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Variations in phenolic constituents of green tea [*Camellia sinensis* (L) O Kuntze] Due to changes in weather conditions

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Abstract
Climate change is impacting agro-ecosystems, crops, and farmer livelihoods in communities worldwide. Climate events in many areas are resulting in a decline in crop yields, the impact on crop quality is less acknowledged, yet it is critical for food systems that benefit both farmers and consumers through high-quality products. This study examines tea (*Camellia sinensis*), the worlds most widely consumed beverage after water, as a study system to measure effects of seasonal variability on crop functional quality. Seasonal variations of total polyphenols (TP) in the range: 205.518 to 113.719 g kg^{-1}, 269.529 to 140.210 g kg^{-1} and 263.223 to 104.759 g kg^{-1} and total catechins (TC) in the range of 110.209 to 40.904 g kg^{-1}, 174.774 to 31.069 g kg^{-1} and 162.619 to 82.673 g kg^{-1} in fresh shoots of Kangra local tea [*Camellia sinensis* (L) O Kuntze] cultivar collected at seven day intervals throughout the harvesting seasons during the year were significant. Summer and rainy flush season's samples of fresh tea shoots had higher levels of TP and TC compared to first and winter flush seasons. Stepwise regression analysis indicated that mechanisms that induced seasonal variations in the levels of phenolic constituents of tea shoots may include one or all the weather parameters which varied markedly across harvesting seasons over the years under investigation.

Keywords: Total polyphenols, Total catechins, Temperature, Relative humidity, Bright sunshine hours, Rainfall and Evaporation

Introduction

The secondary metabolites that contribute to crop quality serve as defense compounds in plants and vary in concentration depending on genetic, environmental, and management conditions. Green tea is one of the most popular beverages consumed worldwide. Like any other crop, the productivity of green tea shoots and synthesis and accumulation of phenolic constituents may be dependent on optimum weather conditions. For example, the concentrations of methylxanthine and polyphenolic catechin compounds in tea plants vary with geographic location, cultivar, herbivory, season, shade, soil, slope, water availability, and management (Lin *et al*. 2003; Ahmed *et al*. 2012; Ahmed *et al*. 2013) [12, 2, 1].

Astill *et al.* (2001) [3] reported that the chemical composition of these two type of tea, China type and Assam type differ significantly in biochemical composition of the fresh shoots. He also reported that fresh green leaves from Assam teas generally have high polyphenol and less pigment content then China type of plant. Young tea shoots contains more than 35% of their dry weight in polyphenols which was known to be one of the main factors in determining the quality of the resulting tea drink (Hara *et al.,* 1995) [10]. Polyphenols had been widely utilized in studying the diversity of tea germplasm. Magoma *et al.* (2000) and Gulati *et al.* (2009) [9] though its concentration also depend on the environment. Fresh tea leaves are rich in flavonoids - a group of phenolic compounds known as catechins. Variations in weather conditions during different harvesting seasons in the region of its cultivation have been reported to affect the synthesis and accumulation of polyphenols in tea shoots (Yao *et al*. 2005; Chen *et al*. 2010) [18, 5]. Tolerance to drought, cold and frost, high solar radiation, and high soil pH, etc. is among the major environmental factors that affect the growth and yield of tea (Ahmed *et al*. 2012) [2]. Temperature is the major environmental factor affecting shoot growth and photosynthesis, hence closely linked to the influence of temperature is the influence of large saturation scarcity. The variation in ecophysiology that exists in the tea germplasm can be used to develop cultivars specifically-suited to different climates (Ahmed *et al*. 2013; Gogoi and Borua 2017) [1, 8].

Keeping this in view, seasonal variations of total polyphenols and total catechins in fresh green tea shoots of local cultivar, as affected by the changes in weather conditions during four flush seasons have been evaluated.

Materials and Methods

Samples of fresh green tea shoots (two leaves and a bud) were collected from Wah Tea Estate, Rajpura, Palampur, Himachal Pradesh, India, at seven days interval during four flush seasons (April to mid May; mid May to June; July to mid September and mid September to October), for the year 2015 given in Table 1. The samples of fresh green tea shoots were always subjected to heat treatment in a microwave oven for three minutes at power 100% (P-HI) in I2FB convection microwave oven (model: 30SC1, 30 L capacity, microwave 1.4 KW, frequency 2450 MHz), within twenty minutes of their plucking and finally dried in a hot air oven maintained at 45 ± 5 °C for 24 h. The dried samples were grounded using MAC Willey Grinder (Arthur H. Thomas Type) to pass through 60 mesh sieve and finally stored in air–tight plastic containers.

Table 1: Week-number, month and treatment of green tea shoots

Week Number	Month	Treatment
W_1	April	The samples of fresh green tea shoots were subjected to heat treatment for 3 minutes in a microwave oven within 20 minutes of their plucking and finally dried in a hot air oven maintained at 45±5 °C. During the year 2009 the samples after microwave heat treatment, were separated into four parts comprising whole leaves (two leaves and a bud), buds, first leaves and second leaves and dried separately.
W_2		
W_3		
W_4		
W_5	May	
W_6		
W_7		
W_8		
W_9		
W_{10}	June	
W_{11}		
W_{12}		
W_{13}		
W_{14}	July	
W_{15}		
W_{16}		
W_{17}		
W_{18}		
W_{19}	August	
W_{20}		
W_{21}		
W_{22}		
W_{23}	September	
W_{24}		
W_{25}		
W_{26}		
W_{27}	October	
W_{28}		
W_{29}		
W_{30}		
W_{31}		

Tea Extracts preparation

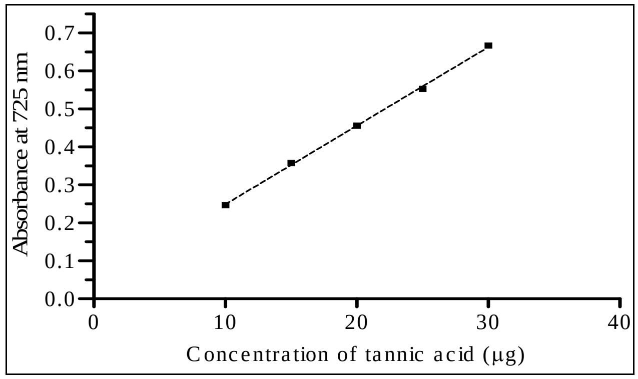
Tea extracts were prepared by taking dried tea sample (300 mg) and 100 mL of pre-boiled hot double distilled water was poured into the flask. The flask was kept in a water bath shaker maintained at 60±5 °C for 20 minutes. The content of the flask was allowed to cool to room temperature and then filtered through Whatman Grade 1 filter and the final volume was made to 100 mL. Total polyphenols were estimated in freshly prepared tea extracts by the method of Makkar (2003) [14] using tannic acid as standard. Total catechins were estimated by the method of Sun *et al* (1998) [17] in freshly prepared tea extracts using catechin as standard.

Meteorological data on weather parameters were collected from the Agro-meteorological Division, Department of Agronomy, College of Agriculture, Himachal Pradesh Agricultural University Palampur, India.

Estimation of total polyphenols and total catechins

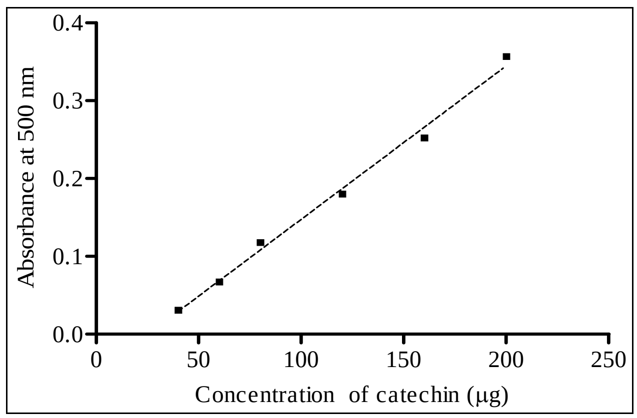
Standard curves

Standard curves for tannic acid and catechin were plotted employing standard techniques prior to quantitative estimation of total polyphenols and total catechins in aqueous tea extracts. Tannic acid and Catechin is used as standards.



Equation of line: y = 0.0207. x + 0.04220
Slope = 0.0207; y-intercept = 0.04220; r^2 = 0.9991

Fig 1: Standard curve for tannic acid



Equation of line: y = 0.001972. x + (– 0.04943)
Slope = 0.001972; y-intercept = (–) 0.04943; r^2 = 0.9933

Fig 2: Standard curve for catechin

Estimation of total polyphenols

Total polyphenols were always estimated in freshly prepared tea extracts by the method of Makkar (2003) [14].

To the freshly prepared tea extract (0.025 mL) was added double distilled water (0.975 mL), 1 N Folin-Ciocalteu's phenol reagent (0.500 mL) and 20% sodium carbonate solution (2.500 mL). The contents were mixed thoroughly with the help of vortex and incubated at 30 °C for 40 minutes. Absorbance was recorded at 725 nm with the help of Merck Spectroquant Pharo 100 spectrophotometer.

Total polyphenols were calculated using following equations:

$$\text{Concentration } (\mu g) = \frac{\text{Absorbance at 725 nm} - (0.04220)}{0.0207} = X\ \mu g$$

The concentration of total polyphenols (TP) was finally expressed in terms of g kg^{-1} of fresh green tea shoots on dry weight basis using following formula:

$$\text{Concentration } (g\ kg^{-1}) = \frac{X\ (\mu g) \times 40 \times 100 \times 10^6}{300 \times (\text{Weight of sample} - \text{Moisture content})}$$

Estimation of total catechins

Total catechins were always estimated in freshly prepared tea extracts by the method of Sun *et al.* (1998) [17].

To the freshly prepared tea extract (0.250 mL) was added methanol (0.750 mL), 1% vanillin reagent (2.500 mL) and 9 M HCl (2.500 mL). The contents were mixed thoroughly with the help of vortex and allowed to incubate in dark at 30°C for 20 minutes. Finally absorbance was recorded at 500 nm with help of Merck Spectroquant Pharo 100 spectrophotometer.

Total catechins were calculated using following equation:

$$\text{Concentration } (\mu g) = \frac{\text{Absorbance at 500 nm} + (0.04943)}{0.001972} = X\ \mu g$$

The concentration of total catechins (TC) was finally expressed in terms of g kg^{-1} in fresh green tea shoots on dry weight basis using following formula:

$$\text{Concentration } (g\ kg^{-1}) = \frac{X \times 4 \times 100}{300 \times (\text{Weight of sample} - \text{Moisture content})} \times 10^{6}$$

Monthly representative samples and preparation of tea powders

Monthly samples of fresh green tea shoots were required to investigate monthly variations of phenolic constituents, preparation of tea powders and evaluation of flavan-3-ols. Samples to represent a particular month were prepared by pooling and mixing thoroughly weekly samples of dried tea shoots/bud/first leaf/second leaf of the month in equal proportions by weight. Total phenols and total catechins were estimated in the samples to determine inter seasonal variations.

Statistical Analysis

All analytical estimations were carried out in triplicate to minimize the experimental error and analyzed statistically using WindoStat software version 8.0. Analysis of variance (ANOVA) followed by analysis of least significant difference ($p<0.05$) among means by the Duncan's Multiple Range Test (DMRT) was performed.

Results and Discussion

Total polyphenols and total catechins contents in fresh green tea shoots of local cultivar were estimated and their seasonal profiles as affected by the changes in weather parameters (temperature, relative humidity, bright sunshine hours, rainfall and evaporation) were evaluated.

Seasonal variations of total polyphenols (TP) and total catechins (TC)

In Tables 2 are given the mean weekly values of TP and TC, respectively along with Critical Difference (CD at 5%) and per cent Coefficient of Variance (CV).

i. Total polyphenols

The mean weekly variations in the levels of TP in the range of 269.529 to 140.210 g kg^{-1} were significant.

During cropping year the mean weekly TP content (269.529 g kg^{-1}) recorded in the sample of fresh green tea shoots harvested on September (W_{23}) was significantly highest and statistically at par with the mean weekly value estimated in sample of June (W_{10}). It was of interest to note that the samples of summer flush season invariably had high level of TP. It could be due to the fact that high temperature and longer day time during summer flush season may help tea shoots to accumulate higher level of TP. The levels of total phenolics in fresh tea shoots were reported to be lower in cooler months that increased throughout the warmer months (Erturk *et al.* 2010) [7].

ii. Total catechins

The mean weekly TC contents varied significantly in the range of 174.774 to 37.185 g kg^{-1}. The variations in the levels of TC were significant over a particular flush season and also over the cropping year. The sample of fresh green tea shoots harvested on June (W_{11}) recorded statistically the highest mean weekly TC content (174.774 g kg^{-1}) given in Table 2. It was evident from the above mentioned results that fresh green tea shoots of summer flush season accumulated highest level of TC; whereas the rainy flush season accounted for lowest TC content. Green teas grown in an area with high temperature, long sun exposure time, and high rainfall were reported to contain lower levels of EC, EGC, EGCG and caffeine than those grown in areas with relatively low temperature, short sun exposure time, and low rainfall (Lee *et al.* 2010) [11].

Table 2: Weekly mean total polyphenols and total catechin content (g kg^{-1}) in fresh green tea shoots

Week number	TP	TC
W_1	$203.931^{m,n}$	108.352^{c}
W_2	$214.639^{k,l,m}$	130.407^{b}
W_3	197.517^{n}	$102.133^{c,d}$
W_4	$203.936^{m,n}$	$96.196^{d,e}$
W_5	$231.611^{g,h,i}$	$87.938^{e,f}$
W_6	$243.234^{d,e,f,g}$	$92.361^{d,e,f}$
W_7	$225.956^{h,i,j}$	110.539^{c}
W_8	$242.310^{d,e,f,g}$	132.604^{b}
W_9	$255.070^{b,c}$	131.312^{b}
W_{10}	267.651^{a}	123.246^{b}
W_{11}	$220.227^{j,k,l}$	174.774^{a}
W_{12}	$251.699^{c,d}$	$83.303^{f,g}$
W_{13}	$248.311^{c,d,e}$	$84.460^{f,g}$
W_{14}	$236.971^{e,f,g,h}$	$90.119^{e,f}$
W_{15}	$236.619^{e,f,g,h}$	$91.768^{d,e,f}$
W_{16}	$238.129^{e,f,g}$	$52.355^{i,j,k}$
W_{17}	$234.376^{f,g,h}$	$43.652^{j,k,l}$
W_{18}	$253.925^{b,c,d}$	$48.930^{i,j,k}$
W_{19}	$230.093^{g,h,i}$	$41.664^{k,l}$
W_{20}	$245.807^{c,d,e,f}$	56.204^{i}
W_{21}	$245.895^{c,d,e,f}$	37.185^{m}
W_{22}	$237.884^{e,f,g}$	37.828^{l}
W_{23}	269.529^{a}	$51.069^{i,j,k}$
W_{24}	$235.405^{f,g,h}$	$50.435^{i,j,k}$
W_{25}	$248.134^{c,d,e}$	$54.036^{i,j}$
W_{26}	$210.370^{l,m}$	$47.925^{i,j,k,l}$
W_{27}	$222.415^{i,j,k}$	$46.709^{i,j,k,l}$
W_{28}	$217.167^{j,k,l}$	$52.354^{i,j,k}$
W_{29}	170.203^{o}	$75.716^{g,h}$
W_{30}	145.288^{p}	$74.053^{g,h}$
W_{31}	140.210^{p}	71.200^{h}
Mean	227.662	79.381
CD (5%)	10.164	10.315
CV (%)	2.736	7.961

The experimental results were analyzed in one–way analysis of variance with Duncan's Multiple Range Test (DMRT) rankings – means within each column followed by the same letter are not significantly different at $P < 0.05$ according to DMRT.

Inter-seasonal variations

In order to assess the inter-seasonal variations of TP and TC in green tea; the weekly samples of fresh green tea shoots were pooled on monthly basis and subjected for analytical estimations for phenolic constituents. Table 3 represent the mean monthly TP and TC in fresh green tea shoots along with CD (at 5%) and per cent CV.

A perusal of Table 3 indicates that mean monthly TP and TC in fresh green tea shoots varied significantly throughout the cropping year. The mean monthly TP levels varied significantly in the range of, 263.100 to 185.194 g kg^{-1}. Mean monthly TC content of fresh green tea shoots varied significantly in the range of 105.878 to 87.125 g kg^{-1}.

Table 3: Monthly mean total polyphenols (g kg^{-1}) and total catechins (g kg^{-1}) contents in pooled samples of fresh green tea shoots of Kangra local cultivar during 2007, 2008 and 2009

Month	TP	TC
April	193.482^d	87.125^c
May	208.978^b	98.079^b
June	263.100^a	103.660^a
July	191.125^d	91.692^c
August	200.637^c	105.878^a
September	186.943^d	101.790^a
October	185.194^d	100.397^b
Mean	202.065	97.517
CD (5%)	7.27	4.72
CV (%)	2.06	2.76

Rankings – means within each column followed by the same letter are not significantly different at $P < 0.05$.

These observations corroborated our earlier results on TP and TC contents in fresh green tea shoots with respect to seasonal variations.

Weather parameters and its effect on phenolic content

Like any other crop, the productivity of green tea shoots and synthesis and accumulation of phenolic constituents may be dependent on optimum weather conditions. Carr (1972) [4] described a 'good growing season' for tea as one having warm days, long sunshine hours, high humidity and adequate rainfall preferably in overnight showers. Weather parameters, especially mean temperature and rainfall were also reported to be beneficial for growth and productivity of tea crop (Sen *et al.*1966) [15].

Since the plucking was done at seven day intervals, therefore, the weekly mean data of the preceding week has been incorporated. In order to visualize the impact of weather parameters on the phenolic profile of green tea; correlations (linear and stepwise) among TP, TC and weather parameters and regression analysis were carried out.

Linear correlation

Linear correlations among TP and TC contents and mean weather parameters are given in Table 4. TP content exhibited a significant positive correlation with minimum temperature, rainfall and a significant negative correlation with bright sunshine hours, whereas TC content exhibited a significant positive correlation with evaporation and a significant negative correlation with relative humidity.

Table 4: Linear correlation coefficient among total polyphenols (TP), total catechins (TC) of fresh green tea shoots of Kangra local cultivar and mean weather parameters*

	TP	TC	Max	Min	RH	BSS	RAIN	EVAP
TP	1.00	NS	NS	0.74^a	NS	-0.46^a	0.44^a	NS
TC		1.00	NS	NS	-0.63^a	NS	NS	0.52^a
Max			1.00	0.36^a	-0.48^a	0.52^a	NS	0.72^a
Min				1.00	0.47^a	-0.53^a	0.58^a	NS
RH					1.00	-0.75^a	0.65^a	-0.88^a
BSS						1.00	-0.82^a	0.71^a
RAIN							1.00	-0.61^a
EVAP								1.00

^{a} – Significant at $P < 0.05$; NS – Not significant.

* Max – Maximum temperature; Min – Minimum temperature, RH – Relative humidity; BSS –Bright sunshine hours; RAIN – Rainfall, EVAP – Evaporation.

It was of interest to note that whereas relative humidity exhibited a significant negative correlation; temperature and evaporation invariably had significant positive correlation with phenolic contents of fresh green tea shoots. Lack of any overt correlation of weather parameter with polyphenolic contents in present study could be due to thenatural interdependency among weather parameters and their distinct differences during the cropping year.

Stepwise regression analysis

Stepwise regression analysis and regression equations of TP, TC contents estimated in samples of fresh green tea shoots and weather parameters were carried out and given in Table 5 along with correlation coefficients incorporating different weather parameters responsible for the observed TP and TC contents in fresh green tea shoots.

During the cropping year when climate was cold with high accumulated seasonal rainfall, variations in the levels of TP were 80% due to mean weekly minimum temperature, mean weekly evaporation (both positively correlated) and mean weekly bright sunshine hours which was negatively correlated. Variations in the levels of TC were 68% due to mean weekly relative humidity and mean weekly bright sunshine hours which were negatively correlated.

However, the impact of weather parameters on the levels of TP and TC in fresh green tea shoots over the years was 74% and 85%, respectively due to mean weekly maximum temperature and mean weekly relative humidity, weekly bright sunshine hours. It was inferred from the above results that the synthesis of TP was assisted by minimum temperature and evaporation. Sharma *et al*. (2010) [16] reported a high positive correlation between catechins content and temperature. Bright sunshine and rainfall seemed to have negative effect on the synthesis and accumulation of TP and TC in tea shoots. Yao *et al*. (2005) [18] and Ercisli *et al*. (2008) [6] held day length, sunlight, and/or temperature responsible for the induction of seasonal variations of phenolic compounds in tea shoots.

Table 5: Correlation and regression among total polyphenols, total catechins of fresh green tea shoots and weather parameters

S. No.	Regression equations[a]	Correlation coefficients	Standard errors of estimates
Total polyphenols			
1.	19Y4NSiG8kkzwWjMD1 7euEaQ5PErpwxWkP	0.80^b	2.633
Total catechins			
2.	$221.570 – 1.658X_3 – 5.439X_4$	0.68^b	3.801

[a] – X_1 = mean weekly maximum temperature, X_2 = mean weekly minimum temperature, X_3 = mean weekly relative humidity, X_4 = mean weekly bright sunshine hours, X_5 = accumulated weekly rainfall, X_6 = mean weekly evaporation. [b] – Significant at $P < 0.05$.

Conclusion

Significant seasonal variations of total polyhenols and total catechins in fresh green tea shoots were due to weather parameters which varied significantly across the harvesting flush seasons over the years. Fresh green tea shoots of summer and rainy flush seasons invariably had higher contents of total catechins. It was reasonable to conclude that the mechanisms that induced seasonal variations in the levels of phenolic constituents of tea shoots may include one or all the weather parameters which varied markedly across harvesting seasons over the years under investigation.

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