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Original Research Article

# Catechin and caffeine content of tea (*Camellia sinensis* L.) leaf significantly differ with seasonal variation: A study on popular cultivars in North East India

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ABSTRACT

Two thirds of the tea growing area of North East India is covered by Tocklai vegetative (TV) cultivars. The present study investigates the principal catechins [(−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), and (+)-catechin (+C)], total catechin (TC) and caffeine content in thirty-one TV cultivars (TV1-TV31) and four popular cultivars, viz. Betjan, Kharijan, S.3A/3, and T.3E/3 in three harvesting seasons i.e., pre-monsoon, monsoon and autumn, along with their genetic diversity. The monsoon harvested crop showed the highest average content of TC, EGCG, ECG, and caffeine. The TC levels varied from 129 ± 0.03 to 214 ± 4.72, 151 ± 4.21 to 238 ± 8.41, and 121 ± 4.45 to 181 ± 5.82 mg $g^{-1}$ in pre-monsoon, monsoon and autumn, respectively. The most predominant catechin EGCG varied from 52.2 ± 1.07 to 111 ± 1.24, 70.4 ± 1.03 to 141 ± 1.35, and 58.4 ± 2.47 to 108 ± 2.15 mg $g^{-1}$in pre-monsoon, monsoon and autumn, respectively. Caffeine content varied from 27.1 ± 0.32 to 48.7 ± 0.50, 35.3 ± 1.56 to 55.0 ± 1.34 and 27.6 ± 1.11 to 40.7 ± 0.42 mg $g^{-1}$ in pre-monsoon, monsoon and autumn, respectively. TC contents in cultivars TV23, TV17 and TV15; and EGCG contents in TV10, TV11, and TV9 were significantly higher ($p \leq 0.05$) than for other cultivars. Environmental factors, viz. day length, sunlight, temperature across seasons might have induced seasonal variation in phenolic composition.

## 1. Introduction

Tea (*Camellia sinensis* L.), the second most consumed beverage in the world, is a rich source of polyphenolic compounds. Tea is processed from the tender shoots of the *Camellia sinensis* (L.) family *Theaceae*, a perennial crop that grows in a warm and humid climate with sufficient rainfall in acidic soil (Carloni et al., 2013; Karak et al., 2014). Polyphenols in tea comprising flavonoids, flavan-3-ols, flavandiols, and phenolic acids constitute up to 30% of dry weight (Massounga Bora et al., 2018). The flavan-3-ols (also known as catechins) are the major phenolics in tea leaves which constitute 70-80% of total content (Zheng et al., 2018). The major catechins include (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), and (+)-catechin (C) (Jin et al., 2014; Sabhapondit et al., 2012).

Tea has gained popularity among the consumers due to its taste,

*Abbreviations:* ACH, Assam China hybrid; AH, Assam hybrid; ANOVA, Analysis of variance; C, Catechin; CH, China hybrid; CI, Catechin index; DHC, Dihydroxy catechin; EC, (-), Epicatechin; ECG, (-), Epicatechin gallate; EGC, (-), Epigallocatechin; EGCG, (-), Epigallocatechin gallate; GaC, Galloylated catechin; GNR, Galloylated to non-galloylated catechin ratio; LOD, Limit of detection; LOQ, Limit of quantification; NGaC, Non-galloylated catechin; SE, Standard error; TC, Total catechin; TE, Tea Estate; TF, Theaflavin; THC, Trihydroxy catechin; TR, Thearubigin; TTRI, Tocklai Tea Research Institute; TV, Tocklai vegetative; UPASI, United Planters' Association of Southern India.

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flavour, aroma and health beneficial effects. Tea consumption can be directly related to a reduced risk of developing life-threatening diseases such as cardiovascular diseases, type 2 diabetes, cancer, obesity, inflammation (Jiang et al., 2019; Karak and Bhagat, 2010; Liu et al., 2020; Massounga Bora et al., 2018; Rho et al., 2019) and increased neuroprotective activities (Rho et al., 2019). The numerous health benefits of tea have been attributed to the potent antioxidant activity of the flavonoids present in it. In a recent study, Bora et al. (2019) reported that catechins form complexes with aluminium (Al) present in tea leaf, thus reducing the risk of Al toxicity.

Tocklai Tea Research Institute (TTRI), Assam, India has released more than 150 cultivars for plantation in the plains of North East (NE) India (Sabhapondit et al., 2012). These varieties are occupying over 70% of the total tea growing areas of NE India plains. Genetic diversity and the environment of the tea growing region play a key role in dictating the regional variation of quality (Sabhapondit et al., 2012). Large scale cultivation of clonal tea in search of high yield and better quality may pose a threat to genetic diversity (Magoma et al., 2000). This necessitates the conservation of germplasm for the sustainability of the tea industry. Biochemical characterisation with emphasis on metabolite profile and assessment of genetic diversity is extremely important for sustainable development of the tea industry as its sensible application can lead to successful breeding and manufacturing of quality products with desired attributes (Punyasiri et al., 2017). Biochemical diversity of the germplasm can be ascertained by biochemical characterization (Kottawa-Arachchi et al., 2019).

The catechin content can be used as a scale to ascertain the quality potential of tea (Kottawa-Arachchi et al., 2014; Sabhapondit et al., 2012). An insight into the catechin profile of different cultivars may prove handy in having information on plant diversity as well as understanding their role as a precursor of quality as the catechin profile significantly influences the formation of theaflavins (TFs) and thearubigins (TRs), the two important characteristic quality attributes of black tea (Sabhapondit et al., 2012). The distinct sensory characteristics such as taste and colour of black tea can be attributed to TF and TR content. Seasonal, genetic and agronomic factors affect the phenolic composition of the tea shoots in the field (Hilton and Palmer-Jones, 1973; Tan et al., 2017; Zheng et al., 2018). Gulati et al. (2009) studied the catechin profile of representative cultivars of Indian tea germplasm including some Tocklai cultivars. Sabhapondit et al. (2012) reported the variation as well as relative expression of catechin profile of extreme varieties of Assam, China and Cambod cultivars grown in NE India. In their study, only selected 13 Tocklai vegetative (TV) cultivars were considered among 31 TV cultivars. Furthermore, the caffeine content of the cultivars was not reported in their study. There is little even no information is available on all the Tocklai cultivars coupled with highly popular commercial genotypes for biochemical characterization. Jayasekera et al. (2014) reported that total polyphenols, as well as related constituent compounds in Sri Lankan tea, are significantly affected by climatic variability. However, there is very little information available on that aspect in the Indian scenario. Furthermore, to our knowledge, the effect of seasonal variability towards biochemical properties of all the vegetative propagated cultivars of Tocklai is very limited. Therefore, the present investigation aimed to study the catechin and caffeine content of all 31 TV cultivars (TV1 to TV31) and four popular cultivars (Betjan, Kharijan, S.3A/3, and T.3E/3) of NE India to understand their seasonal and genetic variation in these biochemical properties.

## 2. Materials and methods

### 2.1. Chemicals

Gallic acid monohydrate (≥98.0%), caffeine (anhydrous, 99%), (−)-epigallocatechin-3-gallate (≥95%), (−)-epicatechin-3-gallate (≥95%, HPLC), (−)-epigallocatechin (≥95%, HPLC), (−)-epicatechin (≥90%, HPLC), and (+)-catechin (≥95%, HPLC) were procured from Sigma-Aldrich, India. Acetic acid (HPLC grade), acetonitrile (HPLC grade), ethylenediaminetetraacetic acid disodium salt, L-ascorbic acid and all other chemicals were obtained from Merck KGaA, Darmstadt, Germany.

### 2.2. Tea samples

The tender leaf samples were collected from Borbheta Experimental Tea Estate (T.E.) of TTRI, Assam, India (longitude: 94°11'54"E, latitude: 26°43'14"N, elevation: 96.5 meters above mean sea level, average precipitation: 2036 mm, Supplementary material Fig. S1). The origin and year of release of different cultivars are depicted in Table S1 (Supplementary material). All the cultivars were grown under identical environmental conditions and nutrient management of growing soil. The plants of all the cultivars under study were chosen in the age range from 30 to 35 years. The tender two leaves and the terminal bud were hand-plucked in three seasons, viz. pre-monsoon (April and May), monsoon (July and August) and autumn (October and November) of 2017 from a total of 35 cultivars, viz. TV1-TV31, Betjan, Kharijan, S.3A/3 and T.3E/3, which include 16 Assam, 14 Cambod, 2 Assam China hybrid (ACH), 2 Assam hybrid (AH) and 1 China hybrid (CH) varieties. The sampling was performed using the procedure mentioned in Fang et al. (2017). A sample from each cultivar in every season consisted of three biological replicates and technical measurements were performed in triplicate for each replicate sample. The fresh samples were subjected to enzyme deactivation by steaming for 90 s and then dried in a hot air oven at 60 ± 2 °C. The samples were ground followed by sieving through a 30-mesh sieve and then used for further analysis. The dry matter contents of the samples were determined after drying at 105 ± 2 °C for six hours and were used for quantification of chemical compounds of interest.

### 2.3. Preparation of the calibration standard

Caffeine standard was used for the determination of individual catechins and caffeine. A caffeine standard stock solution of 1000 μg $mL^{-1}$ was prepared. This stock solution was diluted to obtain standard solutions in the concentration range 5-25 μg $mL^{-1}$. These standard solutions were injected in the UPLC (Dionex, Ultimate 3000). A calibration curve was constructed using the peak areas against the respective concentrations. The regression equation of the calibration curve was: $y = 0.307x - 0.927$, with $R^2 = 0.999$. Slope (S) and standard deviation (δ) of the response were determined for the calibration curve. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 and 10 times of δ/S, respectively as described by Fernando and Soysa (2016). The LOD, LOQ, and recovery for caffeine were 0.50 μg $mL^{-1}$, 1.52 μg $mL^{-1}$, and 98.73%, respectively.

### 2.4. Estimation of catechin and caffeine contents

Catechin and caffeine contents were estimated using International Standard Organization method (ISO 14502-2:2005). Briefly, 0.2 g of ground tea leaf sample was extracted by 5 mL 70% methanol at 70 °C in a water bath for 10 min and this process was repeated once. Then, the volume of supernatant was made up to 10 mL using 70% methanol. One mL supernatant was diluted to 5 mL with a stabilizing agent. The stabilizing agent was prepared by using ascorbic acid (500 μg $mL^{-1}$), EDTA (500 μg $mL^{-1}$), and acetonitrile (25% v/v) in water. Then the diluted extract was filtered through 0.45 μm syringe filters before quantitative estimation using UPLC (Dionex, Ultimate 3000) with Luna 5 μ phenylhexyl Phenomenex column (4.5 mm × 250 mm; Torrance, CA, USA) and UV-Vis detector set at 278 nm. The column temperature was set at 25 ± 0.5 °C. Mobile phase A consisted of 2% (v/v) acetic acid, 9% (v/v) acetonitrile and ultrapure water. Mobile phase B consisted of 80% (v/v) acetonitrile and ultrapure water. The flow rate was 1 mL $min^{-1}$. The gradient elution was set as 100% mobile phase A for 10 min, then a

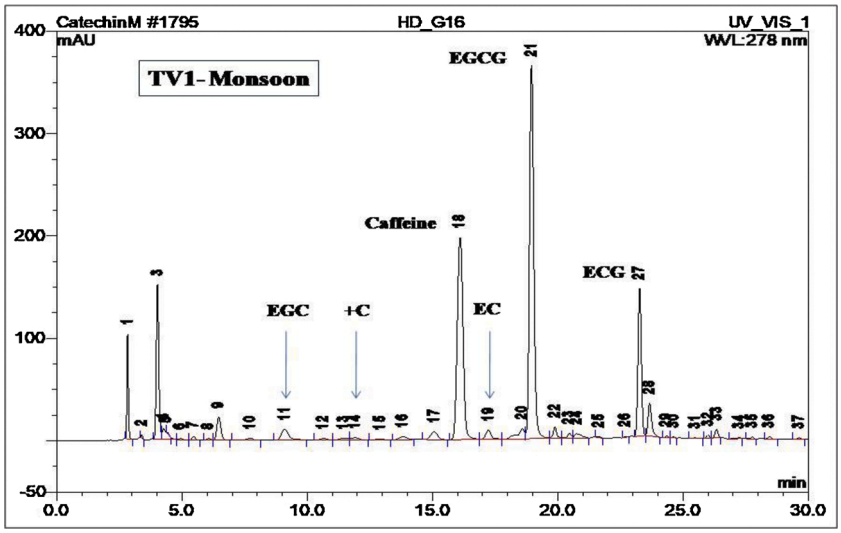



**Fig. 1.** UPLC chromatogram for cultivar TV1 in monsoon season.

linear gradient to 68% mobile phase A, 32% mobile phase B over 15 min and held at this composition for 10 min. Catechin and caffeine peaks were identified by using the standards. The estimation of individual catechins and caffeine were done by using relative response factors of catechins with respect to caffeine as described in ISO 14502-2:2005.

### 2.5. Statistical analysis

Analysis of variance (ANOVA) was performed by using SPSS software version 17.00 (SPSS Inc., Chicago, IL). Tukey's multiple comparison test was used to get the differences between means and the differences were considered significant at $p \leq 0.05$ and $p \leq 0.01$. For each sample, all data were reported as the mean ± standard error (SE) with three replications. Heatmaps of Pearson correlation matrices among the different catechins were generated using the R software package to study the association among them. As visualization is generally easier to understand than reading tabular data, heatmaps are typically used to visualize correlation matrices. Moreover, Pearson's correlations between biochemical parameters for each season have also been computed to check for any significant differences among them.

## 3. Results and discussion

### 3.1. Catechin content

The young tea leaves are a rich source of monomeric flavan-3-ols (also called catechins). The principal catechins, viz. EGCG, ECG, EGC, EC, and + C were estimated. The sum of the content of these individual catechins was presented as total catechin (TC). A representative UPLC chromatogram of cultivar TV1 in monsoon season is presented in Fig. 1.

In pre-monsoon, the EGCG levels ranged from 52.2 ± 1.07 mg $g^{-1}$ in TV1 to 111 ± 1.24 mg $g^{-1}$ in TV10, which expressed a 2.12 fold difference (Table 1). In monsoon, TV11 showed the highest (141 ± 1.35 mg $g^{-1}$) and TV17 showed the lowest level (70.4 ± 1.03 mg $g^{-1}$) of EGCG (Table 3). A 1.83 fold difference was observed between the highest- and lowest-ranked cultivars. In autumn, the EGCG level was in the range between 58.4 ± 2.47 mg $g^{-1}$ in TV24 and 108 ± 2.15 mg $g^{-1}$ in TV10, which expressed a 1.85 fold variation (Table 5). EGCG showed a significant positive correlation ($p \leq 0.01$) with TC (Tables 2, 4 and 6). EGCG contributed 46.0-49.27% to TC content throughout the harvesting seasons. The present study found a significant influence of seasonal variation on catechin concentrations for all cultivars in conformity with previous studies (Dai et al., 2015; Fang et al., 2017; Wakamatsu et al., 2019). The catechin composition of central and southern African tea cultivars are dominated by EGCG at around 50% level (Wright et al., 2000). In a study of seasonal effect on Chinese green tea, EGCG was reported to contribute ~60% to total catechin (Xu et al., 2012). In Kenyan tea cultivars (Owuor and Obanda, 2007), EGCG contributes about 25% to total flavan-3-ol, which is much lower than Tocklai cultivars. The EGCG levels of Sri Lankan tea germplasm (Punyasiri et al., 2017) were reported in the range between 41.45 and 120.86 mg $g^{-1}$. Obanda et al. (1997) reported Kenyan tea germplasm with EGCG levels from 60.46 to 118.45 mg $g^{-1}$. These reports from Sri Lanka and Kenya are consistent with the present findings. However, later on, a Kenyan study reported lower levels of EGCG in the range between 20.12 and 32.65 mg $g^{-1}$ (Owuor and Obanda, 2007). Wei et al. (2011) reported low EGCG levels in Chinese tea cultivars (41.5-59.6 mg $g^{-1}$).

ECG content in pre-monsoon varied from 17.2 ± 0.29 mg $g^{-1}$ in TV30 to 52.6 ± 1.35 mg $g^{-1}$ in TV19, expressing a 3.06 fold variation (Table 1). In monsoon, TV16 possessed the highest ECG content (60.2 ± 0.69 mg $g^{-1}$) whereas TV30 possessed the lowest content (16.8 ± 1.00 mg $g^{-1}$) (Table 3). A 3.58 fold difference was observed between the highest and lowest-ranked cultivars. In autumn, ECG content varied from 14.2 ± 0.69 mg $g^{-1}$ in TV30 to 49.0 ± 1.07 mg $g^{-1}$ in TV19, which represented a 3.45 fold variation (Table 5). There were significant positive correlations ($p \leq 0.01$) between the levels of ECG and that of EC, and TC irrespective of the season (Tables 2, 4 and 6). The ECG levels in the green leaves from different cultivars of Sri Lankan tea (Punyasiri et al., 2017) and Kenyan tea (Obanda et al., 1997) were in the range between 14.36 and 47.42 mg $g^{-1}$ and between 18.31 and 49.15 mg $g^{-1}$, respectively supporting the variation in ECG content with varietal types which confirm with our results. Owuor and Obanda (2007) reported lower ECG levels in Kenyan tea cultivars in the range between 21.68 and 29.86 mg $g^{-1}$ with a mean of 25.24 mg $g^{-1}$. Wei et al. (2011) presented a much lower range (14.7-21.6 mg $g^{-1}$) of ECG levels in Chinese tea cultivars.

The variations of EGC content of the cultivars with the season are presented in Tables 1, 3 and 5. In pre-monsoon, EGC content in TV6 (68.5 ± 4.22 mg $g^{-1}$) was comparatively higher than other cultivars whereas, TV7 (15.9 ± 1.63 mg $g^{-1}$) exhibited the lowest value. In this study, a 4.31 fold difference was observed between the highest and lowest-ranked cultivars. Significant positive correlations ($p \leq 0.01$) were observed between the levels of EGC and that of EC, EGCG, and TC for the pre-monsoon season (Table 2). In monsoon, EGC content ranged from 21.1 ± 0.35 mg $g^{-1}$ in TV31 to 57.4 ± 0.54 mg $g^{-1}$ in T.3E/3, which represented a 2.72 fold variation (Table 3). In autumn, EGC varied from 19.9 ± 0.47 mg $g^{-1}$ in TV19 to 51.2 ± 1.23 mg $g^{-1}$ in T.3E/3, expressing a 2.56 fold variation (Table 5). Significant positive correlations ($p \leq 0.01$) were observed for EGC levels with that of EC and TC (Tables 4 and 6).

**Table 1**
Catechin profile and caffeine content of cultivars in pre-monsoon.

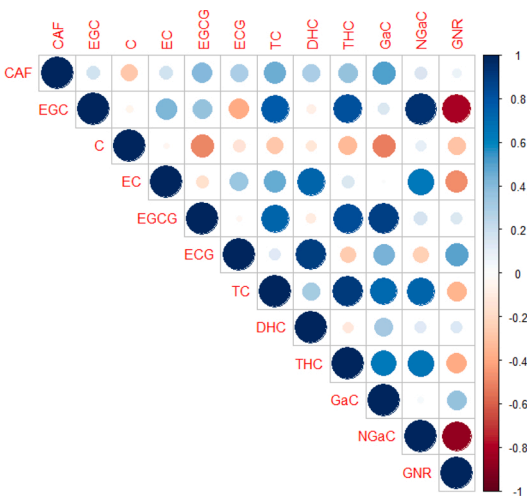
| Cultivar | Parameters* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EGC | +C | EC | EGCG | ECG | GaC | NGaC | DHC | THC | CI | TC | GNR | CAF |
| **TV1** | 20.6 ± 0.41[a, #] | 10.4 ± 0.70[a] | 10.8 ± 0.70[a] | 52.2 ± 1.07[a] | 35.4 ± 1.48[a] | 87.5 ± 2.55[a] | 41.9 ± 1.69[a] | 46.2 ± 2.17[a] | 72.8 ± 1.47[a] | 0.63 ± 0.02[a] | 129 ± 4.23[a] | 2.09 ± 0.02[a] | 31.6 ± 0.18[a] |
| **TV2** | 53.1 ± 0.85[b] | 4.90 ± 0.46[b] | 18.5 ± 1.50[b] | 94.3 ± 4.33[b] | 26.4 ± 1.51[b] | 121 ± 5.83[b] | 76.5 ± 2.14[b] | 44.9 ± 2.75[a] | 147 ± 5.17[b] | 0.30 ± 0.01[b] | 197 ± 7.80[b] | 1.58 ± 0.04[b] | 41.2 ± 1.32[b] |
| **TV3** | 50.8 ± 2.71[b] | 7.90 ± 0.44[c] | 10.1 ± 0.39[a] | 104 ± 4.93[c] | 27.9 ± 2.15[b] | 133 ± 6.49[c] | 68.8 ± 2.68[c] | 38.0 ± 2.31[b] | 155 ± 3.40[c] | 0.24 ± 0.01[c] | 201 ± 5.42[c] | 1.93 ± 0.16[c] | 37.2 ± 0.94[c] |
| **TV4** | 62.3 ± 0.55[c] | 6.60 ± 0.36[c] | 14.2 ± 0.09[c] | 104 ± 4.37[c] | 26.6 ± 0.68[b] | 131 ± 5.04[c] | 83.1 ± 0.36[d] | 40.8 ± 0.68[c] | 167 ± 3.88[d] | 0.24 ± 0.01[c] | 214 ± 4.72[d] | 1.58 ± 0.07[b] | 35.2 ± 0.35[c] |
| **TV5** | 67.2 ± 1.37[c,d] | 7.70 ± 0.22[c] | 12.8 ± 1.19[d] | 90.9 ± 3.20[b] | 22.8 ± 1.51[c] | 114 ± 4.57[d] | 87.7 ± 2.43[d] | 35.6 ± 2.70[d] | 158 ± 3.88[c] | 0.22 ± 0.01[d] | 201 ± 6.99[c] | 1.30 ± 0.07[d] | 35.7 ± 1.17[c] |
| **TV6** | 68.5 ± 4.22[d] | 11.3 ± 0.81[a] | 12.6 ± 0.76[d] | 85.4 ± 5.14[b] | 22.2 ± 0.93[c] | 108 ± 6.06[e] | 92.4 ± 4.46[e] | 34.8 ± 1.39[d] | 154 ± 0.92[c] | 0.23 ± 0.01[d] | 200 ± 1.61[c] | 1.16 ± 0.12[e] | 44.9 ± 0.09[d] |
| **TV7** | 15.9 ± 1.63[e] | 4.40 ± 0.18[b] | 10.5 ± 0.60[a] | 64.7 ± 2.57[d] | 36.8 ± 1.63[a] | 101 ± 1.02[f] | 30.8 ± 1.18[f] | 47.3 ± 2.17[e] | 80.6 ± 4.11[e] | 0.59 ± 0.06[e] | 132 ± 2.09[e] | 3.29 ± 0.10[f] | 37.8 ± 0.52[c] |
| **TV8** | 39.9 ± 0.19[f] | 2.90 ± 0.50[d] | 9.00 ± 0.32[a] | 87.2 ± 3.10[b] | 22.5 ± 0.19[c] | 110 ± 2.98[e] | 51.7 ± 0.58[g] | 31.5 ± 0.49[f] | 127 ± 3.23[f] | 0.25 ± 0.01[c] | 162 ± 2.60[f] | 2.12 ± 0.08[a] | 38.4 ± 0.24[c] |
| **TV9** | 45.4 ± 0.93[g] | 2.40 ± 0.29[d] | 15.0 ± 0.09[c] | 104 ± 4.82[e] | 31.8 ± 0.15[d] | 136 ± 4.71[c] | 62.5 ± 1.02[h] | 46.8 ± 0.09[a] | 149 ± 5.70[b] | 0.31 ± 0.01[b] | 199 ± 5.72[c] | 2.18 ± 0.04[g] | 45.3 ± 1.51[d] |
| **TV10** | 38.8 ± 0.35[f] | 2.70 ± 0.33[d] | 9.50 ± 0.23[a] | 111 ± 1.24[e] | 39.0 ± 0.61[e] | 150 ± 1.19[g] | 50.9 ± 0.23[g] | 48.4 ± 0.39[e] | 150 ± 1.29[b] | 0.32 ± 0.01[b] | 201 ± 1.26[c] | 2.94 ± 0.02[j] | 41.2 ± 0.23[b] |
| **TV11** | 47.3 ± 0.43[g] | 2.30 ± 0.26[d] | 14.8 ± 0.74[c] | 98.1 ± 4.27[e] | 32.2 ± 0.23[d] | 130 ± 4.50[c] | 64.3 ± 1.15[h] | 47.0 ± 0.92[e] | 145 ± 4.71[b] | 0.32 ± 0.01[b] | 195 ± 5.65[b] | 2.03 ± 0.03[a] | 35.0 ± 0.21[c] |
| **TV12** | 51.9 ± 2.02[b] | 3.00 ± 0.26[d] | 11.6 ± 0.71[a] | 106 ± 0.90[e] | 35.4 ± 1.81[a] | 142 ± 0.97[h] | 66.4 ± 1.18[h] | 47.0 ± 1.17[e] | 158 ± 1.15[c] | 0.30 ± 0.01[b] | 208 ± 2.12[g] | 2.13 ± 0.03[a] | 44.3 ± 0.63[d] |
| **TV13** | 46.2 ± 1.91[g] | 7.40 ± 0.38[c] | 11.3 ± 1.11[a] | 98.3 ± 1.26[e] | 26.5 ± 0.70[b] | 125 ± 0.58[b] | 64.9 ± 2.79[h] | 37.9 ± 0.99[b] | 144 ± 2.64[b] | 0.26 ± 0.01[f] | 190 ± 3.21[h] | 1.92 ± 0.08[c] | 40.3 ± 0.78[b] |
| **TV14** | 18.5 ± 0.32[h] | 12.8 ± 0.39[a] | 13.6 ± 0.29[c] | 71.4 ± 0.74[f] | 37.7 ± 0.98[e] | 110 ± 1.21[e] | 44.9 ± 0.47[a] | 51.4 ± 0.94[g] | 89.9 ± 0.93[h] | 0.57 ± 0.01[g] | 154 ± 1.01[i] | 2.43 ± 0.05[k] | 37.7 ± 0.61[c] |
| **TV15** | 53.3 ± 1.79[b] | 2.70 ± 0.35[d] | 21.2 ± 1.59[b] | 92.6 ± 2.08[b] | 39.9 ± 3.93[e] | 133 ± 3.23[c] | 77.2 ± 3.40[b] | 61.1 ± 4.99[h] | 146 ± 0.87[b] | 0.42 ± 0.03[h] | 210 ± 5.06[g] | 1.72 ± 0.08[l] | 48.7 ± 0.50[e] |
| **TV16** | 25.4 ± 0.48[i] | 6.70 ± 0.23[c] | 12.5 ± 1.40[c] | 78.1 ± 2.65[g] | 52.2 ± 2.54[f] | 130 ± 0.67[c] | 44.6 ± 0.92[a] | 64.6 ± 3.93[h] | 104 ± 3.11[i] | 0.63 ± 0.06[a] | 175 ± 1.26[j] | 2.92 ± 0.06[j] | 43.7 ± 1.54[d] |
| **TV17** | 50.4 ± 0.72[b] | 6.30 ± 0.34[c] | 35.0 ± 0.41[e] | 65.5 ± 0.90[d] | 46.1 ± 1.71[g] | 112 ± 2.40[e] | 91.8 ± 1.44[e] | 81.1 ± 1.34[i] | 116 ± 1.04[j] | 0.70 ± 0.01[i] | 203 ± 1.87[c] | 1.22 ± 0.04[m] | 43.5 ± 0.43[d] |
| **TV18** | 27.3 ± 0.87[i] | 2.50 ± 0.20[d] | 7.90 ± 0.41[a] | 88.2 ± 2.53[b] | 25.7 ± 0.82[b] | 114 ± 3.32[e] | 37.7 ± 1.47[i] | 33.6 ± 1.17[f] | 116 ± 3.38[j] | 0.29 ± 0.01[b] | 152 ± 4.74[i] | 3.02 ± 0.04[n] | 42.5 ± 1.40[d] |
| **TV19** | 21.0 ± 0.12[j] | 3.20 ± 0.26[d] | 16.2 ± 0.98[f] | 104 ± 1.08[e] | 52.6 ± 1.35[f] | 157 ± 2.37[i] | 40.4 ± 1.23[i] | 68.8 ± 1.85[h] | 125 ± 1.19[f] | 0.55 ± 0.01[j] | 197 ± 2.96[c] | 3.87 ± 0.12[◦] | 42.2 ± 0.35[d] |
| **TV20** | 36.7 ± 0.61[f] | 2.30 ± 0.23[d] | 10.7 ± 0.39[a] | 111 ± 0.98[e] | 32.3 ± 0.32[d] | 143 ± 0.99[h] | 49.7 ± 1.21[g] | 42.9 ± 0.09[a] | 147 ± 0.97[b] | 0.29 ± 0.01[b] | 193 ± 1.07[b] | 2.88 ± 0.08[j] | 43.0 ± 0.52[d] |
| **TV21** | 44.2 ± 0.64[g] | 2.70 ± 0.12[d] | 7.00 ± 0.67[a] | 79.2 ± 1.67[g] | 21.1 ± 0.35[c] | 100 ± 1.79[f] | 53.9 ± 0.15[g] | 28.2 ± 0.59[j] | 123 ± 1.29[f] | 0.23 ± 0.01[d] | 154 ± 1.85[i] | 1.86 ± 0.03[c] | 33.6 ± 0.44[c] |
| **TV22** | 43.3 ± 1.00[g] | 2.20 ± 0.18[d] | 17.3 ± 1.10[c] | 85.2 ± 1.70[b] | 38.9 ± 0.15[e] | 124 ± 1.74[b] | 62.8 ± 2.21[h] | 56.1 ± 1.16[k] | 129 ± 2.66[f] | 0.44 ± 0.01[h] | 187 ± 3.93[k] | 1.98 ± 0.04[a] | 34.1 ± 0.90[c] |
| **TV23** | 52.4 ± 0.35[b] | 6.30 ± 0.35[c] | 25.3 ± 0.68[g] | 71.4 ± 0.44[f] | 37.8 ± 0.38[e] | 109 ± 0.74[e] | 83.9 ± 1.04[d] | 63.2 ± 0.54[h] | 124 ± 0.12[f] | 0.51 ± 0.01[k] | 193 ± 0.33[b] | 1.30 ± 0.03[d] | 32.4 ± 0.26[c] |
| **TV24** | 32.5 ± 0.93[j] | 7.10 ± 0.38[c] | 14.2 ± 1.01[c] | 61.9 ± 2.55[d] | 32.5 ± 1.95[d] | 94.4 ± 4.36[j] | 53.8 ± 1.53[g] | 46.6 ± 2.47[e] | 94.4 ± 3.10[k] | 0.49 ± 0.01[k] | 148 ± 5.23[l] | 1.76 ± 0.07[l] | 38.0. ± 0.50[c] |
| **TV25** | 38.1 ± 1.71[f] | 3.70 ± 0.38[d] | 12.5 ± 0.43[c] | 104 ± 3.28[e] | 36.5 ± 1.01[e] | 140 ± 3.45[h] | 54.3 ± 2.54[g] | 49.0 ± 1.24[e] | 142 ± 2.59[b] | 0.34 ± 0.01[b] | 195 ± 3.04[b] | 2.59 ± 0.17[b] | 35.0 ± 0.59[c] |
| **TV26** | 35.6 ± 0.93[f] | 2.20 ± 0.12[d] | 10.6 ± 0.99[a] | 94.5 ± 2.17[b] | 19.5 ± 0.57[c] | 114 ± 2.70[e] | 48.3 ± 1.85[g] | 30.1 ± 1.56[j] | 130 ± 2.89[f] | 0.23 ± 0.01[d] | 162 ± 4.36[f] | 2.36 ± 0.06[k] | 34.0 ± 0.57[c] |
| **TV27** | 23.3 ± 0.38[k] | 7.40 ± 0.33[c] | 8.50 ± 0.32[a] | 82.7 ± 1.47[b] | 32.0 ± 0.40[d] | 115 ± 1.51[e] | 39.2 ± 0.50[i] | 40.5 ± 0.67[a] | 106 ± 1.84[i] | 0.38 ± 0.01[l] | 154 ± 1.58[i] | 2.93 ± 0.05[j] | 41.0 ± 0.38[d] |
| **TV28** | 27.1 ± 0.23[i] | 7.90 ± 0.49[c] | 9.20 ± 0.53[a] | 82.0 ± 2.43[b] | 34.8 ± 0.86[d] | 117 ± 1.69[e] | 44.2 ± 0.84[a] | 44.0 ± 1.36[a] | 109 ± 2.51[i] | 0.40 ± 0.02[l] | 161 ± 2.19[f] | 2.64 ± 0.05[b] | 35.0 ± 0.24[c] |
| **TV29** | 28.8 ± 1.13[i] | 2.20 ± 0.09[d] | 13.4 ± 0.52[c] | 105 ± 1.05[e] | 38.3 ± 0.75[e] | 143 ± 0.38[h] | 44.4 ± 1.59[a] | 51.7 ± 1.02[e] | 134 ± 1.70[f] | 0.39 ± 0.01[l] | 188 ± 1.44[k] | 3.23 ± 0.12[f] | 38.0 ± 0.32[c] |
| **TV30** | 23.9 ± 2.14[k] | 10.9 ± 0.98[a] | 9.40 ± 0.12[a] | 67.2 ± 3.30[d] | 17.2 ± 0.29[h] | 84.4 ± 3.15[k] | 44.2 ± 3.15[a] | 26.6 ± 0.41[j] | 91.0 ± 1.20[k] | 0.29 ± 0.01[b] | 129 ± 0.03[a] | 1.91 ± 0.21[c] | 27.1 ± 0.32[e] |
| **TV31** | 19.0 ± 0.17[a] | 3.70 ± 0.09[d] | 9.20 ± 0.39[a] | 93.7 ± 0.96[b] | 44.3 ± 0.62[g] | 138 ± 1.11[h] | 31.9 ± 0.40[f] | 53.6 ± 0.96[e] | 113 ± 0.87[j] | 0.48 ± 0.01[k] | 170 ± 1.04[m] | 4.33 ± 0.07[p] | 33.6 ± 0.26[c] |
| **Betjan** | 48.1 ± 0.41[g] | 2.90 ± 0.12[d] | 12.7 ± 0.28[c] | 100 ± 0.46[e] | 28.2 ± 0.21[b] | 128 ± 0.63[c] | 63.7 ± 0.09[h] | 40.9 ± 0.47[a] | 148 ± 0.70[b] | 0.28 ± 0.01[b] | 192 ± 0.71[b] | 2.01 ± 0.01[a] | 46.5 ± 0.58[e] |
| **Kharijan** | 40.6 ± 1.42[f] | 3.30 ± 0.23[d] | 11.7 ± 0.52[c] | 102 ± 0.70[e] | 36.5 ± 0.39[e] | 138 ± 1.09[h] | 55.6 ± 1.70[g] | 48.2 ± 0.57[e] | 142 ± 0.87[b] | 0.34 ± 0.01[b] | 194 ± 1.04[b] | 2.48 ± 0.09[k] | 42.6 ± 0.68[d] |
| **S.3/A3** | 48.3 ± 0.38[g] | 6.90 ± 0.21[c] | 13.5 ± 0.47[c] | 71.7 ± 0.50[f] | 27.4 ± 0.59[b] | 99.1 ± 0.26[f] | 68.6 ± 0.43[c] | 40.8 ± 1.06[a] | 120 ± 0.89[f] | 0.34 ± 0.01[b] | 168 ± 0.67[m] | 1.44 ± 0.01[q] | 31.2 ± 0.94[c] |
| **T.3/E3** | 61.8 ± 1.32[l] | 2.60 ± 0.26[d] | 23.3 ± 0.42[g] | 76.8 ± 1.55[g] | 29.7 ± 0.51[b] | 107 ± 1.98[e] | 87.7 ± 1.95[d] | 53.0 ± 0.90[e] | 139 ± 2.69[l] | 0.38 ± 0.01[l] | 194 ± 3.84[b] | 1.21 ± 0.01[m] | 36.4 ± 0.27[c] |

* EGC, (−)-epigallocatechin; +C, (+)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechingallate; ECG, (−)-epicatechingallate; GaC, galloylated catechins (EGCG + ECG); NGaC, non-galloylated catechins (EC + EGC); DHC, dihydroxy catechins (EC + ECG); THC, trihydroxy catechins (EGC + EGCG); CI, catechin index

(DHC/THC); TC, total catechin; GNR, galloylated to non-galloylated catechin ratio (GaC/NGaC); CAF, caffeine. All units, except those for CI and GNR, are in mg $g^{-1}$(dry weight basis). Values are 'mean ± SE' of independent triplicate measurements.

[#] Same symbol within a column denotes not significant whereas different symbol denotes significant difference.

**Table 2**
Heatmap of the correlation matrix between catechins and caffeine content in tea leaves harvested in pre-monsoon.




CAF, caffeine; EGC, (−)-epigallocatechin; C, (+)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechingallate; ECG, (−)-epicatechingallate; TC, total catechin; DHC, dihydroxylated catechins; THC, trihydroxylated catechins; GaC, galloylated catechins; NGaC, non-galloylated catechins; GNR, galloylated to non-galloylated catechin ratio.

EGC contents (8.19-54.45 mg $g^{-1}$) of Sri Lankan germplasm (Punyasiri et al., 2017) are consistent with the current study. Kenyan cultivars had higher EGC level (23.92-82.48 mg $g^{-1}$) as reported by Obanda et al. (1997). However, a recent study on Chinese (Wei et al., 2011) and Kenyan (Owuor and Obanda, 2007) tea cultivars reported much lower levels of EGC in the range between 13.3 and 20.4 mg $g^{-1}$ and between 4.26 and 36.14 mg $g^{-1}$, respectively.

The effect of season on EC contents of the cultivars are tabulated in Tables 1, 3 and 5. In pre-monsoon, the EC content varied from 7.00 ± 0.67 mg $g^{-1}$ in TV21 to 35.0 ± 0.41 mg $g^{-1}$ in TV17 expressing a 5.0 fold variation. In monsoon, the variation was between 7.70 ± 1.02 mg $g^{-1}$ in TV10 and 37.2 ± 0.35 mg $g^{-1}$ in TV17. A 4.83 fold difference was observed between the highest and lowest content. In autumn, the EC level varied from 6.20 ± 0.55 mg $g^{-1}$ in TV21 to 25.2 ± 0.85 mg $g^{-1}$ in TV17. The highest and lowest level expressed a 4.06 fold difference. Significant positive correlations ($p \leq 0.01$) between the levels of EC and that of EGC, ECG, and TC were observed irrespective of the season (Tables 2, 4 and 6). EC levels in the tea cultivars from Kenya (Obanda et al., 1997) and Sri Lanka (Punyasiri et al., 2017) were reported in the range between 10.51 and 17.21 mg $g^{-1}$ and between 6.08 and 25.50 mg $g^{-1}$, respectively, which are similar to the findings of the present study. However, Wei et al. (2011) reported much lower EC levels (4.0-7.3 mg $g^{-1}$) in Chinese cultivars. In another study of Kenyan tea, EC levels were reported in a diverse range between 2.60 and 31.93 mg $g^{-1}$ (Owuor and Obanda, 2007).

The +C content in pre-monsoon ranged from 2.20 ± 0.18 mg $g^{-1}$ in TV22 to 12.8 ± 0.39 mg $g^{-1}$ in TV14, which represented a variation of 5.82 fold (Table 1). In monsoon, TV30 had the highest +C content (15.2 ± 0.99 mg $g^{-1}$) and TV9 had the lowest content (3.20 ± 0.21 mg $g^{-1}$). A 4.75 fold difference was observed between the two extremes (Table 3). In autumn, the highest +C content (12.3 ± 1.47 mg $g^{-1}$) was in TV6 and the lowest content was in TV30 (1.40 ± 0.21 mg $g^{-1}$) which was 8.78 fold lower compared to TV6 (Table 5). Fang et al. (2017) reported a higher level of +C in tea cultivars from China in the warm season of Fujian. The +C levels in Chinese (Wei et al., 2011) and Kenyan (Obanda et al., 1997) tea cultivars were reported in the range between 3.4 and 22.7 mg $g^{-1}$, and between 0.01 and 13.70 mg $g^{-1}$, respectively. These results are in good agreement with the present study. In another study, Owuor and Obanda (2007) found +C levels in the range between 1.63 and 8.97 mg $g^{-1}$ relating flavan-3-ol compositions with black tea quality. Yamamoto et al. (1997) reported higher content of EC and +C in green leaves during summer than that of autumn season which is consistent with our results.

The variations of TC content of the cultivars with the season are presented in Tables 1, 3 and 5. In pre-monsoon, TC content varied from 129 ± 0.03 in TV30 to 214 ± 4.72 mg $g^{-1}$ in TV4 with a variation of 1.67 fold. TC content in monsoon varied from 151 ± 4.21 mg $g^{-1}$ in TV30 to 238 ± 8.41 mg $g^{-1}$ in TV23 which had 1.57 fold higher level compared to TV30. In autumn, TC content varied from 121 ± 4.45 mg $g^{-1}$ in TV30 to 181 ± 5.82 mg $g^{-1}$ in TV10 which expressed a 1.49 fold difference. Significant positive correlations ($p \leq 0.01$) were observed between TC and EGC, EC, EGCG, ECG content throughout the harvesting period irrespective of the season (Tables 2, 4 and 6). Xu et al. (2012) observed significant differences in all catechin contents in green tea prepared from spring and summer harvest except for EGC. Compared to our results Wei et al. (2011) reported much lower levels of TC in the range from 92.1 to 119.2 mg $g^{-1}$ in Chinese tea cultivars from different growing locations. In a very recent study, TC level was reported in the range between 72.6 and 200.4 mg $g^{-1}$ in tea samples from Southern Jiangsu region, China (Wen et al., 2020). The tea cultivars released by United Planters' Association of Southern India (UPASI) had TC content (averaged over the seasons) in the range from 138.3 to 203.9 mg $g^{-1}$ (Saravanan et al., 2005). Jin et al. (2014) reported that the total catechin content in 403 Chinese tea cultivars varied from 56.6 to 231.9 mg $g^{-1}$ in which 98.3% cultivars were in the range between 120.1 and 231.9 mg $g^{-1}$.

The variation of catechin content in tea germplasm from different growing regions of the world may be attributed to the difference in the agro-climatic environment (Fang et al., 2017; Wang et al., 2012; Wen et al., 2020; Zheng et al., 2018). Factors like temperature, light intensity, and precipitation significantly influence the catechin level in green tea leaves leading to ambiguity over the most productive harvesting season (Han et al., 2017; Wakamatsu et al., 2019; Wei et al., 2011). Harvesting season significantly affects secondary metabolite (catechins) contents closely related to green leaf quality of tea (Tan et al., 2017; Zeng et al., 2020). Dai et al. (2015) reported that dimeric catechins showed sharp seasonal fluctuations, with a significant increase in summer. The shoots harvested in monsoon had the highest EGCG and ECG contents. This could be due to comparatively higher temperature in monsoon (Supplementary material Fig. S1) and the same metabolic pathway for their biosynthesis as suggested by Bhatia and Ullah (1968) and Singh et al. (1999). Yao et al. (2005) reported a significant positive correlation ($p < 0.05$) between the EGCG level and the temperature of the harvesting season. Increasing sunlight exposure and temperature results in a higher accumulation of catechins in tea leaves (Wen et al., 2020). EGCG and ECG content decreased with decreasing sunlight intensity in autumn. Contrary to our findings, Fang et al. (2017) reported no seasonal variation in EGCG level and the way of response might not be the same for all the cultivars grown in the same environment owing to their genetic variation which influences the biosynthesis of these secondary metabolites. Jayasekera et al. (2014) reported inconsistent variation in phenolic compounds with the season for high grown unfermented tea in Sri Lanka and the effect of season varied with plantation. Wakamatsu et al. (2019) concluded that EGCG and ECG were less abundant in autumn compared to those in monsoon and pre-monsoon. In another report from Japan, EGCG and ECG content of green tea in summer was

**Table 3**
Catechin profile and caffeine content of cultivars in monsoon.

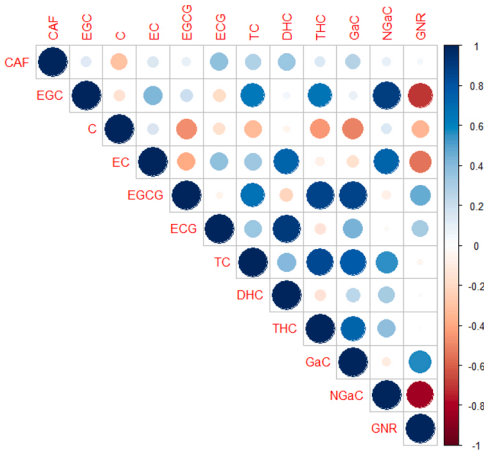
| Cultivar | Parameters* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EGC | +C | EC | EGCG | ECG | GaC | NGaC | DHC | THC | CI | TC | GNR | CAF |
| **TV1** | 24.9 ± 0.38[a,#] | 8.90 ± 0.88[a] | 14.7 ± 0.96[a] | 80.8 ± 2.69[a] | 47.5 ± 0.67[a] | 128 ± 3.30[a] | 48.5 ± 0.30[a] | 62.2 ± 1.63[a] | 105 ± 2.75[a] | 0.59 ± 0.01[a] | 177 ± 3.38[a] | 2.64 ± 0.07[a] | 46.8 ± 1.08[a] |
| **TV2** | 46.4 ± 0.63[b] | 6.00 ± 0.34[b] | 11.4 ± 0.30[b] | 113 ± 4.86[b] | 33.0 ± 1.19[b] | 146 ± 6.00[b] | 63.8 ± 1.08[b] | 44.5 ± 1.44[b] | 160 ± 5.46[b] | 0.28 ± 0.01[b] | 211 ± 7.07[b] | 2.30 ± 0.06[b] | 49.1 ± 0.42[a] |
| **TV3** | 41.8 ± 1.33[c] | 9.50 ± 0.66[a] | 9.10 ± 0.30[c] | 118 ± 2.56[c] | 32.7 ± 2.37[b] | 150 ± 4.76[b] | 60.4 ± 1.71[c] | 41.8 ± 2.12[b] | 160 ± 3.32[b] | 0.26 ± 0.01[b] | 211 ± 5.45[b] | 2.50 ± 0.09[c] | 39.6 ± 1.76[b] |
| **TV4** | 45.9 ± 0.52[b] | 8.30 ± 0.30[a] | 12.6 ± 0.35[b] | 107 ± 3.33[d] | 36.0 ± 0.78[c] | 143 ± 4.01[b] | 66.8 ± 0.33[d] | 48.7 ± 1.13[c] | 153 ± 3.39[c] | 0.32 ± 0.01[c] | 210 ± 4.33[b] | 2.15 ± 0.05[d] | 41.0 ± 0.47[c] |
| **TV5** | 54.9 ± 0.49[d] | 9.20 ± 0.33[a] | 13.0 ± 0.44[b] | 105 ± 4.61[d] | 32.1 ± 0.50[b] | 137 ± 5.10[c] | 77.1 ± 1.25[e] | 45.1 ± 0.67[b] | 161 ± 4.71[b] | 0.28 ± 0.01[b] | 215 ± 5.47[b] | 1.79 ± 0.07[e] | 44.4 ± 0.80[d] |
| **TV6** | 44.6 ± 2.63[b] | 12.7 ± 0.64[c] | 11.5 ± 0.26[b] | 111 ± 5.45[b] | 41.1 ± 2.30[d] | 152 ± 7.32[b] | 68.8 ± 2.77[d] | 52.6 ± 2.53[d] | 156 ± 4.56[c] | 0.34 ± 0.02[c] | 221 ± 5.14[c] | 2.23 ± 0.20[b] | 44.2 ± 0.69[d] |
| **TV7** | 29.5 ± 1.72[e] | 6.80 ± 0.21[b] | 10.4 ± 0.93[c] | 83.0 ± 1.44[a] | 30.5 ± 0.52[b] | 113 ± 1.82[d] | 46.7 ± 2.72[a] | 40.9 ± 0.59[b] | 113 ± 1.46[d] | 0.36 ± 0.01[d] | 160 ± 2.07[d] | 2.43 ± 0.16[f] | 42.3 ± 1.68[c,d] |
| **TV8** | 40.8 ± 2.62[c] | 4.50 ± 0.32[d] | 8.40 ± 0.47[c] | 114 ± 5.78[b] | 34.8 ± 1.60[b] | 149 ± 4.88[b] | 53.7 ± 2.69[f] | 43.2 ± 1.50[b] | 156 ± 3.20[c] | 0.28 ± 0.01[b] | 203 ± 2.21[e] | 2.79 ± 0.22[g] | 38.4 ± 1.05[b] |
| **TV9** | 45.2 ± 0.81[b] | 3.20 ± 0.21[e] | 12.0 ± 0.15[b] | 120 ± 5.32[e] | 33.1 ± 0.43[b] | 153 ± 5.13[b] | 60.4 ± 0.78[c] | 45.1 ± 0.41[b] | 166 ± 4.61[b] | 0.27 ± 0.02[b] | 214 ± 4.51[b] | 2.54 ± 0.11[c] | 55.0 ± 1.34[e] |
| **TV10** | 33.7 ± 1.45[f] | 3.70 ± 0.39[e] | 7.70 ± 1.02[d] | 129 ± 5.15[f] | 35.2 ± 2.29[b] | 163 ± 5.06[e] | 45.0 ± 1.98[g] | 42.9 ± 2.77[b] | 162 ± 5.88[b] | 0.27 ± 0.02 | 209 ± 6.86[b] | 3.64 ± 0.08[h] | 39.7 ± 0.70[b] |
| **TV11** | 38.6 ± 1.23[c] | 3.20 ± 0.15[e] | 11.4 ± 0.54[b] | 141 ± 1.35[g] | 37.4 ± 0.67[c] | 178 ± 2.02[f] | 53.3 ± 1.86[f] | 48.8 ± 1.06[c] | 180 ± 2.50[e] | 0.27 ± 0.01[b] | 236 ± 3.69[f] | 3.35 ± 0.09[h] | 38.4 ± 0.83[b] |
| **TV12** | 50.8 ± 3.91[g] | 3.40 ± 0.34[e] | 11.4 ± 0.15[b] | 113 ± 0.75[b] | 42.8 ± 3.21[d] | 155 ± 3.87[b] | 65.5 ± 4.39[d] | 54.2 ± 3.34[d] | 164 ± 4.64[b] | 0.33 ± 0.01[c] | 221 ± 8.21[c] | 2.37 ± 0.11[b] | 51.6 ± 1.24[a] |
| **TV13** | 43.9 ± 0.80[b] | 8.50 ± 0.38[a] | 10.6 ± 0.55[c] | 118 ± 1.47[c] | 28.5 ± 1.37[e] | 147 ± 2.37[b] | 63.0 ± 1.16[b] | 39.1 ± 1.91[e] | 162 ± 2.11[b] | 0.24 ± 0.01[d] | 210 ± 3.41[b] | 2.33 ± 0.02[b] | 45.0 ± 0.44[d] |
| **TV14** | 26.1 ± 0.47[a] | 7.40 ± 0.48[b] | 9.30 ± 0.78[c] | 82.5 ± 2.26[a] | 46.1 ± 2.24[a] | 129 ± 4.25[a] | 42.8 ± 0.66[h] | 55.4 ± 2.76[d] | 109 ± 1.81[f] | 0.51 ± 0.02[e] | 171 ± 4.88[g] | 3.00 ± 0.06[h] | 48.4 ± 0.99[a] |
| **TV15** | 40.9 ± 3.24[c] | 4.70 ± 0.38[d] | 21.2 ± 2.52[e] | 99.9 ± 3.75[h] | 53.4 ± 2.40[f] | 153 ± 2.50[b] | 66.8 ± 5.64[d] | 74.6 ± 4.83[f] | 141 ± 0.75[g] | 0.53 ± 0.04[e] | 220 ± 4.31[c] | 2.29 ± 0.21[b] | 47.9 ± 0.84[a] |
| **TV16** | 21.7 ± 1.39[h] | 8.90 ± 1.48[a] | 14.5 ± 0.97[a] | 81.4 ± 1.32[a] | 60.2 ± 0.69[g] | 142 ± 1.53[b] | 45.1 ± 1.94[g] | 74.7 ± 0.39[f] | 103 ± 2.46[a] | 0.73 ± 0.02[f] | 187 ± 3.44[h] | 3.14 ± 0.10[h] | 44.4 ± 0.83[d] |
| **TV17** | 55.6 ± 1.87[d] | 8.20 ± 0.41[a] | 37.2 ± 0.35[f] | 70.4 ± 1.03[i] | 52.3 ± 0.52[f] | 123 ± 1.01[g] | 101 ± 1.58[i] | 89.6 ± 0.37[g] | 126 ± 1.91[h] | 0.71 ± 0.01[f] | 224 ± 2.04[c] | 1.21 ± 0.02[i] | 47.2 ± 0.41[a] |
| **TV18** | 33.2 ± 2.65[f] | 5.60 ± 0.24[b] | 10.0 ± 1.38[c] | 101 ± 1.94[h] | 29.6 ± 1.20[e] | 130 ± 2.34[a] | 48.8 ± 3.89[a] | 39.7 ± 1.80[e] | 134 ± 4.59[i] | 0.30 ± 0.01[c] | 179 ± 5.98[a] | 2.67 ± 0.19[c] | 46.8 ± 1.47[a] |
| **TV19** | 23.2 ± 0.72[a] | 5.30 ± 0.38[b] | 12.9 ± 0.46[b] | 122 ± 1.32[e] | 48.7 ± 1.48[a] | 170 ± 2.51[h] | 41.4 ± 1.45[h] | 61.6 ± 1.93[a] | 145 ± 1.91[j] | 0.43 ± 0.01[g] | 212 ± 3.87[b] | 4.11 ± 0.09[j] | 46.6 ± 1.56[a] |
| **TV20** | 45.4 ± 0.74[b] | 3.60 ± 0.22[e] | 10.8 ± 0.36[c] | 120 ± 2.77[e] | 32.3 ± 0.92[b] | 152 ± 2.40[b] | 59.8 ± 1.10[c] | 43.1 ± 1.25[b] | 165 ± 2.11[b] | 0.26 ± 0.01[b] | 212 ± 1.58[b] | 2.54 ± 0.08[c] | 46.5 ± 0.84[a] |
| **TV21** | 50.5 ± 0.96[g] | 7.40 ± 0.55[b] | 10.7 ± 0.87[c] | 84.5 ± 2.33[a] | 21.8 ± 1.02[h] | 106 ± 3.35[i] | 68.6 ± 1.37[d] | 32.5 ± 0.44[h] | 135 ± 3.00[i] | 0.24 ± 0.01[b] | 175 ± 3.84[g,a] | 1.55 ± 0.05[e] | 37.9 ± 0.95[b] |
| **TV22** | 42.2 ± 3.10[b] | 3.40 ± 0.24[e] | 17.2 ± 0.95[g] | 103 ± 2.40[h] | 44.7 ± 0.46[i] | 148 ± 2.70[b] | 62.8 ± 3.76[b] | 61.9 ± 1.20[a] | 146 ± 5.39[j] | 0.43 ± 0.01[g] | 211 ± 6.46[b] | 2.36 ± 0.10[b] | 44.8 ± 1.40[d] |
| **TV23** | 50.6 ± 1.39[g] | 8.70 ± 0.23[a] | 25.3 ± 0.70[h] | 103 ± 3.82[h] | 49.6 ± 2.60[a] | 153 ± 6.31[b] | 84.6 ± 2.11[j] | 74.9 ± 3.22[f] | 154 ± 5.21[c] | 0.49 ± 0.01[h] | 238 ± 8.41[f] | 1.81 ± 0.03[e] | 43.7 ± 1.11[d] |
| **TV24** | 29.0 ± 2.21[e] | 8.10 ± 0.51[a] | 14.3 ± 0.98[i] | 71.2 ± 3.69[i] | 43.6 ± 0.52[i] | 115 ± 4.18[d] | 51.4 ± 3.59[f] | 57.9 ± 1.37[d] | 100 ± 5.90[k] | 0.58 ± 0.02[a] | 166 ± 7.73[i] | 2.23 ± 0.07[b] | 39.9 ± 1.21[b] |
| **TV25** | 41.2 ± 0.93[c] | 6.70 ± 0.46[b] | 10.8 ± 1.15[c] | 116 ± 3.97[b] | 33.6 ± 1.23[b] | 150 ± 4.96[b] | 58.7 ± 2.01[c] | 44.4 ± 2.27[b] | 158 ± 4.84[c] | 0.28 ± 0.01[b] | 208 ± 6.78[b] | 2.55 ± 0.04[b] | 35.7 ± 1.26[f] |
| **TV26** | 44.6 ± 0.44[b] | 3.30 ± 0.28[e] | 11.6 ± 0.71[b] | 98.2 ± 1.19[h] | 33.7 ± 1.70[b] | 132 ± 2.73[a] | 59.5 ± 0.91[c] | 45.3 ± 1.01[b] | 143 ± 1.27[j] | 0.32 ± 0.01[c] | 191 ± 2.10[j] | 2.22 ± 0.07[b] | 39.6 ± 0.62[b] |
| **TV27** | 26.3 ± 0.74[a] | 9.30 ± 0.23[a] | 9.80 ± 1.01[c] | 89.0 ± 4.65[j] | 33.3 ± 0.87[b] | 122 ± 3.78[g] | 45.4 ± 0.84[g] | 43.2 ± 1.63[b] | 115 ± 4.51[d] | 0.38 ± 0.03[i] | 168 ± 3.07[i] | 2.70 ± 0.13[b] | 41.3 ± 0.80[c] |
| **TV28** | 27.2 ± 0.44[a] | 9.70 ± 0.26[a] | 12.6 ± 1.77[b] | 88.5 ± 2.53[j] | 35.3 ± 0.57[b] | 123 ± 2.79[g] | 49.4 ± 2.10[a] | 47.9 ± 2.34[c] | 116 ± 2.35[d] | 0.41 ± 0.02[j] | 173 ± 4.20[a] | 2.51 ± 0.10[b] | 42.9 ± 1.73[d] |
| **TV29** | 32.0 ± 0.38[f] | 4.40 ± 0.34[d] | 12.8 ± 0.74[b] | 105 ± 4.83[h] | 47.8 ± 0.92[a] | 153 ± 5.73[b] | 49.2 ± 1.03[a] | 60.6 ± 1.65[a] | 138 ± 5.03[i] | 0.44 ± 0.01[g] | 203 ± 6.76[b] | 3.12 ± 0.05[h] | 41.2 ± 0.35[c] |
| **TV30** | 25.4 ± 0.75[a] | 15.2 ± 0.99[f] | 15.6 ± 2.51[i] | 78.0 ± 2.29[a] | 16.8 ± 1.00[j] | 94.8 ± 1.42[i] | 56.2 ± 2.98[c] | 32.5 ± 2.83[h] | 103 ± 2.81[k] | 0.31 ± 0.03[c] | 151 ± 4.21[k] | 1.69 ± 0.07[e] | 35.3 ± 1.56[f] |
| **TV31** | 21.1 ± 0.35[h] | 7.10 ± 0.64[b] | 10.8 ± 0.47[c] | 115 ± 1.56[b] | 39.2 ± 2.25[k] | 154 ± 3.40[g] | 39.0 ± 0.32[k] | 50.0 ± 2.44[d] | 137 ± 1.71[i] | 0.37 ± 0.02[i] | 193 ± 3.71[j] | 3.95 ± 0.06[h] | 41.3 ± 0.51[c] |
| **Betjan** | 48.9 ± 0.45[g] | 6.30 ± 0.35[b] | 13.0 ± 0.47[b] | 101 ± 2.47[h] | 40.7 ± 1.30[k] | 142 ± 3.74[b] | 68.1 ± 0.70[d] | 53.7 ± 1.75[d] | 150 ± 2.91[c] | 0.36 ± 0.01[i] | 210 ± 4.36[b] | 2.08 ± 0.04[b] | 42.3 ± 0.84[d] |
| **Kharijan** | 54.8 ± 0.58[d] | 6.70 ± 0.43[b] | 14.2 ± 0.29[i] | 105 ± 2.87[h] | 32.7 ± 0.67[b] | 137 ± 2.65[j] | 75.6 ± 1.16[l] | 46.8 ± 0.67[b] | 160 ± 3.08[b] | 0.29 ± 0.01[b] | 213 ± 3.70[b] | 1.82 ± 0.02[e] | 46.8 ± 1.73[a] |
| **S.3/A3** | 57.1 ± 0.47[i] | 10.0 ± 0.51[a] | 20.8 ± 0.24[e] | 84.3 ± 0.34[a] | 30.1 ± 0.41[b] | 114 ± 0.53[b] | 87.9 ± 0.38[m] | 50.8 ± 0.33[d] | 141 ± 0.23[j] | 0.36 ± 0.01[i] | 202 ± 0.91[b] | 1.30 ± 0.01[e] | 37.6 ± 0.71[b] |
| **T.3/E3** | 57.4 ± 0.54[i] | 5.00 ± 0.18[b] | 22.6 ± 0.35[e] | 95.5 ± 1.91[k] | 27.3 ± 0.53[e] | 123 ± 1.58[g] | 85.0 ± 0.78 | 49.9 ± 0.24[d] | 153 ± 1.46[c] | 0.33 ± 0.01[c] | 208 ± 1.32[b] | 1.44 ± 0.03[e] | 42.3 ± 0.26[d] |

* EGC, (−)-epigallocatechin; +C, (+)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechingallate; ECG, (−)-epicatechingallate; GaC, galloylated catechins (EGCG + ECG); NGaC, non-galloylated catechins (EC + EGC); DHC, dihydroxy catechins (EC + ECG); THC, trihydroxy catechins (EGC + EGCG); CI, catechin index

(DHC/THC); TC, total catechin; GNR, galloylated to non-galloylated catechin ratio (GaC/NGaC); CAF, caffeine. All units, except those for CI and GNR, are in mg $g^{-1}$(dry weight basis). Values are 'mean ± SE' of independent triplicate measurements.

[#] Same symbol within a column denotes not significant whereas different symbol denotes significant difference.

**Table 4**
Heatmap of the correlation matrix between catechins and caffeine content in tea leaves harvested in monsoon.




CAF, caffeine ;EGC, (−)-epigallocatechin; C, (+)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechingallate; ECG, (−)-epicatechingallate; TC, total catechin; DHC, dihydroxylated catechins; THC, trihydroxylated catechins; GaC, galloylated catechins; NGaC, non-galloylated catechins; GNR, galloylated to non-galloylated catechin ratio.

much higher (122.0 and 41.0 mg $g^{-1}$, respectively) than that in spring (88.0 and 28.0 mg $g^{-1}$, respectively) (Chu and Juneja, 1997). This seasonal variation could be due to the active synthesis of these catechins in the plant tissues which gets retarded with a decrease in temperature (Bockuchava and Skobeleva, 1969). This increased level of catechins with increasing daylight and temperature can be associated with significant enhancement of expression of key genes involved in phenylpropanoid metabolism leading to the accumulation of phenylpropanoids (catechins) in response to light in tea callus (Wang et al., 2012). EGC did not follow the same seasonal pattern as those of EGCG and ECG. It showed the highest level in pre-monsoon for most of the cultivars and then slowly decreased with an increase in temperature and day length in the warmer months. This finding conforms with the earlier study of Bockuchava and Skobeleva (1969) where the authors reported that the winter period favours higher level of EGC formation. In contrast, Fang et al. (2017) observed that EGC levels in cultivars from China increased with increase in temperature of the growing region. They reported significantly higher levels of EGC in 12 of the 21 cultivars studied. Fang et al. (2017) also demonstrated that EGC could be used as a marker for seasonal classification of harvests with 61% accuracy. In Australia and central Africa, EGC levels in the tea shoots harvested during the cold season were higher than that harvested in warmer seasons (Hilton and Palmer-Jones, 1973; Yao et al., 2005). Yao et al. (2005) further demonstrated that due to higher biosynthetic activity during the growth of shoots, EGCG level was three times the ECG level which corresponds to the present findings. One more possibility, supporting the results of this investigation, is that esterification of EC and EGC with gallate may lead to the formation of ECG and EGCG, respectively (Harbowy and Balentine, 1997). The increased TC content in warmer months may be justified by the active synthesis of EGCG and ECG in tea shoots. As a significant quantity is used up in EGCG biosynthesis, the EGC level is decreased in summer months (Yao et al., 2005). The result of the present study and the studies of Yao et al. (2005) suggest no possibility of peak levels of these flavanols at the same harvesting period as the biosynthesis of EGC responds to temperature in the opposite way as those of EGCG and ECG. Therefore, black tea quality could be determined predominantly by catechin gallates rather than catechins in tea shoots as the catechin gallates quantitatively dominate the catechins in tea shoots (Yao et al., 2005).

### 3.2. Catechin and genetic diversity

Significant variations in TC content, as well as individual catechins, were observed among the different varieties of tea cultivars (Table 7). Furthermore, a significant influence of seasonal variation on catechin and genetic diversity was observed for all 35 cultivars (Table 8). Conforming to the earlier studies TC content can be conveniently used for discriminative identification of CH variety from the other groups of tea (Jin et al., 2014; Magoma et al., 2000; Sabhapondit et al., 2012). The Assam variety had the highest TC content throughout the harvesting period whereas CH variety had the markedly lowest content. This result is in agreement with the study of Sabhapondit et al. (2012) on the catechin profiles of some NE India tea cultivars. In pre-monsoon, monsoon and autumn, average TC content in shoots were 192 ± 4.27, 210 ± 3.02 and 161 ± 2.86 mg $g^{-1}$; 173 ± 6.02, 194 ± 6.41 and 153 ± 5.12 mg $g^{-1}$; 166 ± 26.1, 200 ± 16.5 and 162 ± 17.6 mg $g^{-1}$; 164 ± 10.4, 179 ± 7.68 and 155 ± 9.98 mg $g^{-1}$; and 132 ± 2.09, 160 ± 2.07 and 133 ± 3.80 mg $g^{-1}$ for Assam, Cambod, ACH, AH, and CH varieties, respectively. The content of EGCG, the most dominant catechin, in the Cambod variety had the highest contribution to TC at 51.72, 52.62 and 54.18% in pre-monsoon, monsoon, and autumn, respectively, whereas that of ACH variety was lowest at 35.35, 37.75, and 42.38% for the respective seasons. ECG is the second largest contributor towards TC after EGCG for Cambod, ACH, AH, and CH varieties, while it is the third largest (after EGCG and EGC) for Assam variety. The highest ECG contribution to TC, among the varieties, was for AH at 27.59% (averaged over the seasons), whereas the lowest was for Assam variety at 15.07% (averaged over the seasons). The Assam variety can be characterised by high EGC content as reported by Sabhapondit et al. (2012). The EGC content in Assam variety contributed 24.26% (averaged over the seasons) to its TC content.

TC content in green leaf and the ratio of dihydroxy catechin (DHC) to trihydroxy catechin (THC), termed as catechin index (CI), were used to express the genetic differentiation in the Kenyan tea germplasm (Magoma et al., 2000). The authors demonstrated THC (sum of EGC and EGCG) level as a useful tool for identification of Chinese variety whereas DHC (sum of EC and ECG) levels could not give any clarity. In the present study, DHC and THC levels could not ascertain any clear picture of genetic diversity. This may be due to the extensive hybridization practices which resulted in varieties like ACH, AH, etc. The common practice of hybridization between different tea taxa leads to difficulty in assigning a cultivar to a particular varietal taxa (Visser, 1969). In the current study, CI was comparatively higher for ACH and AH varieties (0.50-0.67) indicating a high contribution of DHC to TC whereas, CI was lowest for Assam variety as it had a higher content of EGC than ECG, thereby, leading to low contribution of DHC to TC content (Table 7). Hence, low CI can also be seen as a characteristic property of Assam varieties in addition to high EGC level. Previous studies from South India (Saravanan et al., 2005) and Kenya (Magoma et al., 2000) observed the lowest CI (1:4) for Assam variety which conforms to our findings. CI can be applied as a tool for the identification of high-quality cultivars in tea breeding programmes (Punyasiri et al., 2017; Wei et al., 2011). The observed wide ranges of catechin content are the result of the genetically controlled synthesis of these secondary metabolites in different cultivars (Fujimura et al., 2011).

**Table 5**
Catechin profile and caffeine content of cultivars in autumn

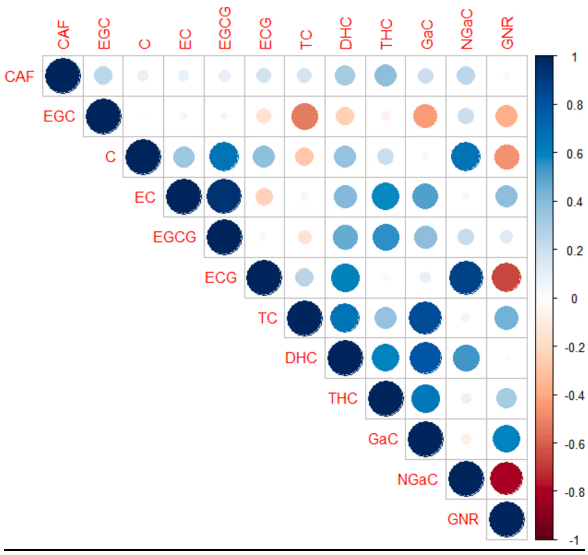
| Cultivar | Parameters* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EGC | +C | EC | EGCG | ECG | GaC | NGaC | DHC | THC | CI | TC | GNR | CAF |
| **TV1** | 23.5 ± 0.57[a,#] | 10.2 ± 0.55[a] | 10.3 ± 0.75[a] | 72.2 ± 2.11[a] | 28.3 ± 1.74[a] | 100 ± 3.46[a] | 44.0 ± 0.90[a] | 38.6 ± 2.49[a] | 95.7 ± 2.19[a] | 0.40 ± 0.02[a] | 144 ± 3.90[a] | 2.29 ± 0.07[a] | 36.8 ± 0.82[a] |
| **TV2** | 41.7 ± 2.35[b] | 4.40 ± 0.27[b] | 10.1 ± 0.20[a] | 95.3 ± 4.53[b] | 17.8 ± 0.82[b] | 113 ± 5.17[a] | 56.2 ± 2.28[b] | 27.8 ± 0.82[b] | 137 ± 2.20[b] | 0.20 ± 0.01[b] | 169 ± 2.90[b] | 2.03 ± 0.18[a] | 40.7 ± 0.42[b] |
| **TV3** | 37.2 ± 2.02[c] | 9.20 ± 0.53[a] | 7.80 ± 0.44[b] | 91.4 ± 2.74[b] | 20.1 ± 0.32[c] | 111 ± 2.69[a] | 54.2 ± 1.23[b] | 27.9 ± 0.74[b] | 128 ± 4.05[c] | 0.22 ± 0.01[b] | 165 ± 3.60[b] | 2.06 ± 0.04[a] | 36.3 ± 0.87[a] |
| **TV4** | 36.6 ± 2.35[c] | 7.30 ± 0.90[c] | 11.2 ± 1.66[a] | 77.3 ± 2.84[c] | 17.9 ± 2.02[b] | 95.2 ± 4.77[b] | 55.1 ± 1.16[b] | 29.1 ± 3.53[b] | 133 ± 1.28[b] | 0.26 ± 0.03[b] | 150 ± 5.10[c] | 1.73 ± 0.09[b] | 35.7 ± 0.61[a] |
| **TV5** | 34.0 ± 2.62[c] | 9.90 ± 0.89[a] | 8.80 ± 0.68[b] | 77.7 ± 5.78[c] | 17.1 ± 0.73[b] | 94.9 ± 6.41[b] | 52.8 ± 1.23[b] | 25.9 ± 1.38[b] | 111 ± 3.44[d] | 0.23 ± 0.01[b] | 147 ± 5.29[a] | 1.80 ± 0.16[b] | 35.8 ± 1.16[a] |
| **TV6** | 39.5 ± 2.46[c] | 12.3 ± 1.47[c] | 10.0 ± 0.78[a] | 80.2 ± 2.95[c] | 20.2 ± 0.67[c] | 100 ± 3.58[a] | 61.8 ± 2.75[c] | 30.2 ± 1.38[b] | 119 ± 2.79[d] | 0.25 ± 0.01[b] | 162 ± 5.29[b] | 1.63 ± 0.07[b] | 38.7 ± 0.99[a] |
| **TV7** | 20.5 ± 0.62[d] | 5.90 ± 0.74[b] | 7.20 ± 0.37[b] | 71.1 ± 3.79[a] | 28.3 ± 0.64[a] | 99.4 ± 3.55[a] | 33.5 ± 0.27[d] | 35.4 ± 0.68[c] | 91.6 ± 4.40[a] | 0.39 ± 0.02[c] | 133 ± 3.80[d] | 2.96 ± 0.08[a] | 38.2 ± 0.50[a] |
| **TV8** | 32.0 ± 3.07[e] | 2.80 ± 0.15[d] | 6.70 ± 0.36[c] | 91.8 ± 2.76[b] | 18.8 ± 0.85[b] | 110 ± 3.30[a] | 41.6 ± 3.20[a] | 25.5 ± 1.19[b] | 123 ± 4.93[c] | 0.21 ± 0.01[b] | 152 ± 5.32[c] | 2.69 ± 0.18[a] | 34.8 ± 1.74[a] |
| **TV9** | 30.6 ± 0.66[e] | 2.40 ± 0.26[d] | 9.10 ± 0.32[a] | 107 ± 2.78[d] | 29.0 ± 0.93[a] | 136 ± 3.55[c] | 42.0 ± 1.07[a] | 38.1 ± 1.25[a] | 137 ± 3.34[b] | 0.28 ± 0.01[b] | 178 ± 4.56[e] | 3.24 ± 0.03[c] | 38.7 ± 1.95[a] |
| **TV10** | 36.6 ± 2.58[c] | 2.40 ± 0.23[d] | 6.80 ± 0.46[c] | 108 ± 2.15[d] | 27.9 ± 2.18[a] | 135 ± 3.89[c] | 45.8 ± 2.42[a] | 34.7 ± 2.54[c] | 144 ± 4.53[e] | 0.24 ± 0.02[b] | 181 ± 5.82[e] | 2.97 ± 0.11[a,c] | 34.7 ± 1.24[a] |
| **TV11** | 39.3 ± 0.57[c] | 2.00 ± 0.15[d] | 10.8 ± 0.99[a] | 98.3 ± 5.22[b] | 28.5 ± 1.96[a] | 126 ± 7.18[c] | 52.1 ± 1.30[b] | 39.3 ± 2.95[a] | 137 ± 5.33[b] | 0.29 ± 0.01[b] | 178 ± 8.32[e] | 2.42 ± 0.09[a] | 30.7 ± 1.44[c] |
| **TV12** | 42.4 ± 2.71[b] | 2.70 ± 0.49[d] | 9.00 ± 0.46[a] | 83.9 ± 2.79[c] | 20.9 ± 0.99[c] | 104 ± 3.59[a] | 54.1 ± 2.85[b] | 28.8 ± 1.44[b] | 126 ± 3.33[c] | 0.24 ± 0.01[b] | 158 ± 4.00[c] | 1.95 ± 0.13[a,b] | 36.0 ± 1.09[a] |
| **TV13** | 35.8 ± 0.95[c] | 6.00 ± 0.49[b] | 13.6 ± 0.84[d] | 80.5 ± 3.63[c] | 20.8 ± 1.22[c] | 101 ± 4.80[a] | 55.4 ± 0.87[b] | 34.4 ± 0.38[c] | 116 ± 4.29[d] | 0.30 ± 0.01[b] | 156 ± 4.22[c] | 1.83 ± 0.11[a,b] | 38.3 ± 0.70[a] |
| **TV14** | 24.3 ± 2.00[a] | 10.1 ± 0.70[a] | 10.4 ± 1.47[a] | 62.7 ± 1.96[e] | 38.4 ± 1.88[d] | 101 ± 3.68[a] | 44.8 ± 2.83[a] | 48.8 ± 3.31[d] | 87.0 ± 2.88[f] | 0.56 ± 0.03[d] | 146 ± 5.38[a] | 2.27 ± 0.14[a] | 31.7 ± 0.64[c] |
| **TV15** | 37.6 ± 2.11[c] | 2.80 ± 0.20[d] | 11.1 ± 0.84[a] | 92.6 ± 4.30[b] | 25.0 ± 0.73[e] | 117 ± 4.63[a] | 51.5 ± 1.98[b] | 36.1 ± 0.83[c] | 130 ± 5.29[c] | 0.28 ± 0.01[b] | 169 ± 5.25[b] | 2.29 ± 0.12[a] | 29.4 ± 0.99[c] |
| **TV16** | 30.8 ± 0.79[e] | 11.0 ± 0.87[a] | 12.0 ± 0.90[a] | 70.2 ± 2.34[a] | 41.8 ± 0.77[f] | 112 ± 3.10[a] | 53.8 ± 1.89[b] | 53.8 ± 1.64[e] | 101 ± 2.86[g] | 0.53 ± 0.01[d] | 165 ± 4.33[b] | 2.09 ± 0.07[a] | 34.4 ± 2.44[c] |
| **TV17** | 42.9 ± 2.03[b] | 6.10 ± 0.34[b] | 25.2 ± 0.85[e] | 65.2 ± 3.00[e] | 40.4 ± 0.27[f] | 105 ± 3.15[a] | 74.2 ± 1.50[e] | 65.5 ± 1.07[f] | 108 ± 1.86[d] | 0.61 ± 0.01[e] | 179 ± 2.14[e] | 1.42 ± 0.07[b] | 38.7 ± 0.64[a] |
| **TV18** | 25.7 ± 0.55[a] | 2.40 ± 0.24[d] | 7.90 ± 1.18[b] | 90.9 ± 6.03[b] | 23.0 ± 2.55[c] | 113 ± 8.29[a] | 35.9 ± 1.34[d] | 30.8 ± 2.96[b] | 116 ± 5.49[d] | 0.26 ± 0.02[b] | 149 ± 7.76[a] | 3.19 ± 0.30[d] | 32.1 ± 1.32[c] |
| **TV19** | 19.9 ± 0.47[d] | 2.40 ± 0.18[d] | 9.50 ± 1.07[a] | 92.7 ± 4.05[b] | 49.0 ± 1.07[g] | 141 ± 4.53[a] | 31.8 ± 1.03[d] | 58.4 ± 2.09[g] | 112 ± 4.41[d] | 0.52 ± 0.02[d] | 173 ± 5.55[e] | 4.45 ± 0.02[e] | 35.5 ± 0.30[a] |
| **TV20** | 34.8 ± 1.43[c] | 1.80 ± 0.15[e] | 9.20 ± 0.55[a] | 83.8 ± 2.58[c] | 25.9 ± 0.23[c] | 109 ± 2.78[a] | 45.8 ± 2.07[a] | 35.1 ± 0.78[c] | 118 ± 4.00[d] | 0.30 ± 0.01[b] | 155 ± 4.83[c] | 2.40 ± 0.05[a] | 33.5 ± 1.82[a] |
| **TV21** | 37.7 ± 0.53[c] | 2.20 ± 0.24[d] | 6.20 ± 0.55[c] | 79.6 ± 2.38[c] | 17.8 ± 0.55[b] | 97.4 ± 1.99[b] | 46.0 ± 1.02[a] | 23.9 ± 0.07[b] | 117 ± 2.81[d] | 0.20 ± 0.01[b] | 143 ± 2.57[a] | 2.12 ± 0.05[a] | 33.5 ± 0.72[a] |
| **TV22** | 33.0 ± 0.98[c] | 1.90 ± 0.09[d,e] | 15.1 ± 0.94[f] | 93.7 ± 3.33[b,c] | 32.8 ± 1.82[h] | 126 ± 4.78[c] | 50.0 ± 1.85[b] | 47.8 ± 2.76[d] | 126 ± 3.91[c] | 0.38 ± 0.01[a] | 176 ± 6.36[e] | 2.53 ± 0.06[a] | 35.0 ± 2.26[a] |
| **TV23** | 38.2 ± 1.79[c] | 2.70 ± 0.33[d] | 14.7 ± 1.05[f] | 83.6 ± 4.58[c] | 38.5 ± 1.30[d] | 122 ± 5.28[c] | 55.7 ± 1.53[b] | 53.2 ± 1.96[e] | 121 ± 3.52[c] | 0.44 ± 0.01[a] | 177 ± 5.63[e] | 2.19 ± 0.11[a] | 36.8 ± 0.61[a] |
| **TV24** | 32.0 ± 1.08[c] | 7.60 ± 0.35[f] | 11.0 ± 0.73[a] | 58.4 ± 2.47[f] | 21.4 ± 3.73[c] | 79.8 ± 2.64[d] | 50.6 ± 1.68[b] | 32.4 ± 1.40[c] | 90.4 ± 1.82[h] | 0.36 ± 0.01[a] | 130 ± 3.03[d] | 1.58 ± 0.07[b] | 28.1 ± 0.23[d] |
| **TV25** | 28.3 ± 0.55[e] | 2.40 ± 0.26[d] | 8.50 ± 1.29[b] | 89.8 ± 2.64[b] | 29.4 ± 1.10[a] | 119 ± 2.43[a] | 39.2 ± 1.91[f] | 37.9 ± 1.42[c] | 118 ± 3.19[d] | 0.32 ± 0.01[a,c] | 158 ± 4.21[c] | 3.04 ± 0.10[d] | 27.6 ± 1.11[d] |
| **TV26** | 33.6 ± 1.31[c] | 1.60 ± 0.18[e] | 8.30 ± 0.90[b] | 88.1 ± 3.99[b] | 20.2 ± 1.71[c] | 108 ± 5.64[a] | 43.5 ± 0.41[a] | 28.4 ± 2.61[b] | 121 ± 3.15[d] | 0.23 ± 0.02[b] | 151 ± 5.54[c] | 2.49 ± 0.14[a] | 30.2 ± 1.05[c] |
| **TV27** | 23.4 ± 0.82[a] | 6.30 ± 0.37[b] | 7.60 ± 1.03[b] | 69.3 ± 1.84[a] | 22.8 ± 0.95[c] | 92.1 ± 2.34[b] | 37.2 ± 1.65[f] | 30.4 ± 1.98[b,c] | 92.7 ± 2.56[h] | 0.33 ± 0.02[c,a] | 129 ± 3.96[d] | 2.48 ± 0.05[a] | 29.3 ± 1.11[c] |
| **TV28** | 24.5 ± 0.62[a] | 7.50 ± 0.51[f] | 8.00 ± 0.84[b] | 68.5 ± 3.88[a] | 25.9 ± 0.22[c] | 94.3 ± 3.73[b] | 40.1 ± 1.53[a] | 33.9 ± 0.70[c] | 93.0 ± 4.41[h] | 0.37 ± 0.01[c] | 134 ± 5.04[d] | 2.35 ± 0.06[a] | 31.5 ± 0.32[c] |
| **TV29** | 23.6 ± 2.06[a] | 2.20 ± 0.18[d] | 9.10 ± 1.19[a] | 93.0 ± 3.25[b,c] | 31.2 ± 4.19[h] | 124 ± 7.20[c] | 34.9 ± 1.10[d] | 40.2 ± 5.38[a] | 116 ± 2.27[d] | 0.34 ± 0.04[c] | 159 ± 6.39[c] | 3.58 ± 0.31[d] | 34.3 ± 2.23[a] |
| **TV30** | 21.2 ± 2.21[a] | 1.40 ± 0.21[e] | 11.9 ± 0.49[a] | 72.9 ± 2.40[a] | 14.2 ± 0.69[i] | 87.1 ± 2.76[e] | 34.5 ± 2.38[d] | 26.0 ± 1.05[b] | 94.1 ± 3.53[h] | 0.28 ± 0.01[b] | 121 ± 4.45[f] | 2.54 ± 0.14[a] | 30.4 ± 2.32[c] |
| **TV31** | 30.5 ± 1.31[c] | 3.30 ± 0.39[g] | 17.7 ± 0.94[g] | 68.6 ± 4.37[a] | 25.6 ± 1.70[c] | 94.3 ± 3.86[b] | 51.5 ± 2.31[b] | 43.3 ± 2.56[a] | 99.1 ± 4.68[a] | 0.44 ± 0.04[a] | 145 ± 4.68[c] | 1.84 ± 0.10[b] | 29.6 ± 0.90[c] |
| **Betjan** | 32.3 ± 2.62[c] | 2.40 ± 0.15[d] | 7.70 ± 0.87[b] | 83.5 ± 1.28[c] | 21.2 ± 0.23[c] | 104 ± 1.52[a] | 42.4 ± 2.02[a] | 28.8 ± 0.97[b] | 115 ± 3.47[d] | 0.25 ± 0.01[b] | 147 ± 3.32[c] | 2.48 ± 0.09[a] | 32.9 ± 0.71[a] |
| **Kharijan** | 40.6 ± 0.69[b] | 3.10 ± 0.20[g] | 8.70 ± 1.48[b] | 90.4 ± 4.90[b] | 23.4 ± 2.62[c] | 113 ± 7.52[a] | 52.4 ± 1.08[b] | 32.0 ± 4.05[c] | 131 ± 4.23[b] | 0.24 ± 0.02[b] | 166 ± 8.23[b] | 2.17 ± 0.12[a] | 37.2 ± 1.76[a] |
| **S.3/A3** | 42.4 ± 0.27[b] | 1.50 ± 0.15[e] | 11.6 ± 0.41[a] | 73.1 ± 1.01[a] | 28.0 ± 0.81[a] | 101 ± 1.63[a] | 55.6 ± 0.60[b] | 39.6 ± 1.09[c] | 115 ± 0.75[d] | 0.34 ± 0.01[c] | 156 ± 1.41[c] | 1.82 ± 0.04[b] | 31.2 ± 0.59[a] |
| **T.3/E3** | 51.2 ± 1.23[f] | 2.20 ± 0.15[d] | 14.7 ± 0.49[f] | 80.7 ± 0.52[c] | 23.4 ± 0.41[c] | 104 ± 0.44[a] | 68.1 ± 1.85[g] | 38.1 ± 0.55[c] | 131 ± 1.65[b] | 0.29 ± 0.01[b] | 172 ± 2.28[e] | 1.53 ± 0.03[b] | 36.0 ± 0.46[a] |

* EGC, (−)-epigallocatechin; +C, (+)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechingallate; ECG, (−)-epicatechingallate; GaC, galloylated catechins (EGCG + ECG); NGaC, non-galloylated catechins (EC + EGC); DHC, dihydroxy catechins (EC + ECG); THC, trihydroxy catechins (EGC + EGCG); CI, catechin index

(DHC/THC); TC, total catechin; GNR, galloylated to non-galloylated catechin ratio (GaC/NGaC); CAF, caffeine. All units, except those for CI and GNR, are in mg $g^{-1}$(dry weight basis). Values are 'mean ± SE' of independent triplicate measurements.

[#] Same symbol within a column denotes not significant whereas different symbol denotes significant difference.

**Table 6**
Heatmap of the correlation matrix between catechins and caffeine content in tea leaves harvested in autumn.




CAF, caffeine ;EGC, (−)-epigallocatechin; C, (+)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechingallate; ECG, (−)-epicatechingallate; TC, total catechin; DHC, dihydroxylated catechins; THC, trihydroxylated catechins; GaC, galloylated catechins; NGaC, non-galloylated catechins; GNR, galloylated to non-galloylated catechin ratio.

### 3.3. Galloylated catechins and non-galloylated catechins

The principal galloylated catechins (GaCs) are EGCG and ECG, whereas EGC, (+)C and EC constitute the non-galloylated catechins (NGaCs). The bioactivity of GaCs is considered to be higher than that of NGaCs (Wolfram et al., 2006). Xu et al. (2012) projected the catechin profile as one of the season discriminating parameters. They reported a higher level of GaCs (78.00 vs 54.88 mg $g^{-1}$) and NGaCs (12.09 vs 8.57 mg $g^{-1}$) in summer compared to spring. The level of total GaCs could be used as a quality indicator of black tea processed from Kenyan tea cultivars (Owuor and Obanda, 2007).

In pre-monsoon, GaCs content varied from 84.4 ± 3.15 mg $g^{-1}$ in TV30 to 157 ± 2.37 mg $g^{-1}$ in TV19 which represented a 1.86 fold variation (Table 1). TV6 presented the highest NGaCs content with 92.4 ± 4.46 mg $g^{-1}$ and that of TV7 was the lowest with 30.8 ± 1.18 mg $g^{-1}$, representing a 3.00 fold difference. The variation in the extent of galloylation, defined by the ratio of GaCs to NGaCs [GNR=(ECG + EGCG)/(EC + EGC)], was observed in the range between 1.16 and 4.33. In monsoon, GaC contents ranged from 94.8 ± 1.42 mg $g^{-1}$ in TV30 to 178 ± 2.02 mg $g^{-1}$ in TV11, which expressed a 1.88 fold variation (Table 3). TV17 had the highest content of NGaCs with 101 ± 1.58 mg $g^{-1}$, whereas TV31 had the lowest value with 39.0 ± 0.32 mg $g^{-1}$. The highest content differed from the lowest with a factor of 2.59. GNR was observed in the range between 1.21 and 4.11. In autumn, GaC contents varied from 79.8 ± 2.64 mg $g^{-1}$ in TV24 to 141 ± 4.53 mg $g^{-1}$ in TV19, which represented a 1.78 fold variation (Table 5). NGaCs content ranged from 31.8 ± 1.03 mg $g^{-1}$ in TV19 to 74.2 ± 1.50 mg $g^{-1}$ in TV17. The two extremes were differed by a factor of 2.33. GNR values were found in the range between 1.42 and 4.45. Punyasiri et al. (2017) reported GNR values in the range between 1.17 and 7.79 in Sri Lankan tea cultivars which was much higher than our results indicating the higher extent of contribution of GaCs to TC than for Tocklai cultivars.

### 3.4. Caffeine

Caffeine, a purine alkaloid, is an important secondary metabolite present in tea plants (Zhu et al., 2019). This alkaloid is accumulated in tender leaves and seeds and its level is observed at 20-50 mg $g^{-1}$ in tea leaves (Zhu et al., 2019). Caffeine contributes to the creaming properties of black tea. Therefore tea with a low caffeine level is considered to be of inferior quality. The effect of season on the caffeine content of the cultivars is presented in Tables 1, 3 and 5. In pre-monsoon, cultivar TV15 had the highest caffeine content (48.7 ± 0.50 mg $g^{-1}$) whereas cultivar TV30 had the lowest content (27.1 ± 0.32 mg $g^{-1}$). The two extreme cultivars represented 1.80 fold variation. In monsoon, caffeine content varied from 35.3 ± 1.56 mg $g^{-1}$ in TV30 to 55.0 ± 1.34 mg $g^{-1}$ in TV9, expressing a variation of 1.56 fold. In autumn, caffeine content varied from 27.6 ± 1.11 mg $g^{-1}$ in TV25 to 40.7 ± 0.42 mg $g^{-1}$ in TV2, expressing a variation of 1.47 fold. Xu et al. (2012) observed an increase of 2.163 mg $g^{-1}$ in caffeine level in green tea processed from summer harvest in comparison to spring. The caffeine content of Sri Lankan (Punyasiri et al., 2017) and Kenyan (Obanda et al., 1997) tea germplasm were reported in the range between 18.11 and 44.93 mg $g^{-1}$ and between 27.6 and 49.7 mg $g^{-1}$, respectively, which are in close agreement with the present study.

## 4. Conclusions

The study demonstrates the phenolic profile of Tocklai tea cultivars in a broad sense manifesting significant variations with seasonal effect. The catechin contents critical to the quality of black tea reached a peak level in the monsoon season. The contents of TC in cultivars TV23, TV17 and TV15; and that of EGCG in TV10, TV11, and TV9 were significantly higher than for other cultivars. A database has been generated to allow selection of tea cultivars with higher catechin content paying adequate attention to the season. Knowledge of specific differences in the catechin profile among tea cultivars is important to identify suitable cultivars for breeding new varieties that thrive better to alleviate climate change effect and processing of quality tea to meet the changing demands of consumers. Marked variation in day length, sunlight and temperature across seasons triggers variation in phenolic composition in tea shoots. Further study in a controlled environment and over a longer period may provide mechanistic insight into the effect of the environmental factors on the biosynthesis of quality precursors in tea shoots.

## CRediT authorship contribution statement

**Himangshu Deka:** Conceptualization, Methodology, Investigation, Validation, Writing - original draft, Writing - review & editing. **Tupu Barman:** Software, Visualization, Writing - review & editing. **Jintu Dutta:** Statistical analysis, Writing - review & editing. **Arundhuti Devi:** Supervision, Writing - review & editing. **Pradip Tamuly:** Resources, Visualization, Supervision. **Ranjit Kumar Paul:** Statistical analysis, Data interpretation, Writing - review & editing. **Tanmoy Karak:** Supervision, Visualization, Data curation, Writing - review & editing.

**Table 7**
Variation and distribution of catechins and caffeine content in different varieties harvested in different seasons

| Parameters* | Assam | | | Cambod | | | Assam China hybrid | | | Assam hybrid | | | China hybrid | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-monsoon | Monsoon | Autumn | Pre-monsoon | Monsoon | Autumn | Pre-monsoon | Monsoon | Autumn | Pre-monsoon | Monsoon | Autumn | Pre-monsoon | Monsoon | Autumn |
| **EGC** | 51.3 ± 2.33[a, #] | 46.6 ± 1.75[b] | 38.5 ± 1.16[c] | 32.4 ± 2.64[d] | 34.7 ± 2.62[d] | 28.5 ± 1.49[e] | 35.5 ± 10.5[d] | 40.2 ± 10.8[c] | 33.2 ± 9.72[d] | 21.9 ± 3.47[f] | 23.9 ± 2.20[f] | 27.5 ± 3.27[e] | 15.8 ± 1.63[g] | 29.5 ± 1.72[e] | 20.5 ± 0.62[f] |
| **EGCG** | 93.9 ± 2.84[a] | 108 ± 3.66[b] | 86.5 ± 2.32[c] | 89.6 ± 4.12[a,c] | 102 ± 4.27[d] | 82.9 ± 3.59[c] | 58.8 ± 4.71[e] | 75.6 ± 3.70[f] | 68.7 ± 3.48[g] | 74.7 ± 3.33[f] | 81.9 ± 0.55[c] | 66.5 ± 3.77[g] | 64.7 ± 2.57[g] | 82.9 ± 1.44[c] | 71.1 ± 3.79[f] |
| **ECG** | 29.0 ± 1.55[a] | 34.9 ± 1.82[b] | 21.8 ± 0.96[c] | 33.8 ± 2.45[b] | 37.2 ± 2.41[d] | 27.7 ± 2.28[a] | 40.7 ± 3.79[d] | 49.9 ± 1.72[e] | 34.3 ± 6.03[b] | 44.9 ± 7.22[d] | 53.1 ± 7.07[e] | 40.1 ± 1.70[b] | 36.7 ± 1.63[b] | 30.5 ± 0.52[a] | 28.3 ± 0.64[a] |
| **EC** | 13.3 ± 1.09[a] | 13.1 ± 1.13[a] | 9.69 ± 0.61[b] | 12.8 ± 1.25[a] | 13.3 ± 1.08[a] | 10.5 ± 0.85[b] | 22.9 ± 8.56[c] | 25.9 ± 7.95[c] | 17.7 ± 7.43[d] | 13.0 ± 0.58[a] | 11.9 ± 2.58[e] | 11.1 ± 0.82[e] | 10.5 ± 0.60[e] | 10.4 ± 0.93[e] | 7.17 ± 0.37[f] |
| **+C** | 4.85 ± 0.68[a] | 6.82 ± 0.68[b] | 4.58 ± 0.83[a] | 4.55 ± 0.75[a] | 6.68 ± 0.89[b] | 3.28 ± 0.58[c] | 8.38 ± 1.45[d] | 8.52 ± 0.25[d] | 8.15 ± 2.02[d] | 9.73 ± 3.03[e] | 8.15 ± 0.78[d] | 10.5 ± 0.43[e] | 4.43 ± 0.18[a] | 6.80 ± 0.21[b] | 5.90 ± 0.74[b] |
| **TC** | 192 ± 4.27[a] | 210 ± 3.02[b] | 161 ± 2.86[c] | 173 ± 6.02[d] | 194 ± 6.41[a] | 153 ± 5.12[e] | 166 ± 26.1[c] | 200 ± 16.5[f] | 162 ± 17.6[c] | 164 ± 10.4[c] | 179 ± 7.68[d] | 155 ± 9.98[e] | 132 ± 2.09[g] | 160 ± 2.07[c] | 133 ± 3.80[g] |
| **DHC** | 42.3 ± 2.09[a] | 48.0 ± 2.28[b] | 31.4 ± 1.26[c] | 46.7 ± 3.18[b] | 50.5 ± 3.02[b] | 38.3 ± 2.54[d] | 63.7 ± 12.3[e] | 75.8 ± 9.68[f] | 52.1 ± 13.4[b] | 58.0 ± 6.63[b] | 65.0 ± 9.65[e] | 51.3 ± 2.52[b] | 47.7 ± 2.17[b] | 40.9 ± 0.59[a] | 35.4 ± 0.68[d] |
| **THC** | 145 ± 3.24[a] | 155 ± 2.65[b] | 125 ± 2.45[c] | 122 ± 4.89[c] | 136 ± 5.70[d] | 111 ± 3.98[e] | 94.3 ± 15.2[f] | 115 ± 7.15[e] | 101 ± 6.23[g] | 96.7 ± 6.80[f] | 105 ± 2.75[g] | 94.0 ± 7.03[f] | 80.5 ± 4.11[h] | 112 ± 1.46[e] | 91.6 ± 4.40[f] |
| **CI** | 0.29 ± 0.01[a] | 0.31 ± 0.02[a] | 0.25 ± 0.01[b] | 0.39 ± 0.03[c] | 0.38 ± 0.02[c] | 0.35 ± 0.02[c] | 0.67 ± 0.02[d] | 0.65 ± 0.04[d] | 0.50 ± 0.10[e] | 0.60 ± 0.03[d] | 0.62 ± 0.11[d] | 0.55 ± 0.01[e] | 0.59 ± 0.06[e] | 0.36 ± 0.01[c] | 0.39 ± 0.02[c] |
| **GaC** | 122 ± 3.81[a] | 143 ± 4.46[b] | 108 ± 2.83[c] | 123 ± 5.46[a] | 139 ± 5.43[b] | 110 ± 5.06[e] | 99.5 ± 8.51[d] | 125 ± 1.98[a] | 103 ± 2.55[d] | 119 ± 10.5[a] | 135 ± 6.52[b] | 106 ± 5.47[d] | 101 ± 1.02[d] | 113 ± 1.82[e] | 99.4 ± 3.55[d] |
| **NGaC** | 69.6 ± 3.33[a] | 66.8 ± 2.79[a] | 52.8 ± 1.68[b] | 49.8 ± 3.55[c] | 54.7 ± 3.03[d] | 42.3 ± 1.98[e] | 66.8 ± 17.6[a] | 74.7 ± 18.5[f] | 59.1 ± 15.1[g] | 44.7 ± 0.15[e] | 43.9 ± 1.17[e] | 49.3 ± 4.52[c] | 30.8 ± 1.18[h] | 46.7 ± 2.72[e] | 33.5 ± 0.27[i] |
| **GNR** | 1.84 ± 0.12[a] | 2.25 ± 0.16[b] | 2.09 ± 0.10[c] | 2.65 ± 0.22[c] | 2.65 ± 0.18[c] | 2.71 ± 0.20[c] | 1.65 ± 0.31[a] | 1.93 ± 0.51[a] | 1.86 ± 0.43[a] | 2.68 ± 0.24[c] | 3.08 ± 0.07[d] | 2.18 ± 0.09[c] | 3.30 ± 0.11[d] | 2.45 ± 0.16[c] | 2.96 ± 0.08[c,d] |
| **CAF** | 39.5 ± 1.25[a] | 42.8 ± 1.08[b] | 35.1 ± 0.76[c] | 37.4 ± 1.34[d] | 42.9 ± 1.34[b] | 32.3 ± 0.90[e] | 37.5 ± 5.95[d] | 46.9 ± 0.20[f] | 37.7 ± 0.98[d] | 40.7 ± 3.00[b] | 46.3 ± 1.98[f] | 33.1 ± 1.37[e] | 37.7 ± 0.52[d] | 42.2 ± 1.68[b] | 38.1 ± 0.50[d] |

*EGC, (−)-epigallocatechin; +C, (+)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechingallate; ECG, (−)-epicatechingallate; GaC, galloylated catechins (EGCG + ECG); NGaC, non-galloylated catechins (EC + EGC); DHC, dihydroxy catechins (EC + ECG); THC, trihydroxy catechins (EGC + EGCG); CI, catechin index (DHC/THC); TC, total catechin; GNR, galloylated to non-galloylated catechin ratio (GaC/NGaC); CAF, caffeine. All units, except those for CI and GNR, are in mg $g^{-1}$(dry weight basis). Values are 'mean ± SE' of independent triplicate measurements.
[#]Same symbol within a row denotes not significant whereas different symbol denotes significant difference.

**Table 8**
Pearson's correlation between catechins and caffeine content during different and seasons

| Pearson's correlation | Parameters# | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Caffeine | EGC | + C | EC | EGCG | ECG | TC | DHC | THC | GaC | NGaC | GNR |
| Pre-monsoon vs Monsoon | 0.573* | 0.810* | 0.791* | 0.848* | 0.789* | 0.706* | 0.839* | 0.813* | 0.859* | −0.196 | 0.142 | −0.752* |
| Pre-monsoon vs Autumn | 0.217** | 0.759* | 0.741* | 0.684* | 0.619* | 0.800* | 0.664* | 0.834* | 0.734* | −0.043 | 0.015 | −0.620* |
| Monsoon vs Autumn | 0.475** | 0.759* | 0.500** | 0.719* | 0.666* | 0.643* | 0.703* | 0.718* | 0.779* | 0.758* | 0.599* | 0.677* |

#EGC, (−)-epigallocatechin; +C, (+)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechingallate; ECG, (−)-epicatechingallate; TC, total catechin; DHC, dihydroxy catechins (EC + ECG); THC, trihydroxy catechins (EGC + EGCG); GaC, galloylated catechins (EGCG + ECG); NGaC, non-galloylated catechins (EC + EGC); GNR, galloylated to non-galloylated catechin ratio (GaC/NGaC).
*and ** denote significant at 1% and 5% level of significance respectively.

## Declaration of Competing Interest

The authors report no declarations of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2020.103684.

## References

Bhatia, I.S., Ullah, M.R., 1968. Polyphenols of tea. IV.-Qualitative and quantitative study of the polyphenols of different organs and some cultivated varieties of tea plant. J. Sci. Food Agric. 19, 535–542. https://doi.org/10.1002/jsfa.2740190911.

Bockuchava, M.A., Skobeleva, N.I., 1969. The chemistry and biochemistry of tea and tea manufacture. Adv. Food Res. 17, 215–292.

Bora, K., Sarkar, D., Konwar, K., Payeng, B., Sood, K., Paul, R.K., Datta, R., Das, S., Khare, P., Karak, T., 2019. Disentanglement of the secrets of aluminium in acidophilic tea plant (*Camellia sinensis* L.) influenced by organic and inorganic amendments. Food Res. Int. 120, 851–864. https://doi.org/10.1016/j.foodres.2018.11.049.

Carloni, P., Tiano, L., Padella, L., Bacchetti, T., Customu, C., Kay, A., Damiani, E., 2013. Antioxidant activity of white, green and black tea obtained from the same tea cultivar. Food Res. Int. 53, 900–908. https://doi.org/10.1016/j.foodres.2012.07.057.

Chu, D.C., Juneja, L.R., 1997. General chemical composition of green tea and its infusion. In: Yamamoto, T., Juneja, L.R., Chu, D.C., Kim, M. (Eds.), Chemistry and Applications of Green Tea. CRC Press, New York, pp. 13–22.

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.). J. Agric. Food Chem. 63, 9869–9878. https://doi.org/10.1021/acs.jafc.5b03967.

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 Chem. 220, 517–526. https://doi.org/10.1016/j.foodchem.2016.09.047.

Fernando, C.D., Soysa, P., 2016. Simple isocratic method for simultaneous determination of caffeine and catechins in tea products by HPLC. Springerplus 5, 970. https://doi.org/10.1186/s40064-016-2672-9.

Fujimura, Y., Kurihara, K., Ida, M., Kosaka, R., Miura, D., Wariishi, H., Maeda-Yamamoto, M., Nesumi, A., Saito, T., Kanda, T., Yamada, K., Tachibana, H., 2011. Metabolomics-driven nutraceutical evaluation of diverse green tea cultivars. PLoS One 6 (8), e23426. https://doi.org/10.1371/journal.pone.0023426.

Gulati, A., Rajkumar, S., Karthigeyan, S., Sud, R.K., Vijayan, D., Thomas, J., Rajkumar, R., Das, S.C., Tamuly, P., Hazarika, M., Ahuja, P.S., 2009. Catechin and catechin fractions as biochemical markers to study the diversity of Indian tea (*Camellia sinensis* (L.) O. Kuntze) germplasm. Chem. Biodivers. 6, 1042–1052. https://doi.org/10.1002/cbdv.200800122.

Han, W.Y., Huang, J.G., Li, X., Li, Z.X., Ahammed, G.J., Yan, P., Stepp, J.R., 2017. Altitudinal effects on the quality of green tea in east China: a climate change perspective. Eur. Food Res. Technol. 243, 323–330. https://doi.org/10.1007/s00217-016-2746-5.

Harbowy, M.E., Balentine, D.A., 1997. Tea Chemistry. CRC. Crit. Rev. Plant Sci. 16, 415–480. https://doi.org/10.1080/713608154.

Hilton, P.J., Palmer-Jones, R., 1973. Relationship between the flavanol composition of fresh tea shoots and the theaflavin content of manufactured tea. J. Sci. Food Agric. 24, 813–818. https://doi.org/10.1002/jsfa.2740240709.

ISO 14502-2, 2005. Determination of substances characteristics of green and black tea-Part 2: content of catechins in green tea-Method using high-performance liquid chromatography.

Jayasekera, S., Kaur, L., Molan, A.L., Garg, M.L., Moughan, P.J., 2014. Effects of season and plantation on phenolic content of unfermented and fermented Sri Lankan tea. Food Chem. 152, 546–551. https://doi.org/10.1016/j.foodchem.2013.12.005.

Jiang, H., Yu, F., Qin, L., Zhang, N., Cao, Q., Schwab, W., Li, D., Song, C., 2019. Dynamic change in amino acids, catechins, alkaloids, and gallic acid in six types of tea processed from the same batch of fresh tea (*Camellia sinensis* L.) leaves. J. Food Compos. Anal. 77, 28–38. https://doi.org/10.1016/j.jfca.2019.01.005.

Jin, J.Q., Ma, J.Q., Ma, C.L., Yao, M.Z., Chen, L., 2014. Determination of catechin content in representative Chinese tea germplasms. J. Agric. Food Chem. 62, 9436–9441. https://doi.org/10.1021/jf5024559.

Karak, T., Bhagat, R.M., 2010. Trace elements in tea leaves, made tea and tea infusion: A review. Food Res. Int. 43, 2234–2252. https://doi.org/10.1016/j.foodres.2010.08.010.

Karak, T., Paul, R.K., Sonar, I., Sanyal, S., Ahmed, K.Z., Boruah, R.K., Das, D.K., Dutta, A. K., 2014. Chromium in soil and tea (*Camellia sinensis* L.) infusion: Does soil amendment with municipal solid waste compost make sense? Food Res. Int. 64, 114–124. https://doi.org/10.1016/j.foodres.2014.06.006.

Kottawa-Arachchi, J.D., Gunasekare, M.T.K., Ranatunga, M.A.B., 2019. Biochemical diversity of global tea [*Camellia sinensis* (L.) O. Kuntze] germplasm and its exploitation: a review. Genet. Resour. Crop Evol. 66, 259–273. https://doi.org/10.1007/s10722-018-0698-2.

Kottawa-Arachchi, J.D., Gunasekare, M.T.K., Ranatunga, M.A.B., Punyasiri, P.A.N., Jayasinghe, L., Karunagoda, R.P., 2014. Biochemical characteristics of tea (*Camellia sinensis* L. spp.) germplasm accessions in Sri Lanka: correlation between black tea quality parameters and organoleptic evaluation. Int. J. Tea Sci. 10, 3–13.

Liu, Z., Bruins, M.E., de Bruijn, W.J.C., Vincken, J.P., 2020. A comparison of the phenolic composition of old and young tea leaves reveals a decrease in flavanols and phenolic acids and an increase in flavonols upon tea leaf maturation. J. Food Compos. Anal. 86, 103385 https://doi.org/10.1016/j.jfca.2019.103385.

Magoma, G.N., Wachira, F.N., Obanda, M., Imbuga, M., Agong, S.G., 2000. The use of catechins as biochemical markers in diversity studies of tea (*Camellia sinensis*). Genet. Resour. Crop Evol. 47, 107–114. https://doi.org/10.1023/A:1008772902917.

Massounga Bora, A.F., Ma, S., Li, X., Liu, L., 2018. Application of microencapsulation for the safe delivery of green tea polyphenols in food systems: Review and recent advances. Food Res. Int. 105, 241–249. https://doi.org/10.1016/j.foodres.2017.11.047.

Obanda, M., Owuor, P.O., Taylor, S.J., 1997. Flavanol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas. J. Sci. Food Agric. 74, 209–215. https://doi.org/10.1002/(SICI)1097-0010(199706)74:2<209::AID-JSFA789>3.0.CO;2-4.

Owuor, P.O., Obanda, M., 2007. The use of green tea (*Camellia sinensis*) leaf flavan-3-ol composition in predicting plain black tea quality potential. Food Chem. 100, 873–884. https://doi.org/10.1016/j.foodchem.2005.10.030.

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., 2017. Genotypic variation in biochemical compounds of the Sri Lankan tea (*Camellia sinensis* L.) accessions and their relationships to quality and biotic stresses. J. Hortic. Sci. Biotechnol. 92, 502–512. https://doi.org/10.1080/14620316.2017.1289070.

Rho, T., Choi, M.S., Jung, M., Kil, H.W., Hong, Y.D., Yoon, K.D., 2019. Identification of fermented tea (*Camellia sinensis*) polyphenols and their inhibitory activities against amyloid-beta aggregation. Phytochemistry 160, 11–18. https://doi.org/10.1016/j.phytochem.2018.12.013.

Sabhapondit, S., Karak, T., Bhuyan, L.P., Goswami, B.C., Hazarika, M., 2012. Diversity of catechin in Northeast Indian Tea cultivars. Sci. World J. 2012 https://doi.org/10.1100/2012/485193.

Saravanan, M., John, K.M.M., Raj Kumar, R., Pius, P.K., Sasikumar, R., 2005. Genetic diversity of UPASI tea clones (*Camellia sinensis* (L.) O. Kuntze) on the basis of total catechins and their fractions. Phytochemistry 66, 561–565. https://doi.org/10.1016/j.phytochem.2004.06.024.

Singh, H.P., Ravindranath, S.D., Singh, C., 1999. Analysis of tea shoot catechins: Spectrophotometric quantitation and selective visualization on two-dimensional

paper chromatograms using diazotized sulfanilamide. J. Agric. Food Chem. 47, 1041–1045. https://doi.org/10.1021/jf9807263.

Tan, J., Engelhardt, U.H., Lin, Z., Kaiser, N., Maiwald, B., 2017. Flavonoids, phenolic acids, alkaloids and theanine in different types of authentic Chinese white tea samples. J. Food Compos. Anal. 57, 8–15. https://doi.org/10.1016/j.jfca.2016.12.011.

Visser, T., 1969. Tea, *Camellia sinensis* (L.) O. Kuntze. In: Ferwarda, F.P., Wit, F. (Eds.), Outlines of Perennial Crop Breeding in Tropics. H. Veenman & Zonen N.V. Wageningen, pp. 459–493.

Wakamatsu, M., Yamanouchi, H., Sahara, H., Iwanaga, T., Kuroda, R., Yamamoto, A., Minami, Y., Sekijima, M., Yamada, K., Kajiya, K., 2019. Catechin and caffeine contents in green tea at different harvest periods and their metabolism in miniature swine. Food Sci. Nutr. 7, 2769–2778. https://doi.org/10.1002/fsn3.1143.

Wang, Y.S., Gao, L.P., Wang, Z.R., Liu, Y.J., Sun, M.L., Yang, D.Q., Wei, C.L., Shan, Y., Xia, T., 2012. Light-induced expression of genes involved in phenylpropanoid biosynthetic pathways in callus of tea (*Camellia sinensis* (L.) O. Kuntze). Sci. Hortic. (Amsterdam). 133, 72–83. https://doi.org/10.1016/j.scienta.2011.10.017.

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. https://doi.org/10.1016/j.foodchem.2010.08.029.

Wen, B., Ren, S., Zhang, Y., Duan, Y., Shen, J., Zhu, X., Wang, Y., Ma, Y., Zou, Z., Fang, W., 2020. Effects of geographic locations and topographical factors on secondary metabolites distribution in green tea at a regional scale. Food Control 110, 106979. https://doi.org/10.1016/j.foodcont.2019.106979.

Wolfram, S., Wang, Y., Thielecke, F., 2006. Anti-obesity effects of green tea: From bedside to bench. Mol. Nutr. Food Res. 50, 176–187. https://doi.org/10.1002/mnfr.200500102.

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 black tea produced in Central and Southern Africa. J. Sci. Food Agric. 1823–1830. https://doi.org/10.1002/1097-0010(200010)80:13<1823::AID-JSFA702>3.0.CO;2-E.

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. J. Agric. Food Chem. 60, 7064–7070. https://doi.org/10.1021/jf301340z.

Yamamoto, T., Juneja, L.R., Chu, D., Kim, M., 1997. Chemistry and application in green tea. CRC Press, Boca Raton, USA.

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*). J. Agric. Food Chem. 53, 6477–6483. https://doi.org/10.1021/jf050382y.

Zeng, C., Lin, H., Liu, Zhixiang, Liu, Zhonghua, 2020. Metabolomics analysis of *Camellia sinensis* with respect to harvesting time. Food Res. Int. 128, 108814 https://doi.org/10.1016/j.foodres.2019.108814.

Zheng, X.Q., Nie, Y., Gao, Y., Huang, B., Ye, J.H., Lu, J.L., Liang, Y.R., 2018. Screening the cultivar and processing factors based on the flavonoid profiles of dry teas using principal component analysis. J. Food Compos. Anal. 67, 29–37. https://doi.org/10.1016/j.jfca.2017.12.016.

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. J. Agric. Food Chem. 67, 3400–3411. https://doi.org/10.1021/acs.jafc.9b0024.