 and six of them (DT 1, DUN 7, S 106, NAY 3, H 1/58 and WT 26) are estate selections having medium-size leaves and rest of the three accessions (VHMOR, INTRI 6 and TRI 777) are introductions. Interestingly, all these estate selections and TRI 777 proved as of high tea quality.[33] High amounts of TF, TCT and BT were detected in VHMOR and INTRI 6, proving that those accessions possess high quality character.

Cluster 2 consists of a mixture of estate selections and the cultivars developed by the TRISL and represented with high quality accessions such as PK 2, N 2, TC 9 and TRI 4079 and moderate quality accessions such as TRI 2142 and TRI 4078. Furthermore, TRI 4078 and TRI 4079 evolved from self pollination of N 2 which is also grouped in the same cluster. Cluster 3 comprised mixture of estate selections and TRI developed cultivars and represents moderate or low quality accessions. Though they do not fall into the category of proven quality, most of the accessions (TRI 2025, TRI 3013, TRI 4052, TRI 4071, CY 9, DN and KEN 16/3) are high yielding having

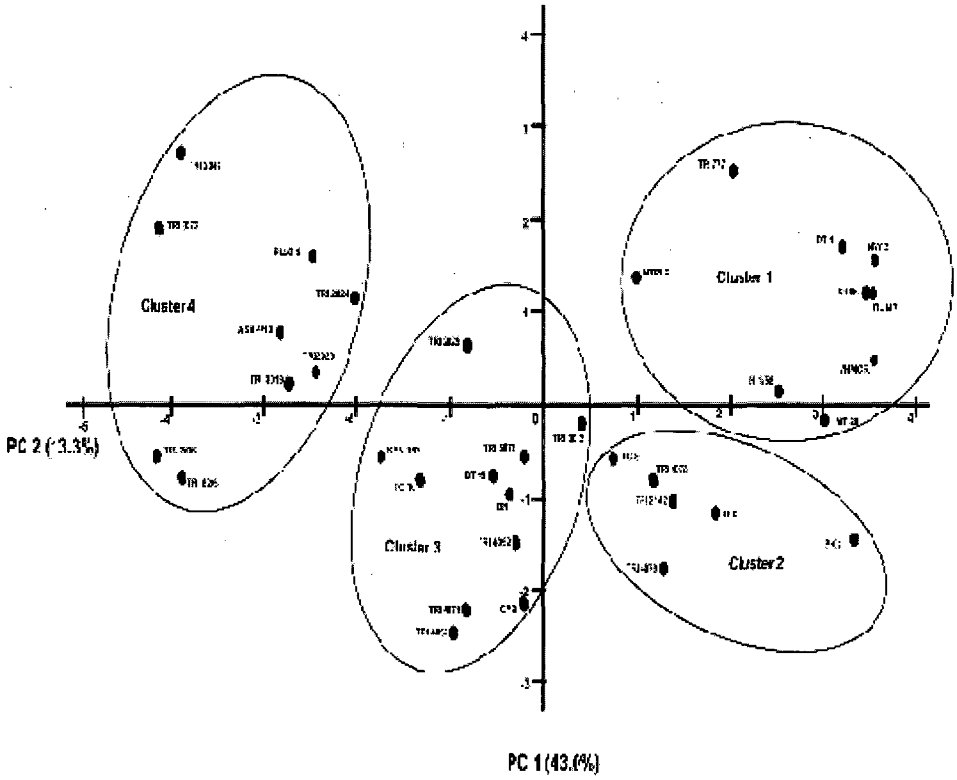

Fig. 2: Principal component analysis bi-plot derived from biochemical and organolaptic data.

predominant Assam type characters.

Nine accessions grouped in cluster four and seven accessions in this cluster (ASM 4/10, TRI 2023, TRI 2024, TRI 62/5, TRI 3019, TRI 3072, and TRI 2016) evolved from open pollinated seeds of ASM 4/10 or have indirect relationship with ASM 4/10. PLLG 2 was selected from existing old seedling at the Poonagala Estate and it showed the lowest amount of TF in both seasons. TRI 2043 showed unique morphological characteristics such as dark purple pigmentation in young shoots as well as young leaf with dense pubescence. Interestingly, all accessions in this group were confirmed as of low quality.

Utilization of Biochemical Compounds

The cultivated tea populatins comprise *C. sinensis* (L.) O. Kuntze, *C. assamica* (Masters) Wight and the intermediate, *C. assamica* ssp. *lasiocalyx* (Planchon ex Watt) Wight (*i.e.* China, Assam and Cambod varieties respectively).[34] These classifications are mostly based on leaf and floral morphology and tree architecture rather than biochemical variations. Recently, however, biochemical compounds in green leaf have also been used to characterize tea germplasm.[35] Present study revealed that processed black tea biochemical variation is important parameter to classify tea varieties in addition to using morphological attributes.

Based on the records available, less than 6% of total accessions preserved in Sri Lankan tea germplasm have been utilized in tea breeding program till 1998 and it has increased up to 13.6% by the end of 2009.[36] Lack of information on genetic variation, mainly biochemical and molecular diversity, of existing collection could be one of the reasons for under-utilization of the existing germplasm in tea breeding.

Significant variations of selected black tea biochemical compounds detected in the present study indicate the presence of adequate genetic diversity of tea germplasm in Sri Lanka and substantiate its potential utilization in future tea crop improvement programmes more objectively. Furthermore, quantification of other groups of biochemical compounds such as carbohydrates, lipids, amino acids and alkaloids is vital to discover their contribution to black tea quality as well as to elicit health benefits present in tea.

Conclusion

The significant variations of selected black tea biochemical compounds detected in the present study indicate the high biochemical diversity of tea germplasm in Sri

Lanka. This provides a useful guideline on use of black tea biochemical compounds such as theaflavins, thearubigins, total polyphenols, total catechins and caffeine in characterizing the Sri Lankan tea germplasm. Use of biochemical parameters to supplement the quality of made tea determined by professional tea taster's using organoleptic evaluation is proposed from the results of this study to derive more meaningful conclusions about black tea quality. In addition, the study confirmed the seasonal fluctuation of biochemical compounds in tea accessions. Findings of the study also elucidate the possibility of using biochemical compounds to categorize accessions into the three main tea taxa, Assam, Cambod and China, to a greater extent.

Information generated on biochemical profiles of tea accessions would also help the tea breeder to identify parents for future breeding programme supported with more meaningful descriptors that would enhance the rationale for breeding new cultivars with diverse traits to cater to ever-changing demand of the consumer in the future.

References

1. Engelhardt UH. 2010. Chemistry of tea – Comprehensive natural products II. *In*: L Mander & HW Liu (Eds), *Chemistry and Biology. Development & Modification of Bioactivity*, Elsevier Ltd: UK.
2. Liang Y, Lu J, Zhang L, Wu S, & Wu Y. 2003. Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions. *Food Chem* 80: 283–290
3. Robertson A. 1992. The chemistry and biochemistry of black tea production – the non-volatiles. *In*: KC Wilson & MN Clifford (Eds), *Tea: Cultivation to Consumption*. Chapman and Hall: London.
4. Yao L, Liu X, Jiang Y, Caffin N, D'Arcy B, Singanusong R, Datta N, & Xu Y. 2006. Compositional analysis of teas from Australian supermarkets. *Food Chem* 94: 115–122.
5. Sharangi AB. 2009. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.) – A review. *Food Res Int* 42: 529–535.
6. Bhuyan LP, Hussain A, Tamuly P, Gogoi RC, Bordoloi PK, & Hazarika M. 2009. Chemical characterisation of CTC black tea of northeast India: correlation of quality parameters with tea tasters' evaluation. *J Sci Food Agric* 89: 1498–1507.
7. Kumar RSS, Muraleedharan NN, Murugesan S, Kottur G, Anand MP, & Nishadh A. 2011. Biochemical quality characteristics of CTC black teas of South India and their relation to organoleptic evaluation. *Food Chem* 129: 117–124.
8. Wang K & Ruan J. 2009. Analysis of chemical components in green tea in relation with perceived quality, a case study with Longjing teas. *Int J Food Sci Tech* 44: 2476–2484.
9. Chen Y, Jiang Y, Duan J, Shi J, Xue S, & Kakuda Y. 2010. Variation in catechin contents in relation to quality of 'Huang Zhi Xiang' Oolong tea (*Camellia sinensis*) at various growing altitudes and seasons. *Food Chem* 119: 648–652.
10. Wang K, Liu F, Liu Z, Huang J, Xu Z, Li Y, Chen J, Gong Y, & Yang X. 2010. Analysis of chemical components in Oolong tea in relation to perceived quality. *Int J Food Sci Tech* 45: 913–920.
11. Kottawa-Arachchi JD, Gunasekare MTK, Ranatunga MAB, Jayasinghe L, & Karunagoda RP. 2012. Analysis of selected biochemical constituents in black tea (*Camellia sinensis*) for predicting the quality of tea germplasm in Sri Lanka. *Tropical Agric Res* 23(1): 30–41 (available online at: DOI: http://dx.doi.org/10.4038/tar.v23i1.4629).
12. Roberts EAH & Smith RF. 1963. The phenolic substances of manufactured tea. IX. The spectrophotometric evaluation of tea liquors. *J Sci Food Agric* 14: 689–699.
13. Obanda M, Owuor PO, Mang'oka R, & Kavoi MM. 2004. Changes in thearubigin fractions and theaflavin levels due to variations in processing conditions and their influence on black tea liquor brightness and total colour. *Food Chem* 85: 163–173.
14. ISO 14502-1. 2005. Content of Total Polyphenols in Tea. Colorimetric Method Using Folin-Ciocalteu Reagent.
15. Swain T & Hillis WE. 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *J Sci Food Agric* 10: 63–68.
16. Yemm EW & Cocking EC. 1955. The determination of amino-acids with ninhydrin. *Analyst* 80: 209–213.
17. Chen L & Zhou ZX. 2005. Variations of main quality components of tea genetic resources [*Camellia sinensis* (L.) O. Kuntze] Preserved in the China National Germplasm Tea Repository. *Plant Foods Hum Nutr* 60: 31–35.
18. Roberts GR & Fernando RSS. 1981. Some observation on the correlation of polyphenol content to the quality of tea clones. *Tea Q* 50: 30–34.
19. Owuor PO, Reeves SG, & Wanyoko JK. 1986. Correlation of theaflavins contents and valuation of

Kenyan black teas. *J Sci Food Agric* 37: 507–513.

20. Lopez SJ, Thomas J, Pius PK, Kumar RR, & Muraleedharan N. 2005. A reliable technique to identify superior quality clones from tea germplasm. *Food Chem* 91: 771–778.

21. Takeda Y. 1994. Differences in caffeine and tannin contents between tea cultivars and application to tea breeding. *Japan Agric Res Q* 28: 117–123.

22. Wei K, Wang L, Zhou J, He W, Zeng J, Jiang Y, & Cheng H. 2011. Catechin contents in tea (*Camellia sinensis*) as affected by cultivar and environment and their relation to chlorophyll contents. *Food Chem* 125: 44–48.

23. Wang YS, Gao LP, Wang ZR, Liu YJ, Sun ML, Yang DQ, Wei CL, Shan Y, & Xia T. 2012. Light-induced expression of genes involved in phenylpropanoid biosynthetic pathways in callus of tea (*Camellia sinensis* (L.) O. Kuntze). *Scientia Hort* 133: 72–83.

24. Biswas AK, Sarkar AR & Biswas AK. 1973. Biological and chemical factors affecting the valuation of north east Indian plains teas III. Statistical evaluation of the biochemical constituents and their effects on colour, brightness and strength of black teas. *J Sci Food Agric* 24: 1457–1477.

25. Yao LH, Jiang YM, Shi J, Tomas-Barberan FA, Datta N, Singanusong R, & Chen SS. 2004. Flavonoids in food and their health benefits. *Plant Foods Hum Nutr* 59: 113–122.

26. Wickremasinghe RL, Perera KPWC, Perera VH, & Kanapathipillai P. 1966. Analysis of polyphenols, amino acids and chlorophyll levels in tea flush at different seasons. *Tea Q* 37: 232–235.

27. Owuor PO & Chavanji AM. 1986. Caffeine contents of clonal tea, seasonal variations and effects of plucking standards under Kenyan conditions. *Food Chem* 20: 225–233.

28. Couzinet-Mossin A, Balayssac S, Gilard V, Malet-Martino M, Potin-Gautier M, & Behra S. 2010. Interaction mechanisms between caffeine and polyphenols in infusions of *Camellia sinensis* leaves. *Food Chem* 119: 173–181.

29. Joshi R, Babu GDK, & Gulati A. 2013. Effect of decaffeination conditions on quality parameters of Kangra orthodox black tea. *Food Res Int* (avaiable online at: DOI: 2013; 10.1016/j.foodres.2012.12.050).

30. Yadav SK & Ahuja PS. 2007. Towards generating caffeine-free tea by metabolic engineering. *Plant Foods Hum Nutr* 62: 185–191.

31. Mohanpuria P, Kumar V, Ahuja PS & Yadav SK. 2011. Producing low-caffeine tea through post-transcriptional silencing of caffeine synthase mRNA. *Plant Mol Biol* 76: 3–34.

32. Vuong QV, Bowyer MC, & Roach PD. 2011. L-Theanine: Properties, synthesis and isolation from tea. A review. *J Sci Food Agric* 91: 1931–1939.

33. Tea Research Institute of Sri Lanka. 2002. *The suitability of tea clones for different regions*. Tea Research Institute Advisory Circular No. PN 01- Serial No. 6/02. Tea Research Institute of Sri Lanka: Talawakelle.

34. Banerjee B. 1992. Botanical classification of tea. *In*: KC Wilson & MN Clifford (Eds), *Tea: Cultivation to Consumption*. Chapman and Hall: London

35. Kottawa-Arachchi JD, Gunasekare MTK, Ranatunga MAB, Punyasiri PAN, & Jayasinghe L. 2013. Use of biochemical compounds in tea germplasm characterization and its implications in tea breeding in Sri Lanka. *J Natn Sci Foundation Sri Lanka* 41(4): 309–318. (available online at: DOI: http://dx.doi.org/10.4038/jnsfsr.v41i4.6252).

36. Gunasekare MTK, Ranatunga MAB, Piyasundara JHN, & Kottawa-Arachchi JD. 2012. Tea genetic resources in Sri Lanka: Collection, conservation and appraisal. A review. *Int J Tea Sci* 8(3): 51–60.