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ORIGINAL ARTICLE

Multivariant Analysis - A Tool For Characterization and Variability Assessment In Teak (*Tectona grandis* L.f) Clones for Dus Testing (Distinct, Uniform and Stable)

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ABSTRACT
Using a statistical method - Multivariant analysis, 122 Teak clones in Institute of Forest Genetics and Tree Breeding (IFGTB) were analyzed to prove the Distinct, Uniform, and Stability (DUS) testing guidelines phonetically based on quantitative traits [14]. Multivariate methods can also be used for identifying genotypic variation based on distinct character or character set. It is mainly used for summarizing information, eliminating noise/error from the data sets, and revealing the structure of the data sets [7, 15]. The results of Multivariant analysis such as Canonical Discriminant analysis, Principal Component Analysis, and Cluster analysis are revealed that all individual clones showed considerable variation in terms of quantitative characteristic traits of leaves alone. Therefore the weightage of the quantitative traits was proven by Canonical Discriminant analysis, three significant discriminant functions, which had Eigen value > 0.7 and these 2 values accounted for a cumulative variation of 39.2%, Principal Component Analysis, three significant principal components (PCs) extracted had Eigen value >1 and out of 12 this three values accounted for a cumulative variation of 92.8%, and in Cluster analysis 122 Teak clones' dendrogram showed 3 distinct clusters using the Agglomerative hierarchical clustering (AHC) –Wards method based on Dissimilarity: Euclidean distance. The dendrogram drawn represented 5 distinct clusters using Agglomerative hierarchical clustering (AHC) - Unweighted pair-group average using Similarity: Pearson correlation coefficient.
Keyword: Cluster analysis, Discriminant analysis, Distinct characters, Mature leaves, Principal component analysis.

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INTRODUCTION

Teak (*Tectona grandis* Linn. f.) is one of the most valuable timber yielding species in the world, which belongs to the Lamiaceae family according to APG IV (ANGIOSPERM PHYLOGENY GROUP 2016) and (The International Plant Names Index, 2014-IPNI). The genus *Tectona* is represented by only three species, namely *Tectona* grandis L.f., *T. hamiltoniana* Wall. and *T. philippinensis* Benth. & H.f., *T.* grandis occurs naturally between 9° to 26° N latitude and 73° to 104° E longitude and a major component of diverse forest types. Geographical distribution of teak spans over southern and central India, Myanmar, Laos People's Democratic Republic, and northern Thailand [1].
In India, teak forests are spread over a large geographic area of 8.9 million ha in very-dry to very-moist types. It grows well from sea level to an elevation of 1000 m, and on areas with an annual precipitation ranging from 1250- 3750 mm and minimum temperature of 13º to 17ºC and maximum temperature of 39º to 43ºC. It is grown most suitable soil is deep, well-drained, fertile alluvial soil with a pH of 6.5-8 and a relatively high calcium and phosphorous content.
The quality of growth, however, depends on the depth, drainage, moisture status, and fertility of the soil. Teak does not tolerate water logging or infertile lateritic soils. Teak is growing as plantations across 36

tropical countries of Asia, Africa, and Latin America. Teak is naturally distributed in different climatic and edaphic zones and has developed different ecotypes during the process of evolution. Wide variation in the performance of different ecotypes has been recognized[2,3].

In India, the Clonal plantation of Teak was not practiced on a large scale, but now it is upcoming and has a great scope in increasing the productivity of teak Clonal forestry increases the productivity significantly compared to the seedling plantation. Significant gains can be achieved in the shortest period 2 to 4 fold enhancement of productivity has been reported in clonal plantation compared to seedling plantation MAI up to 10 m^3 / ha/year has been achieved using superior clonal plants and improved Silviculture. The world's teak supply from natural and planted forests adds up to a total of 2-2.5 million m3 annually. The global demand for teak is expected to grow and will continue to be governed by trends in the Asian markets. At the current average price of 600-1000 USD/m3 for high-quality logs and 350-750 USD/m3 for small dimensional plantation logs of 20-25 yrs, teak is already one of the expensive hardwoods in the world timber market.

Maharashtra forest department has previously developed a national teak germplasm collection with 259 clones with pedigree details of Teak were chosen for the present study.

MATERIAL AND METHODS

Tectona grandis L.**:** Genetic material evaluated in the research included 122 Teak clones collected from National Teak Germplasm at Lohara in Chandrapur district in Maharashtra and 20 clones collected from Walayar in Kerala. Data was collected from clones in experimental trials which were planted in 8×8 m spacing with three ramets with three replications in 5.09 Hectare area for grouping of a characteristic trait. The commercially important mature leaves of selected 122 teak clones leaves are always glabrous often coriaceous, rarely scabrous. Almost always opposite and decussate, only occasionally three at a node or and rarely alternate in seedling moreover easily available in all seasons. Mature leaves were collected and DUS testing was conducted in the laboratory of Institute of Forest Genetics and Tree Breeding, Genetics and Tree Improvement Division, Coimbatore, India. The detailed study for DUS testing was carried out with 122 clones are as follows,

List of selected 122 clones

Clone S. No	All India clone	Arunachal Pradesh	Andhra Pradesh	Maharastra	Madhya Pradesh	Gujarath	Odisha	Karnataka	Kerala	Tamil Nadu	Uttara Pradesh	Westbengal
1	AI0	AC11	APJNB1	MHALA2	PT03	GUJ13	ORANP3	ST11	KLK1	TNT1	UPA	WB4
2	AIA	ACI	APKEA23	MHALA3	PT41	GUJ8	ORANP7	ST14	KLK2	TNT10	UPC	
3	AID	AS4	APKEA24	MHALA4	PT46		ORANR2	ST21	KLN2	TNT11	UPD	
4	AIE		APKEA25	MHALA6	BLC10		ORANR3	ST26	KLN4	TNT13	UPG	
5	AIF		APKEC2	MHALA7			ORANR4	ST27	KLS1	TNT15		
6	AII		APKEN1	MHALA8			ORANR6	ST33	KLS2	TNT16		
7	AIJ		APMN4	MHALP1			ORANR7	ST36	KLS3	TNT17		
8			APNPL1	MHALP2			ORJEK1	ST41	KLS4	TNT18		
9			APNPL10	MHALP3			ORPB17	ST44		TNT2		
10			APNPL2	MHALP4			ORPB18	ST45		TNT20		
11			APNPL3	MHALP5			ORPLM1	ST49		TNT3		
12			APNPL4	MHALP6			ORPUB11			TNT4		
13			APNPL5	MHALP7			ORPUB12			TNT5		
14			APNPL6	MHALP9			ORPUB15			TNT6		
15			APNPL7	MHEMR1			ORPUB21			TNT7		
16			APNPL8	MHSCA1			ORPUB5			TNT8		
17			APNPL9	MHSCA2						TNT9		
18			APT11	MHSCA3								
19			APT13	MHSCJ1								
20			APT14	MHSCJ2								

21			APT15									
22			APT16									
23			APT20									
24			APT22									
25			APT6									
26			APT7									
27			APT8									
28			APT9									
29			SBL1									

Selected quantitative characters: The quantitative character included Mature leaf Area, Length, Breadth, Perimeter, Convex Perimeter, Equivalent diameter, Curve length, Curve width, Roundness, Aspect ratio, Convex area, and Full ratio. Based on the measurement by a single observation of a group of plants or parts of plants the data were collected (Table 1). For assessment and grouping, we followed the DUS Guidelines in the Plant Variety Journal of India. The growth stage code adapted for this observation is 20 and 32. The data were recorded from 6 randomly chosen competitive plants in each replicate. To plot quantitative data or to show distributions of variables, Histograms with ranges of the data grouped into bins or intervals are used (Plate 1 and 2).

Multi-variant analysis: Multi-variant analysis such as Discriminant Analysis, Principal Component Analysis (PCA) was used to classify genotypes and see how big contribution to the character of *Teak* genotype appearance [8, 10]. Genetic diversity and distance based on the similarity and dissimilarity between objects under study were analyzed using cluster analysis. The SPSS 20 for discriminant analysis, XLSTAT 2012 computer software was used for cluster analysis and PAST software for Spread plot method.

RESULT AND DISCUSSION
MULTIVARIANT ANALYSIS OF QUANTITATIVE TRAITS MATURE LEAVES OF 122 TEAK CLONES
Canonical Discriminant analysis of clones based on Mature Leaf Characters
Results of multivariate analysis showed that clones can be discriminated against even with 12 quantitative characters of mature leaves traits of 122 Teak clones with 30 % of accuracy [15]. Classification based on this analysis showed that the samples can be classified into respective clones/ genotypes with only 40% accuracy. The application of such methods may be sufficient for self-pollinated varieties. In this study, Stepwise discriminant analysis was run in 11 steps for distinguishing the species. Eigen value, also called the characteristic roots, is a ratio between the explained and unexplained variation in a model. For a good model, the Eigen value must be more than one. For tree crops, it should be > 0.7 Eigen value. The bigger the Eigen value, the stronger is the discriminating power of the function. In an analysis with three groups, the ratio between two Eigen values indicates the relative discriminating power of one discriminant function over the other. Three significant discriminant functions, which had an Eigen value >0.7, and these 2 values accounted for a cumulative variation of 39.3%. However, the remaining components contributed only 59.7% towards the total diversity for this set of 122 clones. The first function explained the most variability accounted for 21% followed by 18.3 %, respectively from the second towards total variation.[6] According to the variance extraction rule, it should be more than 0.7. If the variance is less than 0.7, then we should not consider that a factor.

The first three Discriminant functions were statistically significant according to the chi-square test at a probability of 0.000 (i.e <0.005). Proper values and the distribution of their variances indicated that the first two functions accounted for more than 39.3% of the total variance. Wilk's Lamba is used to test the significance of the discriminant functions. Mathematically, it is one minus the explained variation and the value ranges from 0 to 1. Unlike the F-statistics in linear regression, when the value lambda for a function is small, the function is significant. In Teak clones Wilks' lambda coefficients for these functions were precisely the lowest, indicating almost perfect discrimination regarding the remaining functions [19,22]. The standardized canonical discriminant coefficients can be used to rank the importance of each variable [16]. A high standardized discriminant function coefficient for a trait might mean that the variable has a greater discriminating ability (Table 2).

The discriminant function coefficients are partial coefficients that reflect the unique contribution of each variable to the classification of the groups in the dependent variable. The standardized canonical discriminant function coefficients for two functions are summarized in Table 3. Convex Perimeter (3.04) and followed by Perimeter (2.9) were responsible for maximum separation along with the first function.

It maximizes the differences between the groups in the dependent variable. Curve width (2.6) and Perimeter (1.6) in the second function were responsible for maximum separation. The second function is orthogonal to the first (uncorrelated with it) and maximizes the differences between the groups in the dependent variable, controlling for the first function. Though mathematically different, each discriminant function is a dimension that differentiates a case into groups in the dependent variable based on its values on the independent variables. Classification based on this analysis showed that the samples can be classified into respective clones/ genotypes with 40% accuracy. The centroids are in a unidimensional space, one center for each group. For three groups in the dependent variable, there are two discriminant functions. Hence, the centroids are in a two-dimensional space. By connecting the centroids a canonical plot can be created depicting a discriminant function space. (Plate 3). The Proximity of group centroids indicates the errors in classification. The distance between group centroids for different clusters is far away which indicates the precision of classification level.

Principal Component Analysis (PCA) of clones based on Mature Leaf Characters
Principal Component Analysis (PCA) involves finding the linear combination of a set of variables that, has more than one variable[11]. In this study, three significant principal components (PCs) extracted had Eigenvalue >1 and three values accounted for a cumulative variation of 92.8% (Fig. 2). However, the remaining components contributed only 7.2% towards the total diversity for this set of 122 genotypes. The first principal component (PC I) explained the most variability accounted for 61% followed by 21.1% components in PC 2, 10.7% components in PC 3 towards total variation (Table 4). The active variables and the observation of individual clones were represented in Figures 3-5. All traits except Roundness and Aspect ratio showed considerable positive factor loadings on PC I this is because of the shape of the leaf lamina. The 2nd PC was related to diversity among clones due to Length, Breadth, Perimeter, Convex Perimeter, Roundness, Aspect ratio, Equivalent diameter, Curve length, with their positive loadings (Plate 5, Fig. 1 & 2). In PC3 Breadth, Convex perimeter, Roundness, Aspect ratio, Equivalent diameter, Curve width, and Full ratio shows positive loadings.

Spread out the plot of clones based on Mature Leaf Characters
For determining distinctness for quantitative characteristics different approaches depending on the method of observation of a variety are used. The PCA is also used to describe genotypic data to use quantitative characters that have a high contribution towards variability in DUS Descriptor within the bi-plot. It is used to determine the contribution of one character on variability to easily determine the character that can represent a genotype [4]The scatter plots are shown in a graphic biplot as determined by the PC values that give the highest contribution to variability[6,17].
The PC 2 and PC 3 are the component values that give the highest contribution towards the variability of a character. The variability values from the two components for each genotype are scattered within the biplot forming 4 quadrants (Plate 5). It shows that the different *Teaks* genotypes are found in four different quadrants based on the active variables. The study shows that there are characters in the PC that have relatively high variability and are important in separating genotypes [20, 21]. Quadrant 2 and 3 have more variables. And the spread out the plot of the principal component I and 3 showed that 1st, 8th, 65th, 121st, 122nd, 51th, and 16th (AIO, AII, MHSCA1, UPG, WB4, MHALA3, and APMN4 respectively) were more diversified so these quantitative characteristics have latent to used, to show Distinctness of clones/genotypes based on the morphological characters, i.e., only quantitative characters[13]. Therefore this study provides evidence that quantitative leaf characteristics determined by image analysis techniques can be used for taxonomic differentiation of the *Teak* clones[22].

Clustering of clones based on quantitative characters of Mature Leaf
Based on 12 quantitative traits of Mature leaf, 122 clones were classified into Main major and small minority groups. cluster analysis based on similarity using the Pearson correlation coefficient method to group the 25 Eucalyptus clones into three main groups[5]. Cluster "A" "B" and "C" are composed of nine, eight, and eight genotypes, respectively[9,12]. In *Teaks* clones also the dendrogram drawn depicted three distinct clusters based on the Dissimilarity using Euclidean distance, i.e. Agglomerative hierarchical clustering (AHC) –Wards method. Cluster I comprised 75 clones (Table.6) Cluster II consists of 10 clones cluster III showed 37 clones (Plate 5).
The dendrogram drawn depicted five distinct clusters based on the Similarity: Pearson correlation coefficient, clones were grouped using Agglomerative hierarchical clustering (AHC) -Unweighted pair-group (Plate 5). Cluster I comprised of 9 clones (Table. 7), Cluster II consists of 71 clones, cluster III showed 22 clones, Cluster IV showed 19 clones, and cluster 5 has only one monophyletic group (KLK1). Though the clonal variability i.e. Distinctness was proved by both PCA and CDA, the diversity of the clones were represented in the dendrogram also. As per the multivariant analysis, the selected 122 clones

showed a significant result in all 12 quantitative characteristic traits of mature leaf and it can be recommended for Morphological characterization of *Teak*.

Table 1. Descriptive statistical analysis report of 12 quantitative characters of Mature leaves Teak clones.

Descriptive statistics	Mean	Std. Error of Mean	Std. Deviation	Variance	Skewness	Std. Error of Skewness	Kurtosis	Std. Error of Kurtosis	Range	Minimum	Maximum
Full Ratio	.9306	.00040	.02722	.001	-1.028	.036	1.984	.073	.26	.75	1.00
Convex Area cm2	521.6348	3.49815	236.14471	55764.325	.995	.036	1.346	.073	1425.22	85.02	1510.24
Curve Width cm	11.9338	.04607	3.09486	9.578	.573	.036	.341	.073	21.21	3.67	24.88
Curve Length cm	37.6643	.13236	8.88781	78.993	.709	.036	.572	.073	54.11	16.03	70.13
Equivalent Diameter cm	23.6744	.07232	4.85111	23.533	.406	.037	-.085	.073	28.24	9.75	38.00
Aspect Ratio	1.56	0.004	0.26	0.07	4.29	0.037	34.36	0.07	3.56	1.08	4.63
Roundness value	1.69	0.004	0.32	0.10	3.95	0.03	28.11	0.07	5.09	1.11	6.20
Convex Perimeter cm	81.1811	.24215	16.27474	264.867	.469	.036	.188	.073	103.54	34.93	138.46
Perimeter cm	99.3841	.31804	21.36107	456.295	.570	.036	.266	.073	143.95	43.83	187.78
Breadth cm	20.8078	.06978	4.68673	21.965	.440	.036	-.089	.073	26.94	8.70	35.64
Length cm	31.9520	.09556	6.42238	41.247	.454	.036	.356	.073	43.80	13.17	56.97
Area cm2	434.6266	2.73002	181.17115	32822.987	.514	.037	-.168	.074	873.85	74.72	948.57

Table 2. Eigen value of first 3 canonical discriminant functions, canonical correlation, Wilks lambda, and chi-square values

Function	Eigen value	% of Variance	Cumulative %	Canonical Correlation	Wilks' Lambda	Chi-square	Sig.
1	.837a	21.0	21.0	.675	.040	13141.361	.000
2	.728a	18.3	39.2	.649	.073	10663.103	.000
3	.545a	13.7	52.9	.594	.126	8432.960	.000
4	.528a	13.3	66.2	.588	.195	6659.260	.000
5	.305a	7.7	73.8	.484	.298	4929.702	.000
6	.270a	6.8	80.6	.461	.390	3843.914	.000
7	.263a	6.6	87.2	.457	.495	2868.451	.000
8	.205a	5.2	92.4	.413	.625	1915.140	.000
9	.176a	4.4	96.8	.387	.754	1153.644	.000
10	.128a	3.2	100.0	.337	.886	492.669	.000

Table 3. The Standardized Canonical Discriminant Function coefficients for 3 function of traits

	Function									
	1	2	3	4	5	6	7	8	9	10
Area	.240	-.286	-1.003	-.932	-4.063	-1.719	-1.181	-1.355	2.013	1.218
Length	.005	-1.213	3.306	2.046	-3.970	-2.025	-1.049	2.149	-5.511	2.746
Breadth	.508	-.245	.184	1.407	3.526	2.395	3.319	-2.984	1.547	7.462
Perimeter	2.938	1.627	-1.271	2.668	2.379	6.709	-10.589	-12.388	-2.345	1.786
ConvxPerim	3.047	-.027	-11.230	1.919	1.346	-1.615	2.412	-1.077	3.930	-9.568
Roundness	-.772	.159	-.132	-.147	-.935	-1.054	1.636	4.573	.702	.027
AspectRatio	.080	.244	.922	.048	1.970	1.607	.801	-1.464	2.278	1.778
EquivDiam	-6.410	-1.775	11.491	-9.568	1.838	-5.608	9.716	15.968	1.007	-3.265
CurveWidth	.429	2.673	-1.315	3.187	-.314	2.194	-3.602	-1.009	-.300	.880
ConvexArea	.535	.357	.096	-.273	-.376	.502	.355	-.011	-.114	-.040

Table 4. Results of Principal Component Analysis >1 Eigenvalue of PCA 1, PCA 2 and PCA 3

PCA	Eigen value	% variance	Area	Length	Breadth	Perimeter	Convex Perimeter	Equivalent Diameter	Curve Length	Curve Width	Convex Area	Full Ratio
1	7.32	61.01	0.90	0.93	0.96	0.91	0.98	0.98	0.86	0.80	0.66	0.09
2	2.54	21.14	**-0.37**	0.29	0.03	0.34	0.18	0.08	0.35	**-0.18**	**-0.25**	**-0.59**
3	1.29	10.74	**-0.11**	**-0.08**	0.20	**-0.11**	0.03	0.16	**-0.27**	0.51	**-0.30**	0.76

Table 5. Results of Cluster analysis using Agglomerative hierarchical clustering (AHC) –Wards method based on Dissimilarity: Euclidean distance

Class	1	2	3
Objects	75	10	37
Sum of weights	75	10	37
Within-class variance	31418.185	41271.572	11113.706
Minimum distance to the centroid	14.479	120.652	24.447
Average distance to the centroid	144.128	182.606	95.802
Maximum distance to the centroid	438.104	346.146	198.573

Table 6: Results of Agglomerative hierarchical clustering (AHC) -Unweighted pair-group average using Similarity: Pearson correlation coefficient

Class	1	2	3	4	5
Objects	9	71	22	19	1
Sum of weights	9	71	22	19	1
Within-class variance	10968.824	37446.130	72576.153	10872.582	0.000
Minimum distance to centroid	23.024	21.110	32.055	22.474	0.000
Average distance to centroid	83.994	154.450	205.185	88.232	0.000
Maximum distance to centroid	168.562	640.183	666.268	217.847	0.000

Plate 1. Histogram showing the frequency of Area, Length, Breadth, Perimeter, Convex Perimeter, and Equivalent diameter
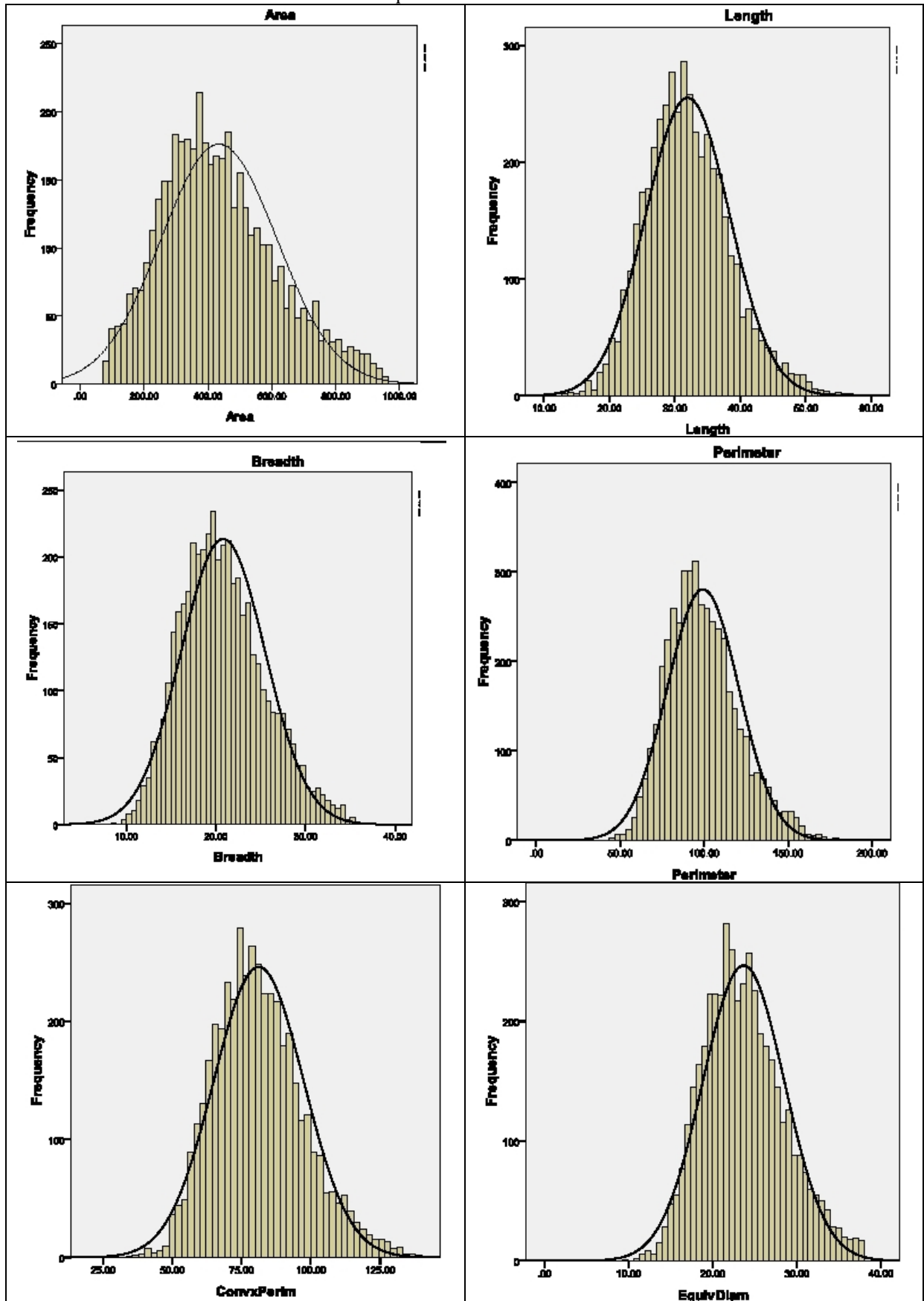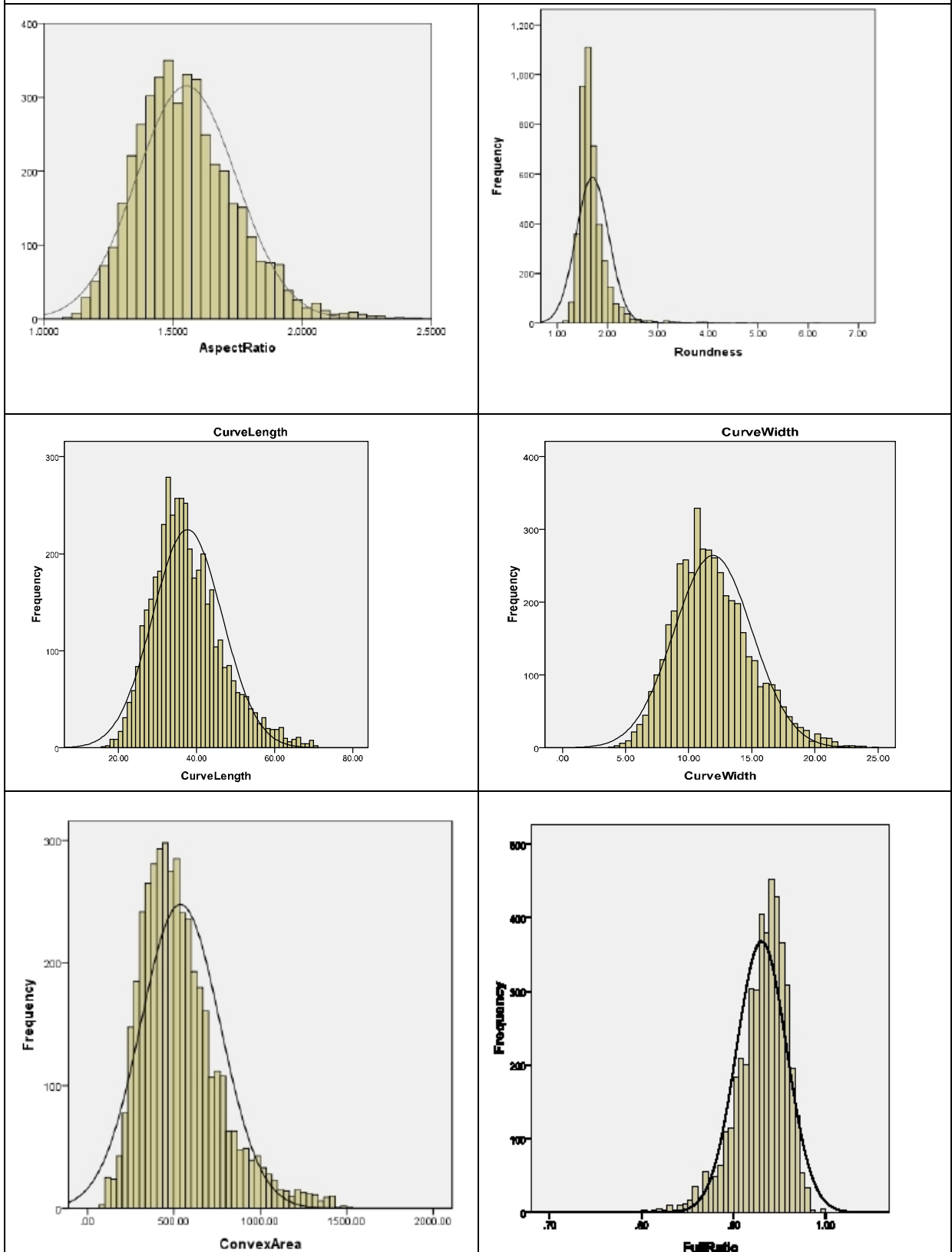
Plate 2. Histogram showing the frequency of Aspect ratio, Roundness, Curve Length, Curve width, Convex Area and Full ratio

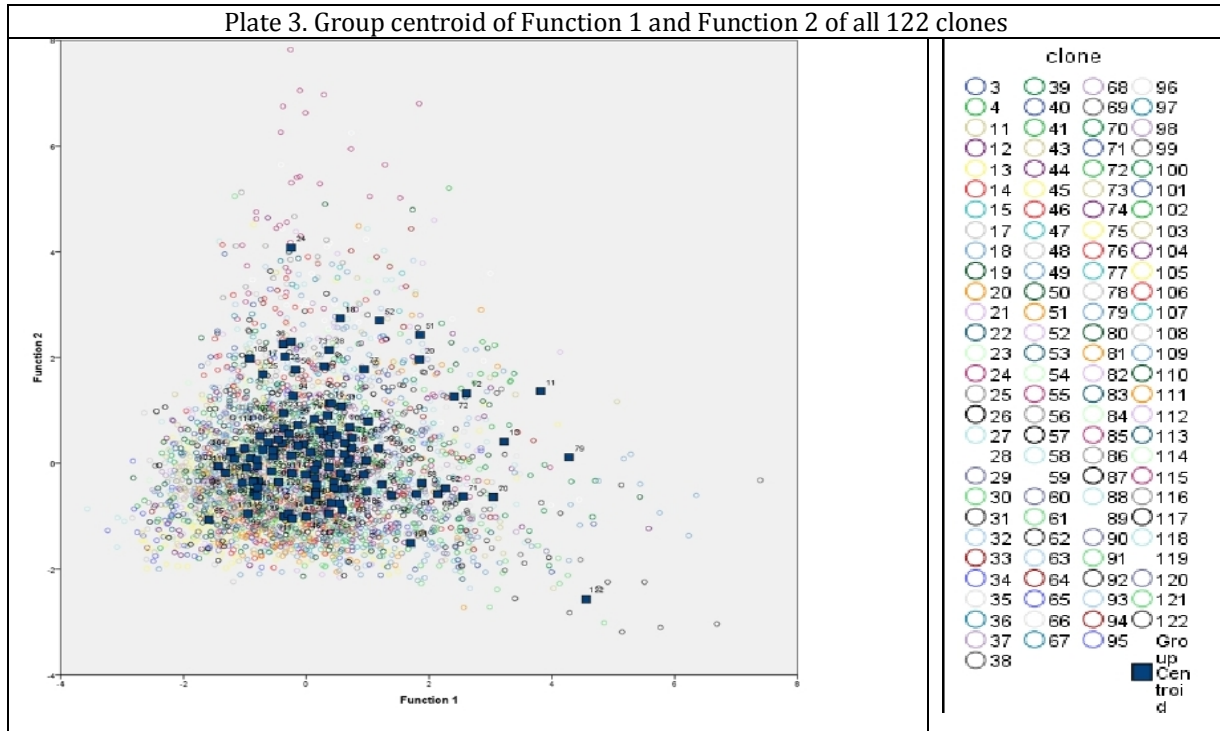Plate 3. Group centroid of Function 1 and Function 2 of all 122 clones

Plate 4. Results of Principal Component analysis showing the distribution of all 122 Teak clones and variables & Factors loading based on 12 quantitative characters
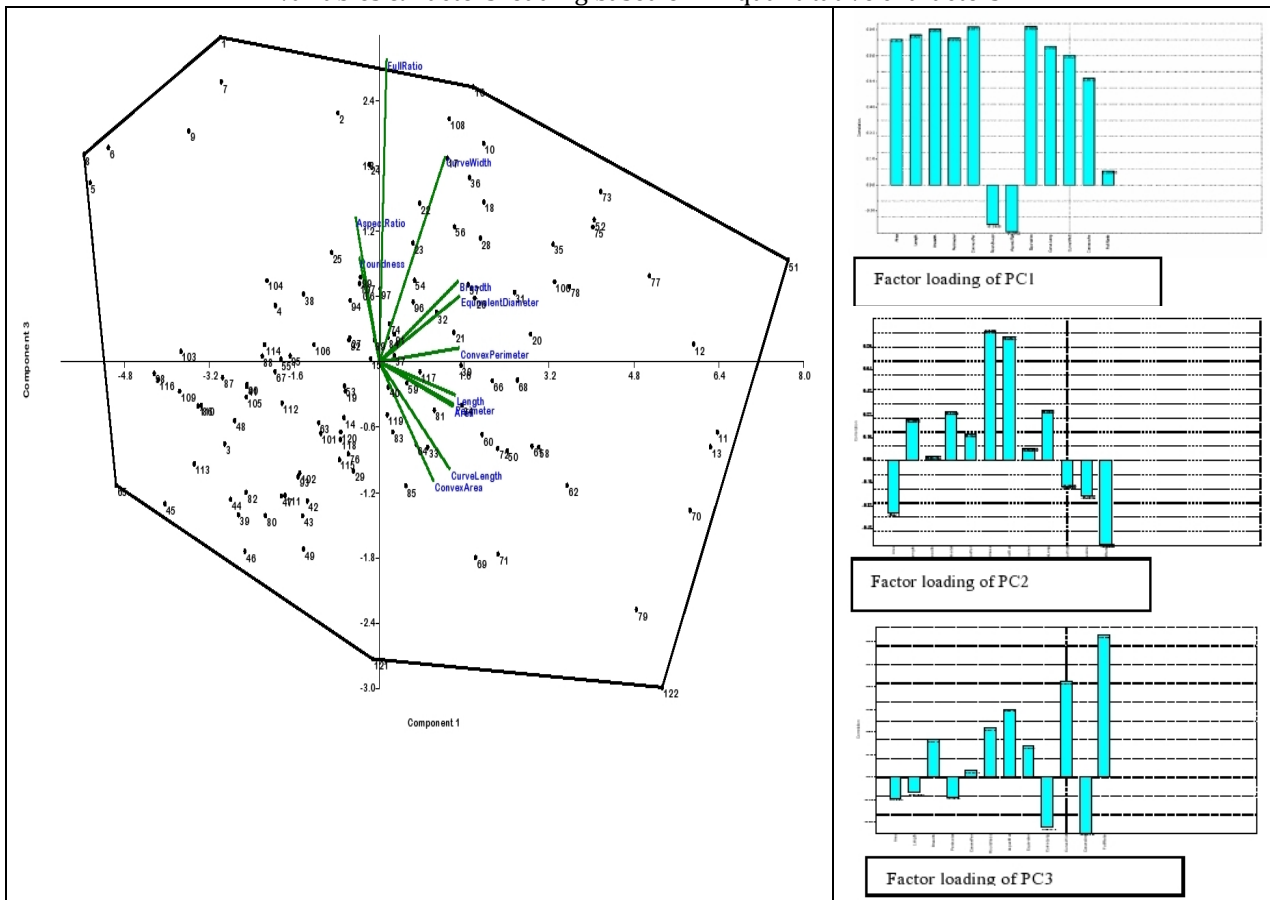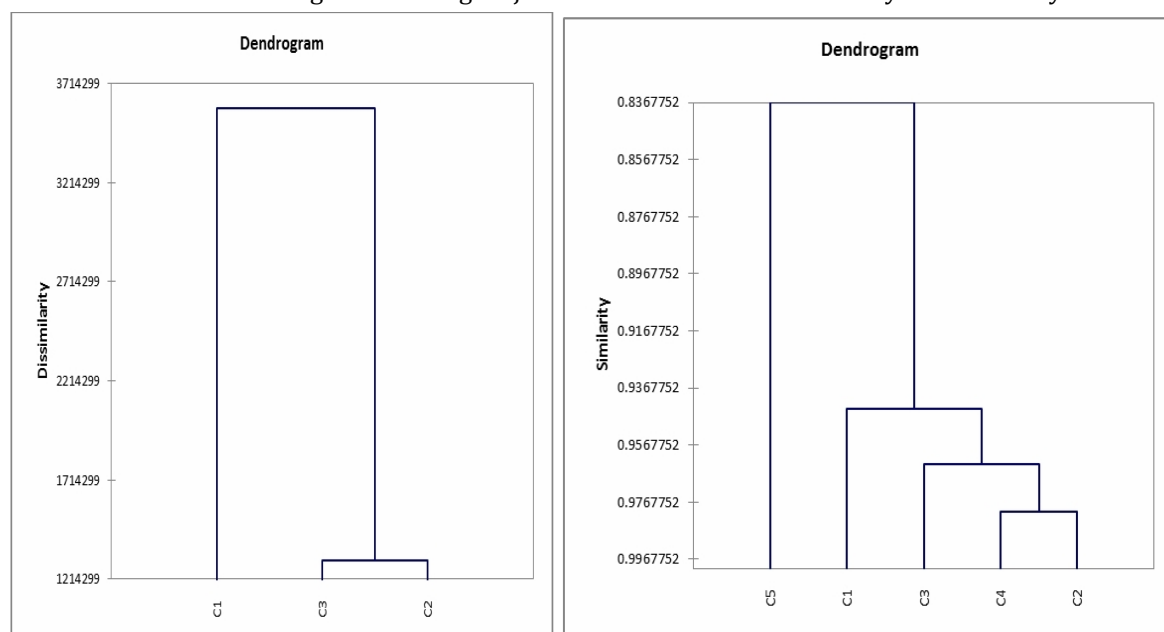
Plate 5. Dendrogram showing major clusters based on dissimilarity and similarity

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