Original Research Article

Characterization of Some Perennial Pigmented Tea Accessions of Assam

ABSTRACT

Tea leaves has high antioxidant activity mainly due to presence of high amount of flavonoids and to some extent presence of pigments and has been a potential important raw material for the pharmaceutical industry. Therefore a study had been under taken to evaluate twenty (20 nos.) pigmented tea accessions exhibiting morphological variations of Experimental Garden for Plantation Crops, Assam Agricultural University, Assam to profile based on shoot growth and biochemical characters by field survey and laboratory investigation during the period 2017-18. Post hoc analysis was used to test the significance level and correlation similarity co-efficient matrix was used to analyze the genetic relationship among clones.

The plucking point density which is an important criteria from yield point of view was found to be highest in THT 028 (0.158 g) with maximum fresh weight of young shoot for both Light Prune (LP) and Medium Skiff (MS) years whereas the maximum dry weight was found in THT 035 (0.036 g) followed by THT 028 (0.035 g) in both LP and MS years. An increasing trend of, antioxidant and total catechin content observed from rain flush to autumn flush in all the germplasms. The germplasms with purple young shoot colour THT 024, THT 032 and THT 039 had the highest anthocyanin content (39 mg/l, 43.7 mg/l and 40.56 mg/l respectively) and they can be further studied for development of specialty tea. The germplasms with higher catechin content (THT 027, THT 030 and THT 034) can be studied for development of parent material for green tea manufacturing. On hierarchical cluster analysis based on dendogram, THT 024, THT 032 and THT 039 formed discriminated clusters on the basis of shoot characters and biochemical characters which can be selected for future breeding programme.

Key words: Tea, Germplasm, Anthocyanin, Antioxidant, Catechin

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1. INTRODUCTION

Tea is an evergreen, perennial, cross-pollinated [1] tree species, young leaves of which are processed to prepare a nonalcoholic beverage globally known as ‘tea’. Tea is drunk in almost every country around the world and has reached a ceremonial status in many places both as a social and medicinal beverage. Based on the environmental conditions as well as the historical and scientific documents, tea has been a valuable perennial industrial crop in terms

of economy, pharmaceutical materials, environment protection, and also socio-culture aspects of many regions of the world. Tea is a potential commercial crop in India which gives a reasonable foreign currency income. Indian tea is among the finest in the world owing to strong geographical indications, heavy investments in tea processing units, continuous innovation, augmented product mix and strategic market expansion. The main tea growing regions in India are Northeast India including Assam, Darjeeling district and the Dooars region of north Bengal and in the Nilgiris of South India. Tea industry has largely contributed to the economy of Assam. Of the agriculture-based industries, tea occupies an important place in Assam. Assam produces some of the finest and expensive teas and has its own variety Camellia assamica. In production, India is the second largest producer of tea next to China producing around 1257.53 Million kg of made tea as per Tea Board, 2020. India also has a significant share in the international tea market with a 11 % share of world tea exports in 2020.

Tea has a number of biochemical compounds which are found to have a large number of health benefits. Tea contains bioactive polyphenols, mainly the catechins, anthocyanins etc. which play an important role in green and black tea quality [2]. This pharmacological value of tea heavily relies on its antioxidative properties known to surpass that of major antioxidants such as vitamins C and E [3]. Tea leaves has high antioxidant activity mainly due to presence of high amount of flavonoids and to some extent presence of pigments and has been a potential important raw material for the pharmaceutical industry. It has been suggested that green and black tea may protect against cancer [4] or other diseases such as obesity [5] or Alzheimer's disease [6] for the antioxidant property of tea.

However, compared to other flavonoid groups from tea such as the catechins, little is known about the bioavailability and pharmacological benefits of tea anthocyanins (ACN) despite the fact that anthocyanins from other sources have been associated with a broad spectrum of health benefits including cardiovascular, neurological, urinary tract and ocular protection, as well as anti-carcinogenic, anti-diabetic, anti-aging, antioxidant and anti-inflammatory properties [7]. The health benefits of a diet rich in phytochemicals is attributed to the complex mixture of phytochemicals present in it, an observation which clearly suggests that to improve their nutrition and health, consumers should take antioxidants from diverse sources.

Tea germplasm is presently one of the most valuable fundamental materials for tea breeding and biotechnology, with valuable potential for the whole tea industry in the future. India especially the North East India have a long-standing tradition and customs of drinking, processing and planting tea. North East India is also believed to be highly rich in genetic diversity of tea and the genetic diversity within and between local cultivated tea accessions. Amongst the available accessions of this region, some are found with annual purple pigmentation. However some purple colouration also occurs in leaves of some tea varieties in winter season, which may be due to increase in anthocyanin content in the leaves.

But at present the widespread cultivation of clonal tea would loss of genetic diversity due to focus on material with high yield, eventually resulting in low adaptability to prevailing erratic climatic conditions this has led to deterioration of existing plants in terms of yield and quality. Therefore, rational utilization of the available germplasm in breeding programmes with an understanding of the morphological and biochemical diversity among the germplasm accessions is important if the best results are to be obtained from crop improvement programmes.

From commercial point of view, to increase the foreign currency income from tea production, introduction of improved varieties with high production and quality is the need of the hour. Possible solution is to look for planting material that could retain or at least compensate this loss in the yield and quality of tea under the prevailing climatic conditions through making foray into specialty tea market such as pigmented tea.

In the Experimental Garden for Plantation Crops, Assam Agricultural University, Jorhat there are some tea accessions which show seasonal and perennial purple pigmentation. It is

expected that the presence of anthocyanins in addition to the catechins would contribute to new and unique tea products for developing planting materials for specialty tea. Therefore looking into its importance, a study had been under taken to evaluate and document the characteristics of available accessions of tea plants based on shoot growth and autumnal and perennial colorations in conformity with the descriptor and can be used for profiling of various tea cultivars to estimate the suitability for manufacturing different forms of made tea like black tea or green tea.

2. **MATERIAL AND METHODS**

Twenty (20 nos.) unidentified germplasm exhibiting morphological variations from others were marked at the Experimental Garden for Plantation Crops of AAU, Jorhat. These were then numbered as THT-021 to THT-040 and utilized in the present investigation to study the shoot growth and biochemical assessment. Clone TV 1 was taken as a standard for the study. The present study was conducted during the period 2017-2018. All the studied germplasm had followed the same pruning cycle i.e. light pruned - medium skiff during the period. In the study, due to the homogeneity of local climatic conditions and the similarity of farming techniques applied, the effect of those factors on the morphological characteristics was not considered or interpreted. The germplasms were light pruned in the month of December 2016 and were medium skiffed in the month of January, 2018.

2.1 Shoot characters

Two leaves and a bud with a little portion of internode below the second leaf was considered to be a young shoot. Numerical taxonomic studies were carried out using both dimensional method and morphological descriptors as per the recommendation of the International Plant Genetic Resources Institute Rome, Italy, 1997 [8]. The shoot characters studied were young shoot colour, young shoot pubescence, plucking point density and shoot weight. Young shoot colour and young shoot pubescence were measured as per the recommendation of IPGRI descriptors are presented in Table 1.

Table 1: Plant morphological descriptors of Camellia species and range of variation used for diversity analysis of young shoot characters of tea

Sl. No.	Characters	Weightage
1.	Young shoot colour	1. Green 2. Bronze 3. Red 4. Purple
2.	Young shoot pubescence	3. Sparse 5. Intermediate 7. Dense

Shoot growth parameters viz; plucking point density and shoot weight were recorded in both light pruned year and the medium skiffed year. Tea shoots were plucked on weekly basis during the month July, 2017 when the number of pluckable shoots was maximum to study the plucking point density of shoot. Plucking point density was measured as the total number of pluckable shoots present per unit area. The shoots were plucked from a 45cm x 45 cm area of the central zone of the bush and plucking density was expressed as number of shoots per 30cm x 30 cm plucking table area. The fresh weight of the young shoot was measured in gram and average value was calculated. These shoots were again oven-dried to record the dry weight and average value was calculated in gram.

2.2 Biochemical characters

For studying biochemical characters, the plant material plucked as a standard of two leaves and a bud from the studied germplasm during July to October for 2017. The estimations were done for two plucking season with three replications for three biochemical characters viz.total anthocyanin, total antioxidant and catechin

Total Anthocyanin Content (TAC) was assayed by using the pH differential method. About 5 g fresh tea leaf samples was extracted three times with 15mL methanol: 1 M HCl (85:15, v/v) using a shaker under dark conditions, and then centrifuged for 15 min at room temperature. The clear supernatants were anthocyanin extracts. Before determination of TAC, two solutions were prepared, one buffer at pH 1.0 (1.49% KCl water buffer, acidified with HCl) and the other buffer at pH 4.5 (1.64% sodium acetate water buffer, acidified with HCl). Samples were diluted 10 times to a final volume of 2 ml. The absorbance of each sample was measured both at 520 nm and 700 nm. TAC was expressed as cyanidin-3 glucoside equivalent, and calculated as follows:

Monomeric anthocyanin pigment (mg/l) = A ×MW×DF ×1000/(€×L)

1000 is the conversion from milliliter to liter.

Where :

A is the absorbance calculated as:

A = (A520nm-700nm) pH 1.0 - (A520nm-A700nm) pH 4.5

MW is the molecular weight for cyanidin-3-glucoside (449.2 g/mol),

DF is the dilution factor

€ is the molar absorbance of cyanidin-3-glucoside (26, 900 L/(cm ×mol)

L is cell path length (1 cm),

The antioxidant method was determined by the method gave by Molyneux in 2004 [9]. Free radical scavenging ability of DPPH (1,1-diphenyl-2-picrylhydrazyl) was determined on methanolic extracts of oven dried tea leaf samples. One gram of oven dried tea leaves sample was extracted in 10 ml methanol, centrifuged at 10000 rpm for 20 min and the supernatant was used for the assay, after making up volume to 10 ml by methanol. To 50 µl – 700 µl of methanolic sample extract methanol was added to make up the volume to 1 ml. To it 1 ml of DPPH (0.1Mm in methanol) was added and the mixture was incubated at room temperature in dark for 30 min. The absorbance was measured at 517 nm taking methanol as blank. A mixture of equal volume of methanol and DPPH reagent served as control. A decreasing intensity of the purple colouration was taken as increasing scavenging activity. Antioxidant activity of L- ascorbic acid and quercentine were also assayed as standard.

The inhibition of DPPH radicals by the sample was calculated as-

$$\text{DPPH inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

The amount of sample necessary to decrease the absorbance of DPPH by 50 percent i.e. IC50 was derived from the percent disappearance vs. concentration plot.

Catechin content in tea samples was estimated by following the method of ISO 14502-2 (ISO, 2005). Approximately 0.2 g of powdered material accurately weighed is extracted with 5 ml of 70% aqueous methanol refluxed in a water bath, set at 70˚C for 10 min. After cooling the extracts were centrifuged at 3000 rpm for 10 min. The supernatant is transferred to a 10 ml volumetric flask. The process is repeated with another 5 ml extraction solvent. 1 ml of the extract was diluted to 5 times with stabilizing agent prepared from 10% acetonitrile, 25 µg/ml ascorbic acid and EDTA each. The estimation of individual catechins was carried out with a High Performance Liquid Chromatography (Dionex UHPLC system, fitted UV- visible detector). 10µl of the diluted extract was injected into a phenomenox Luna 5 micron phenyl hexyl column (250 mm × 4.5mm). The column temperature was kept at 25˚C using a column oven. The mobile phase A was 2% acetic acid, 9% acetonitrile and solvent B was 80% acetonitrile. The programme for elution was 100% solvent A for initial 10 min followed by a gradient to 37% B over a period of 15 min and another 10 min with this condition. The flow rate was 1 ml/ min. The UV/ VIS detector was set at 276 nm.

The individual catechins were identified by comparing retention times from sample chromatograms with those obtained from the standard solutions of catechins obtained under the same chromatographic conditions. Quantifications of catechins were carried out from the anhydrous concentrations (µg/ml) against the peak areas of the standard catechin. The individual catechins were expressed as percentage by mass on a sample dry matter basis.

2.3. Statistical analysis

The differences among the mean values were detected by using one way analysis of variance (ANOVA). The pearson correlation coefficient and the correlation similarity proximity matrix with squared Euclidean Distance method was used to see the genetic relationship amongst the germplasms. Hierarchical Cluster analysis was done to group the germplasms into different clusters with similar morphological, physiological and biochemical characters. The statistical analyses were performed using Statistical Package for Social Science (SPSS) 23.0.

3. RESULTS AND DISCUSSION

3.1 Young shoot characters

Young shoot colour and pubescence showed significant variation amongst the germplasm. Rajanna et al. 2011 [10] reported that, twelve accessions from the germplasm collection centre at Valparai, Tamil Nadu were characterized based on 15 morphological descriptors of IPGRI guidelines using leaf and young shoot characters. In the present experiment, the germplasm THT 024, THT 032 and THT 039 were found to have purple colour young shoot while THT 026, THT 028, THT 031, THT 035, THT 038 and THT 040 had bronzed coloured young shoot (Table 2). Pigmented teas from *Camellia* genus contain red and purple anthocyanins. which are related to similar anthocyanin compounds found in blueberries, raspberries, purple grapes, and other common foods that contribute to their characteristic colors and health benefits. Anthocyanin supplements (proanthocyanins) are widely marketed for their health enhancing properties. The other different products that can be produced from the purple tea leaf include extracted catechins, anthocyanins, anthocyanidinins (which are used as drug supplements, preservatives and other industrial uses), tea polyphenol extracts for pharmacological and industrial uses, manufacture of instant teas, Ready To Drink (RTD) tea, and other fast moving consumer goods such as health care products, foods and confectionaries. Six germplasms (THT 026, THT 028, THT 031, THT 035, THT 038 and THT

040) produced bronze coloured young shoots and three germplasms (THT 024, THT 032 and THT 039) had purplish young shoot colour which can used for making of speciality tea like purple tea. This statement can be supported by the reports by Yang et al. 2011[11] that drinking tea can yield the positive health effects due to presence of high amount of flavonoids and to some extent presence of pigments.

The rest of the germplasm showed green colour young shoot which was similar with that of TV 1. Ravichandran and Parthiban, 1998 [12] reported that tea bushes with light colour indicated lower quality tea. However, Bera *et al.*, 2012 [13] stated that leaves with more chlorophyll produced lower quality tea. In an earlier experiments, Dev Choudhury and Bajaj in 1980 [14] reported that shoots with higher chlorophyll content produced low quality (inferior) tea which usually contained a grassy odour. The clones with green colour may be selected for producing an emerald-green, smooth finished green tea product.

In case of shoot pubescence, variation was prominent with three distinct categories- dense, medium and sparse. Amongst the twenty germplasm, two germplasm viz, THT 024 and THT 039 exhibited dense pubescence while THT 026, THT 027, THT 028, THT 030, THT 032, THT 034, THT 035, THT 036, THT 037, THT 038 and THT 040 were found to have intermediately distributed young shoot pubescence (Table 2). It was also observed that the young shoot pubescence was intermediate in most of the germplasm. Jin, *et.al*., in 2005 [15] in one of his work reported that the amount of pubescence on the leaf epidermis is considered as an important morphological marker for the quality of the green tea. According to literature, the shoots with dense and intermediate pubescence produce better tea than sparse ones irrespective of leaf colour [16]. The germplasm THT 021, THT 022, THT 023, THT 024, THT 025, THT 029, THT 033 including TV 1 possessed sparse pubescence.

In shoot growth, the plucking point density, fresh weight of young shoot and dry weight of young shoots were measured two times: firstly in the light pruned year and secondly in the medium skiffed year. It was observed that in the light pruned year the plucking point density was highest in THT 028 followed by THT 030 and THT 040 with 36, 35 and 34 pluckable shoots per 45 cm^2 area of the plucking surface of the bush (Table 2). In the medium skiffed year also the plucking point density was found to be highest in THT 028 followed by THT 030 with 41 and 40 pluckable shoots per 45 cm^2 area of the pucking surface of the bush respectively. Plucking point density increased from light prune to medium skiff year in all the germplasms and it was also observed that the minimum shoots per unit area and the maximum unit weight of shoot was observed in light prune year. They tend to increase in number and decrease in unit weight with lighter forms of skiff [17]. The decrease in weight has been explained by Portsmouth in 1957 [18]. He considered it to be due to increasing complexity of branching at the plucking surface resulting with interference of the movement of water and nutrients to the growing apices. Light pruned bushes also produced more fine shoots compared to skiffing operations. Fine shoots with fine plucking gaves more quality tea because of the presence of high levels of high levels of polyphenols and caffeine and low levels of chlorogenic acids [19]. Barua and Dutta in 1971 [20] reported that the plucking point density was the most important criteria for selection or rejection of mother bushes by eye judgment and this was true for all bushes irrespective of vigour.

Table 2. Weightage assigned to germplasms on young shoot characters viz, young shoot colour and young shoot pubescence on basis of IPGRI descriptors and mean of fresh shoot weight and dry shoot weight and plucking point density of the germplasms

Characters / Germplasms	Young shoot colo	Young shoot pubescence	Plucking point density (nos/30cm^2 area)		Fresh weight of young shoots (g)		Dry weight of young shoots (g)	
			After LP	After MS	After LP	After MS	After LP	After MS

	ur							
THT 021	1	3	33±2.83	38±2.12	0.157±0.03	0.134±0.04	0.028±0.011	0.027±0.012
THT 022	1	3	25±5.66	27±1.41	0.128±0.05	0.092±0.03	0.021±0.013	0.020±0.008
THT 023	1	3	31±5.66	34±2.12	0.151±0.05	0.124±0.05	0.023±0.013	0.021±0.013
THT 024	4	3	30±2.83	39±1.41	0.152±0.03	0.142±0.05	0.030±0.007	0.028±0.008
THT 025	1	3	24±5.66	29±1.41	0.148±0.03	0.135±0.02	0.024±0.009	0.022±0.008
THT 026	2	5	24±2.83	28±2.12	0.140±0.03	0.103±0.03	0.021±0.007	0.019±0.009
THT 027	1	5	23±5.66	29±1.41	0.141±0.02	0.098±0.03	0.021±0.011	0.020±0.009
THT 028	2	5	36±4.24	41±0.71	0.158±0.02	0.135±0.03	0.035±0.008	0.034±0.005
THT 029	1	3	22±7.07	29±2.12	0.144±0.04	0.124±0.03	0.022±0.007	0.021±0.011
THT 030	1	5	35±5.66	40±2.12	0.145±0.05	0.127±0.03	0.031±0.007	0.029±0.011
THT 031	2	7	25±1.41	27±0.71	0.134±0.05	0.121±0.03	0.023±0.008	0.022±0.015
THT 032	4	5	33±7.07	38±1.41	0.147±0.04	0.123±0.05	0.032±0.008	0.031±0.007
THT 033	1	3	27±7.07	34±6.36	0.147±0.03	0.120±0.04	0.034±0.008	0.033±0.007
THT 034	1	5	32±1.41	38±1.41	0.145±0.02	0.115±0.05	0.024±0.008	0.024±0.013
THT 035	2	5	28±4.24	23±0.71	0.154±0.04	0.132±0.02	0.036±0.012	0.035±0.008
THT 036	1	5	24±4.24	26±2.12	0.141±0.03	0.099±0.02	0.030±0.008	0.029±0.007
THT 037	1	5	25±2.83	30±2.12	0.145±0.03	0.119±0.04	0.029±0.005	0.028±0.008
THT 038	2	5	21±2.83	24±1.41	0.146±0.03	0.122±0.03	0.022±0.008	0.020±0.009
THT 039	4	7	23±2.83	27±2.12	0.147±0.02	0.125±0.03	0.032±0.008	0.031±0.007

THT 040	2	5	342±4.24	39±2.12	0.146±0.03	0.120±0.02	0.035±0.009	0.034±0.008
TV 1	1	3	25±4.24	32±1.41	0.143±0.02	0.123±0.02	0.031±0.015	0.030±0.008
			C.D. at P=0.005 Between germplasms (C) : 0.000					

The lowest plucking point density was found in THT 038 with 21 pluckable shoots per 30 cm^2 area of the plucking surface of the bush. The plucking point density of the remaining germplasms were found to be in the range of 22 to 33 pluckable shoots per 30 cm^2 area of the plucking surface of the bush. Post hock analysis (tukey) showed that there was a significant difference between the germplasms in plucking point density. With respect to plucking point density four homogeneous subsets are formed where all the subsets were found to have almost equally distributed number of germplasms.

In the light pruned year the fresh weight of young shoots was found to be highest in THT 028 (0.158 g) followed by THT 021 (0.157 g) and THT 035 (0.154 g) and the lowest fresh weight was observed in THT 022 (0.128 g). In the medium skiffed year the fresh weight of young shoots was found to be highest in THT 024 (0.142 g) followed by THT 025 (0.135 g) and THT 028 (0.135 g) and the lowest fresh weight was observed in THT 022 (0.092 g) (Table 2). In post hoc analysis, the germplasms having the maximum and minimum fresh weight formed two distinct subsets which are highly significant from each other.

In the light pruned year the dry weight of young shoots was found to be highest in THT 035 (0.036 g) followed by THT 028 (0.035 g) and THT 040 (0.035 g) and the lowest dry weight was observed in three germplasms viz, THT 022, THT 026 and THT 027 (0.021 g). In the medium skiffed year the dry weight of young shoots was found to be highest in THT 035 (0.035 g) followed by THT 040 (0.034 g) and THT 028 (0.034 g) and the lowest fresh weight was observed in THT 026 (0.0190 g) (Table 2).

From the experiment it was observed that the fresh weight and dry weight of the germplasms showed significant difference. Germplasm THT 028 exhibited the highest fresh weight (0.158 g) in the light pruned year and also the highest fresh weight in medium skiffed year (0.135 g). The Assam type of tea has bigger shoot size with higher shoot weight compared to China type of plant [21]. So the clones with higher shoot weight can be related to Assam type of tea. But the dry weight was found to be highest in THT 035 in both the light pruned and medium skiffed year (0.036 g and 0.035 g respectively). A research work done by Rajanna et al. in 2011[10] used young shoot characters to study the genetic diversity in South Indian tea clones. He reported that the qualitative characters especially the weight of harvested shoots showed a wide range of variations in his study. Again Barua,1989 [21] recorded that the fresh weight of plucked shoots were positively correlated at certain predetermined tipping height. From the experiment it can be stated that all the studied clones were good with respect the physical characters considered for the experiment. Bezbaruah and Hussain in1975 [22] observed that although higher percentage of dry matter results in higher out- turn of made tea from same amount of leaves, all clones with higher dry matter content might not be always higher yielder. Again generally flushes from the less rapid growth accumulate more dry matter and it was only the quicker regeneration and growth of shoots which, in the long run contributes to the total yield of the crop. From the study it was also observed that both the fresh weight and dry weight of the shoots remains higher in the light pruned year and they reduce slightly during the medium skiffed year.

3.2 Biochemical characters

The biochemical constituents of young tea shoot (two leaves and a bud) were determinant for standard specification of a beverage crop- tea [23]. From earlier experiments it was found that the chemical composition of China type and Assam type tea plant differ significantly in biochemical composition in the fresh shoots. In the present experiment, all the twenty germplasms of tea for two harvesting seasons i.e. rain flush (July to mid September) and autumn flush (mid September to October) were analyzed for total anthocyanin content, antioxidant activity and total catechin content in their young shoot for the year 2017. During the rain flush, a wide variation was recorded among the germplasm based on their anthocyanin content.

During the rain flush the total anthocyanin content was recorded highest in the germplasm THT 032, followed by THT 019 and THT 024 with anthocyanin content of 43.70 mg/l, 40.56 mg/l and 39.30 mg/l respectively whereas lowest anthocyanin content was recorded in the germplasm THT 034 with anthocyanin content of 2 mg/l. The anthocyanin content of remaining germplasm was recorded between 3.32 mg/l to 37.43 mg/l (Table 3). During the autumn flush the total anthocyanin content was recorded highest in the germplasm THT 032, followed by THT 039 and THT 024 with anthocyanin content of 44.13 mg/l, 41.76 mg/l and 40.57 mg/l respectively whereas lowest anthocyanin content was recorded in the germplasm THT 034 with anthocyanin content of 2.08 mg/l. The anthocyanin content of remaining germplasm was recorded between 3.67 mg/l to 38.15 mg/l (Table 3).

Zheng *et al*., in 2009 [24] determined biochemical components in the shoot of purple tea bud varieties. He found that the purple depth of tea varieties was related to the anthocyanins content and the total content of anthocyanin, polyphenols are higher in varieties of purple buds. In another study done by Hsu et al., 2012 [25] stated that purple-shoot tea extract contains higher anthocyanin and anthocyanidins than that of ordinary tea. Hence the biochemical composition of fresh tea shoots can be used for profiling of various tea cultivars into their basic kinds and suitability for manufacturing different types of specialty tea like purple tea or green tea.

From the present study, it was also observed that most of the germplasm showed higher anthocyanin content in autumn flush compared to rain flush. Das et al. in 2016 [26] worked on screening and determination of anthocyanin in pigmented tea germplasm of India and observed that the amount of anthocyanin increases during the autumn flush in all the selected germplasms.

Table 3. Total Anthocyanin, Antioxidant activity and Catechin content in the germplasms.

Characters / Germplasms	Anthocyanin (mg/l)		Total Antioxidant (IC 50 mg)		Total Catechin (% dry mass basis)	
	Rain flush	Autumn flush	Rain flush	Autumn flush	Rain flush	Autumn Flush
THT 021	8.02	9.45	1.65	1.63	5.26	5.89
THT 022	9.45	10.21	1.67	1.64	5.62	6.09
THT 023	13.00	14.33	1.61	1.58	6.32	6.65
THT 024	39.30	40.57	1.36	1.33	2.96	3.10
THT 025	32.09	34.42	1.54	1.47	3.90	4.10
THT 026	36.44	37.23	1.43	1.37	3.26	3.99
THT 027	3.32	3.81	1.72	1.71	12.36	12.45

THT 028	33.12	33.73	1.67	1.66	3.62	3.90
THT 029	21.20	22.56	1.57	1.60	6.51	7.12
THT 030	11.33	12.78	1.64	1.61	7.38	7.90
THT 031	25.65	26.00	1.56	1.55	4.12	4.88
THT 032	43.70	44.13	1.38	1.37	2.51	3.01
THT 033	7.26	3.67	1.50	1.49	6.11	7.000
THT 034	2.00	2.08	1.74	1.71	7.97	8.23
THT 035	34.45	35.24	1.50	1.48	3.21	3.85
THT 036	16.33	16.86	1.58	1.55	5.58	6.60
THT 037	10.11	11.21	1.62	1.61	5.52	6.31
THT 038	35.23	36.54	1.45	1.39	4.22	5.00
THT 039	40.56	41.76	1.37	1.35	1.37	1.88
THT 040	37.43	38.15	1.42	1.38	3.84	4.20
TV 1	4.21	4.98	1.72	1.70	6.32	6.70
C.D. at P=0.05 Between germplasms (C) :0.000						

During the rain flush the antioxidant activity was recorded highest in the germplasm THT 024, followed by THT 039 and THT 032 with IC_{50} value of 1.36 mg, 1.37 mg and 1.38 mg respectively whereas lowest antioxidant activity was recorded in the germplasm THT 034 with IC_{50} value of 1.74 mg. The antioxidant activities of remaining germplasm were recorded with IC_{50} value in between 1.42 mg to 1.72 mg. (Table 3). Post hoc analysis (tukey) showed that there is a significant difference between the germplasms with respect to antioxidant activity in the rain flush. During the autumn flush the antioxidant activity was recorded highest in the germplasm THT 024, followed by THT 039 and THT 032 with IC_{50} value of 1.33 mg, 1.35 mg and 1.37 mg respectively whereas lowest antioxidant activity was recorded in the germplasm THT 034 and THT 027 with IC_{50} value of 1.71 mg. The antioxidant activity of remaining germplasm was recorded with IC_{50} value in between 1.38 mg to 1.66 mg. (Table 3).

Fresh tea leaf contains four major catechins: epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate, colorless, water-soluble compounds that contribute bitterness and astringency to green tea. Yamamoto *et al*. in 1997 [27] reported the pharmacological importance of catechins present in tea. Saravanan *et al*. in 2005 [28] studied on Genetic diversity of 26 UPASI tea clones of South India (*Camellia sinensis* (L.) O. Kuntze) on the basis of total catechins and their fractions.

Post hoc analysis (tukey) showed that there was a significant difference between the germplasms with respect to total catechin content in the rain flush. During the rain flush the total catechin content was recorded highest in the germplasm THT 027, followed by THT 034 and THT 030 with total catechin content of 12.36%, 7.97% and 7.38% respectively whereas lowest total catechin content was recorded in the germplasm THT 039 with total catechin content of 1.37 %. During the autumn flush the total catechin content was recorded highest in the germplasm THT 027, followed by THT 034 and THT 030 with total catechin content of 12.45%, 8.23% and 7.90% respectively whereas lowest total catechin content was recorded in the germplasm THT 039 with total catechin content of 1.88 %.

Chen *et al*, 2005 [29] in China worked on identification of green tea's (*Camellia sinensis* (L.) quality level according to measurement of main catechins by HPLC and support vector

classification pattern recognition. They said that clones with high catechins content produces better green tea. Till now in North East India, no clones have been established as good planting material for green tea manufacturing. So from the present study, the clones with high catechins content (THT 027, THT 034 and THT 030) can be recommended for further study for developing planting materials for green tea manufacturing.

The studied germplasm also can be categorized as Assam type or China type on the basis of catechins content. Gulati et al. 2009 [30] reported that China hybrids produce low level of total catechins compared to Assam and Cambod types. It was observed from the study that the clones with high catechins have green coloured shoots. So these clones may be proposed to be helpful to classify them according to the future application as black tea, green tea, oolong tea or specialty tea.

3.3 Cluster analysis

The correlation similarity proximity matrix with squared Euclidean Distance method utilizing the biochemical parameters has been presented in Table 5. From the table it can be observed that the similarity value ranges from 0.118 to 1.000 indicating a high degree of variation amongst the germplasm. The highest similarity value (0.118) was observed between THT 039 and THT 034 whereas lowest similarity value i.e. 1.000 could be found between the germplasm THT 021 and THT 022, THT 025 and THT 028 and between THT 026 and THT 040.

Hierarchical cluster analysis of the twenty germplasms based on shoot characters and biochemical parameters and the dendogram was presented in Figure 1. According to the results of the average linkage cluster analysis, germplasms studied were grouped into five different clusters (Table 4). Out of the twenty germplasms, cluster I was the largest cluster incorporating eleven number of germplasm. But Cluster II coming out to be the most promising cluster with THT 024, THT 032 and THT 039 were having highest anthocyanin and antioxidant content with purple coloured young shoots. They were also with dense to intermediate pubescence. They can be used for producing the new and unique tea product, “the purple tea” for exploring the export market for specialty tea and can make a green alternative for tea drinkers due to its health benefits. Cluster V (THT 028, THT 035 and THT 040) & Cluster III (THT 026, THT 031 and THT 038) included the germplasms which have light purple to bronze coloured shoot with average good total catechin content.

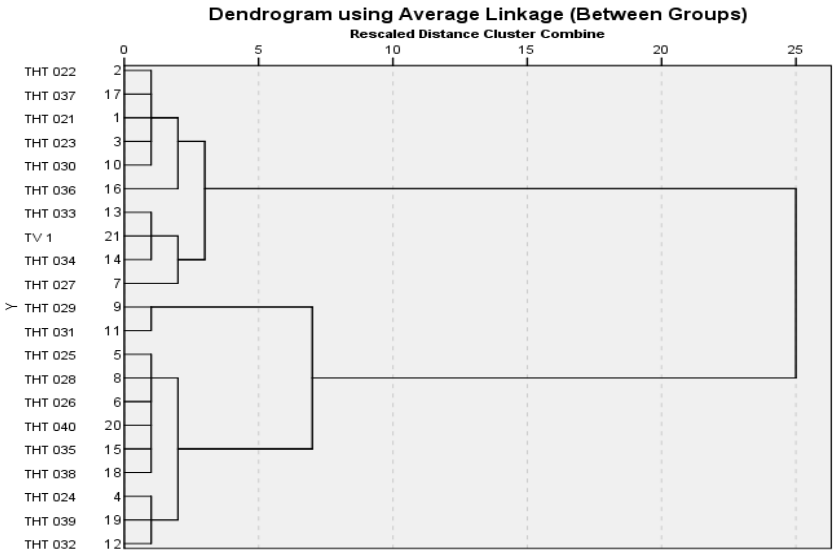

Figure 1. Dendrogram using shoot characters and biochemical characters

Table 4. Grouping of germplasm to different clusters based on the dendrogram of shoot and biochemical characters

Cluster	Germplasm
I	THT 021, THT 022, THT 023, THT 025, THT 029, THT 030, THT 033, THT 034, THT 034, THT 036, THT 037, TV 1
II	THT 024, THT 032, THT 039
III	THT 026, THT 031, THT 038
IV	THT 027
V	THT 028, THT 035, THT 040

The cluster V and III were also found with intermediate pubescence. Cluster IV is consisting of only one germplasms (THT 027) which was having highest total catechin content and can be suitable for producing high quality green tea with intermediate pubescence. Plucking point density was found highest in the germplasm of cluster I, II and V. The germplasm of Cluster I also showed good fresh weight and dry weight of shoots. The germplasm THT 028 which was a pigmented germplasm found to posses good plucking point density with high fresh weight and dry weight of shoot.

Table 5. Proximity matrix based on shoot characters and biochemical characters of the germplasm

Case	Rescaled Squared Euclidean Distance																				
	THT 021	THT 022	THT 023	THT 024	THT 025	THT 026	THT 027	THT 028	THT 029	THT 030	THT 031	THT 032	THT 033	THT 034	THT 035	THT 036	THT 037	THT 038	THT 039	THT 040	TV 1
THT 021	.000	.033	.013	.544	.373	.479	.095	.341	.159	.010	.202	.687	.024	.028	.390	.078	.037	.483	.650	.464	.042
THT 022		.000	.025	.517	.300	.403	.047	.374	.082	.059	.148	.681	.010	.062	.351	.025	.000	.382	.561	.475	.013
THT 023			.000	.388	.238	.326	.110	.232	.079	.011	.110	.516	.043	.076	.256	.031	.024	.327	.473	.327	.062
THT 024				.000	.047	.027	.804	.045	.224	.456	.132	.013	.665	.808	.018	.328	.492	.057	.035	.014	.707
THT 025					.000	.007	.522	.079	.075	.319	.038	.112	.429	.578	.010	.154	.282	.009	.049	.066	.451
THT 026						.000	.647	.083	.130	.409	.067	.072	.549	.707	.008	.226	.377	.003	.015	.053	.575
THT 027							.000	.620	.201	.128	.308	1.000	.029	.053	.591	.113	.050	.606	.842	.744	.021
THT 028								.000	.186	.257	.098	.065	.477	.557	.034	.235	.351	.124	.130	.010	.526
THT 029									.000	.145	.023	.346	.159	.271	.118	.017	.074	.111	.230	.222	.166
THT 030										.000	.164	.574	.059	.057	.319	.076	.055	.421	.567	.369	.086
THT 031											.000	.218	.238	.344	.050	.049	.127	.064	.135	.123	.256
THT 032												.000	.842	.979	.059	.462	.649	.116	.059	.022	.894
THT 033													.000	.021	.479	.073	.017	.529	.731	.603	.000
THT 034														.000	.608	.151	.068	.698	.910	.709	.031
THT 035															.000	.193	.326	.026	.039	.022	.511
THT 036																.000	.017	.208	.347	.301	.079
THT 037																	.000	.356	.526	.448	.020
THT 038																		.000	.024	.092	.547
THT 039																			.000	.077	.759
THT 040																				.000	.652
TV 1																					.000

This is a dissimilarity matrix

3.4. Correlation studies:

The correlation analysis carried out among the biochemical properties of fresh tea shoots were presented in Table 6. There was a significant positive correlation (r=0.930) between anthocyanin and total antioxidant. Whereas total catechins content showed highly significant but negative correlation with anthocyanin and total antioxidant.

Table 6: Correlation analysis among the biochemical properties of fresh young tea shoots

Pearson Correlation Sig. (2-tailed)	Anthocyanin	Total antioxidant	Total catechin
Anthocyanin	1		
Total antioxidant	.930**	1	
	.000		
Total catechin	-.861**	-.895**	1
	.000	.000	

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

Antioxidant potency of tea products was significantly by the total anthocyanin content of the purple leaf coloured cultivars. Hai-Peng et al. 2015 [31] in China worked on identification of the anthocyanins from the purple leaf coloured tea cultivar Zijuan (*Camellia sinensis* var. *assamica*) and characterization of their antioxidant activities. Tea products from the purple leaf coloured tea cultivars have high levels of antioxidant activities. Anthocyanins from other sources have been associated with a broad spectrum of health benefits including anti-carcinogenic, anti-diabetic, anti-aging, antioxidant and anti-inflammatory properties [32].

Wei et al., 2011[33] observed that the rise of pigment content during young leaf development is associated the decline of catechin. From the correlation study, it was also observed that the anthocyanin and antioxidant content of the germplasms showed positive correlation between them whereas both the antioxidant and anthocyanin content showed negative correlation with catechin. The germplasms with higher anthocyanin content viz, THT 032, THT 039 and THT 024 also showed higher antioxidant activity and on the other hand germplasms with lower anthocyanin and antioxidant activity viz, THT 027, THT 034 and THT 030 contained higher catechin content. Kerio et al., 2011 [34] in Kenya worked on characterization of anthocyanin in Kenyan teas. They stated that the anthocyanin content and catechin content of tea were negatively correlated.

4. CONCLUSION

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. The germplasms with high chlorophyll content will be good for producing an emerald- green, smooth finished green tea product while the germplasms with purple coloured young shoots may be selected for producing specialty tea like purple tea. There was an increasing trend of plucking point density from light prune to medium skiff year. The high fresh weight and dry weight along with good plucking point density was observed in pigmented tea germplasm THT 028 which can be further studied for specialty tea production with high yield.

In order to increase the export market of tea, introduction of improved varieties with high production and quality is the need of the hour. Possible solution is to look for planting material that could retain or at least compensate this loss in the yield and quality of tea under the prevailing climatic conditions through making foray into specialty tea market such as pigmented tea. It is also important to note that the parameters that were taken for the present study may be subjected to environmental factors. so more elaborated field trial may be required for these germplasms to assess their yield and quality parameters before selecting them as parent materials for any breeding programmes.

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