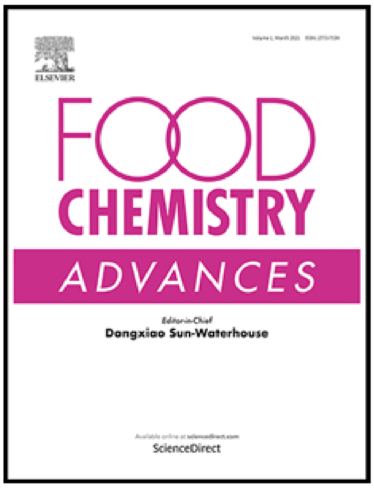
Journal Pre-proof



Variation of catechin and caffeine content in exotic collection of tea [Camellia sinensis (L.) O.Kuntze] in Sri Lanka and potential implication in breeding cultivars with enhanced quality and medicinal properties

Jeevan Dananjaya Kottawa-Arachchi , M.A.B. Ranatunga , A.M.T. Amarakoon , M.T.K. Gunasekare , R.N. Attanayake , R.K. Sharma , H.K Chaudhary , V.K. Sood , R. Katoch , D.K. Banyal , K.G.N.P. Piyasena , E.N.U. Edirisinghe

PII: S2772-753X(22)00096-X
DOI: https://doi.org/10.1016/j.focha.2022.100108
Reference: FOCHA 100108

To appear in: *Food Chemistry Advances*

Received date: 2 June 2022
Revised date: 26 August 2022
Accepted date: 21 September 2022

Please cite this article as: Jeevan Dananjaya Kottawa-Arachchi , M.A.B. Ranatunga , A.M.T. Amarakoon , M.T.K. Gunasekare , R.N. Attanayake , R.K. Sharma , H.K Chaudhary , V.K. Sood , R. Katoch , D.K. Banyal , K.G.N.P. Piyasena , E.N.U. Edirisinghe , Variation of catechin and caffeine content in exotic collection of tea [Camellia sinensis (L.) O.Kuntze] in Sri Lanka and potential implication in breeding cultivars with enhanced quality and medicinal properties, *Food Chemistry Advances* (2022), doi: https://doi.org/10.1016/j.focha.2022.100108

Highlights:

- The comprehensive seasonal and genotypic variations of exotic tea (*Camellia sinensis*) accessions in Sri Lanka were determined using HPLC method.
- Exotic accessions with Indian origin recorded high catechins and caffeine content which is important for production of high quality black tea
- Several exotic tea accessions were identified as potential genetic resources for the development of low caffeine speciality tea.
- The selected exotic tea accessions are rich in bioactive compounds and have promising potentials in producing speciality teas with enhanced quality and medicinal properties.

Variation of catechin and caffeine content in exotic collection of tea [Camellia sinensis (L.) O.Kuntze] in Sri Lanka and potential implication in breeding cultivars with enhanced quality and medicinal properties

Jeevan Dananjaya Kottawa-Arachchi [1,2*], M.A.B. Ranatunga[1], A.M.T. Amarakoon[3], M.T.K. Gunasekare[4], R.N. Attanayake[3], R.K. Sharma[5], H.K Chaudhary[2], V.K. Sood[2], R. Katoch[6] and D.K. Banyal[7], K.G.N.P. Piyasena[8], E.N.U. Edirisinghe[8]

1. Plant Breeding Division, Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka
2. Department of Genetics and Plant Breeding, Collage of Agriculture, Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India
3. Faculty of Science, University of Kelaniya, Sri Lanka
4. Ministry of Plantations, Sethsiripaya, Battaramulla, Sri Lanka
5. Biotechnology Department, Institute of Himalayan Bioresource Technology, Palampur, India
6. Department of Biochemistry, Collage of Basic Sciences, Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India
7. Department of Plant Pathology, Collage of Agriculture, Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India
8. Biochemistry Division, Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka

* **Corresponding to**: J.D. Kottawa-Arachchi, Plant Breeding Division, Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka. E mail address: jeevan.dananjaya@gmail.com

Abstract:

Tea leaves are rich in diverse metabolites with medicinal importance. The quality of made tea is largely depended on the qualitative and quantitative attributes of key metabolites. The aim of the present study was to quantify flavan-3-ols and caffeine of exotic tea germplam and explore the medicinal properties. Fresh leaf flavan-3-ols; catechin, epicatechin (EC), epicatechin gallate (ECg), epigallocatechin (EGC) and epigallocatechin gallate (EGCg), caffeine and gallic acid of 131 accessions (87 exotic and 44 improved cultivars) were performed by High Performance Liquid Chromatography. Variations in metabolites among cultivars as well as between seasons were determined. Among the flavan-3-ols, EGCg was the most abundant followed by EGC, ECg and EC. Hierarchical clustering of 131 accessions based on metabolite diversity resulted two major clusters. Indian introductions clustered with well-known high quality cultivars indicating the potential utilization of high quality black tea production. Exotic accessions with low caffeine contents (< 20 mg g^{-1}) were grouped separate cluster indicating the promising genetic resources for the development of low caffeine tea. Present study revealed that selected exotic tea accessions rich in bioactive compounds such as catechins and caffeine content could be utilized in producing speciality teas with enhanced quality and medicinal properties.

Keywords: tea quality, *Camellia sinensis*, flavan-3-ols, caffeine, seasonal variations

1. Introduction

Tea (*Camellia sinensis* L.), the second most consumed beverage in the world, is a rich source of polyphenolic compounds. Young tea leaves are extremely rich in polyphenolic compounds and the total polyphenols including flavan-3-ols (catechins) in tea ranges from 20 to 30% of the dry weight (Robertson, 1992). Polyphenols and caffeine, the major non-nutrient components in tea, have pharmacological effects and health benefits such as anti-oxidant, antimicrobial and anticancer properties (Sharangi, 2009). Polyphenols, particularly flavan-3-ols and their oxidation products are responsible for the quality of black tea (Engelhardt, 2010). Five major types of flavan-3-ols; (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin gallate (ECg), (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate

(EGCg) have been reported in tea leaves (Sabhapondit et al., 2012). Out of these, flavan-3-ols is a precursor for the formation of two important compound of black tea, theaflavins (TFs) and thearubigins (TRs) (Robertson, 1992). Caffeine is the most abundant alkaloid in black tea and gives briskness and creaming properties to black tea (Balantine et al., 1998). Caffeine is an important ingredient of tea as stimulates mental activity, reduce fatigue and drowsiness. On the other hand, low caffeine tea has a high demand in children and the elderly due to its side effects such as inflammation of the digestive organ, insomnia and arrhythmia with excessive intake of caffeine (Nurminen et al., 1999).

Sri Lanka established its commercial tea plantation nearly one and half century ago and secure its position as highest quality orthodox black tea producer in the world. More than 600 tea germplasm accessions representing two major varietal types (Banerjee, 1992), China type (*C. sinensis* var. *sinensis*) and Assam type (*C. sinensis* var. *assamica* (Masters) Chang) and hybrid Cambod tea (*Camellia sinensis* ssp. *lasiocalyx* (Planchon ex Watt)] are maintained at the Tea Research Institute of Sri Lanka (TRI). Extensive hybridization among three types resulted sharing of mixed characteristics and it is unable to identify pure types in cultivations (Raina et al., 2012). The sustainability and profitability of the tea industry primarily depend on the availability of quality planting materials with desirable traits. However, the narrow genetic base of tea cultivations in Sri Lanka renders it more vulnerable to various biotic and abiotic stresses including pests, diseases and drought (Gunasekare, 2012). Therefore, collection and introduction of diverse exotic tea cultivars are routine practises to sustain the tea cultivation. Evaluation of biochemical attributes is a vital step for the selection and identification of potential parental groups to expedite the breeding of quality tea cultivars.

High throughput techniques such as gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), high performance liquid chromatography (HPLC), and nuclear magnetic resonance spectroscopy (NMR) have been widely used for biochemical characterization of germplasm. HPLC analysis is one of the acceptable methods to define biochemical diversity of various leaf, root, tuber, and fruit crops (Cevallos-cevallos et al., 2009; Price et al., 2020). Comprehensive characterisation of the phenolic profile of different crops has been applied in the selection and evaluation of new cultivars. Polyphenols are considered as a key determinant of food quality and market value of certain food crops. Variations in polyphenolic compounds have been successfully used as chemical markers in African Plum (*Prunus salicina* Lindl.) (Venter et al., 2013) and European grape (*Vitis vinifera*) (Lampíř, 2013; Liang et al., 2011) population in diversity and selection studies. Further, these chemical markers were useful in authentication of the pedigree and determining genetic diversity of grapes (Muccillo et al., 2014). Systematic biochemical and genetic diversity assessment studies of beverage crops like tea, coffee and cocoa is extremely important to identify accessions for variety development targeting quality attributes (Crozier et al., 2011).

Tea Research Institute of Sri Lanka (TRI) has recently established an exotic collection with tea seed batches received from different sources and developed through vegetative propagation by TRI. The origin of the seeds belongs to wild types, cultivated varieties and varieties used for green and Oolong tea production. A recent taxonomic study revealed that this exotic germplasm mostly represented China types with less numbers of Assam types (Ranatunga et al., 2015). However, since most of the cultivated teas in Sri Lanka are predominantly Assam type, accessions of the exotic germplasm with desirable traits could be used to produce new cultivars with a broad genetic variation. Although a few exotic accessions have been previously studied in terms of biochemical profiling (Punyasiri et al., 2017), flavan-3-ols and caffeine content of more than 80% of total exotic germplasm collection remains unknown to date. Effect of seasonal variations on the metabolite profiles and their correlations have been reported and information is useful in producing speciality teas (Deka et al., 2020; Jayasekera et al., 2014). Therefore, it is worthwhile to quantify the major flavan-3-ols and caffeine contents and their seasonal variations in the Sri Lankan exotic tea germplasm.

The major objective of the present study was to discover the variation of the five types of flavan-3-ols; (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin gallate (ECg), (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCg), gallic acid and caffeine in tea accessions with diverse origins selected from tea germplasm maintained at TRI. Long term aim of the project is to effectively exploit them and utilize for breeding purposes targeting speciality teas with health benefits.

2. Materials and methods

2.1. Plant material

A total of 131 tea (*Camellia sinensis* L.) accessions from different sources and developed through vegetative propagation by TRI were selected based on morphological, floral traits and the countries of origin (Ranatunga et al., 2017). Out of 131 accessions, 15 accessions which grow as small trees with robust branches and large, glossy leaves resemble to Assam types whereas 67 accessions are resembled to China types that grow in big shrubs, with thick, hard and small leaves. Rest of 49 accessions are resembled to Cambod (Assam/China hybrids) types. All accessions are maintained as a living collection at the field gene bank at the TRI, Talawakelle, Sri Lanka (St. Coombs Estate; located Lat 6.94N, Long 80.66E, altitude 1382m a.m.s.l.)

2.2. Preparation of tea samples for biochemical analysis

Tender tea shoots (two apical leaves and the bud) from each progeny was collected (*ca*. 50 g) and brought to the laboratory at 4 °C. Samples were immediately stored at –80 °C and then freeze-dried for 24 h (Labconco® Corporation, MS, USA). Freeze-dried leaves were ground in a laboratory mill until a fine powder was obtained and sealed in triple-laminated aluminium foil packages and stored at room temperature until further use as described in (Punyasiri et al., 2015). The sampling was done in wet season (June and July 2019) and in dry season (February and March 2020). The dry matter content was determined by the loss of moisture in mass at 103 °C (ISO-1573, 1980).

2.3. Quantification of flavanols and caffeine content

Caffeine, C, EC, ECg, EGC, EGCg, and gallic acid were quantified using the ISO 14502-2 (2005) method. Briefly, 0.200 g of finely ground tea sample was extracted with 5 ml of 70% methanol at 70 °C for 10 min and this process was repeated once. The two extractions were combined and made up to 10 ml with 70% methanol. One ml of the sample extract was topped up to 5 ml with freshly prepared stabilizing solution containing 500 μg ml^{-1} of EDTA and 500 μg ml^{-1} ascorbic acid in 10% volume fraction of acetonitrile. An aliquot was filtered through 0.45 μm filter (Agilent, HPLC grade) and 10 μL was injected to the HPLC. Quantification was done using Agilent 1260 HPLC Infinity System on a phenyl-bonded column with UV detection at 278 nm (Punyasiri et al., 2015). Analysis was carried out in duplicate. Peak identity was confirmed with catechin and caffeine standards. Caffeine was used as a standard in conjunction with individual flavan-3-ols considering the Relative Response Factors (RRFs) established by an ISO international inter-laboratory test. All chemicals and solvents used in the experiments were of HPLC grade (Sigma-Aldrich, USA).

$$\text{Individual component content (mg g}^{-1}\text{)} = \frac{(A_{\text{samp}} - A_{\text{int}}) \times F_{\text{std}} \times V_{\text{samp}} \times d \times 100 \times 10}{S_{\text{caff}} \times m_{\text{samp}} \times 10000 \times w_{\text{DM, samp}}}$$

A_{samp} is the peak area of the individual component in the test sample

A_{int}	is the peak area at the point the standard calibration line intercept the y-axis
F_{std}	is the Relative Response Factor, measured with respect to caffeine for the individual component
V_{samp}	is the sample extraction volume, in millilitres
d	is the sample dilution factor
S_{caff}	is the caffeine calibration line slope
m_{samp}	is the mass in grams of the sample test portion
$w_{DM, samp}$	is the dry matter content expressed as mass fraction in percent of the test sample determined

2.4. Statistical Analysis

All statistical analyses were carried out using R software and biplots were drawn using PAST3 software. Box plots of seasonal variations and Pearson correlation analysis were carried out using R software. Heatmap was generated using ClustVis, online software at http://biit.cs.ut.ee/clustvis. The data were log (x+1) transformed, and similarity assessment for clustering was based on the Euclidean distance and Ward's minimum variance method.

3. Results

3.1. Flavan-3-ols, gallic acid and caffeine

The HPLC chromatogram for the standard mixture (Figure 1(A)) with that of *C. sinensis* extract (Figure 1(B)) revealed that the tea extract contained major flavan-3-ols and caffeine. The concentration of gallic acid, caffeine, major flavan-3-ols, *viz*. EGC, EC, EGCg, ECg, C, and total catechins (TC) in the extract of 131 accessions are presented in supplementary Table S1.

Figure 1. (A) HPLC chromatogram of a standard solution containing caffeine, + C, EC, EGC, EGCg, ECg, and gallic acid. (HPLC peaks: 1 – gallic acid, 2 – epigallocatechin, 3 – catechin, 4 – caffeine, 5 – epicatechin, 6 – epigallocatechin gallate, 7 – epicatechin gallate).
(B) HPLC chromatogram of an extract of a representative tea sample. (HPLC peaks: 1 – gallic acid, 2 – epigallocatechin, 3 – catechin, 4 – caffeine, 5 – epicatechin, 6 – epigallocatechin gallate, 7 – epicatechin gallate)

Among the major flavan-3-ols detected in the extracts, EGCg was the most abundant followed by EGC, ECg, EC and +C. In dry season, means of all biochemical compounds are significantly different among China, Assam and Cambod type accessions. Except EGC and EGCg, the means of rest of other biochemical constituents were significantly different among three types in wet season. The minimum and maximum concentrations, means, and other statistical parameters of the catechins and caffeine present in wet and dry seasons are given in Table 1.

Table 1: Summary statistics of selected biochemical constituents in China, Assam and Cambod type accessions

Among the accessions studied, high yielding cultivar TRI 3069 possessed the highest EGCg content in both dry and wet seasons as 122.82 and 142.92 mg g^{-1}, respectively (Supplementary Table S1). Conversely, TRI 2043 possessed the lowest EGCg contents in both seasons. Further, majority of Assam and Cambod type accessions showed higher amount of EGCg than China types. In dry seasons, EGC was the second most abundant flavan-3-ols and the mean values of China types were significantly higher than Assam and Cambod types. TRI 4067 (61.48 mg g^{-1}) and INTRI 8 (59.67 mg g^{-1}) showed the highest EGC content in dry and wet seasons, respectively. Introductions ASM4/10 (14.19 mg g^{-1}) and PBGT71 (14.05 mg g^{-1}) recorded the lowest content in dry and wet seasons, respectively.

Irrespective of the season, the mean values of ECg content in China types were significantly lower than Assam and Cambod types. The highest concentration of ECg was observed in TRI 777 and PBGT118 in dry and wet seasons, respectively. The lowest ECg content was recorded in PBGT63 in wet season whereas PBGT124 in dry season. The range of ECg content recorded in the exotic accessions of the present study was consistent with previous studies conducted with recommended tea varieties of Sri Lanka (Punyasiri et al., 2017) and India (Deka et al., 2020).

Irrespective of the seasons, means of EC content were significantly different among three varietal types. The introductions TRI 777 and AI6 recorded the highest values of EC in dry and wet seasons. Although, mean values of minor components such as gallic acid and + catechin were lower, their coefficient of variation (CV %) was higher indicating the high genetic variations among the selected accessions (Table 1). Mean values of sum of all individual flavan-3-ols (total catechins) content in dry season were 144.98, 162.57 and 167.44 mg g^{-1} and in wet season 150.26, 168.82 and 173.70 mg g^{-1} in China, Assam and

Cambod types respectively. The mean values of total catechins of Assam and Cambod type accessions were significantly higher than China types in dry seasons.

Similarly, Deka et al., (2020) observed higher mean values of TC in monsoon period irrespective of varieties. Another study reported that the total flavan-3-ol content in 403 Chinese tea cultivars varied from 56.6 to 231.9 mg g^{-1} which is similar to our observation (Jin et al., 2014). In India, the higher TC content was recorded in Assam types (mean 231 mg g^{-1}) than China types (mean 157 mg g^{-1}) (Sabhapondit et al., 2012). In the present study, Assam introductions and TRI developed varieties which are morphologically grouped in Assam types showed higher TC content.

Caffeine is an important alkaloid in tea. Irrespective of the seasons, it is observed that the means of caffeine content of Assam and Cambod types was significantly higher than China types. In other tea growing countries, the caffeine content varied between 12 – 59 mg g^{-1} in China (Chen & Zhou, 2005), and 19.6 - 43.7 mg g^{-1} in Kenyan (Muthiani et al., 2016) tea germplasm. In addition to low EGCg content which predicted blister blight resistance character, PBGT35, PBGT48, PBGT53 and PBGT71 recorded lowest content of caffeine in both seasons (Figure 2).

Figure 2: The biplot of 131 tea germplasm accessions showing the relationship of caffeine contents between dry and wet seasons.

Gallic acid is an important phenolic carboxylic acid found in tea leaf (Berkowitz et al., 1971) and forms esters with flavan-3-ols. ECg and EGCg are galloyl esters (Ashihara et al., 2010) of EC and EGC, respectively. The relationship between DTR (ratio of dihydroxycatechins to trihydroxycatechins) and GNR (ratio of gallated to non-gallatedcatechins) of 131 germplasm accessions present in Figure 3. The hydroxyl substitution pattern is characterized by the ratio of dihydroxycatechins (EC + ECg) to trihydroxycatechins (EGC + EGCg), and is defined as DTR, i.e. (EC + ECg)/(EGC + EGCg) and also termed as catechin (flavan-3-ol) index (CI). In both seasons, high amount of EC and ECg contents were recorded in AI-6, SI-1, SI-9 and PBGT100. Besides, the extent of galloylation is given by the ratio of gallated to non-gallated flavan-3-ols (GNR) which is defined as (ECg + EGCg/EC + EGC). Several introductions AI-5, AI-6 and AI-8 are grouped with well-established high quality cultivars such as TRI 777, TRI 5001, TRI 5002 showing as potential accessions for production of high quality black tea.

Figure 3: The biplot showing relationship between DTR (ratio of dihydroxycatechins to trihydroxycatechins) and GNR (ratio of gallated to non-gallatedcatechins) of 131 tea accessions

3.2. Correlation analysis and overall variation of selected metabolites

Pearson correlation analysis of catechins and caffeine is presented in Figure 4. There were significant positive correlations of TC with EGCg, and ECg in both seasons. EGCg contributed 35 - 67% and 26 - 71% to TC content in dry and wet seasons, respectively. In previous studies, EGCg was reported to contribute ~50% and ~60% to total catechin in Central and Southern African teas (Wright et al., 2000) and Chinese green teas (Xu et al., 2012), respectively. More or less, EGC in both seasons exhibited negative correlations with all constituents except TC and EC in in wet season. However, Deka et al., 2020 observed strong negative correlations of EGC with TC and ECg in tea leaves harvested in autumn. Caffeine has significant positive correlation with TC and ECg in both seasons.

Figure 4: Correlation matrix between individual catechins and caffeine content in both seasons. (W: wet season, D: dry season) intensity of colour is representative of the significance of the correlation (darker the higher significance). Blue colour indicates positive and red colour indicates negative correlations.

3.3. Cluster analysis

A heatmap was generated to identify grouping pattern of germplasm accessions based on the flavan-3-ols and caffeine content in both dry and wet seasons using hierarchical cluster analysis (Figure 5). The heatmap was generated using Euclidean distance and Ward's linkage clustering method. With Ward's linkage method, the distance between two clusters is the sum of squared deviations from points to centroids. In the hierarchical cluster analysis, accessions studied were grouped into two main clusters (A and B) (Figure 5).

Cluster A consist China type accessions except single Assam (AI-8) and Cambod (TRI 3055) type accessions. Interestingly, out of 37 accessions having Korean origin, 34 were group in cluster A. Further, out of 67 China type accessions, 41 were included in cluster A. All catechins except EGC, negatively contributed for grouping of accessions in cluster A whereas all catechins more or less positively contributed for grouping of accessions (except PBGT 71, ASM4/10, TRI 2025 and TRI 2043) in cluster B.

Except two accessions (AI-8 and TRI 3055), all Assam and Cambod accessions were grouped in Cluster B. Majority of exotic accessions having different origins *viz.* Azerbaijan, Japan and accessions of unknown origin were formed adjacent three sub-clusters in cluster B. Besides, mixture of TRI developed cultivars, Estate selections and accessions having Indian origins in cluster B indicating the close ancestral relationship among them.

The accession TRI 2043 was unique due to its origin, high pubescence density, pigmented leaves and tolerant to blister blight disease. This accession has been separated from other accessions and formed a single sub cluster in Cluster B. EGC, EGCg and total catechins of TRI 2043 in both seasons were negatively contributed to separate from main cluster B. Most of the Indian origins were grouped with well-known high quality cultivars such as TRI 777 and DT1. It is worthwhile to determine the black tea quality potential of Indian introductions and other exotic accessions which are grouped in same cluster. The result of heatmap revealed that the exotic accessions originated from Korea in cluster A could be genetically distinct from the clusters B.

It is interesting to note that majority of Korean originated accessions which are suitable for green tea and Oolong tea production were grouped in clusters A. Further, exotic accessions grouped in clusters A which showed low caffeine content (< 20 mg g^{-1}) in both seasons can be considered as potential genetic resources for development of low caffeine tea.

Figure 5: The heatmap generated using Euclidean distance and Ward's linkage clustering method. Red and blue colour patterns indicate positive and negative contributions of individual catechins and caffeine for clustering of 131 tea accessions, respectively. (W: wet season, D: dry season)

4. Discussion

4.1. Genotypic and seasonal variations of flavanols and caffeine in exotic tea accessions

This is the first comprehensive study report high genetic and seasonal variations of flavan-3-ols and caffeine among exotic tea accessions in Sri Lanka. The newly characterized exotic accessions have high potential of developing as suitable material for producing specialty teas introducing new directions to the Sri Lankan tea industry. Biochemical characterization of tea germplasm using high throughput techniques is very useful in utilization of accessions to improve the existing cultivars. In the present study, a significant influence of genetic variation on individual catechins, caffeine and genetic diversity was observed for all accessions, exotic as well as TRI improved cultivars.

Tea shoots are extremely rich in polyphenolic compounds and flavan-3-ols are the major phenolic constituents of fresh tea leaves. The most important reaction during the manufacturing of black or oolong teas is the conversion of flavan-3-ols into theaflavins (TFs) and thearubigins (TRs) (Stodt & Engelhardt, 2013) which are responsible for the important liquor characteristics such as taste, strength, briskness, astringency and colour (Hara et al., 1995). Various studies reported considerable variations in biochemical constituents in tea leaves due to genotypic, seasonal and geographical variations (Deka et al., 2020; Jayasekera et al., 2014; Kottawa-Arachchi et al., 2013; Lubanga et al., 2021; Punyasiri et al., 2017; Wu et al., 2012). However, this is the first comprehensive study targeting exotic tea germplasm collection in Sri Lanka.

The results of the present study found a significant genotypic variation of flavan-3-ols which is in conformity with previous studies in other tea growing countries (Dai et al., 2015; Deka et al., 2020; Fang et al., 2017). Among individual catechins, ECg showed constant pattern in both seasons indicating less environment influence. Several studies indicated ECg contents in fresh leaves could be a reliable marker for identifying high-quality black tea producing accessions (Yao et al., 2005).

4.2 Health promoting constituents in tea

Today, tea has received considerable attention due to the health benefits of the compounds it contains. Caffeine and polyphenols, the major non-nutrient components in tea, have pharmacological effects. Caffeine is well known for its stimulant properties. Recent investigations showed that anti-oxidative, antimicrobial and other health effects of tea beverages are caused by the interaction between caffeine and polyphenols. Furthermore, Caffeine attributes to intellectual activity sustainment and decrease reaction time, stimulates the heart and cardiovascular activity thus increases the circulatory blood flow rate in many organs in the human body and also increases fatigue force of skeleton muscle contraction thus leads to maintenance of exercise ability (Tazzeo et al., 2012). Caffeine contributes to the creaming properties of black tea. Therefore tea with a low caffeine level is considered to be of inferior quality (Robertson, 1992). All Indian introductions and TRI developed varieties showed relatively higher amount of caffeine (~30 mg g^{-1}) than exotic accessions indicating the suitability for black tea production. The threshold level of caffeine toxicity found to be 400mg/day, meanwhile the recent investigations indicate that above 300mg/day caffeine consumption is associated with lower birth weight (Chin et al., 2008), cancer risk, male infertility, insomnia, calcium imbalance, and etc. and also adult who are highly susceptible to caffeine intake due to genetic variation in enzyme which associated with caffeine metabolism (Kuribara, 2016). Therefore, the demand for decaffeinated or caffeine-less tea is being raised due to its various health benefits and it is higher from Europe, Asia Pacific and North America. Further, caffeine less tea would be a suitable alternative for caffeine sensitive people to acquire medicinal properties of tea. There are several industrial methods available for caffeine-less tea production including CO_2 decaffeination, ethylene acetate decaffeination, water decaffeination, and methylene chloride decaffeination. Several attempts have been made to lower the caffeine content in manufactured tea using genetic engineering (Mohanpuria et al., 2011) methods. However, natural breeding of caffeine-less tea cultivar would be a promising and cost effective way to obtain less caffeine tea with existing quality (Ogino et al., 2019).

The biosynthesis of caffeine is regulated by the expression of tea caffeine synthase (*TCS*) genes (Li et al., 2008). According to the previous studies, caffeine metabolism could be grouped into five categories i.e. *de novo*, purine salvage, caffeine degradation, caffeine synthesis and methyl donor synthesis (Ashihara et al., 2013). Further, different expression patterns of these unigenes are associated between the related gene expression in caffeine metabolism and purine alkaloids content in tea. Zhu et al., (2019) mentioned that the decreased caffeine content in the low-caffeine plants has probably correlated with high expression of related genes in metabolic pathways.

Further, numerous key genes involved in caffeine metabolism have been associated with many transcription factors (TFs), indicating that the caffeine metabolism in tea is complicated. Previous studies observed that the accumulation of secondary compounds in higher concentration has influence caffeine biosynthesis pathways that are induced under abiotic stress conditions (Maritim et al., 2015; Cheptot et al., 2019). Present study revealed that the molecular studies on the effect of caffeine biosynthesis genes under the biotic stress condition for different accessions are paramount. Further, selection of less caffeine accessions with other desirable quality attributes from existing genetic stock have been reported in Japan and China (Nagata & Sakai, 1984; Ogino et al., 2009; Wu et al., 2012). The study facilitated identification of low caffeine exotic accessions *viz* PBGT 10, PBGT 27, PBGT 35, PBGT 71, PBGT 48, PBGT 53 and PBGT 54 which could be useful in developing less caffeine tea cultivars through breeding.

Catechins are biosynthesized by different branches of phenylpropanoid biosynthesic pathway (Punyasiri et al., 2004; Wei et al., 2015). Several studies observed that both accumulation and composition of catechins depend on the expression levels of relative biosynthetic genes. In tea plants under the different conditions, enzymatic catalysis of chalcone isomerase (CsCHI), flavanone 3-hydroxylase (CsF3H), dihydroflavonol 4-reductase (CsDFR), anthocyanidin synthase (CsANS) and anthocyanidin reductase (CsANR), plays a key role in determining gallated and non-gallated catechin compositions ((Punyasiri et al., 2004; Wu et al., 2014). Among catechins reported in tea, epigallocatechin-3-O-(3″-O-methyl)-gallate (EGCG3″Me) and epicatechin-3-O-(3″-O-methyl)-gallate (ECG3″Me) exhibited antiallergic effects specially of histamine release inhibition (Yamamoto et al., 2012). The study further facilitated identification of exotic accessions with high EGCg content. EGCg exhibits anti-cancer effects through a variety of mechanisms (Isemura, 2015), multi anti-diabetic activities and anti-obesity actions through suppressing fat accumulation in humans (Sayama, 2015). Hence, accessions AI-1, AI-4, AI-10 and SI-1 that are characterized with high EGCg content could be immensely useful in future tea breeding programmes to use as parents to develop tea cultivars with enhanced medicinal properties.

4.3. Variations of gallated and non-gallated forms of Flavan-3-ols

It has been reported that the relative amounts of the various flavan-3-ols and their gallated forms are vital for development of new cultivars which could produce high quality black tea. Further, several studies suggested that the EC and ECg can be used as biochemical markers to select cultivars that have the potential to produce high-quality black tea (Sabhapondit et al., 2012; Wright, 2000). In other crops also flavan-3ol levels have been used to distinguish varieties. Lampíř (Lampíř, 2013) showed that +C and EC were the best discriminants and were able to classify all varietal white wines 100% correctly. Therefore, these individuals could be used in the breeding programme to produce cultivars with better black tea quality.

Wei et al., (Wei et al., 2011) suggested that the DTR and total flavan-3-ol content could be used as indicators for superior quality in tea breeding programmes. Based on DTR and GNR ratios, exotic accessions AI-6, AI-8, AI- 9, SI-9 and PBGT100 (Figure 3) could be considered as potential black tea quality accessions and further investigation is needed for confirmation with black tea organoleptic evaluation.

Clustering pattern of heatmap revealed that, it is worthwhile to determine the genetic variations and phylogenetic relationship of exotic collections of Azerbaijan origin with high-throughput molecular marker techniques. With the increasing consumption of green tea and oolong tea, the exotic accessions grouped in clusters A can be incorporated in to the cultivar development programme. It also indicated that the accessions in cluster B are more suitable for black tea production whereas accessions in clusters A are appropriated for the development of green teas and speciality teas with health promoting aspect.

4.4. Implications of biochemical diversity for development of diverse tea cultivars

Previous study on genetic structure of Sri Lankan tea germplasm revealed that it is predominantly represented by Cambod type accessions followed by Assam types (Ranatunga et al., 2017). Further, availability of less number China type accessions has been identified as a major gap and it was emphasized to acquire more China type accessions and develop China hybrids in order to broaden genetic base of the Sri Lankan tea germplasm. Exotic germplasm collection comprises with introduced seed stocks from many countries may have the potential for widen the genetic base. We report the first comprehensive study on characterization of exotic tea germplasm accessions for their variability in catechin and caffeine content. Interestingly, 67 exotic accessions which are having taxonomic affinity towards China type show discrete levels of caffeine and EGCg two important metabolites in tea. Therefore, these accessions could be immensely useful in future hybridization programmes as potential parents for generating diverse progenies. These progenies will be the foundation for developing future tea cultivars with genetic diversity.

Currently, tea breeding programme in Sri Lanka focus on developing cultivars suitable for producing specialty teas *ie*. low caffeine, high EGCg etc with enhanced medicinal properties. Hence, exotic accessions identified in the study could utilize in breeding programmes to develop such cultivars.

As pointed out in previous studies, recurrent use of the same parents in tea breeding in Sri Lanka contributed to narrow genetic diversity in the cultivated gene pool (Gunasekare, 2012). Hence, exotic collection is a valuable resource for tea breeding programmes as a new source of variation.

5. Conclusion

Genotypic variations of green leaf flavan-3-ol and caffeine contents were detected in the selected exotic tea accessions with different origins in the field gene bank of Sri Lanka. In addition, the study confirmed the seasonal fluctuation of biochemical compounds in tea accessions. Most of the accessions which are resembled to Assam types cluster together with TRI developed cultivars indicating the close ancestral relationships between those two groups. The results indicated that further investigation of black tea quality potential of accessions belongs to Indian origins would be much useful for finding high quality tea cultivars. Several exotic accessions which are resembled to China types could be useful to identify less caffeine tea. Findings of the study also elucidate the possibility of using green leaf flavan-3-ols and caffeine contents to categorize accessions into the tea taxa, mainly Assam and China, to a greater extent. Information generated in this study would help to identify diverse exotic accessions as parents for breeding new cultivars with enhanced medicinal properties to cater to ever-changing demand of the consumer in the future.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

The authors thank the directorate of the Tea Research Institute of Sri Lanka for giving permission to carry out this study.

Author contributions

JDK: contributed to the study design, sampling, wet lab experiments, data generation and writing manuscript. MABR and RNA: conceptualization of the idea, acquisition of research funds, data curation, experimental design, statistical analysis, and manuscript wiring and editing. AMTA, MTKG and RKS: conceptualization of the idea, acquisition of research funds, data curation, experimental design and manuscript editing. NPP and ENUE: contributed to the wet lab experiments, biochemical data generation and interpretation. HK Chaudhary, VK. Sood, R. Katoch and DK. Banyal: contributed to supervision of the research, manuscript editing and proofreading.

Funding

The postgraduate programme of the first author and the research were funded by the Sri Lanka Council for Agricultural Research Policy (SLCARP) and Ministry of Science, Technology and Research under Indo-Sri Lanka Joint Research Programme, respectively.

Abbreviations:

HPLC, High Performance Liquid Chromatography; GC–MS, gas chromatography–mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; +C, catechin; EC, epicatechin; ECg, epicatechin gallate, EGC, epigallocatechin; EGCg, epigallocatechin gallate; TC, total catechins; TFs, theaflavins; TRs, thearubigins.

References

Ashihara, H., Yokota, T., & Crozier, A. (2013). Biosynthesis and catabolism of purine alkaloids. *Advances in Botanical Research*, *68*, 111-138.

Ashihara, H., Deng, W. W., Mullen, W., & Crozier, A. (2010). Distribution and biosynthesis of flavan–3–ols in *Camellia sinensis* seedlings and expression of genes encoding biosynthetic enzymes. *Phytochemistry*, *71*, 559–566.

Balantine, D. A., Harbowy, M. E., & Graham, H. N. (1998). Tea: the plant and its manufacture, chemistry and consumption of the beverage. In G. A. Spiller (Ed.), *Caffeine* (pp. 35–72). CRC Press: Boca Raton, New York.

Banerjee, B. (1992). Botanical classification of tea. In K. C. Wilson & M. N. Clifford (Eds.), *Tea cultivation to consumption* (pp. 555–601). Chapman & Hall publication, London.

Berkowitz, J. E., Coggon, P., & Sanderson, G. W. (1971). Formation of epitheaflavic acid and its transformation to thearubigins during fermentation. *Phytochemistry*, *10*, 2271–2278.

Cevallos-cevallos, J. M., Corcuera, J. I. R., Etxeberria, E., Danyluk, M. D., & Rodrick, G. E. (2009). Metabolomic analysis in food science : a review. *Trends in Food Science & Technology*, *20*(11–12), 557–566. https://doi.org/10.1016/j.tifs.2009.07.002

Chen, L., & Zhou, Z. X. (2005). Variations of main quality components of tea genetic resources [*Camellia sinensis* (L.) O. Kuntze] preserved in the China national germplasm tea repository. *Plant Food for Human Nutrition*, *60*, 31–35.

Cheptot, L., Maritim, T., Korir, R., Kipsura, E., Samson, K., Matasyoh, L., & Muoki, R. (2019). Seasonal variations in catechins and caffeine profiles among tea cultivars grown in Kenya. *International Journal of Tea Science*, *14*, 56-61

Chin, J. M., Merves, M. L., Goldberger, B. A., Sampson-Cone, A., & Cone, E. J. (2008).

Caffeine Content of Brewed Teas. *Journal of Analytical Toxicology*, *32*(8), 702–704. https://doi.org/10.1093/jat/32.8.702

Crozier, A., Ashihara, H., & Tomás-Barbéran, F. (Eds.). (2011). *Teas, Cocoa and Coffee*. Wiley-Blackwell. https://doi.org/10.1002/9781444347098

Dai, W., Qi, D., Yang, T., Lv, H., Guo, L., Zhang, Y., Zhu, Y., Peng, Q., Xie, D., Tan, J., & Lin, Z. (2015). Nontargeted analysis using Ultraperformance Liquid Chromatography–Quadrupole time-of-flight mass spectrometry uncovers the effects of harvest season on the metabolites and taste quality of tea (*Camellia sinensis* L.). *Journal of Agricultural and Food Chemistry*, *63*(44), 9869–9878. https://doi.org/10.1021/acs.jafc.5b03967

Deka, H., Barman, T., Dutta, J., Devi, A., Tamuly, P., Paul, R. K., & Karak, T. (2020). Catechin and caffeine content of tea (*Camellia sinensis* L.) leaf significantly differ with seasonal variation: A study on popular cultivars in North East India. *Journal of Food Composition and Analysis*, *96*, 103684. https://doi.org/10.1016/j.jfca.2020.103684

Engelhardt, U. H. (2010). Chemistry of Tea. In L. Mender & H. W. Liu (Eds.), *Comprehensive Natural Products II: Chemistry and Biology* (pp. 999–1032). Elsevier, United Kingdom.

Fang, R., Redfern, S. P., Kirkup, D., Porter, E. A., Kite, G. C., Terry, L. A., Berry, M. J., & Simmonds, M. S. J. (2017). Variation of theanine, phenolic, and methylxanthine compounds in 21 cultivars of *Camellia sinensis* harvested in different seasons. *Food Chemistry*, *220*, 517–526. https://doi.org/10.1016/j.foodchem.2016.09.047

Gunasekare, M. T. K. (2012). Tea Plant (*Camellia sinensis*) breeding in Sri Lanka. In L. Chen & Z. Apostolides (Eds.), *Global Tea Breeding–Achivements, Challenges and Perspectives* (pp. 125–176). Springer-Verlag Berlin Heidelberg/Zhejiang press.

Hara, Y., Luo, S. J., Wickremasinghe, R. L., & Yamanishi, T. (1995). Special issue on tea. *Food Review International*, *11*(3), 371–545.

Isemura, M. (2015). Anti cancer effects - Basic and human studies. In *Scientific evidences for the health benefits of green tea* (pp. 8–13). Japan Tea Central Public interest Incorporated Association.

ISO-1573. (1980). *Tea. Determination of loss in mass at 103°C*.

Jayasekera, S., Kaur, L., Molan, A., Garg, M. L., & Moughan, P. J. (2014). Effects of season and plantation on phenolic content of unfermented and fermented Sri Lankan tea. *Food Chemistry*, *152*, 546–551.

Jin, J. Q., Ma, J. Q., Ma, C. L., Yao, M. Z., & Chen, L. (2014). Determination of catechin content in representative Chinese tea germplasms. *Journal of Agricultural and Food*

Chemistry, *62*, 9436-9441.

Kottawa-Arachchi, J. D., Gunasekare, M. T. K., Ranatunga, M. A. B., Punyasiri, P. A. N., & Jayasinghe, L. (2013). Use of biochemical compounds in tea germplasm characterization and its implications in tea breeding in Sri Lanka. *Journal of the National Science Foundation of Sri Lanka*, *41*(4), 309–318. https://doi.org/10.4038/jnsfsr.v41i4.6252

Kuribara, H. (2016). Caffeine intake in the daily life: mechanism of action and safety assessment. *Bulletin of Tokyo University and Graduate School of Social Welfare*, *6*, 109–125.

Lampíř, L. (2013). Varietal differentiation of white wines on the basis of phenolic compounds profile. *Czech Journal of Food Sciences*, *31*(2), 172–179. https://doi.org/10.17221/270/2012-CJFS

Li, Y., Ogita, S., Keya, C. A., & Ashihara, H. (2008). Expression of caffeine biosynthesis genes in tea (*Camellia sinensis*). *Zeitschrift Für Naturforschung. 63* (3-4), 267.

Liang, Z., Owens, C. L., Zhong, G., & Cheng, L. (2011). Polyphenolic profiles detected in the ripe berries of Vitis vinifera germplasm. *Food Chemistry*, *129*(3), 940–950. https://doi.org/10.1016/j.foodchem.2011.05.050

Lubanga, N., Massawe, F., & Mayes, S. (2021). Genomic and pedigree-based predictive ability for quality traits in tea (Camellia sinensis (L.) O. Kuntze). *Euphytica*, *217*, 32–47.

Maritim, T.K., Kamunya, S.M., Mireji, P., Mwendia, C., Muoki, R.C., Cheruiyot, E.K. & Wachira, F.N. (2015). Physiological and biochemical responses of tea (*Camellia sinensis* L. O Kuntze) to water-deficit stress. *Journal of Horticultural Science & Biotechnology 90*, 395–400

Mohanpuria, P., Kumar, V., Ahuja, P. S., & Yadav, S. K. (2011). Producing low-caffeine tea through post–transcriptional silencing of caffeine synthase mRNA. *Plant Molecular Biology*, *76*, 3–34.

Muccillo, L., Gambuti, A., Frusciante, L., Iorizzo, M., Moio, L., Raieta, K., Rinaldi, A., Colantuoni, V., & Aversano, R. (2014). Biochemical features of native red wines and genetic diversity of the corresponding grape varieties from Campania region. *Food Chemistry*, *143*, 506–513. https://doi.org/10.1016/j.foodchem.2013.07.133

Muthiani, M. A., Wanyoko, J. K., Wachira, F. N., Kamunya, S. M., Chalo, R. M., Kimutai, S., Khalid, R., & Karori, S. (2016). Potential use of Kenyan tea cultivars in development of high value diversified products potential use of Kenyan tea cultivars in development of high value diversified products. *International Journal of Tea Science*, *12*(November), 30–48. https://doi.org/10.20425/ijts.v0iof.9599

Nagata, T., & Sakai, S. (1984). Differences in caffeine, flavonols and amino acids contents in leaves of cultivated species of Camellia. *Japan Journal of Breeding*, *34*, 459–467.

Nurminen, M. L., Niittynen, L., Korpela, R., & Vapaatalo, H. (1999). Coffee, caffeine and blood pressure: a critical review. *European Journal of Clinical Nutrition*, *53*(11), 831–839. https://doi.org/10.1038/sj.ejcn.1600899

Ogino, A., Tanaka, J., Taniguchi, F., Yammamoto, M. P., & Yamada, K. (2009). Detection and characterization of caffeine–less tea plants originated from interspecific hybridization. *Breeding Science*, *59*, 277–283.

Ogino, A., Taniguchi, F., Yoshida, K., Matsumoto, S., Fukuoka, H., & Nesumi, A. (2019). A new DNA marker CafLess-TCS1 for selection of caffeine-less tea plants. *Breeding Science*, *69*, 393–400. https://doi.org/10.1270/jsbbs.18161

Price, E. J., Drapal, M., Perez-fons, L., Amah, D., Bhattacharjee, R., Heider, B., Rouard, M., Swennen, R., Lopez-Lavalle, L. A. B., & Fraser, P. D. (2020). Metabolite database for root , tuber, and banana crops to facilitate modern breeding in understudied crops. *The Plant Journal*, *101*, 1258–1268. https://doi.org/10.1111/tpj.14649

Punyasiri, P. A. N., Abeysinghe, I. S. B., Kumar, V., Treutter, D., Duy, D., Gosch, C., Martens, S., Forkmann, G. & Fischer, T. C. (2004). Flavonoid biosynthesis in the tea plant Camellia sinensis: properties of enzymes of the prominent epicatechin and catechin pathways. Archives of Biocheistry and Biophysics. 431, 22–30. doi: 10.1016/j.abb.2004.08.003

Punyasiri, P. A. N., Jeganathan, B., Kottawa-Arachchi, J. D., Ranatunga, M. A. B., Abeysinghe, I. S. B., Gunasekare, M. T. K., & Bandara, B. M. R. (2015). New sample preparation method for quantification of phenolic compounds of Tea (*Camellia sinensis* L. Kuntze): A polyphenol rich plant. *Journal of Analytical Methods in Chemistry*, *2015*(Article ID 964341). https://doi.org/10.1155/2015/964341

Punyasiri, P. A. N., Jeganathan, B., Kottawa-Arachchi, J. D., Ranatunga, M. A. B., Abeysinghe, I. S. B., Kumudini, M. T., & Bandara, B. M. R. (2017). Genotypic variation in biochemical compounds of the Sri Lankan tea (*Camellia sinensi*s L .) accessions and their relationships to quality and biotic stresses. *The Journal of Horticultural Science and Biotechnology*, *92*(5), 502–512. https://doi.org/10.1080/14620316.2017.1289070

Raina, S. N., Ahuja, P. S., Sharma, R. K., Das, S. C., Bhardwaj, P., Negi, R., Sharma, V., Singh, S. S., Sud, R. K., Kalia, R. K., Pandey, V., Banik, J., Razdan, V., Sehgal, D., Dar, T. H., Kumar, A., Bali, S., Bhat, V., Sharma, S., … Mandi, S. S. (2012). Genetic structure and diversity of India hybrid tea. *Genetic Resources and Crop Evolution*, *59*(7), 1527–1541. https://doi.org/10.1007/s10722-011-9782-6

Ranatunga, M. A. B., Kottawa-Arachchi, J. D., Gunasekare, M. T. K., Yakandawala, D., Punyasiri, P. A. N., Jeganathan, B., Abeysinghe, I. S. B., & Bandara, B. M. R. (2015). Alkaloids and flavonols as chemotaxonomic markers: Potential applications in Camellia sinensis (Tea) breeding. *Postgraduate Institute of Science Research Congress*.

Ranatunga, M. A. B., Kottawa-Arachchi, J. D., Gunasekere, M. T. K., & Yakandawala, D. M. D. (2017). Floral diversity and genetic structure of tea germplasm of Sri Lanka. *International Journal of Biodiversity*, *2017*(Article ID 2957297), 1–11.

Robertson, A. (1992). The chemistry and biochemistry of black tea production - the non-volatiles. In K. C. Willson & M. N. Clifford (Eds.), *Tea: Cultivation to consumption* (pp. 555–601). Chapman & Hall publication.

Sabhapondit, S., Karak, T., Bhuyan, L. P., Goswami, B. C., & Hazarika, M. (2012). Diversity of catechins in Notheast Indian tea cultivars. *The Scientific World Journal*, *2012*(Article ID 485193), 1–8.

Sayama, K. (2015). Preventive effects of obesity by green tea and its components. In *Scientific evidences for the health benefits of green tea* (pp. 68–76). Japan Tea Central Public interest Incorporated Association.

Sharangi, A. B. (2009). Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L .) – A review. *Food Research International*, *42*(5–6), 529–535. https://doi.org/10.1016/j.foodres.2009.01.007

Stodt, U., & Engelhardt, U. H. (2013). Progress in the analysis of selected tea constituents over the past 20years. *Food Research International*, *53*(2), 636–648. https://doi.org/10.1016/j.foodres.2012.12.052

Tazzeo, T., Bates, G., Roman, H. N., Lauzon, A. M., Khasnis, M. D., Eto, M., & Janssen, L. J. (2012). Caffeine relaxes smooth muscle through actin depolymerization. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *303*(4), L334–L342.

Venter, A., Joubert, E., & Beer, D. D. (2013). Characterisation of phenolic compounds in South African plum fruits (*Prunus salicina* Lindl.) using HPLC coupled with diode-array, fluorescence, mass spectrometry and On-Line antioxidant detection. *Molecules*, *18*, 5072–5090. https://doi.org/10.3390/molecules18055072

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 Chemistry*, *125*, 44–48.

Wei, K., Wang, L., Zhang, C., Wu, L., Li, H., Zhang, F., & Cheng, H. (2015). Transcriptome analysis reveals key flavonoid 3'-hydroxylase and flavonoid 3',5'-hydroxylase genes in affecting the ratio of dihydroxylated to trihydroxylated catechins in *Camellia sinensis*. *PLoS ONE* 10:e0137925. doi: 10.1371/journal.pone.0137925

Wright, L. P., Mphangwe, N. I. K., Nyirenda, H. E., & Apostolides, Z. (2000). Analysis of caffeine and flavan-3-ol composition in the fresh leaf of *Camellia sinensis* for predicting the quality of the black tea produced in Central and Southern Africa. *Journal of the Science of Food and Agriculture*, *80*, 1823–1830.

Wu, C., Xu, H., Héritier, J., & Andlauer, W. (2012). Determination of catechins and flavonol glycosides in Chinese tea varieties. *Food Chemistry*, *132*, 144–149. https://doi.org/10.1016/j.foodchem.2011.10.045

Wu, Z. J., Li, X. H., Liu, Z. W., Xu, Z. S., & Zhuang, J. (2014). De novo assembly and transcriptome characterization: novel insights into catechins biosynthesis in *Camellia sinensis*. *BMC Plant Biology*. *14*:277. doi: 10.1186/s12870-014-0277-4

Xu, W., Song, Q., Li, D., & Wan, X. (2012). Discrimination of the production season of Chinese green tea by chemical analysis in combination with supervised pattern recognition. *Journal of Agricultural and Food Chemistry*, *60*, 7064-7070. https://doi.org/https://doi.org/10.1021/jf301340z

Yamamoto, M. M., Ema, K., Monobe, M., Tokuda, Y., & Tachibana, H. (2012). Epicatechin-3-*O*-(3″-*O*-methyl)-gallate content in various tea cultivars (*Camellia sinensis* L.) and its *in vitro* inhibitory effect on histamine release. *Journal of Agricultural and Food Chemistry, 60 (9)*, 2165-2170

Yao, L., Caffin, N., D'Arcy, B., Jiang, Y., Shi, J., Singanusong, R., Liu, X., Datta, N., Kakuda, Y., & Xu, Y. (2005). Seasonal variations of phenolic compounds in Australia-grown tea (Camellia sinensis). *Journal of Agricultural and Food Chemistry*, *53*, 6477–6483.

Zhu, B., Chen, L. B., Lu, M., Zhang, J., Han, J., Deng, W. W., & Zhang, Z. Z. (2019). Caffeine content and related gene expression: novel insight into caffeine metabolism in *Camellia* plants containing low, normal and high caffeine concentrations. *Journal of Agricultural and Food Chemistry*. 67(12), 3400–3411

Figure Captions

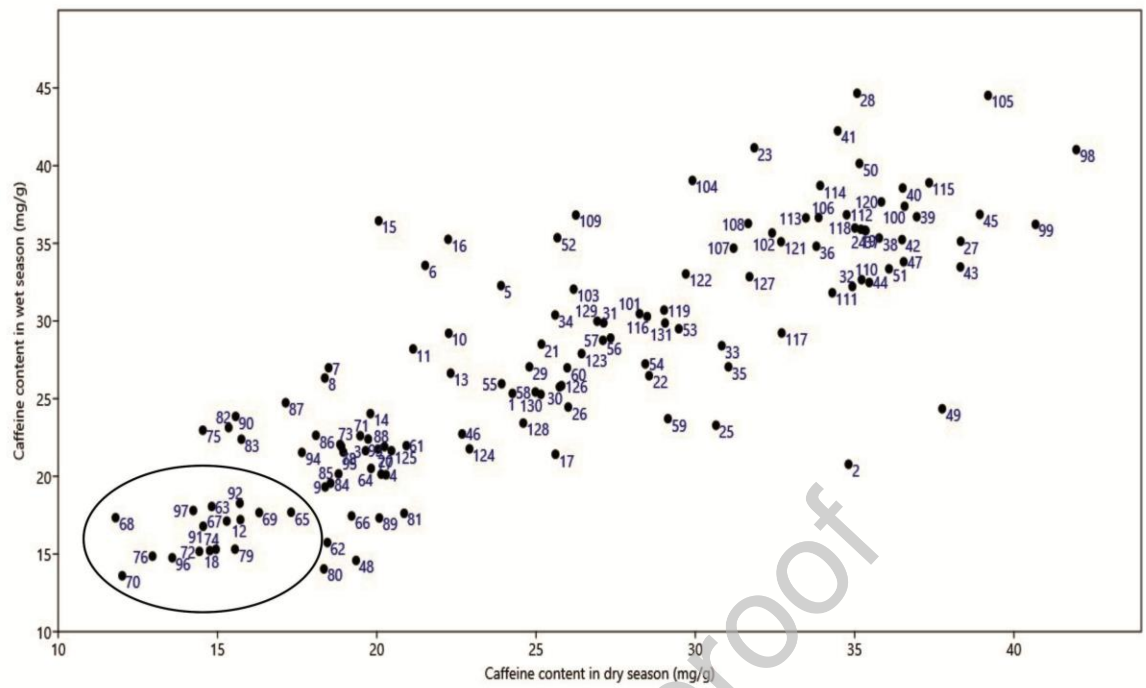
Figure 1. (A) HPLC chromatogram of a standard solution containing caffeine, + C, EC, EGC, EGCg, ECg, and gallic acid. (HPLC peaks: 1 – gallic acid, 2 – epigallocatechin, 3 – catechin, 4 – caffeine, 5 – epicatechin, 6 – epigallocatechin gallate, 7 – epicatechin gallate).
(B) HPLC chromatogram of an extract of a representative tea sample. (HPLC peaks: 1 – gallic acid, 2 – epigallocatechin, 3 – catechin, 4 – caffeine, 5 – epicatechin, 6 – epigallocatechin gallate, 7 – epicatechin gallate)

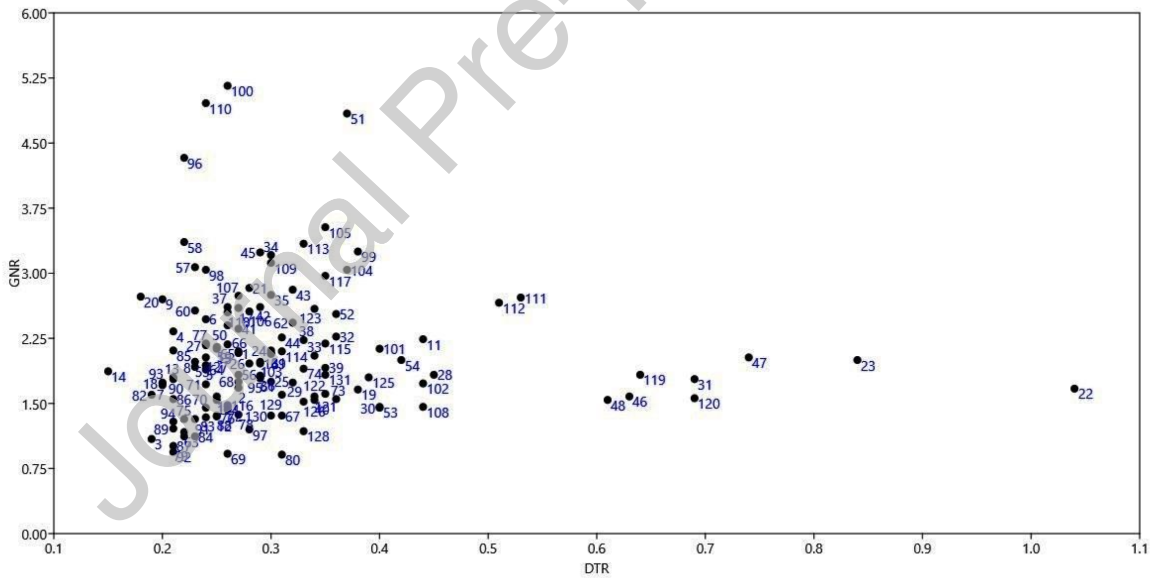
Figure 2: The biplot of 131 tea germplasm accessions showing the relationship of caffeine contents between dry and wet seasons.

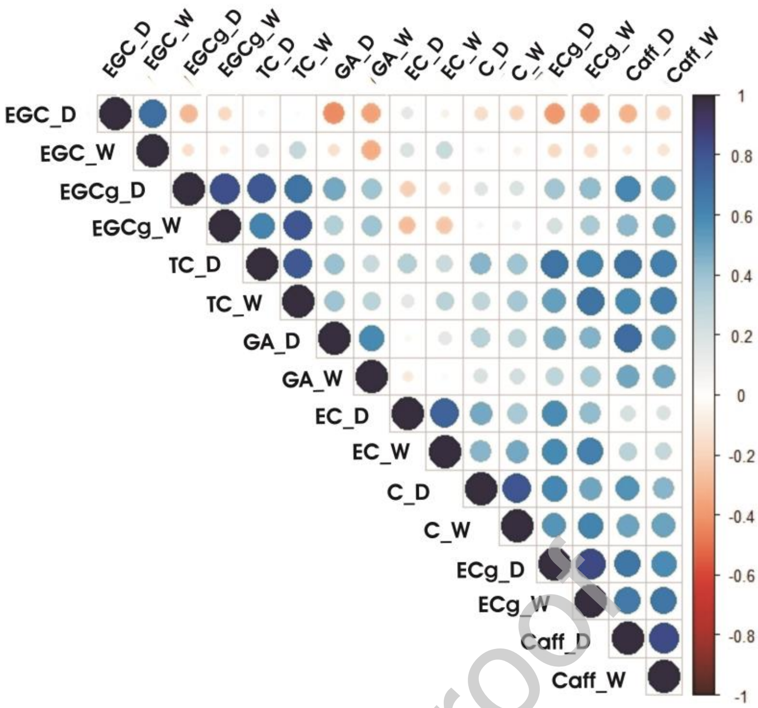
Figure 3: The biplot showing relationship between DTR (ratio of dihydroxycatechins to trihydroxycatechins) and GNR (ratio of gallated to non-gallatedcatechins) of 131 tea accessions

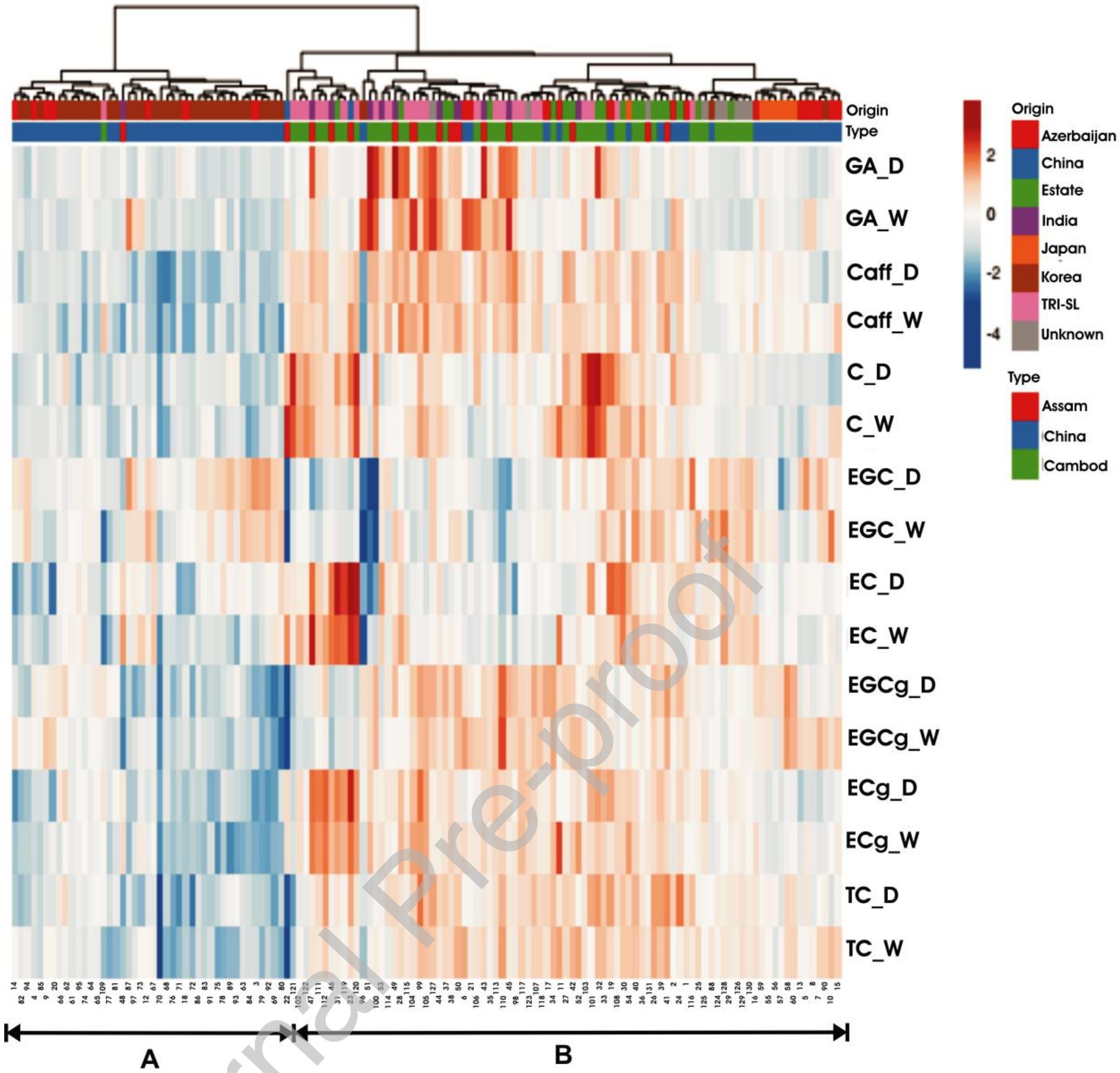
Figure 4: Correlation matrix between individual catechins and caffeine content in both seasons. (W: wet season, D: dry season) intensity of colour is representative of the significance of the correlation (darker the higher significance). Blue colour indicates positive and red colour indicates negative correlations.

Figure 5: The heatmap generated using Euclidean distance and Ward's linkage clustering method. Red and blue colour patterns indicate positive and negative contributions of individual catechins and caffeine for clustering of 131 tea accessions, respectively. (W: wet season, D: dry season)


Caffeine content in wet season (mg/g)
Caffeine content in dry season (mg/g)
10
15
20
25
30
35
40
45


GNR
DTR
0.00
0.75
1.50
2.25
3.00
3.75
4.50
5.25
6.00
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1


EGC_D
EGC_W
EGCg_D
EGCg_W
TC_D
TC_W
GA_D
GA_W
EC_D
EC_W
C_D
C_W
ECg_D
ECg_W
Caff_D
Caff_W
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1


Origin
Type
GA_D
GA_W
Caff_D
Caff_W
C_D
C_W
EGC_D
EGC_W
EC_D
EC_W
EGCg_D
EGCg_W
ECg_D
ECg_W
TC_D
TC_W
2
0
-2
-4
Origin
Azerbaijan
China
Estate
India
Japan
Korea
TRI-SL
Unknown
Type
Assam
China
Cambod
A
B

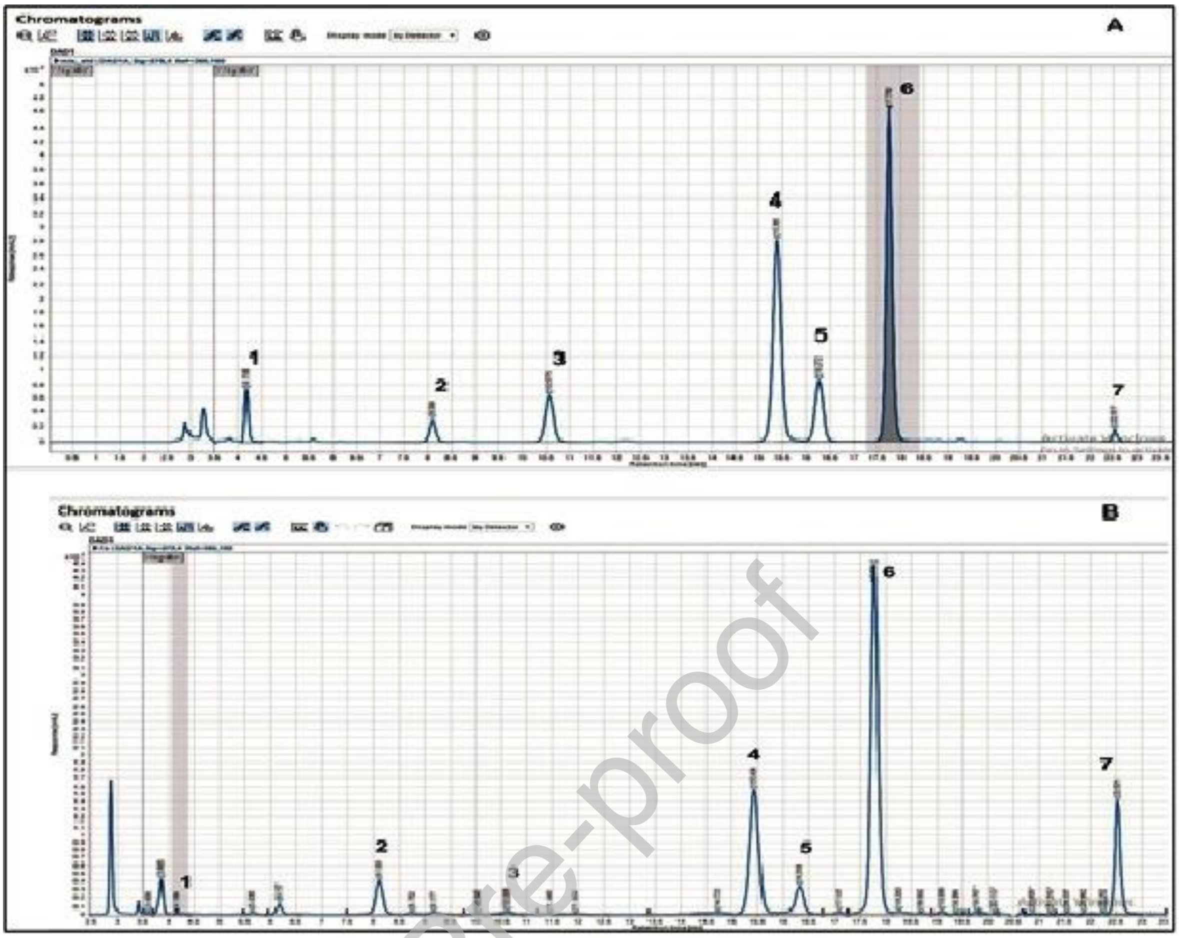
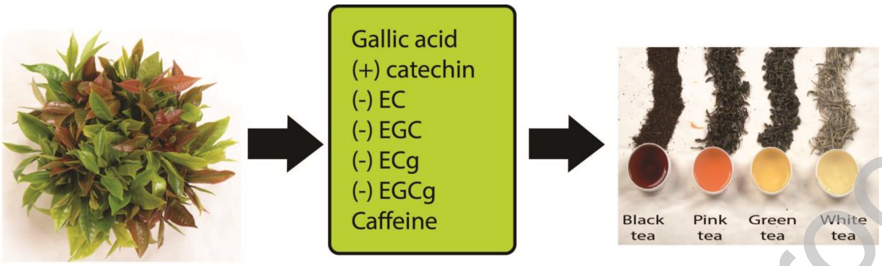

Chromatograms
A
1
2
3
4
5
6
7
Chromatograms
B
1
2
3
4
5
6
7

Table 1: Summary statistics of selected biochemical constituents in Assam/Cambod types and China types accessions tested in exotic germplasm

	China types (n=67)					Assam types (n=15)					Cambod types (n=49)				
	Min (mg g^{-1})	Max (mg g^{-1})	Mean* (mg g^{-1})	SD	CV%	Min (mg g^{-1})	Max (mg g^{-1})	Mean* (mg g^{-1})	SD	CV%	Min (mg g^{-1})	Max (mg g^{-1})	Mean* (mg g^{-1})	SD	CV%
Dry season															
GA	0.12	0.38	0.20^{c}	0.05	26.55	0.22	0.85	0.40^{a}	0.18	45.85	0.16	0.68	0.34^{b}	0.14	40.47
EGC	19.24	57.32	41.03^{a}	8.55	20.85	17.84	44.83	31.02^{b}	7.40	23.86	14.19	61.48	37.21^{a}	9.76	26.24
C	0.87	4.14	1.71^{b}	0.56	32.93	1.69	3.95	2.47^{a}	0.60	24.45	1.33	5.29	2.70^{a}	0.99	36.53
Caff	11.80	36.95	20.18^{b}	5.56	27.56	19.35	38.93	32.68^{a}	5.79	17.71	20.46	41.96	31.26^{a}	4.96	15.86
EC	7.50	24.72	13.85^{b}	2.93	21.16	11.56	33.49	17.21^{a}	5.78	33.58	7.53	30.21	16.02^{a}	4.88	30.47
EGCg	42.45	113.11	72.14^{b}	16.51	22.88	36.49	108.59	82.11^{a}	22.44	27.33	52.79	122.82	84.06^{a}	15.38	18.30
ECg	8.52	35.78	16.26^{b}	5.69	34.99	23.63	58.47	29.77^{a}	9.45	31.75	13.36	45.36	27.45^{a}	7.15	26.05
TC	98.07	201.18	144.98^{b}	20.48	14.13	100.93	197.33	162.57^{a}	25.15	15.47	114.81	203.54	167.44^{a}	16.66	9.95
Wet season															
GA	0.16	0.56	0.28^{b}	0.10	34.47	0.18	0.62	0.38^{a}	0.13	34.56	0.19	0.62	0.33^{a}	0.11	33.31
EGC	14.05	59.74	38.38^{a}	8.39	21.86	11.14	48.39	34.91^{a}	9.05	25.93	16.79	59.67	37.98^{a}	9.95	26.19
C	0.76	4.04	1.63^{b}	0.59	36.03	1.41	4.99	2.60^{a}	0.88	33.77	1.27	4.85	2.56^{a}	0.91	35.52
Caff	13.60	36.71	22.59^{b}	5.95	26.32	14.59	42.24	32.52^{a}	7.72	23.73	21.65	44.66	32.53^{a}	5.50	16.91
EC	5.76	23.60	12.40^{c}	3.13	25.26	12.72	30.52	18.18^{a}	5.89	32.41	6.62	27.40	15.66^{b}	4.47	28.54
EGCg	44.03	120.38	81.25^{a}	17.97	22.12	22.76	114.00	83.56^{a}	26.14	31.29	52.79	122.82	84.06^{a}	15.38	18.30
ECg	8.11	51.94	16.60^{b}	7.33	44.14	21.02	44.09	29.57^{a}	6.80	23.00	14.59	43.14	27.12^{a}	6.19	22.82
TC	89.97	201.82	150.26^{b}	24.99	16.63	86.98	198.98	168.82^{a}	29.06	17.21	122.06	204.78	173.70^{a}	17.55	10.10

* Mean values among China, Assam and Cambod types followed by different letters are significantly different at p =0.05 according to t test. SD: standard deviation of population mean; CV%: coefficient of variance of population


Gallic acid
(+) catechin
(-) EC
(-) EGC
(-) ECg
(-) EGCg
Caffeine
Black tea
Pink tea
Green tea
White tea