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Profiling of few of the tea accessions on physiological growth and stress tolerance

ABSTRACT

Tea [*Camellia sinensis*(L) O Kuntz] is one of the oldest, most widely consumed and least expensive natural beverages, known to the world over for its heritage brew with various flavor and antioxidant properties. The productivity of tea in world as well as in India has been raised by developing high yielding clones and seed stocks but the genetic base of the existing diversity of the crop is rapidly narrowing down. In present scenario, apart from yield and quality, physiological parameters and abiotic stress tolerance are also raising up to be the most important environmental indicator impacting on sustainability and productivity of tea. In the published research work, an effort was made to characterize and estimate the variation of 20 tea germplasm named as THT1 to THT20, growing in the Experimental Garden for Plantation Crops, AAU, Jorhat on the basis of growth parameters and abiotic stress tolerance capability of tea.

In the experiment all the germplasm showed highly significant difference amongst them regarding growth parameters, pigment content and on abiotic stress tolerance. THT 1, THT 10, THT 3 contained higher total chlorophyll and THT 4, THT 10, THT 20 showed high carotene content. In case of growth parameters THT 11, THT 7, THT5 showed higher plucking point density whereas THT 11, THT 17, THT 7 showed good abiotic stress tolerance with high value of proline content, relative turgidity, water saturation deficit and epicuticular wax content.

When clustering was done based on abiotic stress tolerance, pigment and growth parameters respectively, THT11 and THT 17 are found to form a discriminated cluster in the dendogram. The parameters taken especially for abiotic stress tolerance study may be subjected to environmental factors for which more elaborated field trial may be required for these clones to assess their yield and quality parameters.

Keywords: tea, germplasm, pigment, epicuticular wax, proline

1. INTRODUCTION

The tea plant *Camellia sinensis* (L.) O. Kuntze has been used to produce the oldest and most popular non-alcoholic soft beverage across the world and is one of the most important cash crops of many countries, including India. It has a refreshing, astringent flavour which many people enjoy. With a longstanding history of cultivating and consuming tea, North East India is believed to be highly rich in genetic diversity of tea. Myanmar is the origin of Assam type plant and is rich in germplasm of tea with maximum diversity in respect of genetically and morphological characters. Experimental tea cultivation was started in Assam by importing seeds from China, however, the successful tea cultivation began only after 1823 after the discovery of indigenous Assam plant. Due to the development of high yielding clones and seed stocks, the productivity of tea has increases. However, the introduction of selected clonal materials and clonal seeds tends to result in the extinction of a wide range of genetic materials due to continuous uprooting programes of old tea sections [1]. In present scenario, apart from yield and quality, physiological parameters and abiotic stress tolerance are also raising up to be the most important environmental indicator impacting on sustainability and productivity of tea.

In the Experimental Garden for Plantation Crops under the Department of Tea Husbandry and Technology, Assam Agricultural University, genotypically different tea plants are growing which were collected from different areas by Britishers and planted. However, no detailed study on the growth parameters and abiotic stress tolerance capability of these materials had been studied in the past, although some initial work has been just started recently. Therefore, looking into its importance the

present study has been undertaken with the objectives to study the physiological parameters and stress tolerance capacity of unidentified tea germplasm in Field Gene Bank maintained in Section No 14 of Experimental Garden for Plantation Crops. The study will help to understand the important physiological parameters and stress tolerance capacity within the selected tea accessions and will provide the important informative scientific basic for future tea breeding programs.

2. MATERIAL AND METHODS

An attempt has been made to describe the procedures adopted for systematic assessment of the germplasm accessions present in the Experimental Garden for Plantation Crops, Department of Tea Husbandry &Technology, Assam Agricultural University, Jorhat. A total of twenty germplasms accessions were characterized on the basis of their growth parameters and abiotic stress tolerance capacity.

2.1 Plant materials

Twenty (20 numbered) unidentified germplasm exhibiting morphological variations from others were marked in section no. 14 of the Experimental Garden for Plantation Crops of AAU, Jorhat. These were then numbered as THT 1 to THT 20 and utilized in the present investigation.

2.2 Physiological parameter

2.2.1 Plucking point density

Tea shoots were plucked on weekly basis during the month July, 2016 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 30 cm x 30 cm area of the central zone of the bush and plucking density was expressed as number of shoots per 30 x 30 cm2 plucking table area.

2.2.2 Specific leaf weight

The specific leaf weight was measured as dry weight per unit leaf area. Three (3) mature 5th leaves without petiole from the sample plant were taken at random. The leaf area as well as the dry weight of the samples were recorded. The mean of specific leaf weight for each plant sample was presented in milligram per cm2.

2.2.3 Pigment content

The extraction was done with methanol following the method of Taylor and McDowell,1990 [2] to analyze chlorophyll and carotenoid pigments. Two leaves and bud exposed to full sun light were sampled and homogenized 0.5g of tissue in a pestle and mortar with 25 ml under dark condition and centrifuged at 5000rpm for 5 minutes. Then 2 ml of supernatant was diluted to 10 ml of methanol.

Then spectrophotometric observation of diluted solution were recorded in three different wavelength, viz., 470 nm for total carotene (tc), 653 nm for chlorophyll-a and 666 nm for chlorophyll-b. The spectrophotometric values are converted into the actual quantities of chl-a, chl-b, and Tc by using the standard formulae as follows

$$Chl\text{-}a = 15.65A666 - 7.34A653$$

$$Chl\text{-}b = 27.05A653 - 11.21A666$$

2.3 Abiotic stress tolerance capacity

Morpho-physiological characteristics and chemical composition are directly related to superior competitive ability of crops to respond to various abiotic stresses. In this experiment, the parameters taken for measuring the drought stress were relative turgidity, water saturation deficit, proline content

and epicuticular wax content. Level of moisture stress was quantified by measuring soil moisture content prior to collection of leaf samples for determining the degree of stress in the aforesaid industry clones. The temperature during the period of experimentation ranged from 25.00°C -30.3°C (maximum) and 9.7°C -19.8°C (minimum) with relative humidity ranging from 95-100 percent and to a minimum 58-79 percent, respectively. To study the parameters, three number of observation per germplasm were noted.

2.3.1 Relative turgidity and water saturation deficit

Relative turgidity was determined by the following method described by Weatherley,1960[3] with certain modifications. At very first collect the 3rd or 4th leaf of a tea plant and were cut it in 10 circular dishes of uniform diameter with the help of a cork borer. And fresh weight was taken. Immerse the leaf dishes in separate petriplates containing distilled water and was kept them for 24 hours and then recorded the turgid weights.

They were kept in oven at 80°C temperature for 12 hours and then dry weight was recorded. Relative turgidity and saturation deficits were expressed in percentage and determined using the following formula:

2.3.2 Proline content

Free proline was estimated following the method suggested by Bates *et al.*,1973[4]. Fully expanded 5th leaves of tea shoots exposed to full sunshine were sampled and homogenized 0.5 g of tissue in a pestle and mortar with 10 ml of 3% aqueous sulphosalicylic acid and centrifuged at 4000 rpm for 10 minutes. From the supernatants solution, 2 ml was taken for analysis in a test tube to which 2 ml glacial acetic acid and 2 ml of the acid ninhydrin mixture were added. Then the mixture was kept in a boiling water bath for 1 hour following which the reaction was terminated by placing the mixture in ice bath. After cooling, the reaction mixture was extracted with 4 ml toluene in a separating funnel. The chomophase contains toluene was separated and allowed to stand for some time.

The absorbency was read at 520 nm using toluene as blank in a spectrophotometer. A standard curve was prepared from pure proline (range 0.1-36 mole). The estimation of leaf proline content was done with the help of standard curve and expressed as moles/g on fresh tissue.

2.3.3 Epicuticular wax content

Epicuticular wax was estimated from the 5th leaf of fresh tea shoots collected randomly from the each of twenty germplasms following chloroform extraction method as described by Fernandes *et al.*,1964 [5]. For quantification of epicuticular wax, sampled leaves were kept in a pre-weighed petridish and immersed in 100 ml of chloroform for 30 seconds, at the end of which chloroform was allowed to evaporate completely and the petri dishes were weighed again to obtain the content of epicuticular wax and expressed in mg/cm^2.

2.4 Statistical Analysis and Interpretation of Data

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 germplasm, 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) 21.

3. RESULTS AND DISCUSSION

3.1 Physiological parameters

3.1.1 Plucking point density

The plucking point density (PPD) had been considered as yield indicator in the experiment and the mean values estimated for the twenty germplasm were presented in the Table 1. Post hoc (Tukey) analysis showed that there was a significant difference ($p<0.05$) between the germplasm. Highest

plucking point density had been observed in THT11 (45.67 nos. of pluckable shoots per 30 cm^2 area) followed by THT7 (43.33 no. of pluckable shoots per 30 cm^2 area) (Fig.1). And the lowest plucking point density had been observed in THT19 (9.33 no. of pluckable shoots per 30 cm^2 area). Plants with higher plucking point density are more preferable for commercial cultivation of tea. 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 [6]. Plucking point density in the studied germplasms showed a high significant difference. Plucking point density is directly related with high extension rate and shoot growth rate. In this experiment plucking point density was observed in rainy season when plucking point density normally found to be higher than the other seasons.

Table 1. Mean of plucking point density (PPD) and specific leaf weight (SLW) of the germplasm

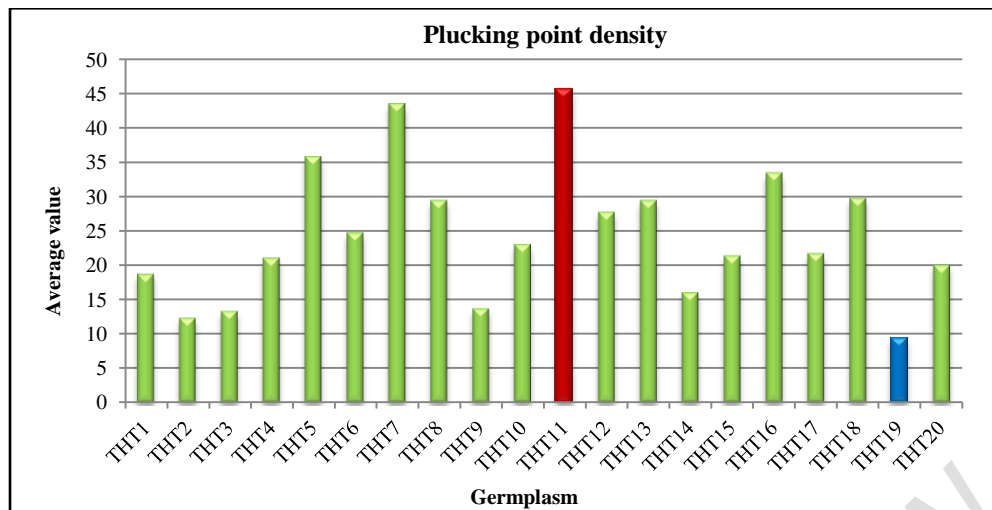| Germplasm		PPD (nos/30cm^2 plucking table area)	SLW (mg/mm^2)
THT 1		18.67±2.62	0.36±0.00
THT2		12.33±1.70	0.52±0.00
THT3		13.33±0.47	0.51±0.00
THT4		21.00±0.81	0.53±0.00
THT5		35.67±1.70	0.50±0.00
THT6		24.67±0.94	0.51±0.01
THT7		43.33±1.70	0.52±0.02
THT8		29.33±1.24	0.53±0.01
THT9		13.66±1.24	0.69±0.03
THT10		23.00±1.41	0.64±0.00
THT11		45.67±0.47	0.76±0.00
THT12		27.67±0.94	0.54±0.00
THT13		29.33±1.24	0.74±0.00
THT14		16.00±0.81	0.53±0.00
THT15		21.33±1.24	0.46±0.00
THT16		33.33±1.70	0.47±0.00
THT17		21.66±1.24	0.71±0.00
THT18		29.66±0.47	0.78±0.02
THT19		9.33±0.47	0.57±0.00
THT 20		20.00±0.86	0.50±0.00
Germplasm	df 19	35.667**	295.986**
Error	df 40	33.133	2.467
CD		5.84	11.89

Fig. 1. Variation in plucking point density of different germplasm
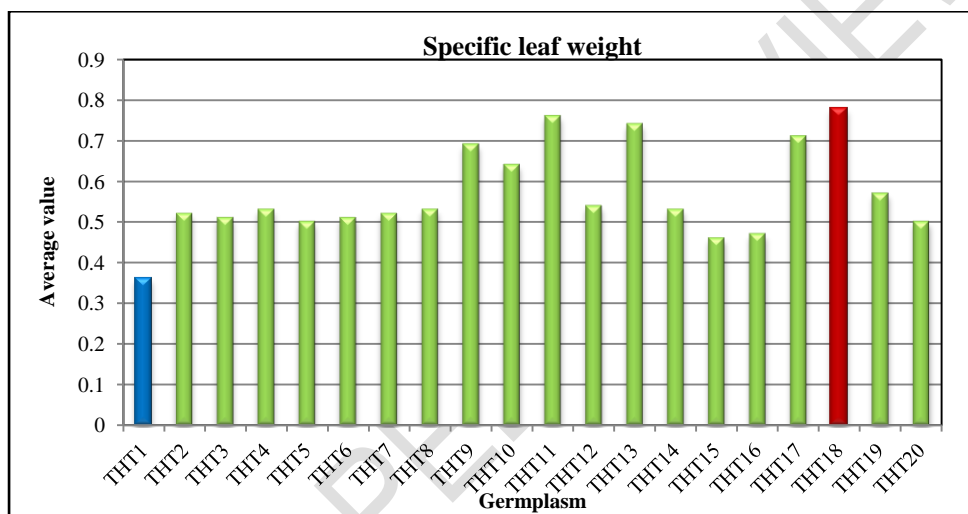

Fig. 2. Variation in specific leaf weight of different germplasm

3.1.2 Specific leaf weight

Specific leaf weight (SLW) is positively related to relative growth rates, leaf turnover rates, foliar nutrient concentrations and photosynthetic capacities [7, 8]. The mean value on the specific leaf area (SLA) for the twenty germplasm was presented in the Table 1. Higher amount of specific leaf weight were recorded in THT18, THT11, THT 13, THT17, THT9 and THT10 ranging from 0.78 mg/mm2 to 0.64 mg/mm2 which can be considered as more photosynthetically active germplasm. There is a linear and positive relationship between the specific leaf weight and photosynthetic rate when studied the relationship between specific leaf weight and photosynthetic rate at panicle initiation stage in super hybrid rice [9]. On the other hand THT1, THT15, THT16, THT5, THT20 and THT3 were recorded with lower SLW which range from 0.36 mg/mm2 to 0.51 mg/mm2 (Fig. 2). In this experiment leaf with larger size showed higher specific leaf weight. These findings were supported by the earlier findings which stated that on an average, large leaves of a given species tend to have higher SLW and lower specific leaf area [10].

3.1.3 Co-efficient of correlation of specific leaf weight with plucking point density

Co-efficient of correlation study was done to know the relationship of specific leaf weight with yield of the planting materials (Table 2). The major contribution to shoot yield is made by the leaves and unit weight of the leaf but when the specific leaf weight of the 5th leaf was attempted to correlate with plucking point density of the studied germplasm, a non-significant but positive correlation between specific leaf weight with plucking point density (0.221) was observed. The yield is the function of various other factors like number of shoots per unit area on plucking table, rate of leaf unfolding and uniformity in flushing behavior apart from the size and weight of the shoots [11].So, the criteria of specific leaf weight alone could not be given due importance so far as the yield performance of a bush is concerned as indicated by the results of present investigation.

Table 2. Co-efficient of correlation of specific leaf weight with yield

Plant Characters	Correlation Co-efficient with yield (g fresh weight)
Specific leaf weight	0.221[NS]

NS = Non-significant

3.2 Pigment content

Different pigments present in germplasms were analyzed sprectophotomatrically and a high significant difference was observed amongst the germplasm. Chlorophyll plays a significant role in tea blackness that is one of the most important factors in commercial evaluation in tea [12]. In present experiment the maximum value for chl-a, chl-b, and total chlorophyll content had been recorded in THT1 which was 1.42 mg/g, 0.761 mg/g and 2.17 mg/g, respectively (Table 3). The lowest value for chlorophyll-a, chlorophyll-b and total chlorophyll had been found in THT 7 which is 0.40 mg/g, 0.15 mg/g and 0.55 mg/g, respectively (Fig.3, Fig 4, Fig.5). The leaves with more chlorophyll indicate lower quality tea [13]. However, the higher chlorophyll content would lead to higher total liquor colour [14]. It gives the blackness of made tea which is an important character in the commercial evaluation of tea [12].

Aroma is one of the most important factors in the evaluation of the quality of final black tea. Carotenoids are involved in the formation of aroma complex of tea [15, 16]. Carotenoids are yellow pigments present in the fresh leaf and their degradation during manufacturing leads to the formation of terpenoid flavour compounds in black tea [17].Carotenoid content in different germplasm also showed high significant variation. In the present experiment carotenoid content was found highest in THT4 (0.27 mg/g) and lowest in THT2 (0.07 mg/g) amongst the twenty tea germplasms (Table 3, Fig.6). The higher concentration of carotenoid adds to the formation of flavoury compound [18]. So THT4 can be considered as good planting material with regard to aroma formation and to manufacture high quality black teas. An increase in endogenous carotene content also enhanced all the quality parameters of tea, the VFC (volatile flavor compounds) index, almost being doubled [19].

Based on the investigations conducted on the relation of chlorophyll and carotenoid content of the twenty tea germplasm, it can be stated that germplasm with more chlorophyll and carotenoid content

may be more important when it comes to commercial tea production as they add to quality of the made tea. Again germplasm having high total chlorophyll content germplasm may be selected for producing an emerald-green, smooth finished green tea product while the germplasm with high carotenoid content may be preferred for quality black tea production. Earlier experiment reported that significant and positive correlation exists between the quality of green tea and the chlorophyll content [20]. The results may be used for profiling the germplasm for black and green tea manufacturing. The application of pigment (chlorophylls and carotenoids etc.) profiles for the selection of breeding material and for fingerprinting of clones/jats should be emphasis.

In many tea growing countries, work had been done on characterization of their tea germplasms using biochemical constituents like total catechins and their fractions, total polyphenols, chlorophylls, carotenoids and caffeine in the fresh leaf as discriminative markers to evaluate diversity and genetic potential warehoused in the germplasm [21,22, 23]. The fresh green leaves from Assam cultivar are generally low in chlorophyll content than china cultivars [24]. The studied germplasms can be categorized into these three basic kinds of tea on the basis of their pigment properties. The china *jats* of tea cultivars possess high pigment content specially the total chlorophyll and carotenoids [25]. So the germplasm with high total chlorophyll and carotenoid (THT1 and THT10) content can be characterized as China type of tea plant. On the other hand low chlorophyll content was recorded in THT 7 (0.55 mg/g) which can be categorized as Assam or Cambod type of tea plant. When the correlation similarity proximity matrix was studied with Squared Euclidean Distance method utilizing the pigment parameters, the lowest similarity value (1.000) was observed between the germplasm THT1 and THT7 which indicates that they are the two types of plants which are opposite to each other in pigment characters.

Table 3. Mean of chlorophyll-a, chlorophyll-b, total chlorophyll and total carotenoid content of the germplasm

Germplasm	Chl a (mg/g)	Chl b (mg/g)	Total chl (mg/g)	Carotenoid (mg/g)
THT 1	1.41±0.02	0.76±0.06	2.17±0.05	0.12±0.05
THT2	0.87±0.02	0.41±0.08	1.29±0.06	0.07±0.05
THT3	0.93±0.06	0.49±0.05	1.42±0.11	0.14±0.01
THT4	1.10±0.02	0.44±0.14	1.55±0.14	0.28±0.07
THT5	1.14±0.04	0.42±0.05	1.56±0.02	0.18±0.02
THT6	1.02±0.01	0.50±0.02	1.52±0.02	0.14±0.02
THT7	0.40±0.02	0.14±0.08	0.55±0.06	0.07±0.05
THT8	0.71±0.07	0.24±0.04	0.95±0.03	0.18±0.03
THT9	0.71±0.02	0.24±0.00	0.95±0.01	0.18±0.01
THT10	1.42±0.06	0.40±0.05	1.82±0.02	0.27±0.03
THT11	0.73±0.02	0.17±0.10	0.90±0.05	0.15±0.03
THT12	0.60±0.05	0.26±0.05	0.87±0.04	0.10±0.03
THT13	0.88±0.02	0.52±0.01	1.40±0.01	0.11±0.02
THT14	0.88±0.04	0.52±0.09	1.40±0.05	0.11±0.06
THT15	0.83±0.10	0.32±0.09	1.15±0.11	0.12±0.05
THT16	0.68±0.01	0.27±0.01	0.96±0.00	0.14±0.02
THT17	0.91±0.01	0.41±0.01	1.33±0.03	0.14±0.01

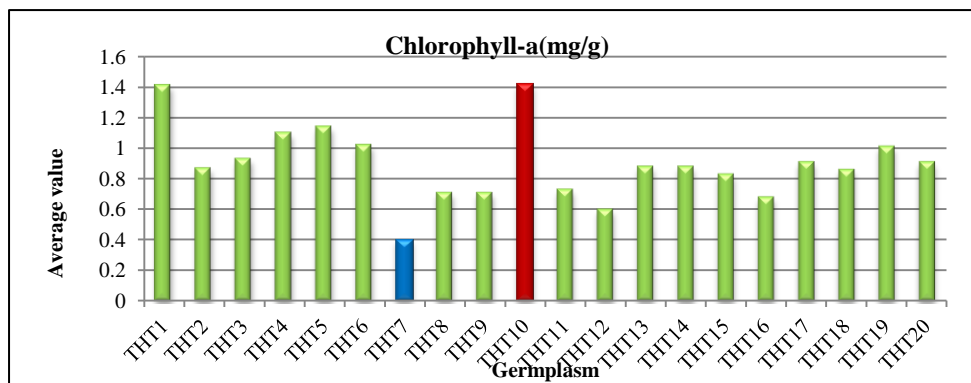|					
THT18		0.86±0.06	0.39±0.09	1.25±0.03	0.14±0.05
THT19		1.01±0.03	0.47±0.01	1.48±0.03	0.08±0.01
THT 20		0.91±0.02	0.34±0.05	1.26±0.06	0.20±0.01
Germplasm	df 19	4.577**	1.556**	85.309**	.233**
Error	df 40	.081	.168	85.117	.049
CD		0.47	0.68	8.78	0.37

"**" highly significant

Fig. 3. Variation in chlorophyll-a content of different germplasm (in fresh weight)
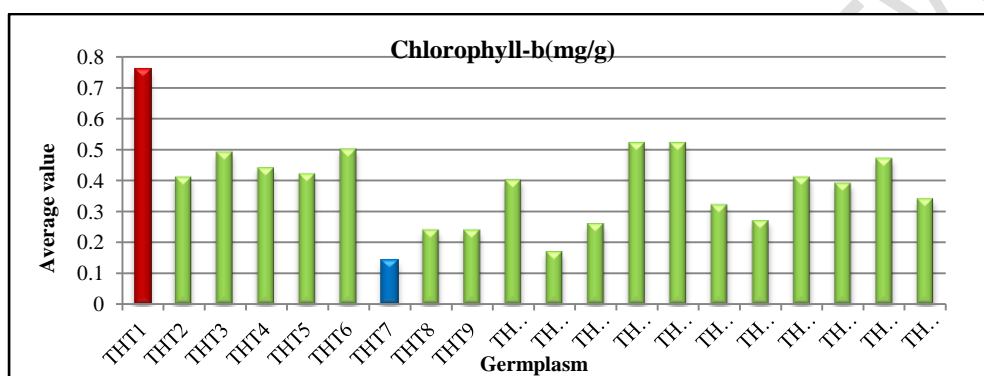
Fig. 4. Variation in chlorophyll-b content of different germplasm (in fresh weight)
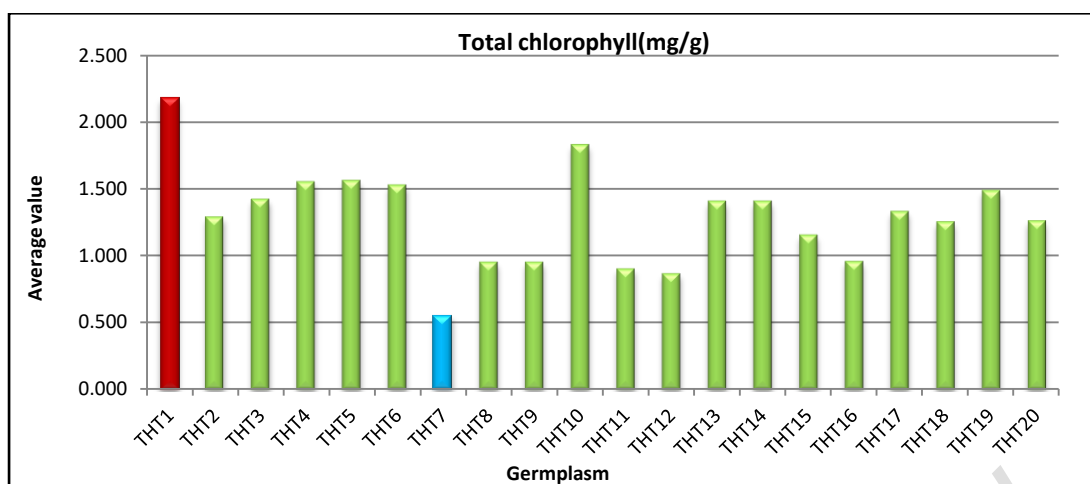
Fig. 5. Variation in total chlorophyll content of different germplasm (in fresh weight)
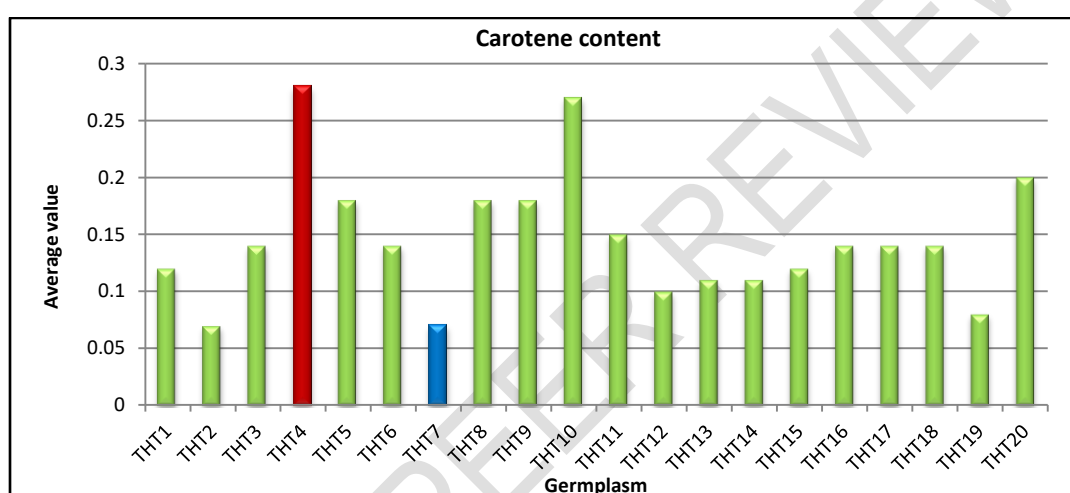
Fig. 6. Variation in total carotene content of different germplasm (in fresh weight)

3.2.1 Genetic relationship and cluster analysis

The correlation similarity proximity matrix with squared Euclidean Distance method utilizing the pigment parameters are presented in Table 4. The highest similarity value was observed between the germplasm THT9 and THT8 (0.000) and the lowest similarity value was observed between the germplasm THT1 and THT7 (1.000).

Again based on the dendrogram,(Fig. 7) when hierarchial clustering of the twenty tea germplasm accessions was done on pigment characters, studied accessions were grouped into four main clusters(Table 5). It was observed that germplasm THT1 and THT10 formed two discriminated clusters and thus group individually. THT1 and THT10 were showing high total chlorophyll content with high carotenoid content in THT10. Again the cluster III was representing six germplasm (THT7, THT8, THT9, THT11, THT12 and THT16) with low total chlorophyll content ranging from 0.55 mg/g to 0.96 mg/g which can be categorized as Assam type of plant. This clone can be considered as good clone for making black tea as high chlorophyll content may give a grassy odor in made tea [13].

The significant variation of the selected pigment compounds detected in the present study can be used for biochemical profiles of the germplasm and identify potential parents for future breeding programes. A research work done in Sri Lanka showed the genetic diversity of thirty five tea cultivars on the basis of biochemical components present in the fresh shoots of tea [26].

Table 4. Pearson similarity coefficient matrix utilizing pigment content data

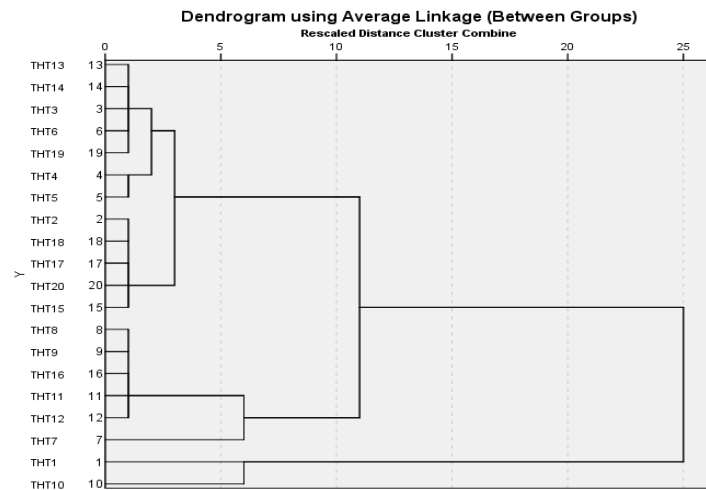|	THT1	THT2	THT3	THT4	THT5	THT6	THT7	THT8	THT9	THT10	THT11	THT12	THT13	THT14	THT15	THT16	THT17	THT18	THT19	THT20
THT1	0.000	0.295	0.216	0.15	0.141	0.158	1.000	0.557	0.557	0.066	0.600	0.644	0.230	0.230	0.388	0.555	0.266	0.318	0.178	0.312
THT2		0.000	0.007	0.040	0.038	0.022	0.209	0.045	0.045	0.153	0.058	0.068	0.007	0.007	0.008	0.042	0.002	0.002	0.015	0.006
THT3			0.000	0.017	0.017	0.005	0.287	0.081	0.081	0.106	0.101	0.115	0.001	0.001	0.027	0.079	0.003	0.01	0.004	0.013
THT4				0.000	0.003	0.007	0.402	0.138	0.138	0.044	0.16	0.192	0.026	0.026	0.067	0.142	0.026	0.041	0.013	0.033
THT5					0.000	0.006	0.409	0.144	0.144	0.039	0.163	0.196	0.027	0.027	0.068	0.146	0.026	0.043	0.009	0.036
THT6						0.000	0.364	0.122	0.122	0.067	0.144	0.165	0.009	0.009	0.052	0.121	0.014	0.028	0.002	0.028
THT7							0.000	0.07	0.07	0.686	0.06	0.039	0.274	0.274	0.144	0.067	0.236	0.191	0.335	0.205
THT8								0.000	0.000	0.321	0.002	0.006	0.079	0.079	0.016	0.001	0.053	0.034	0.107	0.036
THT9									0.000	0.321	0.002	0.006	0.079	0.079	0.016	0.001	0.053	0.034	0.107	0.036
THT10										0.000	0.345	0.402	0.126	0.126	0.204	0.328	0.128	0.162	0.079	0.144
THT11											0.000	0.007	0.100	0.100	0.024	0.004	0.068	0.046	0.126	0.048
THT12												0.000	0.107	0.107	0.034	0.004	0.083	0.058	0.146	0.066
THT13													0.000	0.000	0.027	0.075	0.005	0.011	0.007	0.016
THT14														0.000	0.027	0.075	0.005	0.011	0.007	0.016
THT15															0.000	0.016	0.012	0.004	0.041	0.006
THT16																0.000	0.052	0.033	0.106	0.038
THT17																	0.000	0.002	0.01	0.004
THT18																		0.000	0.021	0.002
THT19																			0.000	0.023
THT20																				0.000

Fig. 7. Dendrogram done by using pigment characters

Table 5. Grouping of germplasm based on dendrogram using pigment characters

Cluster I	Cluster II	Cluster III	Cluster IV
THT1	THT2	THT 7	THT 10
	THT3	THT 8	
	THT 4	THT 9	
	THT 5	THT 11	
	THT 6	THT 12	
	THT 13	THT 16	
	THT 14		
	THT 15		
	THT 17		
	THT 18		
	THT 19		
	THT 20		

3.3 Abiotic stress tolerance capacity

3.3.1 Relative turgidity and water saturation deficit

Tea plant is a perennial crop which is subjected to different environmental stress amongst which moisture stress is one of the important abiotic stress and this problem is often being handled by the tea planters. In N.E. India generally tea suffers from drought during November to April. Drought effect on tea has been reported in numerous tea growing areas of the world with yield penalty ranging from 14 to 33% [27,28]. Natural drought with reduced soil moisture is an ideal time to screen the crop varieties to measure their degree of tolerance to drought [29].To measure the abiotic stress capacity with reference to drought tolerance, relative turgidity, water saturation deficit, proline and epicuticular wax content were studied for the twenty germplasm and the data recorded were tabulated in the Table 4.6.All the parameters had been measured during low soil moisture content (12.98%) where there is no rainfall consecutively for 45 days during November-December. But all the germplasm studied had some degree of tolerance to drought in field moisture stress condition. During the period no germplasm showed any major stress symptoms of drought such as defoliation, die back of shoot tips or death of plants.

In drought tolerant cultivars, values of relative turgidity (RT) is high and value of water saturation deficit (WSD) is low than in susceptible ones [30].RT and WSD are negatively correlated and higher RT and lower WSD is a special character for selection of tolerant variety. In this experiment all the germplasm showed a highly significant difference (p=0.05) (Table 6). The highest RT was recorded in THT12, (88.24%) followed by THT8 (79.84%), THT20 (79.84%) and THT1 (79.60%) (Fig.8). This might be an indicator to consider these clones for growing in drought prone areas. The tissues that can maintain high relative leaf water content and leaf water potential are reported to have a high tolerance to drought. The results are in conformity with the observations of Khan and Stoddard , 2005 [31]. The lowest RT was recorded in THT15 (40.745%). In case of WSD highest value for saturation deficit among the 20 germplasm was observed in THT15 (59.25%) followed by THT4 (56.86%) and THT9 (51.86%) whereas the lowest value was observed in THT12 (11.76%). From the findings we may suggest that germplasm THT12, THT 20 and THT8 have a good capacity for stress tolerance amongst the twenty studied germplasm.

Table 6.Mean difference of relative turgidity (RT), water saturation deficit (WSD), proline content and epicuticular wax content of the germplasm

Germplasm	RT (%)	WSD (%)	Proline (µmole/g)	Epicuticular wax (µg/cm^2)
THT 1	74.20±2.55	25.80±2.55	18.51 ±1.53	0.42±2.62
THT2	67.38±2.08	32.63±2.08	47.26 ±1.69	0.37±1.70
THT3	49.86±1.04	53.53±2.72	61.84 ±1.20	0.79±0.47
THT4	43.06±0.23	57.41±1.47	48.23 ±1.38	0.48±0.82
THT5	67.60±2.54	32.40±2.54	69.84 ±0.79	0.95±1.70
THT6	55.39±1.30	44.61±1.30	50.96±2.58	0.89±0.94
THT7	73.18±2.30	26.00±1.97	79.40 ±0.63	0.84±1.70

|					
THT8		79.84±3.57	20.16±3.57	75.07 ±3.29	0.93±1.25
THT9		48.78±1.21	50.99±1.34	61.64 ±0.65	0.39±1.25
THT10		66.77±2.29	33.23±2.29	73.57 ±1.02	0.74±1.41
THT11		49.85±0.71	49.92±1.01	105.53 ±1.24	1.05±0.47
THT12		88.24 ±2.42	11.76±2.42	54.13 ±0.49	0.93±0.94
THT13		53.95±4.68	46.05±4.68	41.62 ±2.02	0.96±1.25
THT14		73.51±0.83	26.55±0.71	66.59 ±0.51	0.47±0.82
THT15		40.74±1.79	59.25±1.79	55.75 ±0.65	0.82±1.25
THT16		69.55±3.27	30.45±3.27	43.45±1.37	0.44±1.70
THT17		73.58±2.44	26.42±2.44	124.73 ±0.67	0.89±1.25
THT18		67.95±0.55	31.49±0.90	74.16 ±2.08	0.83±0.47
THT19		69.43±1.57	30.24±1.80	43.70 ±1.83	0.80±0.47
THT 20		80.28±1.47	20.40±3.62	36.36 ±1.10	0.66±0.82
Germplasm	df 19	526.62**	526.61**	1851.67**	0.14**
Error	df 40	38.16	38.16	16.08	0.003
CD		10.19	10.19	6.62	0.05

"**" highly significant
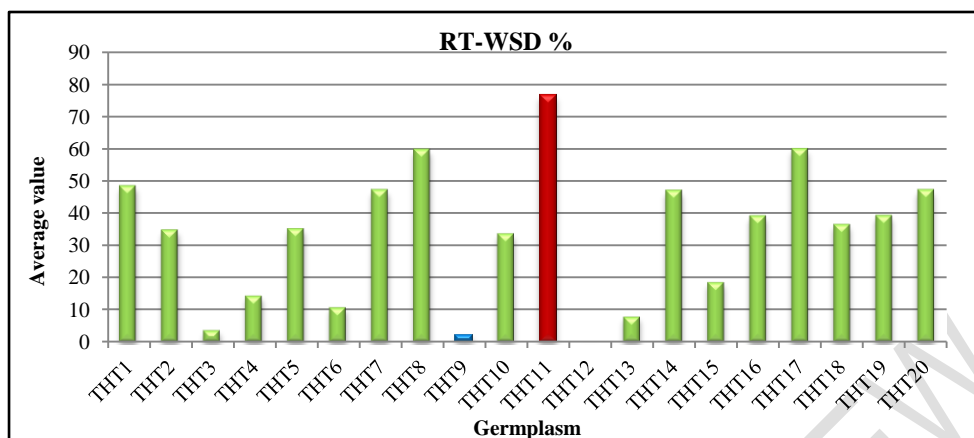
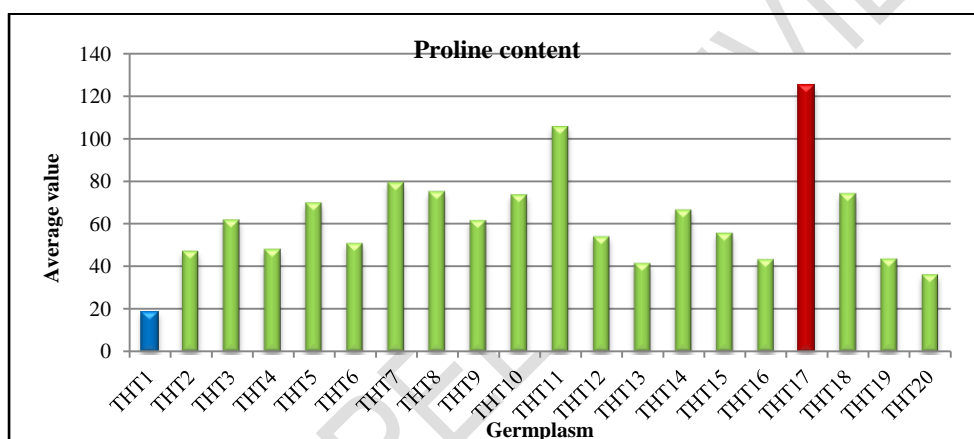**Fig. 8. Variation in RT-WSD % of different germplasm**

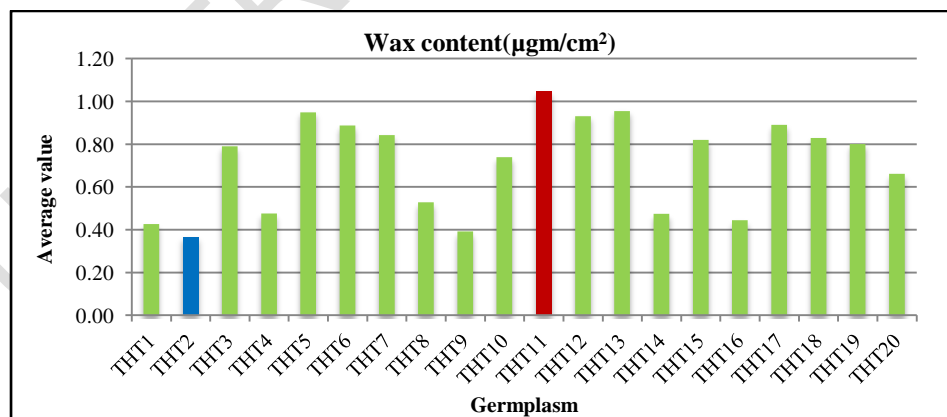**Fig. 9. Variation in proline content of different germplasm**

Fig. 10. Variation in epicuticular wax content of different germplasm

3.3.2 Proline

Enhancement of proline and waxes from non stress condition to stress condition helps to thwart the ill effect of drought and helps in maintaining the metabolic activities of these plants. Similar observations in tea have been reported by other workers [32,33]. In this experiment proline content showed a significant difference among the 20 germplasm. Proline content was found highest in THT17 (124.73 μmol/g) followed by THT11 (105.53 μmol/g) whereas the lowest amount of proline was found in THT1 (18.51 μmol/g) (Table 6). The higher proline and epicuticular wax content of tolerant clones confer them an advantage over the susceptible clones to resist the ill effects of drought more effectively [34]. (Fig. 9).

3.3.3 Epicuticular wax

In case of epicuticular wax content amongst the 20 germplasms, highest epicuticular wax was recorded in THT11 (1.05 mg/cm^2) followed by THT13 (0.96 mg/cm^2) and THT5 (0.95 mg/cm^2) (Table 6, Fig.10). The lowest value for epicuticular wax had been recorded in THT2 (0.37 mg/cm2). Epicuticular waxes on tea leaf surfaces play an important role in protecting the plant from non stomatal water loss from the leaf surface and thus protect the plant from dehydration damage under drought induced stress [35]. Mohamed et al. in1986 [36] observed that leaves of drought resistant clones had a comparatively higher cuticular resistance as compared to leaves of drought susceptible clones and it is possible that this is associated with high epicuticular wax content.

It had been observed from the results that THT11 had high proline and epicuticular wax content which might be the added advantage to tolerate the moisture stress. It is also important to note that in the experiment moisture stress was not induced, but the plants were under natural stress condition which might have triggered the augmented production of proline due to anti stress mechanism in the plant. Proline acts as an osmoprotectant and greater accumulation of proline in THT11 and THT17 suggests a genotypic tolerance of tea to low soil moisture condition. Similarly the enhancement of proline and waxes may alleviate the ill effect of waterlogging and helps to maintain the metabolic activities of tea plants [37].

Handique and Manivel in 1986 [27] reported that the accumulation of proline and epicuticular wax in the leaves of drought tolerant clones were higher than in the susceptible ones under soil moisture deficit condition. When correlation study was done, a significant positive correlation was found between proline and epicuticular wax content of the leaf of the twenty germplasm in the present study. Again epicuticular wax showed non-significant but positive correlation with relative turgidity which may justify that epicuticular wax helps in maintaining the water relations and prevents membrane distortion by reducing the transpirational loss.

3.3.4 Genetic relationship and cluster analysis

The correlation similarity proximity with squared Euclidean Distance method utilizing the physiological and biochemical parameters used for analysis of abiotic stress is shown in Table 7. It is observed that the germplasm THT16 and THT19 have high similarity (0.000) followed by higher similarity proximity value between THT9 and THT3 (0.001). Again as the germplasm THT11 and THT17 may be inferred to be the best germplasm amongst all the studied germplasm with respect to parameters for measuring stress tolerance, but no other germplasm showed high similarity value with THT11 except THT17 (0.131).

Again when hierarchical clustering of the twenty tea germplasm accessions was done based on abiotic stress tolerance, the studied germplasm were grouped into four main clusters (Fig. 11). The distribution of the germplasm into different clusters of dendrogram using average linkage was presented in Table 8. It was observed that germplasm THT11 and THT17 which were showing high proline and epicuticular wax content formed two discriminated clusters and thus group individually. Again THT11 can be categorized as China type of plant on the basis of sclereid morphology with very narrow lumen. In support of this, Singh and Handique in 1993 [30] reported that the domesticated species, cultivars of China hybrid origin with erect to semi erect leaves were found to be the most resistant to drought followed by Cambod hybrids, whereas light leaf Assam hybrids showed least resistance to drought. Again the cluster I was representing seven germplasm including THT1, THT2, THT8, THT12, THT16, THT19 and THT20 which had low proline and epicuticular wax content. Cluster II was found to be the largest, incorporating eleven germplasm. Earlier research [39,40] had shown that drought tolerance varies considerably between genotypes of tea, which provides a good basis to screen the available germplasms for tolerance to drought and to develop germplasm through conventional breeding approaches.

Table 7. Pearson similarity coefficient matrix abiotic stress tolerance data

Case	THT 1	THT2	THT3	THT4	THT 5	THT 6	THT 7	THT 8	THT 9	THT 10	THT 11	THT 11	THT 13	THT 14	THT 15	THT 16	THT 17	THT 18	THT 19	THT 20
THT 1	0.000	0.082	0.287	0.253	0.241	0.156	0.329	0.014	0.278	0.278	0.775	0.147	0.120	0.205	0.321	0.059	1.000	0.281	0.060	0.034
THT2		0.000	0.085	0.107	0.045	0.027	0.098	0.060	0.079	0.061	0.355	0.081	0.035	0.040	0.132	0.002	0.539	0.064	0.002	0.039
THT3			0.000	0.022	0.073	0.020	0.143	0.279	0.001	0.074	0.170	0.290	0.043	0.116	0.014	0.111	0.465	0.085	0.111	0.237
THT4				0.000	0.150	0.029	0.254	0.279	0.022	0.159	0.300	0.369	0.026	0.197	0.006	0.129	0.686	0.174	0.129	0.257
THT5					0.000	0.058	0.014	0.181	0.068	0.001	0.168	0.097	0.104	0.007	0.145	0.062	0.273	0.002	0.061	0.126
THT6						0.000	0.130	0.152	0.018	0.068	0.269	0.192	0.008	0.080	0.040	0.041	0.541	0.077	0.040	0.126
THT7							0.000	0.240	0.136	0.011	0.159	0.095	0.195	0.015	0.241	0.117	0.182	0.007	0.116	0.171
THT8								0.000	0.270	0.214	0.690	0.073	0.135	0.139	0.339	0.040	0.835	0.212	0.040	0.006
THT9									0.000	0.069	0.171	0.279	0.040	0.109	0.015	0.105	0.461	0.080	0.105	0.228
THT10										0.000	0.141	0.115	0.120	0.012	0.148	0.082	0.240	0.000	0.080	0.153
THT11											0.000	0.494	0.365	0.232	0.235	0.409	0.131	0.146	0.407	0.583
THT12												0.000	0.222	0.052	0.400	0.072	0.480	0.107	0.071	0.040
THT13													0.000	0.123	0.049	0.043	0.680	0.130	0.044	0.122
THT14														0.000	0.200	0.050	0.300	0.010	0.049	0.088
THT15															0.000	0.160	0.613	0.164	0.160	0.306
THT16																0.000	0.588	0.084	0.000	0.024
THT17																	0.000	0.232	0.585	0.699
THT18																		0.000	0.083	0.151
THT19																			0.000	0.024
THT 20																				0.000

Dendrogram using Average Linkage (Between Groups)

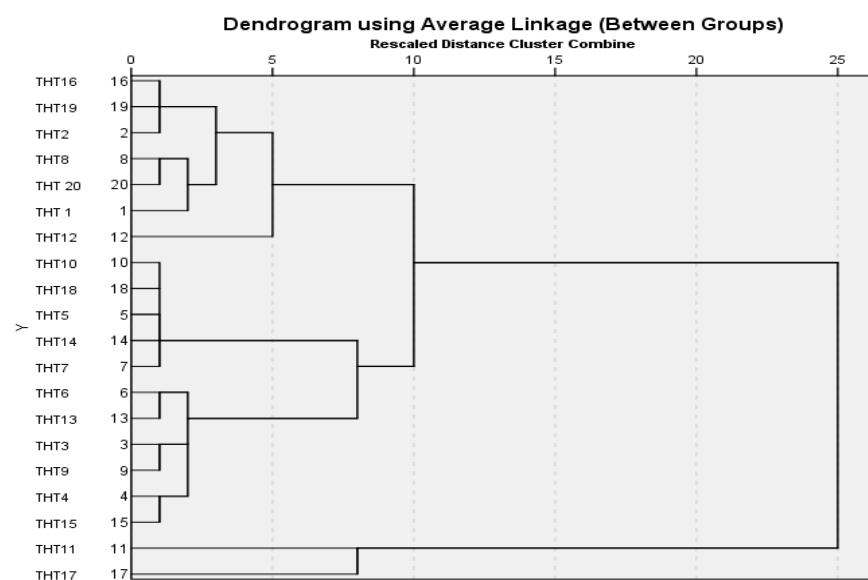Rescaled Distance Cluster Combine

Fig. 11. Dendrogram base on abiotic parameters

Table 8. Grouping of studied Germplasm based on dendrogram done by using abiotic stress tolerant parameter

Cluster I	Cluster II	Cluster III	Cluster IV
THT1	THT3	THT11	THT17
THT2	THT4		
THT8	THT5		
THT12	THT6		
THT16	THT7		
THT19	THT9		
THT20	THT10		
	THT13		
	THT14		
	THT15		
	THT18		

3.3.5 Correlation studies

In Table 9 presents the correlation analysis carried out among the leaf physiological and biochemical properties to measure the abiotic stress. There exists a significant but negative correlation between relative turgidity and water saturation deficit for all the clones. Significant positive correlation exists between proline and epicuticular wax content. Again proline has negative non-significant correlation with relative turgidity but positive non significant correlation with water saturation deficit.

Table 9. Correlation analysis among the leaf physiological and biochemical properties to measure the abiotic stress

Pearson Correlation Sig. (2-tailed)	RT	WSD	Epicuticular Wax	Proline
Relative turgidity	1			
Water Saturation deficit	-1.000[**]	1		
	.000			
Epicuticular Wax	.015	-.015	1	
	.908	.908		
Proline	-.111	.111	.318[*]	1
	.399	.399	.013	

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

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

4. CONCLUSION

The germplasms with higher amount of plucking point density with higher specific leaf weight (THT11, THT7 and THT5) can be considered as a good planting material for production. The germplasms with higher carotene (THT4, THT10, THT20) and chlorophyll content (THT1, THT10, THT3, THT11, THT17) can be selected for black tea and green tea production respectively. The germplasm THT11 with high plucking point density also exhibited higher chlorophyll content which can be selected for developing yield clone for green tea manufacturing. The results may be use for profiling the germplasm for black tea and green tea manufacturing.

In the present experiment the germplasm THT1, THT17 and THT7 were found to have high relative turgidity of leaf, epicuticular wax content and proline content under low field moisture

condition. They also exhibited higher plucking point density which can be selected for future breeding programme for developing yield clone with good stress tolerant capacity. It is also important to note that the parameters taken especially for abiotic stress tolerance study may be subjected to environmental factors for which 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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