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RESEARCH ARTICLE

Cytological and anatomical assessment of a few planting materials of tea (*Camellia* spp.)

■ **Alija Burhagohain and Aditi Smith Gogoi**

SUMMARY

In the present investigation, an effort was made to characterize and estimate the variation of 20 tea germplasm on the basis of cytological and anatomical characteristics. All the germplasm showed highly significant difference amongst them in all the parameters except the ploidy level. Here, all the germplasm were observed to be diploid having 2n=30 no. of chromosome. From the anatomical parameters it was observed that THT8 followed by THT5, THT7, THT13 showed characteristic more likely towards Cambod type also recorded with higher phloem index. The planting material noted with higher phloem index in the present study can be considered as hybrid population which may be a good parent material for breeding programme.

Key Words : Germplasm, Cytological, Anatomical, Phloem index

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Tea [*Camellia sinensis* (L.) O Kuntz] germplasm is the most important basis which promote the sustainable development of tea.The available diversity of genetical base is needed to be characterized and preserved for future crop improvement programmes which may constitute the fundamental support for the industry. Now the knowledge of genetic organization of tea is required to increase productivity and efficient use of germplasm for their cultivation or breeding programme. It has been reported that the cultivated tea remained as diploid all through its long history of cultivation, although polyploidy of common occurrence can be found in other ornamental species of *Camellia* (Longley, 1956). Research studies on available germplasm based on morphological characteristic, cytological, anatomical studies like morphology of sclereids and phloem index of different tea cultivars are different which may help in characterization of tea germplasm.

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In the experimental garden of tea under the Department of Tea Husbandry and Technology, Assam Agricultural University (AAU), Jorhat, Assam genotypically different plants are growing which were collected from different areas by Bruisers and planted. However, no detailed study on the cytological and anatomical variation in these materials had been attempted in the past, although some initial works have been just started recently. Therefore, looking into its importance the present study has been undertaken to study the cytological and anatomical characteristics of unidentified Tea Germplasm in Field Gene Bank maintained in Experimental Garden for Plantation Crops, AAU, Jorhat

MATERIAL AND METHODS

Plant materials:

Twenty (20 nos.) 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-001 to THT-020 and utilized in the present investigation to study the cytological and anatomical assessment.The whole experiment was conducted during the time period from June, 2016 to March, 2017.

Method for recording cytological character:

Cytological character was studied only by doing ploidy level study. The ploidy level study of twenty germplasm was done following the technique of Sharma and Sharma (1980).

Anatomical character:

Under anatomical character following parameters were taken for study.

Sclereid morphology:

For sclereid morphology, the method given by Barua and Dutta (1959) was followed. Lumen shape was measured and determined as per the IBPGR descriptors. For measuring length, breadth and number of spicules per sclereid, dimensional method was used by taking ocular micrometer.

Phloem index:

Phloem index is the average frequency of calcium oxalate crystals in the phloem parenchyma of a complete cross section of a petiole. The expanded leaves of comparable age were collected and transverse section of 20µm thickness was cut from each petiol. A count of all the visible crystal of calcium oxalate in the whole of the phloem parenchyma of the section was made with a low power objective.

Stomatal frequency and stomatal index:

The frequency of stomata and stomatal index was measured in the mid position of lower leaf surface taking the help of the impression methods (Beakbane and Mazumder, 1975).

Differences among the mean values were detected by using one-way analysis of variance (ANOVA). The correlation similarity proximity matrix with squared euclidean distance method was used to see the genetic relationship amongst the clone. The statistical analyses were performed using Statistical Package for Social Science (SPSS) 17.0.

RESULTS AND DISCUSSION

Twenty numbers of tea germplasms designated as THT1 to THT20 growing in the Experimental Garden for Plantation Crops, Assam Agricultural University, Jorhat were characterized on the basis of their cytological, anatomical and stress bearing characters. The experimental findings obtained by using physical method, dimensional method and morphological descriptor reflect less variation among the germplasm as well as the standards *i.e.* China, Assam and Cambod type.

Cytological character:

Different studies reported that the somatic chromosomes of different species and varieties of *Camellia sinensis* showed constant diploid number (2n=30) of chromosome (Bezbaruah, 1968 and 1975; Jayasurya and Govindarajulu, 1975; Dutta and Choudhuri, 1975 and Rahman *et al.,* 2010). In cytological study, ploidy level study had been performed on the twenty germplasms. Each germplasm had been studied with three replications. In the present investigation, no distinct variation had been observed in the chromosome numbers of the germplasm. All the clones studied had been found to have diploid with chromosome number 2 n=30 (Plate 1).

Anatomical characters:

Sclereid morphology:

Earlier researchers stated that sclereid anatomical

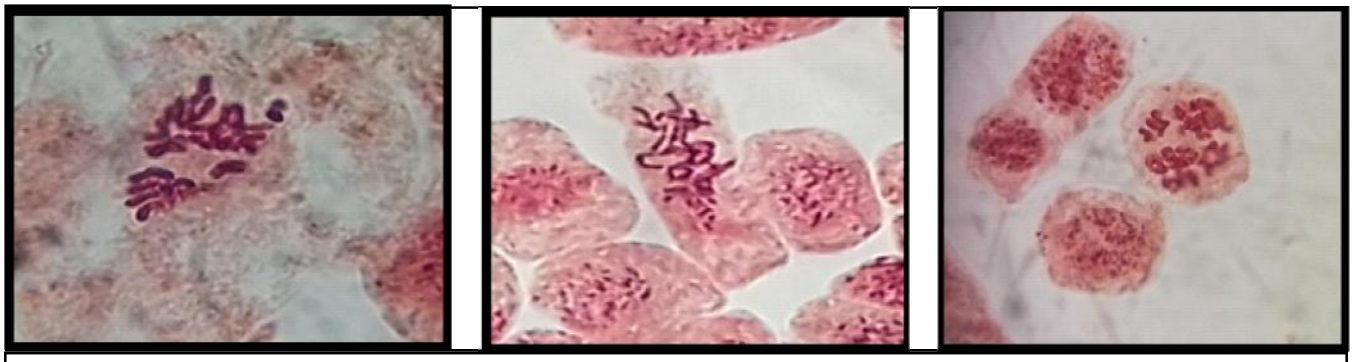

Plate 1: Ploidy level study of three different germplasm

studies were helped in distinguishing the varieties where phenotypic variation is narrow (Barua and Dutta, 1959 and Banerjee, 1992). Barua (1963) provided anatomical description of the three races of tea on the basis of sclereid morphology. In the present investigation, three anatomical characters *i.e.* sclereids, stomatal frequency and phloem index were considered to determine the variation among the twenty germplasms.

The sclereid morphology were observed by recording size of sclereid (length and breadth) (Fig. 1 and 2) in micron, number of spicules per sclereid and lumen shape for the studied germplasm and the data were tabulated in the Table 1. There were a high significant (p=0.000) variation in all the parameters of sclereid

Table 1: Mean length, breadth, number of spicules and lumen shape of sclereid of the germplasm

Germplasm		Sclereid			
		Length (µm)	Breadth (µm)	Number of spicules (Nos)	Lumen shape
THT 1		11.97±1.04	3.201±0.197	4.667±0.471	WC***
THT2		13.92±2.08	3.479±1.096	4.000±0.816	CL***
THT3		12.25±0.79	5.010±0.000	5.333±0.471	WC
THT4		14.47±3.15	3.340±0.000	4.333±0.471	CL
THT5		19.48±0.79	5.340±0.000	6.667±1.247	WC
THT6		30.34±1.42	2.340±0.000	2.000±0.816	VN***
THT7		22.27±0.79	4.175±0.000	5.333±0.471	WC
THT8		22.27±1.57	4.505±0.000	6.333±0.943	VN
THT9		13.92±0.79	4.453±0.787	4.333±0.471	CL
THT10		25.05±2.73	2.732±0.197	2.000±0.000	VN
THT11		18.37±0.00	5.010±0.000	2.333±0.471	VN
THT12		20.04±2.36	2.871±0.197	4.333±0.471	VN
THT13		17.81±1.57	3.010±0.000	4.333±0.471	CL
THT14		20.60±2.84	6.541±0.197	4.333±0.471	WC
THT15		13.92±2.08	3.062±0.394	5.333±0.943	CL
THT16		20.04±1.36	3.340±0.000	4.333±0.471	CL
THT17		15.59±0.79	3.505±0.000	5.333±0.471	CL
THT18		20.60±0.79	4.453±0.787	3.333±0.471	CL
THT19		17.81±1.57	3.062±0.394	3.667±0.471	WC
THT 20		13.64±1.04	2.783±0.394	3.667±0.471	CL
Germplasm	df 19	66.065**	3.388**	3.869**	
Error	df 40	4.265	.230	.583	
CD		1.97	0.46	0.73	

"**" highly significant

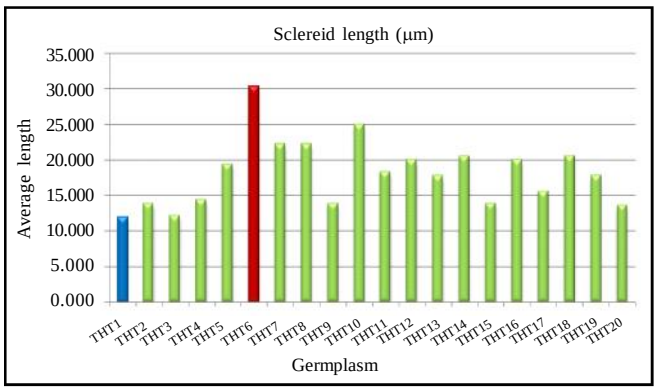

Fig. 1: Variation in the sclereid length («m) of different germplasm

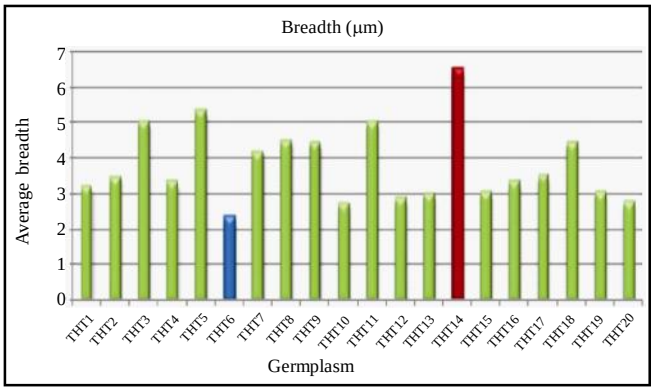

Fig. 2: Variation in the sclereid breadth («m) of different germplasm

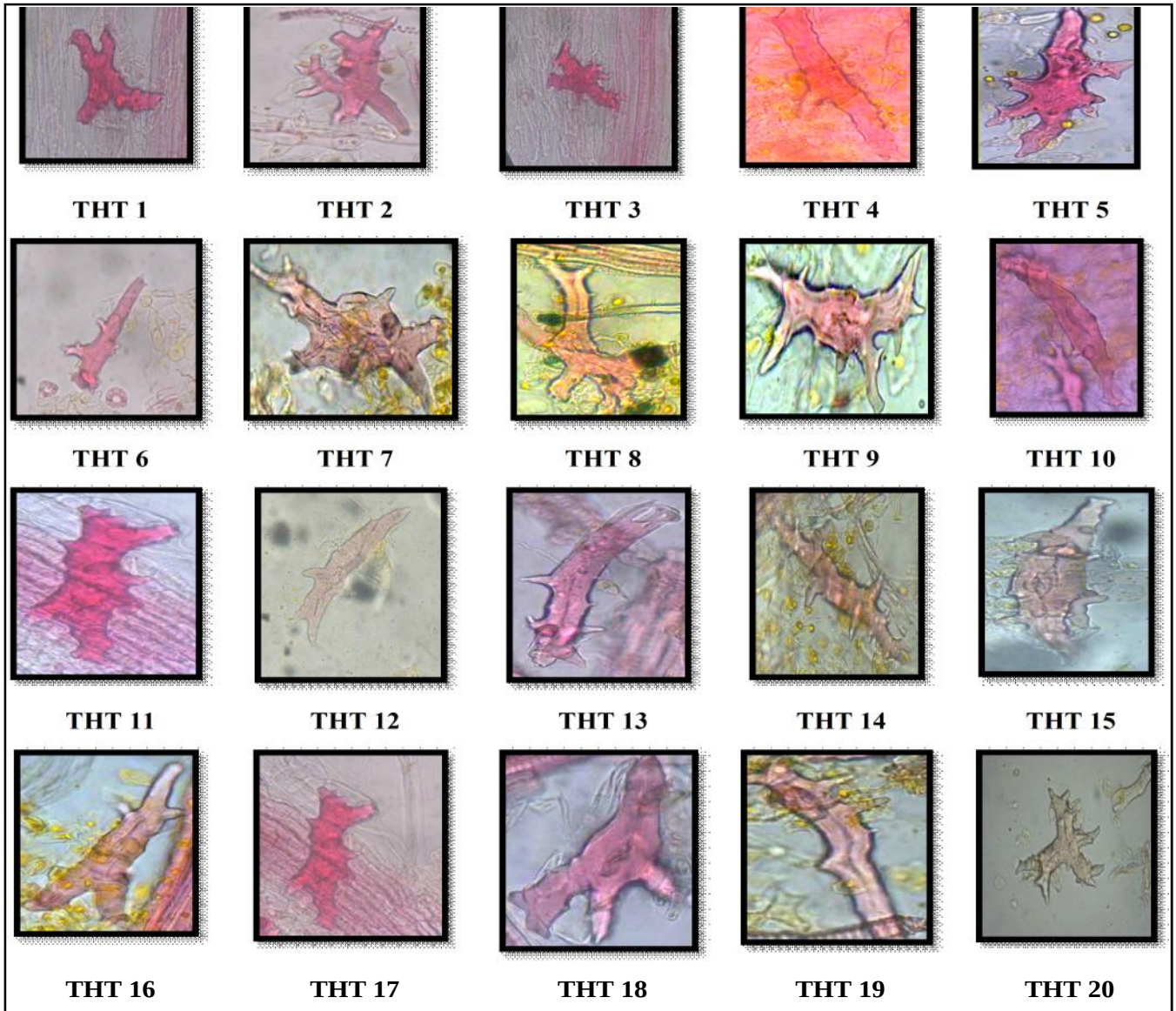

Plate 2: Sclereid morphology of the germplasm

morphology studied on the twenty germplasm.

Sclereid morphology exhibited significant variation among all the germplasms. It was observed that the germplasm THT1, THT3, THT5, THT7 and THT18 showed higher number of spicules (Fig. 3) with broader lumen which is the characteristics of cambod type of tea whereas THT6 THT4 THT10 and THT11 showed very narrow lumen with minimum spicules which is the characteristics features of the China type of tea plant. Other gemplasm *i.e.* THT2 THT4 THT9 THT12 THT13 THT14 THT15 THT16 THT17 showed constricted lumen with limited spicules like Assam type of tea plant. in case of length and beadth of sclereids germplasm showed highest variation. The maximum length was found in THT6 and shortest in THT1. Again in case of breadth maximum was found in THT14 and minimum in THT8 and THT17. Studies like morphology of sclereids and phloem index of different tea cultivars are different which may help in characterization of tea germplasm (Plate 2).

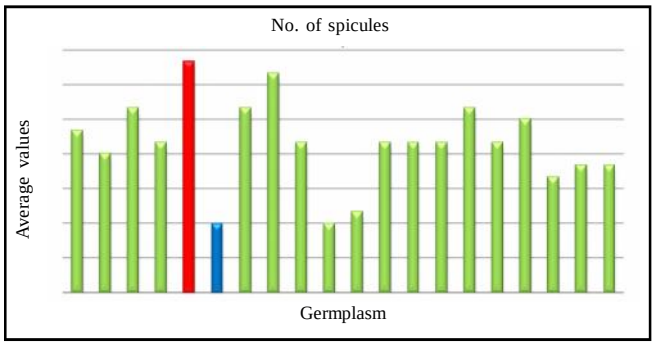

Fig. 3: Variation in number of spicules of different germplasm

Phloem index:

Wight (1959) reported that the mean crystal frequency vary in hybrids populations and has a strong genetic basis. In the present study significant variation was observed amongst the germplasms. The germplasm THT8, THT5, THT9, THT15, THT16 and THT17 showed higher amount of phloem index than

Table 2 : Mean of phloem index, stomata frequency and stomatal index (SI) of the germplasm

Germplasm		Phloem index (no.)	Stomata frequency (no. per unit area)	SI (%)
THT 1		50 ±1.874	3±0.471	30.20±2.48
THT2		41 ±1.231	2±0.471	19.81±2.31
THT3		72 ±0.471	4±0.471	36.66±4.71
THT4		36 ±0.966	4±0.816	36.01±4.76
THT5		211 ±1.345	5±0.471	39.89±2.50
THT6		46 ±2.095	7±0.471	54.29±3.40
THT7		265 ±1.414	2±0.000	15.87±1.12
THT8		280 ±2.273	10±0.471	83.76±0.60
THT9		181 ±2.490	2±0.471	27.85±2.10
THT10		88 ±0.816	3±0.000	29.09±1.28
THT11		103 ±1.686	10±0.47	37.57±1.71
THT12		93 ±1.325	4±0.94	59.21±0.55
THT13		96 ±0.850	4±0.00	47.95±5.47
THT14		44 ±2.009	3±0.47	41.48±2.09
THT15		193 ±1.231	2±0.00	18.51±2.61
THT16		121 ±2.502	8±0.47	33.33±0.00
THT17		195 ±1.304	9±2.16	58.25±4.54
THT18		94 ±2.717	2±0.47	61.15±3.58
THT19		86 ±0.992	8±0.47	39.28±2.52
THT 20		41 ±1.280	4±0.94	75.91±3.03
Germplasm	df 19	20947.312**	23.891**	1011.939**
Error	df 40	15.988	.350	13.165
CD		6.60	0.56	3.46

"**" highly significant

the others. Banerjee (1992) observed that *C. assamica* hybrids lose their specificness and their phloem index increased but intermediates generally had the highest phloem index. Hybrids nearer to *C. sinensis*, however, exhibited a lower index. In the present study, the germplasms with lower phloem index (THT1, THT2, THT4, THT6, THT14 and THT 20) can be considered as China type of tea. Again the germplasms noted with higher phloem index in the present study (THT8 THT5 THT19 THT15 THT16 and THT17) might be considered as similar to Cambod or hybrid population which can be the good parent material for breeding. (Table 2 and Fig. 4).

Stomatal frequency:

In the present experiment also, significant variation in stomata frequency and stomata index were observed. Stomatal frequency found highest in THT 8 (10 nos. per microscopic field) where lowest stomatal frequency found in THT2 (2 nos. per microscopic field) and THT7 (2 nos. per microscopic field). Again, highest stomatal index was recorded THT8 (83.76%) and the lowest in

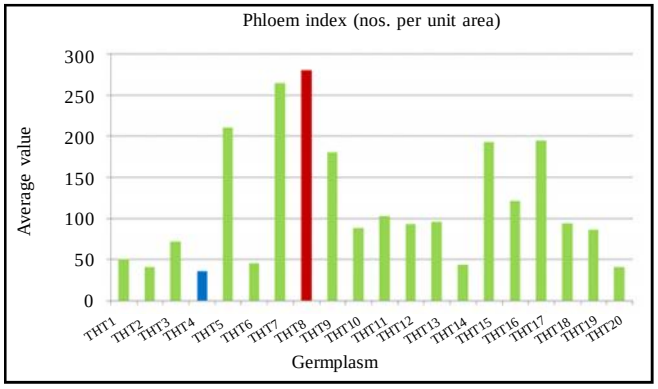

Fig. 4: Variation in phloem index of different germplasm

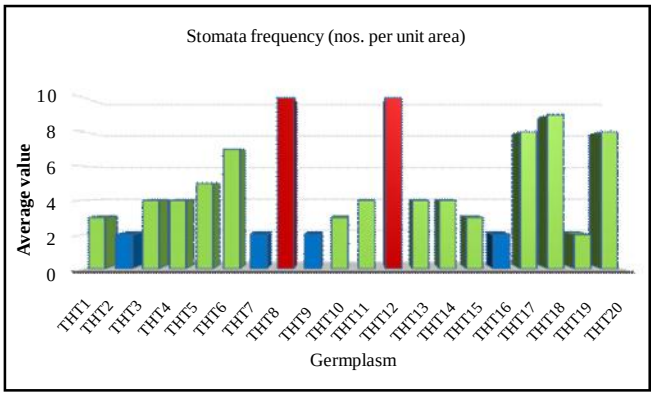

Fig. 5: Variation in stomata frequency of different germplasm

Table 3 : Pearson similarity co-efficient matrix utilizing anatomical data

Case	Rescaled squared euclidean distance																			
	THT1	THT2	THT3	THT4	THT5	THT6	THT7	THT8	THT9	THT10	THT11	THT12	THT13	THT14	THT15	THT16	THT17	THT18	THT19	THT20
THT1	0	0.003	0.007	0.003	0.054	0.013	0.646	0.909	0.741	0.022	0.04	0.039	0.034	0.003	0.286	0.071	0.303	0.042	0.02	0.031
THT2		0	0.017	0.004	0.074	0.021	0.697	0.989	0.8	0.034	0.058	0.06	0.053	0.007	0.321	0.092	0.35	0.064	0.033	0.044
THT3			0	0.018	0.022	0.018	0.524	0.754	0.607	0.007	0.014	0.014	0.01	0.012	0.208	0.034	0.217	0.016	0.003	0.035
THT4				0	0.079	0.01	0.734	1.000	0.834	0.04	0.063	0.053	0.052	0.002	0.346	0.101	0.358	0.056	0.035	0.023
THT5					0	0.063	0.337	0.523	0.404	0.01	0.001	0.01	0.004	0.063	0.1	0.002	0.103	0.011	0.009	0.087
THT6						0	0.687	0.908	0.78	0.034	0.051	0.033	0.038	0.004	0.321	0.086	0.311	0.034	0.028	0.011
THT7							0	0.082	0.006	0.437	0.371	0.437	0.411	0.687	0.073	0.292	0.094	0.435	0.452	0.748
THT8								0	0.05	0.666	0.569	0.603	0.596	0.935	0.219	0.481	0.163	0.596	0.664	0.933
THT9									0	0.519	0.441	0.505	0.481	0.783	0.109	0.356	0.116	0.502	0.53	0.832
THT10										0	0.005	0.014	0.007	0.029	0.156	0.016	0.172	0.015	0.002	0.063
THT11											0	0.008	0.002	0.049	0.118	0.005	0.124	0.009	0.004	0.074
THT12												0	0.002	0.038	0.163	0.021	0.145	0.000	0.007	0.042
THT13													0	0.038	0.143	0.012	0.138	0.003	0.002	0.053
THT14														0	0.316	0.083	0.321	0.04	0.025	0.018
THT15															0	0.075	0.022	0.162	0.165	0.367
THT16																0	0.085	0.021	0.017	0.115
THT17																	0	0.142	0.17	0.333
THT18																		0	0.008	0.043
THT19																			0	0.047
THT20																				0

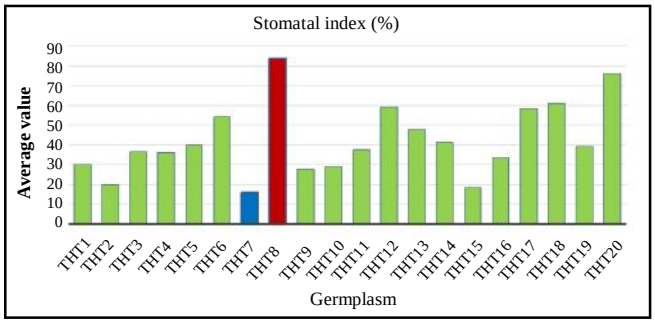

Fig. 6: Variation in stomatal index of different germplasm

THT15 (18.52), (Table 2, Fig. 5 and 6).

From all anatomical characteristics, it was observed that THT6 THT10 and THT11 exhibited very narrow lumen and low phloem index value. On the basis of this, these three germplasm can be said nearer to China type tea. In the proximity matrix also they had very high similarity value. Again THT5 and THT7 were observed to have sclereid morphology similar with cambod type of tea. They were also recorded with higher phloem index (211 and 265 nos., respectively) which is a characteristic of intermediate species (Banerjee, 1992).

Genetic relationship and cluster analysis:

The correlation similarity proximity matrix with squared euclidean distance method was utilizing for cluster analysis of the anatomical parameters and was presented in Table 3. From the table it was observed that similarity value ranges from 0.000 to 1.000 indicating a high degree of variation amongst the germplasm. The highest similarity value (0.000) was observed between the germplasm THT12 and THT18 (0.000) and the lowest similarity value (1.000) was found between the germplasm THT4 and THT8. There was also high similarity value between THT5 and THT11 (0.001).

Based on the Fig. 7 all the germplasms were grouped into four different cluster combinations. The distribution of different germplasm in different clusters of dendrogram has been presented in Table 4. It was observed that THT7 and THT8 formed separate clusters. Cluster II was found to be the largest cluster incorporating eight germplasm.

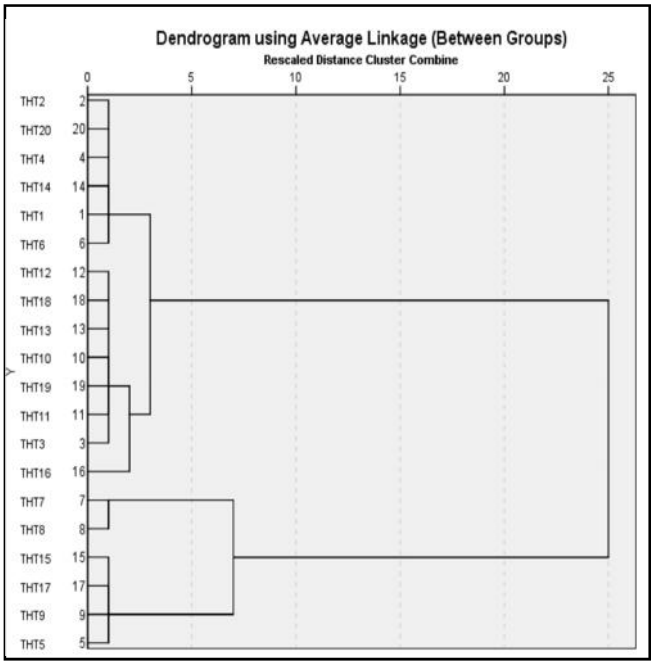

Fig. 7: Dendrogram done by using anatomical characters

Conclusion:

The present investigation was carried out during April 2016 to May 2017 in the Department of Tea Husbandry and Technology of Assam Agricultural University, Jorhat. The study was aimed to access the variability among 20 unidentified germplasm based on cytological and anatomical characteristics. Results obtained are summarized as follows:

Table 4 : Grouping of germplasms based on dendrogram using anatomical characters

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

Cytological study:

No variation was observed in all the germplasm regard to chromosome number. All the germplasm contain same ploidy level containing 2 n=30.

Anatomical study:

On the basis of anatomical study some germplasm can be characterized as Cambod type (THT3 THT5 THT7 THT8 THT9) type but it was difficult to identify the gemplasm into two distinct group of China and Assam type.

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