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PROFILING OF TOTAL POLYPHENOLS AND PIGMENTS IN TEA (CAMELLIA SINENSIS (L.) O. KUNTZE) IN VARIOUS SEASONS FOR MANUFACTURING BLACK TEA AND GREEN TEA

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Tea is receiving increased interest from food scientists due to its antioxidant properties and the presence of bioactive substances like polyphenols which give nutraceutical values to well brew cup of tea. Profile of total polyphenols and pigments in fresh tea shoots consisting of one apical bud and two leaves sampled from fourteen (14) industry tea clones grown in Upper Brahmaputra Valley Zone of Assam was investigated for four harvesting seasons. Total polyphenols were determined according to the method given by Bray and Thorpe (1954). Pigments were determined according to the method of Taylor (1992). Significant seasonal variation was observed in total polyphenol content showing the highest value in second flush (25.11%) followed by autumn flush (23.54%), first flush (22.74%) and rain flush (22.46%). Significant seasonal variation was also observed in total chlorophyll content showing the highest value in rain flush (2.41 mg/g) followed by second flush (1.80 mg/g), autumn flush (1.43 mg/g) and first flushes (1.08 mg/g). The results of present study may be used for profiling the estate clones for black and green tea manufacturing purpose and also for future breeding programme for developing new clones.

Keywords: Tea, Polyphenols, Pigments, Seasonal variation, Industry clone

INTRODUCTION

Tea is one of the world's oldest beverages produce from the shoot tips (two leaves and a bud) of the evergreen tea plant *Camellia sinensis* (L.) O. Kuntze. Most of the tea plants were originated in the Yunnan Province of South-West China and its neighbouring area of Assam, India. There are two main varieties of the tea plant-variety with small leaf known as *Camellia sinensis* (China type), thrives in the cool, high mountain regions of central China and Japan and the broad leaf variety, known as *Camellia assamica* (Assam type), grows best in the moist, tropical climates found in Northeast India (Mendilcioglu, 2000). Tea is non-alcoholic beverage prepared by pouring boiling hot water over processed leaves of *Camellia sinensis* plant. Tea has been known from the ancient time to possess many medicinal properties, particularly for its phenolics – tea is claimed to be antidiabetic, antiallergic, antioxidative, antihypertensive, antiviral, antibacterial antiaging, etc. (Bajaj, 1975; and Penman and Gordon, 1997).

These biochemical constituents are determinant for standard specification of a beverage crop-tea (Mahanta, 1993). Astill *et al.* (2001) 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

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was known to be one of the main factors in determining the quality of the resulting tea drink (Hara *et al.,* 1995). Polyphenols had been widely utilized in studying the diversity of tea germplasm. Magoma *et al.* (2000) and Gulati *et al.* (2009) though its concentration also depend on the environment (Mariya *et al.,* 2003). Certain green plant pigments especially carotenoids appears to be significant in black tea quality determination (Taylor *et al.*, 1992), particularly considerable impact on the flavor and appearance of black tea (Taylor and McDowell, 1990). Earlier workers (Sanderson *et al.*, 1971; and Mahanta and Hazarika, 1985; and Narmaniya and Lagvilava, 1989) reported that pigments (total chlorophyll and carotenoids) had positive influence on final leaf colour and development of the volatile flavour complex ('aroma') of the manufactured tea. Plant pigments also have role in the selection of clonal material in plant breeding trials. Chlorophyll content variation due to different factors, such as genotype of leaf (Wickremasinghe, 1974), increase in rain-fall (Wickremasinghe *et al.,* 1966), shade and low elevation (Barua, 1964) and different seasons (Nagao, 1969). Bajaj (1975) emphasized the ap-plication of pigment (chlorophylls and carotenoids, etc.), profiles for the selection of breeding material and for fingerprinting of clones/*jats*.

Though experimental tea cultivation was started by the British East India Company in Assam by importing seeds China, however, the successful tea cultivation began only after 1823 after the discovery of indigenous Assam plant which was used by a local Siphung tribe of Assam. The better cup quality produced by the Assam tea plant had popularized the Assam variety as an important planting material in the tea industries of the country and it produces some of the finest and expensive teas with a distinctive strength, colour and flavour. The introduction and cultivation of Chinery type along with Assam plants resulted in the development of hybrid plants as tea is highly out breeding crop characterized by self incompatibility (Baruah, 1965; and Bezbaruah, 1975). Initially those genetically diversified population commonly known as seed *jats* were used as only source of propagation until the discovery of the vegetative method of producing planting materials of tea. From the selected plants from old seed *jats* and progenies of biclonal hybrids, 33 vegetative clones, 15 biclonal seed stocks and 153 TRA-Garden series clones were developed for the tea industry. Apart from these planting materials, few tea estates developed their own planting materials by adopting selection method from their old plantations. The plants were selected on the basis of yield and quality parameters and were further propagated by vegetative methods (single leaf internode cutting) to maintain the quality and homogeneity of the mother plant. These planting materials were approved by tea board and released as industry clones. There are more than 100 numbers of industry which are under cultivation and few of them are performing outstanding with regard to yield and quality.

At present India is the second largest producer of tea next to China producing around 1208.78 Million kg of made tea and Assam alone produces 52% of the total tea produced in India and about 1/7th of the tea produced in the world (Tea Board, 2013-14). But the amount of export was slightly decreasing from 2010 (222.02 mkg) to 2015 (199.00 mkg) and the portion of cultivated land for tea growing cannot be expanded unlimitedly. In order to increase the foreign currency income from tea production, a feasible method practiced in many countries is the utilization of new highly productive tea cultivars/clones in tea production. Due to its specific characteristics, i.e., woody perennial, highly heterogeneous and self-incompatible, tea breeding is costly, therefore selection based on natural populations play an important role in introducing new potential cultivars/clones. With a longstanding history of cultivating and consuming tea, North East India is believed to be highly rich in genetic diversity of tea. Furthermore, amongst the existing heterogeneous species, some better quality traits, much desired for better and higher, tea production may still exist. In many tea gardens various industry clones with diversified genetic base are still cultivated due to their high qualitative and quantitative characters which can be selected as parent material in future breeding programme to develop superior clones for the tea industry. But these heterogeneous species are almost on the verge of extinction due to the rapid uprooting of old tea sections. Also due to the planting of limited number of genetically similar clones and seed stocks for higher yield, the genetically base of the tea crop has been rapidly narrowing down which may lead to vulnerability of various biotic and abiotic stress (Konwar,1999).

Therefore looking into its importance, a study had been under taken to evaluate and document the identified genetically diverse tea species on the basis of biochemical characters and can be used for profiling of various tea cultivars into their basic kinds and suitability for manufacturing different forms of made tea like black tea or green tea.

MATERIALS AND METHODS

Study Area

The study was carried out in Dibrugarh district situated in eastern part of Assam under the Agro climatic zone of Upper Brahmaputra Valley zone. The Average annual rainfall of the district is around 2,076 mm. The minimum temperature of the district goes to 9.5 °C in the month of January and maximum to 36.6 °C in the month of August. The predominantly soil of the district is clay loam belongs to alluvial type having pH range of 4.5-6.0.

Plant Materials

Fourteen cultivated industry clones viz. P_{126}, P_{18}, P_{38}, P_{195}, Keyhang, N_{436}, S_3A_3, T_3E_3, Teenali-17, N_{325}, N_{305} and L_{807} cultivated in the tea estates of Upper Brahmaputra Valley zone of Assam for profiling the clones on the basis of total polyphenols and pigments contents in fresh tea shoots. The fresh shoots taken for the present investigation were constited of one apical bud and two leaves. The study was done for four harvesting seasons, first flush (March-April), second flush (mid May-mid June), rain flush (June-July) and autumn flush (mid September-October) during 2014 and 2015. The age of the cultivated industry clones were ranges between 35 to 40 years and had followed the same pruning cycle, i.e., light pruned-unpruned–deep skiff during the period of three years investigation.

Polyphenols Extraction and Estimation Procedure

Total polyphenols were determined according to the method given by Bray and Thorpe (1954). Estimation of polyphenols with Folin-Ciocatteu reagent is based on the reaction between phenols and an oxidizing reagent phosphomolybate which results in the formation of blue complex. First samples were oven dry at 40 °C for 15 minutes to stop the enzymatic (polyphenol oxidize) activity. After the oven dried, the sample were crushed to make powder. From the crushed powder, 1 g of sample was weighed accurately and extracted with 10 ml 80% ethanol. Centrifuged at 10,000 rpm for 20 minutes and pooled the supernatant. After evaporating the supernatant to dryness, dissolved the residue in 10 ml of distilled water. Then 0.5 ml of Folin-Ciocalteu reagent was added to 1 ml of extract followed by adding 2 ml of 20% $NaCO_3$ solution and mixed well. Then volume was made to 5.5 ml by adding 2 ml distilled water. Contents in the test tubes were heated in a boiling water bath for 1 minute and cooled under running water and absorbance was measured at 650 nm in a spectrophotometer. A standard curve was prepared from different concentrations of catecol. The value of total polyphenol was expressed in per cent. Estimation was done on four occasions, i.e., in first flash, second flash, rain flush and autumn flash.

Chlorophyll Extraction and Estimation Procedure

The extraction was done with methanol following the method of Taylor (1992) to analyze chlorophyll and carotenoid pigments. Two leaves and bud exposed to full sun light were sampled and homogenized 0.5 g of tissue in a pestle and mortar with 25 ml under dark condition and centrifuged at 5000 rpm for 5 minutes. Then 2 ml of supernatant was diluted to 10 ml of methanol. Then spectrophotometric observation of diluted solutions were recorded in three different wavelengths, *viz.*, 470 nm for total carotene (Tc), 653 nm for chlorophyll-a and 666nm for chlorophyll-b. The spetrophotometric values are converted into the actual quantities of chl-a, chl-b and Tc by using the standard formulae as follows-

$$\text{Chl-a} = 15.65A_{666} - 7.34A_{653}$$

$$\text{Chl-b} = 27.05A_{653} - 11.21A_{666}$$

$$Tc = \frac{1000A_{470} - 2.86Chl-a - 129.2Chl-bTc}{245}$$

The statistical significance of difference was assessed by doing ANOVA and the comparison of means was done by calculating Critical Difference (CD) at 5% probability level. To construct a dendrogram representing the relationship among the clones, the accessions were grouped by cluster analysis using the Unweighted Pair Group Method Analysis (UPGMA) based on the Proximity matrix of Squared Euclidean Distance. The correlation was carried out using the Statistical Package for Social Science (SPSS) 18.

RESULTS AND DISCUSSION

Total Polyphenols

The phenolic compounds that are present in young tea shoots are known to be one of the main factors in determining the quality of made tea (Hara *et al*., 1995). Misra *et al*. (2008) reported that the fresh tea shoots were extremely rich in phenolic compounds which can constitute up to 300 mg/g of dry leaves. In the present study, the total polyphenols content (Table 1) in fresh tea shoots showed significant difference amongst the clones and also amongst

Table 1: Comparisons Between the Industry Clones on Total Polyphenol (TPP) Content, Chlorophyll-a, Chlorophyll-b, Total Chlorophyll and Cerotenoids for Four Different Seasons

Parameter	Clone	First	Second	Rain	Autumn	Mean	CV (%)
TPP (%)	R_{94}	23.97±0.12	27.52±0.10	23.24±0.14	24.99±0.18	24.93	6.68
	P_{126}	18.18±0.17	21.69±0.19	17.57±0.32	18.87±0.42	19.08	8.61
	N_{436}	21.11±0.10	24.13±0.12	22.03±0.29	23.29±0.11	22.64	5.29
	M_1	22.16±0.09	24.69±0.06	21.85±0.14	22.95±0.16	22.91	4.97
	P_{38}	22.76±0.23	24.94±0.29	21.97±0.13	23.48±0.24	23.29	4.9
	P_{195}	22.37±0.06	24.23±0.05	22.35±0.19	23.64±0.18	23.15	3.65
	P_{18}	22.31±0.19	23.97±0.25	22.28±0.24	22.72±0.30	22.82	3.25
	Keyhang	23.48±0.11	24.97±0.14	22.58±0.36	24.07±0.12	23.77	3.85
	N_{325}	23.67±0.11	25.95±0.13	23.43±0.06	24.59±0.19	24.41	4.19
	N_{305}	22.83±0.23	24.75±0.13	22.08±0.31	23.85±0.17	23.38	4.52
	L_{807}	24.08±0.25	26.59±0.15	24.77±0.36	24.32±0.26	24.94	4.16
	S_3A_3	24.93±0.10	27.50±0.15	24.66±0.16	26.10±0.06	25.8	4.48
	T_3E_3	22.32±0.12	24.13±0.16	22.22±0.17	22.47±0.17	22.79	3.58
	Teenali-17	24.14±0.06	26.41±0.16	23.35±0.28	24.23±0.01	24.53	4.81
	Season Mean	22.74	25.11	22.46	23.54		
	Significance (C.D. at P=0.05)						
	Between cultivars © : 0.000						
	Between seasons (S) : 0.000						
	Interactions (C x S) : 0.000						
Chl a (mg/g)	R_{94}	0.79±0.01	0.89±0.02	1.05±0.02	0.84±0.02	0.89	11.15
	P_{126}	0.87±0.03	0.90±0.02	1.02±0.01	0.92±0.02	0.93	6.45
	N_{436}	0.77±0.02	0.87±0.03	0.94±0.01	0.83±0.02	0.85	7.88
	M_1	0.76±0.01	0.82±0.01	0.86±0.01	0.78±0.01	0.81	5.38
	P_{38}	0.75±0.01	0.82±0.02	0.83±0.01	0.79±0.03	0.81	4.03
	P_{195}	0.92±0.01	0.93±0.02	1.05±0.02	0.89±0.02	**0.95**	6.63
	P_{18}	0.87±0.01	0.92±0.01	0.97±0.03	0.83±0.01	0.9	5.74
	Keyhang	0.79±0.01	0.93±0.01	1.02±0.01	0.96±0.01	0.93	9.39
	N_{325}	0.76±0.01	0.86±0.01	0.94±0.01	0.83±0.01	0.85	7.75
	N_{305}	0.75±0.01	0.79±0.02	0.91±0.01	0.77±0.01	0.81	7.71
	L_{807}	0.72±0.01	0.91±0.01	0.98±0.01	0.81±0.01	0.85	12.07
	S_3A_3	0.72±0.01	0.81±0.02	0.92±0.01	0.76±0.02	**0.8**	9.88
	T_3E_3	0.81±0.01	0.84±0.01	0.95±0.02	0.77±0.02	0.84	8.58
	Tinali-17	0.87±0.02	0.92±0.02	1.06±0.01	0.82±0.01	0.92	9.99
	Mean	0.8	0.87	0.96	0.83		
	Significance (C.D. at P=0.05)						
	Between cultivars © : 0.000						
	Between seasons (S) : 0.000						
	Interactions (C x S) : 0.000						

Table 1 (Cont.)

Parameter	Clone	First	Second	Rain	Autumn	Mean	CV (%)
Chl b (mg/g)	R_{94}	0.24±0.005	0.32±0.022	0.40±0.013	0.23±0.018	**0.3**	24.21
	P_{126}	0.22±0.005	0.24±0.015	0.34±0.008	0.22±0.007	0.26	20.26
	N_{436}	0.23±0.013	0.26±0.043	0.36±0.013	0.23±0.020	0.2	22.67
	M_1	0.21±0.005	0.22±0.009	0.27±0.015	0.18±0.011	0.22	16.65
	P_{38}	0.23±0.004	0.24±0.022	0.25±0.014	0.23±0.007	0.24	6.06
	P_{195}	0.20±0.005	0.20±0.022	0.24±0.008	0.22±0.011	0.21	10.08
	P_{18}	0.15±0.008	0.21±0.011	0.22±0.011	0.17±0.016	**0.18**	16.81
	Keyhang	0.17±0.012	0.24±0.005	0.29±0.011	0.21±0.016	0.23	19.86
	N_{325}	0.15±0.011	0.19±0.005	0.26±0.011	0.19±0.009	0.2	16.65
	N_{305}	0.19±0.007	0.24±0.004	0.27±0.008	0.18±0.004	0.22	17.42
	L_{807}	0.15±0.005	0.19±0.008	0.23±0.004	0.19±0.004	0.18	15.92
	S_3A_3	0.16±0.009	0.19±0.010	0.25±0.004	0.21±0.004	0.2	16.16
	T_3E_3	0.18±0.011	0.18±0.004	0.21±0.007	0.19±0.008	0.19	7.72
	Tinali-17	0.16±0.007	0.21±0.008	0.25±0.011	0.18±0.009	0.2	18.42
	Mean (seasonwise)	0.19	0.22	0.27	0.2		
	Significance (C.D. at P=0.05)						
	Between cultivars © : 0.000						
	Between seasons (S) : 0.000						
	Interactions (C x S) : 0.000						
Total Chl (mg/g)	R_{94}	1.04±0.02	1.20±0.02	1.45±0.03	1.06±0.06	1.18	14.34
	P_{126}	1.11±0.07	1.13±0.03	1.36±0.02	1.17±0.09	**1.2**	9.72
	N_{436}	0.99±0.02	1.11±0.04	1.30±0.01	1.06±0.03	1.12	10.74
	M_1	0.97±0.04	1.04±0.02	1.14±0.02	0.96±0.07	1.02	7.41
	P_{38}	0.98±0.05	1.05±0.02	1.07±0.08	1.02±0.08	1.03	3.93
	P_{195}	1.12±0.04	1.13±0.05	1.39±0.07	1.11±0.02	1.16	6.89
	P_{18}	1.02±0.07	1.13±0.02	1.17±0.05	1.11±0.02	1.08	6.9
	Keyhang	0.96±0.02	1.17±0.04	1.30±0.02	1.17±0.02	1.15	11.04
	N_{325}	0.90±0.04	1.05±0.03	1.19±0.02	1.01±0.03	1.04	10.17
	N_{305}	0.91±0.02	1.05±0.03	1.19±0.03	0.94±0.03	1.02	11.13
	L_{807}	0.86±0.03	1.10±0.02	1.20±0.03	1.00±0.03	1.04	12.66
	S_3A_3	0.92±0.04	0.98±0.03	1.15±0.02	0.98±0.04	**1.01**	8.94
	T_3E_3	0.99±0.04	1.02±0.05	1.10±0.02	0.95±0.02	1.03	7.95
	Teenali-17	1.02±0.02	1.14±0.02	1.32±0.03	1.01±0.03	1.12	11.31
	Mean	0.99	1.09	1.24	1.04		
	Significance (C.D. at P=0.05)						
	Between cultivars © : 0.000						
	Between seasons (S) : 0.000						
	Interactions (C x S) : 0.000						

Table 1 (Cont.)

Parameter	Clone	First	Second	Rain	Autumn	Mean	CV (%)
Carotinoids (mg/g)	R_{94}	0.091±0.03	0.107±0.02	**0.175**±0.02	0.103±0.02	0.119	29.42
	P_{126}	0.109±0.02	0.123±0.03	0.174±0.02	0.118±0.03	**0.131**	20.98
	N_{436}	0.124±0.02	0.118±0.04	0.167±0.05	0.106±0.04	**0.129**	19.49
	M_1	0.114±0.07	0.126±0.04	0.159±0.02	0.111±0.04	**0.127**	16
	P_{38}	0.114±0.06	0.114±0.04	0.140±0.05	0.093±0.02	0.115	15.52
	P_{195}	0.102±0.02	0.093±0.03	0.153±0.04	0.105±0.05	0.113	21.47
	P_{18}	0.099±0.03	0.091±0.02	0.120±0.03	0.085±0.05	**0.099**	14.07
	Keyhang	0.099±0.04	0.112±0.05	0.153±0.03	0.098±0.04	0.116	20.21
	N_{325}	0.108±0.06	0.109±0.07	0.159±0.05	0.086±0.03	0.116	24.09
	N_{305}	0.104±0.05	0.115±0.04	0.146±0.06	0.100±0.04	0.116	30
	L_{807}	0.108±0.03	0.147±0.05	0.166±0.04	0.088±0.03	**0.127**	25.05
	S_3A_3	**0.082**±0.04	0.110±0.04	**0.170**±0.03	0.096±0.03	0.115	30.26
	T_3E_3	0.096±0.03	0.092±0.03	0.164±0.04	0.117±0.03	0.117	25.09
	Teenali-17	0.103±0.03	0.120±0.02	0.166±0.03	0.096±0.03	0.121	24.83
	Mean	0.104	0.113	0.147	0.1		
	Significance (C.D. at P=0.05)						
	Between cultivars © : 0.000						
	Between seasons (S) : 0.000						
	Interactions (C x S) : 0.000						

the seasonal. The highest value for total polyphenols content amongst the fourteen industry clones was found in S_3A_3 (25.79 %) followed by L_{807} (24.93%) and R_{94} (24.93%). The lowest total polyphenols content was recorded in P_{126} (19.08). The content of total phenolics in fresh tea shoots grown in Kenyan plain tea quality parameters and clones with low total phenolic content produced low quality black teas (Obanda *et al*., 1997). So the industry clones found with higher total polyphenols content in the present study can be selected for black tea manufacturing.

The weather parameters, *i.e.*, temperature, rainfall, evaporation and bright sunshine hours also induced the variations in biosynthesis and accumulation of phenolic compounds in tea. Harbowy and Balentine (1997) reported that the biosynthesis of phenolic compounds can be effectively induced by sunlight. When seasonal variation is considered for all the fourteen industry clones, the maximum total polyphenol content was found in second flush (25.11%) followed by autumn flush (23.54%), first flush (22.74%) and the lowest was in the rain flush (22.46%). On the basis of above result, the second flush tea shoot could be considered as the best harvest amongst the four harvesting seasons in respect of quality tea production. These results are in agreement with the findings of Yao *et al*. (2005) who observed more phenolic compound occurred during bright sunny months in the tea shoots. During second flush season, sunshine hours are more with minimum rainfall which is fall during the month of May-mid of June in the climatic condition of Upper Brahmaputra Valley Zone. Day length and the mean temperatures have been reported to be the only climatic factors significantly correlating with the tea productivity in North-East India (Sen *et al.*, 1966). In the study, the total polyphenols content results would indicated that there was a potentiality to produce better quality black tea from all the studied clones though $L_{807,}$ S_3A_3 and R_{94} showed higher total polyphenols content amongst all the studied clones.

The Pigments

The leaf pigment contents *viz.* chlorophyll a, chlorophyll b, total chlorophylls and carotenoids showed significant differences ($p<0.05$) for all the industry clones. The maximum value for chlorophyll a, b and total chlorophyll content were found in P_{195} (0.95 mg/g), R_{94} (0.30mg/g) and P_{126} (1.20 mg/g), respectively (Table 1). The lowest value for chlorophyll

a (0.80 mg/g), chlorophyll b (1.01 mg/g) was found in S_3A_3 and total chlorophyll content (0.18 mg/g) in P_{18} respectively. The highest carotenoids content was found in P_{126} (0.131 mg/g) and the lowest was in P_{18} (0.099 mg/g). Liyanage *et al.* (1993) reported that chlorophyll plays a significant role in tea blackness that is one of the most important factors in commercial evaluation of tea. After undergoing a lot of changes during oxidization process, chlorophyll turns into pheophytin (black colour) and pheophorbide (brown color). Ravichandran *et al.* (2002) reported that tea bushes with light colour indicated lower quality tea. However, Bera *et al.* (2012) stated that leaves with more chlorophyll produced lower quality tea. Earlier experiments had shown that shoots with higher chlorophyll content produced low quality (inferior) tea which usually contained a grassy odour (Dev Choudhury and Bajaj, 1980). They reported that the conversion of chlorophylls to phaeophytin during oxidation and firing contributed to the black appearance of tea. But in the same time when chlorophyll content is higher, the theaflavins and thearubigins content were reduced and tasters variation was also low (Dev Choudhury and Bajaj, 1980). However Sud and Baru (2000) reported that the higher chlorophyll content would lead to higher total liquor colour. It contributes to the 'blackness' of made tea which is considered to be one of the important criteria in the commercial evaluation of tea (Liyanage *et al.*, 1993).

The highest amount of pigment development (chlorophyll a, chlorophyll b, total chlorophylls and carotenoids) was observed during rain flush (0.96 mg/g, 0.27 mg/g, 1.24 mg/g and 0.147 mg/g respectively) for all the industry clones. The present finding was well supported by many earlier findings (Hazarika and Mahanta, 1984; and Sud and Baru, 2000). Accumulation of higher chlorophyll during rainy season (Table 2) could have reflected positively on the total liquor colour of rainy season teas. Sud and Baru (2000) reported that the higher chlorophyll content would lead to higher total liquor colour.

While estimating the amount of carotenids in fresh green tea leaves in the present study, it was observed that carotenoids value was ranged from 0.082 to 0.175 mg/g fresh weight. The maximum carotenoids content was found in P_{126} (0.131 mg/g). Aroma is one of the most important factors in the evaluation of the quality of final black tea. The higher concentration of carotenoid adds to the formation of flavoury compound (Hazarika and Mahanta, 1983). Concentrations of carotenoids also showed significant variation over the seasons. The maximum amount of carotenoid content was found in rain flush (0.147 mg/g) followed by second flush (0.113 mg/g) for all the estate clones. These observations are in contradiction with the reports for Assam conditions (Hazarika and Mahanta, 1984); where they have reported that the carotenoid concentrations are minimum in monsoon periods and maximum during summer periods.

Table 2: Grouping of Estate Clones to Different Clusters Based on the Dendogram

Cluster I	Cluster II	Cluster III	Cluster IV
R_{94}		N_{436}	
N_{325}		M_1	
L_{807}		P_{38}	
Teenali-17	P_{126}	P_{195}	S_3A_3
		P_{18}	
		Keyhang	
		N_{305}	
		T_3E_3	

Genetic Relationship and Average Linkage Cluster Analysis

In the present study an attempt has been made to explore the biochemical composition of fresh green tea shoots and also the seasonal variation of the biochemical parameters of the fourteen industry tea clones so that the clones can be characterized on the basis of biochemical descriptors. In many tea growing countries, work had been done on characterizing their tea germplasms using biochemical constituents like total total polyphenols, chlorophylls, carotenoids and caffeine in the fresh leaf as discriminative markers for to evaluate diversity and genetic potential warehoused in the germplasm (Magoma *et al.*, 2003; Chen and Zhou, 2005; Lopez *et al.*, 2005; and Sabhapondit *et al.*, 2012). Gulati *et al.* (2009) reported that China hybrids produce low level of total polyphenol compared to Assam and Cambod types. In the experiment, the high polyphenol content was found in fresh tea shoots of S_3A_3, Teenali-17 and R_{94} which could be classified under Assam type. Saravanan *et al.* (2005) mentioned that the total polyphenols and its components could be used to classify naturally hybridized tea populations of different types/*jats*. Again Ullah (1979) reported that china *jats* of tea cultivars posses high pigment content specially the total chlorophyll and carotenoids. So the clones with high total chlorophyll and

carotenoid content P_{126} N_{436} M_1 and L_{807} can be characterized as china type of plant. Study conducted at Central Africa (Anon, 1997) on quality potential in tea clones by measuring chlorophyll fluorescence revealed that analysis of chlorophyll fluorescence can be used as selection tool for quality potential in tea clones. The technique will also help quick selection of tea seedlings in field for high quality genotypes with which genetic base of the breeding programmes can be broadened.

According to the results of the average linkage cluster analysis (Figure 2), accessions studied were grouped into four main clusters. Out of fourteen clones, eight clones (N_{436} M_1 P_{38} P_{195} P_{18} Keyhang N_{305} and T_3E_3) were grouped into cluster III and were with average quality characters on the basis of biochemical properties. It was observed that S_3A_3 and P_{126} formed two discriminated clusters and thus group individually (Table 2). The squared Euclidean Distance method which was used to estimate the correlation similarity proximity matrix with the total polyphenol and pigments, also showed the lowest similarity value between the clone S_3A_3 and P_{126} (Table 3). The clone P_{126} grouped under Cluster II exhibited unique biochemical characteristics with lowest amount of total polyphenols and high pigments (Chlorophyll a, chlorophyll b, total chlorophyll and Carotenoids) content which may be selected for producing an emerald-green, smooth finished green tea product. The cluster IV was formed by the single clone S_3A_3 which was found with high Polyphenol and low pigment contents and

Figure 1: Change of Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotinoids in the Fourteen Estate Clones

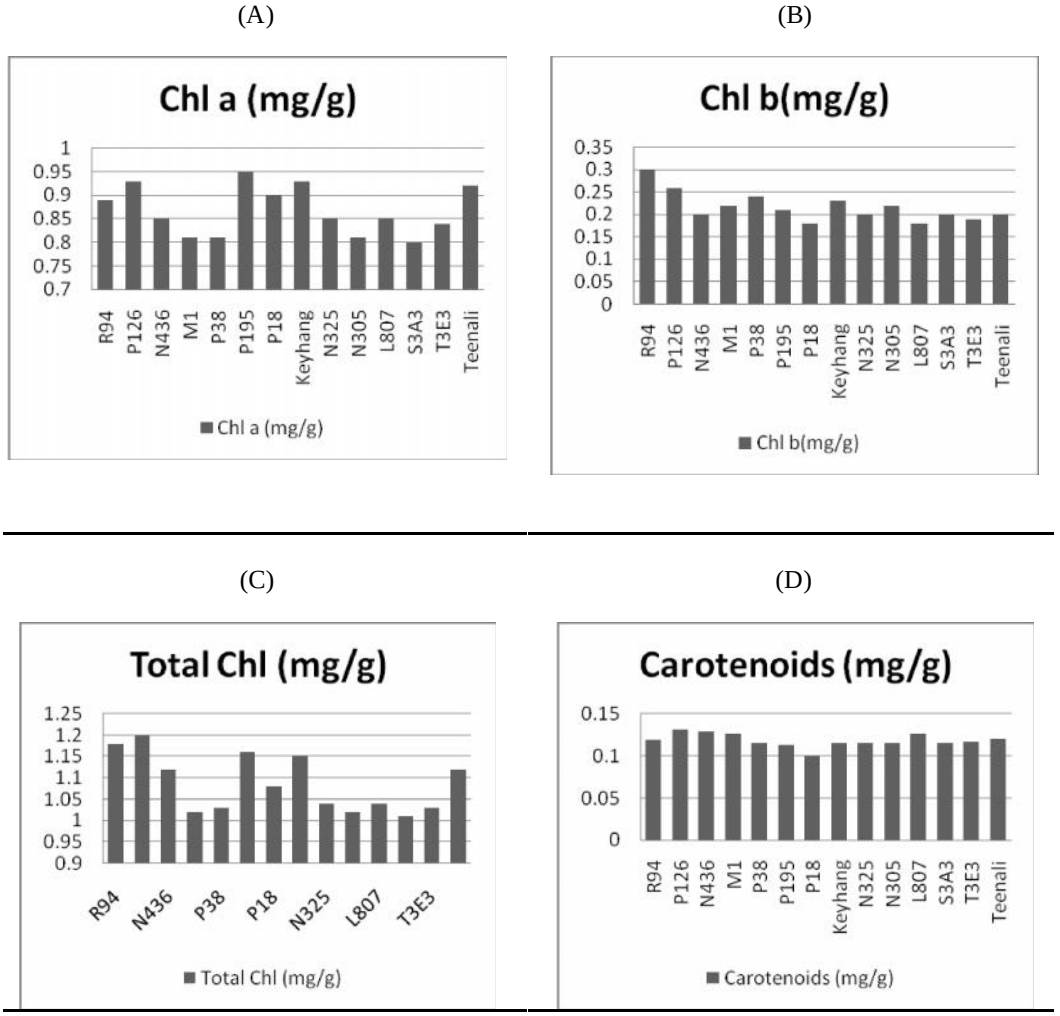
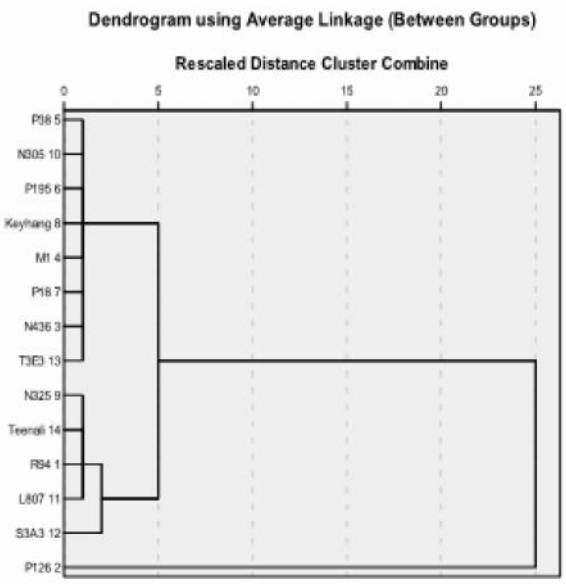


Table 3: Pearson Similarity Coefficient Matrix Utilizing Total Polyphenol and Pigment Content Data

Case	Rescaled Squared Euclidean Distance													
	R_{94}	P_{126}	N_{436}	M_1	P_{38}	P_{195}	P_{18}	Keyhang	N_{325}	N_{305}	L_{807}	S_3A_3	T_3E_3	Teenali-17
R_{94}	0	0.76	0.118	0.097	0.062	0.072	0.103	0.031	0.008	0.055	0.014	0.025	0.103	0.011
P_{126}		0	0.281	0.325	0.393	0.366	0.309	0.487	0.63	0.41	0.766	1	0.307	0.657
N_{436}			0	0.003	0.009	0.006	0.001	0.028	0.069	0.012	0.123	0.224	0.001	0.081
M_1				0	0.005	0.004	0.001	0.019	0.053	0.007	0.099	0.185	0.004	0.058
P_{38}					0	0.001	0.005	0.006	0.028	0	0.066	0.142	0.006	0.036
P_{195}						0	0.003	0.008	0.036	0.002	0.077	0.159	0.004	0.044
P_{18}							0	0.021	0.057	0.008	0.106	0.198	0.002	0.065
Keyhang								0	0.01	0.004	0.039	0.095	0.022	0.015
N_{325}									0	0.023	0.012	0.048	0.058	0.005
N_{305}										0	0.06	0.133	0.008	0.033
L_{807}											0	0.029	0.109	0.013
S_3A_3												0	0.207	0.036
T_3E_3													0	0.072
Teenali-17														0

Figure 2: Dendrogram of Average Linkage Cluster Analysis Based on Biochemical Parameters



has relation with Assam type of cultivars. This clone can be considered as good clone for making black tea. A parameter of fresh tea leaf that correlates with black tea quality is highly desired. The biochemical constituents may serve as criterion for selection of cultivars for black tea with high polyphenol content (Ikeda *et al.,* 1995) or for green tea with low polyphenol and high chlorophyll content. Again clones S_3A_3 and P_{126} can also be used as parent materials for developing better quality content.

Table 4: Correlation Analysis Among the Total Polyphenols and Pigments of Fresh Young Tea Shoots

Pearson Correlation Sig. (2-tailed)	TPP	Chl-a	Chl-b	Total chl	Carotinoids
TPP	1				
Chl-a	-.189**	1			
	0.002				
Chl-b	-.214**	.542**	1		
	0	0			
Total chl	-.246**	.933**	.790**	1	
	0	0	0		
Carotinoids	-.261**	.610**	.634**	.689**	1
	0	0	0	0	

Note: **. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Correlation Among Total Polyphenols and Total Pigments

The results of correlation among the biochemical parameters indicated that there was a negative correlation among the

amount of pigments and quality factors, *i.e.*, total polyphenols. Carotenoid has a positive correlation with chlorophyll but a negative one with polyphenols that cause transparency and color in tea. Wei *et al*. (2011) observed that the rise of chlorophyll-*a* content during young leaf development was associated with the decline of catechin. The more the amount of chlorophyll, the more the amount of carotenoid will be (Hazarika and Mahanta, 1983; and Hazarika and Mahanta, 1984). However, there was a significant positive correlation among all the pigment parameters, i.e., chlorophyll a, chlorophyll b, total chlorophyll and carotenoid at 1% probability. But as stated above, the chlorophyll add to colour of the liquor of the black tea and carotenoids enhance tea flavor, so from the point of colour and aroma, rain flush may be are considered as best flush for black tea manufacture.

CONCLUSION:

To providing a useful tool for studying the effects of cultivar on black tea quality, the establishment of a quantitative relationship between the plant pigment composition of the green leaf and 'quality' could provide a useful tool for selecting breeding materials for plant development programme. These also will help in studying the effects of agronomic practices, environmental factors and manufacturing conditions on the quality of black tea. In the present study an attempt has been made to explore the biochemical composition of fresh green tea shoots and also the seasonal variation of the biochemical parameters of the fourteen industry tea clones so that the clones can be characterized on the basis of biochemical descriptors. Again the industry clones (P_{126}) with low polyphenol content and high total chlorophyll content may be selected for producing an emerald-green, smooth finished green tea product while the estate clones (S_3A_3, Teenali-17 and R_{94}) with high polyphenol and low carotinoid content may be preferred for quality black tea production.

Higher chlorophyll and carotenoid content enhance the antioxidant activities in tea. Based on this, it also can be stated that when medical and health properties are more concerned than quality, clones with more pigments such as P_{126} will be more suitable planting material. The results may be used for profiling the industry clones for black and green tea manufacturing on the basis of pigments and total polyphenol contents which again may be used for the selection of breeding material and for fingerprinting of clones/*jats*.

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