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Research article

Classification of selected 25 *Eucalyptus* clones based on qualitative, pseudo-qualitative traits and quantitative traits using numerical taxonomy for distinct, uniform and stability (DUS) testing

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Abstract: This study aimed to evaluate the genetic variability of the selected 25 *Eucalyptus* clones from germplasm bank of Institute of Forest Genetics and Tree Breeding (IFGTB), using morphological traits which covers qualitative, pseudo-qualitative and quantitative traits by numerical taxonomic methods. All were evaluated in a randomized complete block design with five replicates, and each plot was composed of three ramets. Data collected during the present investigation is concerned with the trend of morphological variation both within and between clones were assessed based on conventional taxonomic practice and analyzed their variability using SPSS. Due to significance of leaves as taxonomic entities is that it can be conserved for longer periods of time used for quantitative characters for discrimination. For that results of Principle component analysis and Cluster analysis of morphological characters have been used to distinguish the different taxonomic units in the field.
Keywords: Eigen value - Juvenile character - Mature leave character - Multi variant analysis - Tree characters.

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INTRODUCTION

India is one of the largest country planted with *Eucalyptus* (4 million ha) in the world. In early 1999, *Eucalyptus* plantations were raised mainly through seedlings of *Eucalyptus camaldulensis* Dehnh and *Eucalyptus tereticornis* Sm and as the plantations were raised through seeds, the variability was high resulting in low yield over the years. So as to increase the yield per unit area, clonal forestry in Eucalyptus was adopted through shoot cutting methods (macro, mini and micro propagation technique by IFGTB) in Coimbatore. The socio-economic and commercial importance of these species demanded launching systematic breeding programmes even in the early 1990s. These programmes have built up a wide genetic base of those species and additionally commenced presenting pedigree-known seed orchard seeds and clones. Since clonal plantations are the most productive and the describing characters are uniform among different ramets of the same clones, it was chosen for the study. IFGTB have been released, ten *Eucalyptus* clones since 2009 and their number is expected to increase in future by other breeders. Considering all these aspects it's considerably necessary to broaden the descriptors and DUS Testing Guidelines for *Eucalyptus*.

Distinct means a clone should be clearly distinguishable by one or more essential characteristics from other existing clones. The clone is deemed Uniform if it is sufficiently uniform in its relevant characteristics, subject to variation that may be expected from the particular features of its population. After repeated propagation, if its relevant characteristics remain unchanged then the variety is said to be **S**table. Consequently Distinct, Uniform & Stable (DUS) is a procedure for describing a variety's morphological characteristics and a means of establishing whether these are expressed uniformly within a variety and consistently from generation to

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generation.

This study was carried out to describe an outline of the procedures used for conducting DUS tests in conformity to the International Union for the Protection of New Plant Varieties (UPOV) and revealed the role of numerical taxonomy in DUS Testing. In 1950, numerical taxonomy analysis blooms in corresponding with the development of computers (Sneath 2001) and validated comparison of large numbers of phenotypic characteristic traits for large numbers of taxa and the generated datasets were the first to be analyzed using computers. Nowaday's numerical taxonomy has been applied to cultivated plants only. Datasets in terms of matrices showed the degree of similarity among each pair of the taxa and clusters showed resulting dendrograms which revealed a clear depiction of the phenotypic traits with a particular group of taxa. Thereafter studies with cultivated plants were emerged with the aim to investigate the suitable numerical taxonomic techniques for classification or characterization. The term 'characteristic' is known as descriptors (with descriptor states and notes) and describing a clone based on such descriptor (in terms of number as a note) is known as 'characterization'. These characteristics are chosen as being known by experience to be least affected by the environment. Keeping these points in view, the present study was undertaken to develop the descriptors for *Eucalyptus* clone based on the phonetic characters. For *Eucalyptus*, these characteristics have been described by the International Union for the Protection of New Plant Varieties (UPOV) with the help of IFGTB, DUS Centre along with the procedures for conducting DUS tests. The descriptors published by IFGTB may differ slightly with regard to the number of characters proposed here, though there were many similarities too. Morphological descriptors to determine the morphological characteristics of *Eucalyptus* clones, about 25 samples from each clone composed of young, developing and developed plant parts were collected. The evaluations were separated into categorise as quantitative (32), qualitative (11) and pseudoqualitative (16). For characterizing the morphological expression, we used the studies carried out by Vidal & Vidal (2003) and the International Union for Protection of New Varieties of Plants (UPOV 2010). The evaluations and observations were accompanied by photographic records. The determination of the morphological traits was adapted from Miranda (2013) who worked with the morphological characterization and evaluation of the initial development of teak (*Tectona grandis* L.f.) clones. The choice of the traits to be evaluated was based on the descriptor table for *Eucalyptus* and *Hevea* (Brasil 2011); and in similar studies carried out with other species of agronomic interest by Lyngdoh *et al.* (2007), Alcantara & Souza (2007), Nascimento (2008), Andrade *et al*. (2009), Gomes Filho *et al*. (2010), Pinto *et al*. (2010), Chimello *et al*. (2017).

MATERIALS AND METHODS

The study on distinct, uniform and stable (DUS) descriptor development in *Eucalyptus camaldulensis* & *E. tereticornis* clones was carried out on the base population available with the Institute of Forest Genetics and Tree breeding at various agro climatic zones within Coimbatore, South India. The experimental material comprised 25 clones in coimbatore, planted in a field with spacing of 3 × 2 m and 1.0 × 1.5 m in randomized block design with five replications with 3 ramets. The morphological analysis is considered as a first approach towards the assessment of genetic diversity in a plant species (Boubaya *et al.* 2009). Prior to data collection, the available clones were carefully examined and the choices of characters were determined. According to Numerical Taxonomy the classification is the first step, accordingly variation in morphological characters in the trunk, branch, scar, bark, both juvenile & adult leaf, petiole, flower and fruit were studied. Operational Taxonomic Units (OTUs) are known as the objects (selected 25 clones) to be classified. The characters are numerically recorded either in the form of appropriate numbers as notes.

Then studies were also conducted in replicated clonal trials for identification of distinctness, uniformity and stability of the selected morphological traits in 3 different agro climatic zones around Coimbatore. For the last step discrimination process, an extensive work has been done in the field of plant identification using leaf samples. Earlier such works only focused on the basic morphological characteristics of a leaf shape such as area, perimeter, eccentricity, maximum length and maximum width. But, discrimination of these basic morphological parameters are beneficial for the broader classification of leaves and do not endow with sufficient information about defining the insignificant details of the shape of a leaf So this study concentrate full ratio, aspect ratio, convex area, convex perimeter, vein angle at base and middle along with basic parameters). In general heteroblastic development is a advanced, environmentally independent change in size and structure of consecutive organs (Nobel & Walker 1985), resulting in distinctly different young juvenile and mature adult stage shoots and leaves. Photographic documentation of visually assessed characters of both adult and juvenile leaf was done. Wherein for measurement like leaf, a random sample of 60 for each Eucalyptus clone were evaluated as per the descriptors prescribed by the International Union for Protection of New Varieties of Plants (UPOV 1986), International Board of Plant Genetic Resources (Huaman *et al*. 1977). The image analysis program Leica Q win V 3 was used to measure some parameters like; the total length, leaf perimeter (the outline of the lamina), average leaf breadth and scanned area of each clone (measured characters) and some derived parameters such as convex perimeter, convex area, aspect ratio, fullratio, roundness, curve length, curve width . The objective of this work is to evaluate the weight-age of 30 quantitative leaf traits in the discrimination process using Multi-variant analysis for DUS Testing. The PCA and cluster analysis are preferred tools for morphological characterization of genotypes and their grouping on similarity basis based on this approach (Peeters & Martinelli 1989, Mohammadi & Prasanna 2003). In numerical taxonomy, the cluster analysis technique for set of clones follows various procedures. On the basis of a set of attributes sharing, among OUT's, they are divided into two or more subgroups (clusters)

RESULTS AND DISCUSSION

Classification and identification of clones based on morphometric characters

One of accepted infrageneric classification systems of the genus *Crotalaria* was based on morphometrics (Bisby 1973, Bisby & Polhill 1973). Morphological characters, both vegetative and generative, were used for constructing classifications (Agyeno *et al*. 2014a). Similarly, **Jayeola** (2001) reported the efficiency of utilizing vegetative and floral parts in numerical evaluation of similarities among taxa. This study also shows that the classification of *Eucalyptus* clones based on number of state representation/attributes of morphometric characterization (variability assessment) of trunk, branch, bark, both juvenile and adult leaves, flower and fruits characters results as 2 out of 59 characteristics were polymorphic (more than three characteristic traits) 17 were dimorphic (Presence of two different characteristic traits / states of expression) among the 25 clones studied, while 40 characteristics traits were trimorphic. And it can be grouped into qualitative & pseudo qualitative and quantitative descriptor for DUS Testing for tranquil assessment (Appendix I). For identification of the individual clone, distinctness was noted and uniformity and stability was calculated manually using data sheets for 4 years. Out of 25 *Eucalyptus* clones, distinctness could be established for all clones by using the combination of 59 morphological characteristics. Similar attempts for establishment of distinctness were made in soybean (Ravikumar & Naraayanswamy 1999), oat (Kumar *et al*.. 2002), rapeseed-mustard (Gupta *et al*. 2003, Yadav 2004), pearl millet (Kumar *et al*. 2004), rice (Joshi *et al*. 2007, Patra *et al*. 2010), jute (Kumar *et al*., 2008) and maize (Yadav & Singh, 2010). All the morphological DUS descriptors did not show any variation in their states of expression over four years data. Further less number of off-types was observed in both three locations in four consecutive years. But in most of the crops, acceptance probability of 95% has been recommended for the variety is considered as stable. Moreover, uniformity and stability are same for the vegetatively propagated plants. Therefore, it may be inferred that all these 25 clones were uniform and stable. The identification keys to clones were provided using qualitative and pseudo- qualitative characters. As per numerical taxonomy for quantitative characters clonal discriminations was done using multivariant analysis like principal component analysis and cluster analysis to achieve identification of clones for DUS Testing.

Key to clones based on Qualitative and Pseudo-qualitative characters of all traits

1A. Clear bole height above top 1/3rd total height …………………………………………………………………. 2

1B. Clear bole height within middle 1/3rd total height …………………………….…………………………… 15

2A. Scar periphery projection flat ..……………………………………… ………………...…..….. **Clone 101**

2B. Scar periphery projection other than flat ………………..……………………………………….………. 3

3A. Scar periphery projection Horizontal ..……………………………… ………………...…………...……… 4

3B. Scar periphery projection downward or depressed ……...……………………...…………………...………5

4A. Self pruning present …………………..……………………………………………………..………… **Clone 94**

4B. Self pruning absent ……………………………………………………………………………...…. **Clone 187**

5A. Peeling type strip ……………………………………………………………………………...……. **Clone 14**

5B. Peeling type flake ……………………………………………………………………...…………..... 6

6A. Fresh bark colour light grey …………………………………………….…………………...…… **Clone 124**

6B. Fresh bark colour light green ……………………………………………...…………..……….……. 7

7A. Juvenile leaf rounded at apex ……..……………………………………………..……..…. **Clone 115**

7B. Juvenile leaf acute at apex ………………………………………………………...…………………… 8

8A. Anthocyanin strong in juvenile branch stem ……………………………...…...………………….. **Clone 116**

8B. Anthocyanin weak or medium in juvenile branch stem ….……………………...………………………….. 9

9A. Juvenile leaf shape ovate ………………………………………………...……………….……...…… 10

9B. Juvenile leaf shape lanceolate …..…………………………………………………….……………………. 11

10A. Juvenile leaf entire at margins ……………………………………………….……….......... **Clone 196**

10B. Juvenile leaf slightly wavy at margins …………………………………………….…………..…. **Clone 19**

11A. Branch attitude horizontal …..……………………………………………………………….. **Clone 10**

11B. Branch attitude upward or drooping ………………………………………………..………..…… 12

12A. Branch attitude upward ………………………………………………………………………………… 13

12B. Branch attitude drooping ……………………………………………………………..…..………….. 14

13A. Mature leaf cuneate at base ……………………………………………………………..…...…. **Clone 63**

13B. Mature leaf obtuse at base …………………………………………………………...…………. **Clone 66**

14A. Scar type open; branch thickness thick …..………………………………………...….……. **Clone 186**

14B. Scar type close; branch thickness medium ……………………………………….....….……. **Clone 188**

15A. Bark peeling type mixed …...………………………………………………………….…..... **Clone 198**

15B. Bark peeling type flake …………………………………………………………………..……..…. 16

16A. Operculum shape elongated ……..…………………………………………………….…...….. **Clone 111**

16B. Operculum shape hemispherical apiculate or conical ………………………………...………..…… 17

17A. Operculum shape conical ……...……………………………………………………….………..... 18

17B. Operculum shape hemispherical apiculate ………………………………………………………...…… 19

18A. Juvenile leaf acute at apex; juvenile leaf anthocyanin present ……….……………...….……….. **Clone 191**

18B. Juvenile leaf rounded at apex; juvenile leaf anthocyanin absent or weak …………………...…... **Clone 207**

19A. Scar type open ……...……………………………………………………………...…………. **Clone 26**

19B. Scar type close ………………………………………………………………………..….....……..……. 20

20A. Scar primary projection flat ……..…………………………………………………………… **Clone 17**

20B.Scar primary projection downward or depressed …………………………………...……………..…… 21

21A. Scar primary projection downward ……..…………………………………………………...……… 22

21B. Scar primary projection depressed ……………………………………………………………...…… 23

22A. Juvenile leaf shape ovate; rounded at apex ……..……………………………………......…. **Clone 1**

22B. Juvenile leaf shape lanceolate; acute at apex ……………………………………............. **Clone 100**

23A. Scar shape inverted 'V'; juvenile leaf base shape acute ..……………… …………………………..…… 24

23B. Scar shape spherical; juvenile leaf base shape convex ……………………………………….…**Clone 53**

24A. Crown shape lanceolate; branch thickness small ………………………….……………..………. **Clone 16**

24B. Crown shape columnar; branch thickness medium ……………...……………………..…… **Clone 23**

Discrimination of quantitative characters using Principal Component Analysis (PCA)

Most of the variation in a large group of variables can be captured with only 17 few principal components and out of this three values accounted for a cumulative variation of 88.62% for juvenile leaves based on the mean data of Appendix II and III. However, in Eucalyptus clones the remaining components contributed only 11.37% towards the total diversity for this set of 25 genotypes. It shows better result than Velázquez-Ventura *et al*. (2018). They used 131 collections of wild peppers to obtain a total of 23 plant, flower and fruit variables and he used only 16 character state to obtained 65.2% cumulative variations. The first principal component (PC I) explained the most variability accounted for 43.36% followed by 30.46 and 14.79% components towards total variation (Table 1). PCA can help to identify the main factors affecting the dependent variable. All traits except roundness ratio and vein angle II like showed considerable positive factor loadings on PC I. The 2nd PC was related to diversity among clones due to breadth, curve width, full ratio, mid width, widest width, vein angle I, vein angle II, base angle I, base angle II and intramarginal width with their positive loadings. The PC III was explained by variation among genotypes due to length, perimeter, convex perimeter, roundness ratio, curve length, convex area, vein angle I, vein angle II, base angle I, base angle II with their positive loadings (Fig. 1). The results indicate that all characters state used here showed variability, which is useful for clonal discrimination.

Table 1. Principal components with Eigen values >1 with % of Variance of Leaf traits.

Juvenile leaf traits	PCA 1	PCA 2	PCA 3	Mature leaf trait	PCA 1	PCA 2
Area	0.98	-0.04	-0.02	Area	0.31	0.92
Length	0.63	-0.70	0.17	Length	0.96	0.2
Breadth	0.88	0.37	-0.21	Breadth	0.08	0.96
Perimeter	0.85	-0.47	0.15	Perimeter	0.94	0.32
Convex Perimeter	0.80	-0.56	0.10	Convex Perimeter	0.95	0.30
Roundness	-0.16	-0.84	0.35	Roundness	0.80	-0.53
Curve Length	0.63	-0.68	0.24	Aspect Ratio	0.68	-0.65
Curve Width	0.80	0.49	-0.24	Curve Length	0.98	0.16
Convex Area	0.86	-0.	0.04	Curve Width	-0.39	0.89
Full Ratio	0.28	0.91	-0.19	Convex Area	0.91	0.38
Mid width	0.86	0.40	-0.23	Full Ratio	-0.82	0.55
Widest width	0.88	0.35	-0.20	Petiole length	-0.12	0.26
Vein Angle I	0.03	0.57	0.62	Eigen value	6.61	4.11
Vein Angle II	0.02	0.67	0.62	% variance	55.15	34.32
Base Angle I	0.34	0.34	0.78	Cumulative %	55.15	89.47
Base Angle II	0.34	0.46	0.71			
Intra Marginal Width	0.41	0.29	-0.33			
Eigenvalue	7.37	5.17	2.51			
% Variance	43.36	30.46	14.79			
Cumulative %	43.36	73.82	88.62			

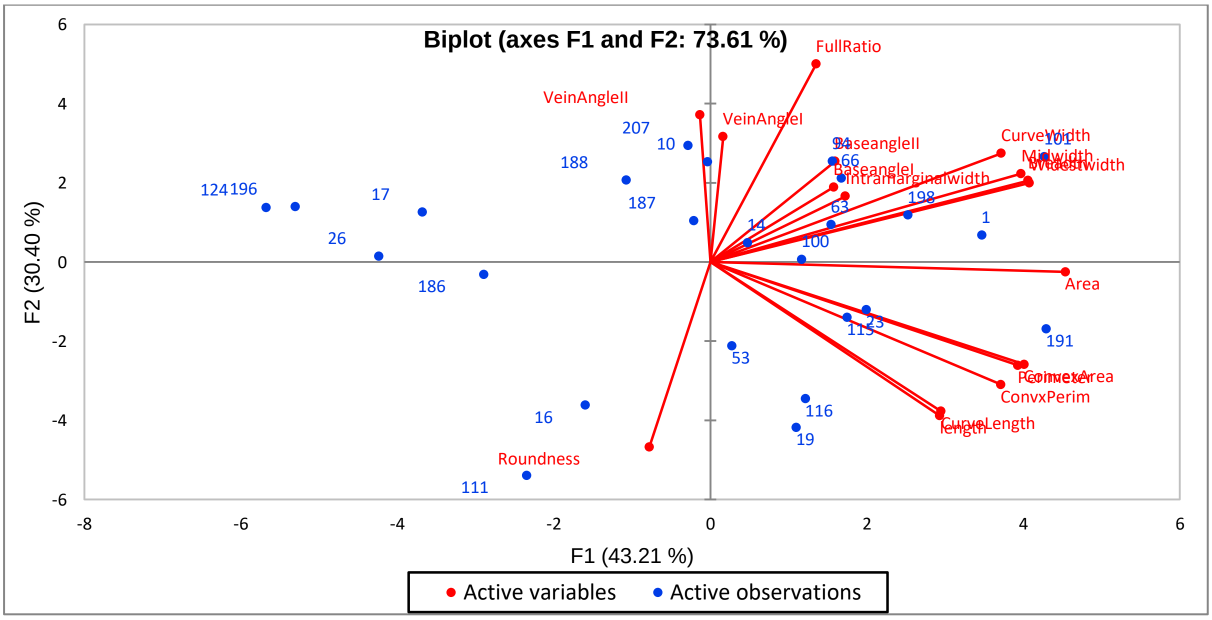

Figure 1. Scatter plot based on Active observation and variables of F1 against F2 of Juvenile leaf.

In this study on mature leaves, based on the Kaiser-Guttman criterian, two significant principal components (PCs) extracted, which had Eigen value >1 and out of 12 this two values accounted for a cumulative variation of 89.47%. However, the remaining components contributed only 10.53% towards the total diversity for this set of 25 genotypes. The first principal component (PC I) explained the most variability accounted for 55.15% followed by 34.32% components in PC 2 towards total variation (Table 1). All traits except Curve width, Full ratio and Petiole length showed considerable positive factor loadings on PC I. The 2nd PC was related to diversity among clones due to area, length, breadth, perimeter, convex perimeter, curve length, curve width, full ratio and petiole length with their positive loadings (Figs. 2 & 3).

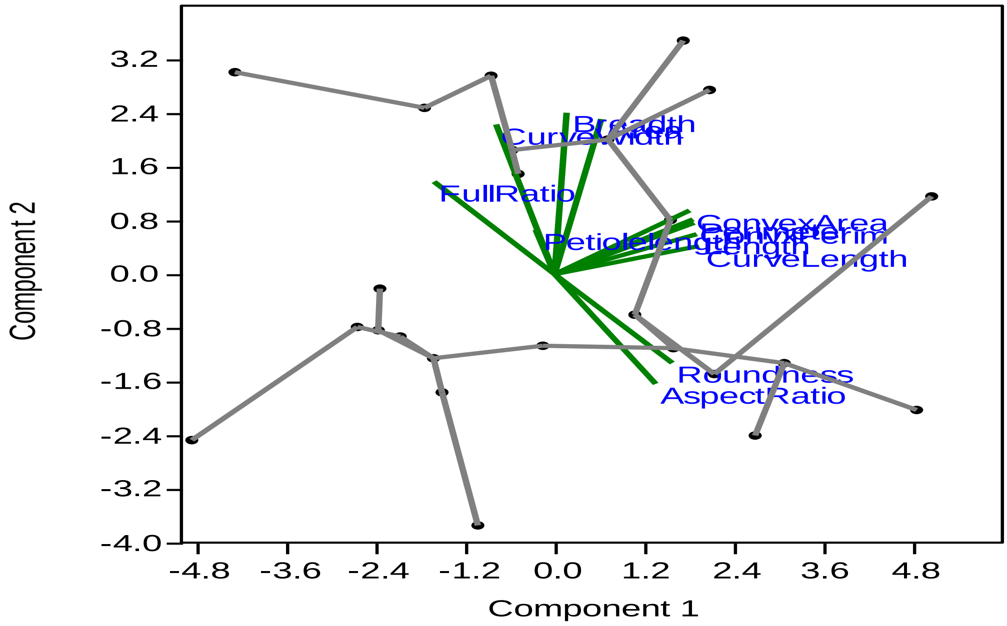

Figure 2. Scatter plot based on Active variables of F1 against F2 of Adult leaf.

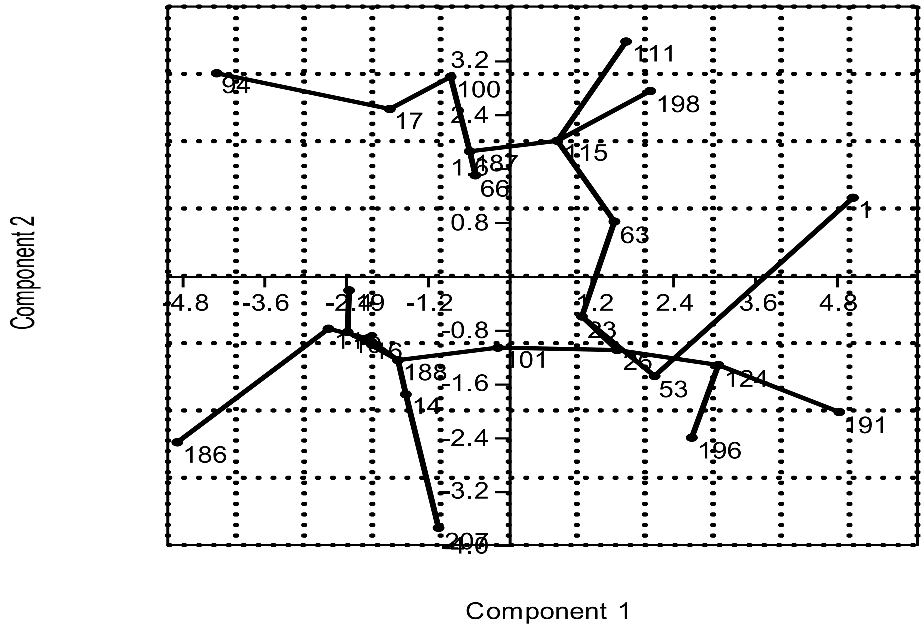

Figure 3. Scatter plot based on Active observation of F1 against F2 of Adult leaf.

Spread out plot

Based on juvenile leaves data, one of the most informative graphical representations of a multivariate dataset is via a spread out plot. And the spread out plot of the principal component I showed that C111, C19, C191, C101, C207, C196 and C124 were more diversified so these quantitative characteristics have latent to used, to show Distinctness of 25 clones/genotypes based on the morphological marker of juvenile leaf traits (Fig. 1).

It shows that, the different Eucalyptus genotypes are found in four different quadrants. In the study by Worede *et al*. (2014), shows that there are characters in the PC that have relatively high variability and are important in separating genotypes. And the spread out plot of the principal component I showed that C94, C186, C1, C191, C196 and C207 were more diversified so these quantitative characteristics have latent to used, to show Distinctness of 25 clones/genotypes based on the morphological marker of mature leaves. It also depicts that Distribution pattern of 12 quantitative morphometric characters of 25 Eucalyptus clones (Figs. 2 & 3).

Clustering based on similarity

The dendrogram drawn depicted four distinct clusters based on the similarity in juvenile leaf data using Pearson correlation coefficient method on Juvenile leaf characters. The Cluster I comprised of eight groups with 10 clones (Clone 1, 14, 23, 53, 63, 66, 100, 101, 115, 187), Cluster II consists of five subgroups with 8 clones (Clone 10, 17, 26, 124, 186, 188, 196, 207), Cluster III showed 2 groups with 4 clones (Clone 16, 94, 111 and 198) and cluster 4 have two groups with 3 clones (Clone 191, 19, 116) (Table 2; Fig. 4). As research conducted by Esmail *et al*. (2008) indicates that cluster analysis based on Euclidean distance using yield characters to group the 21 cotton genotypes into two main groups. Cluster "A" and "B" composed of eleven and ten genotypes, respectively.

Table 2. Results of clustering of different Eucalyptus genotypes based on Similarity using Pearson correlation coefficient of quantitative traits of juvenile leaf Characters.

	Clusters			
	1	**2**	**3**	**4**
Objects	10	8	4	3
Sum of weights	10	8	4	3
Within-class variance	94.62	235.25	124.85	240.56
Minimum distance to centroid	6.63	5.51	6.92	6.72
Average distance to centroid	9.08	12.98	9.52	11.98
Maximum distance to centroid	11.81	26.69	11.68	16.74
Number of clones recorded in each clusters based on the similarity using Pearson correlation coefficient method	C1 C14 C23 C53 C63 C66 C100 C101 C115 C187	C10 C17 C26 C124 C186 C188 C196 C207	C16 C94 C111 C198	C19 C116 C191

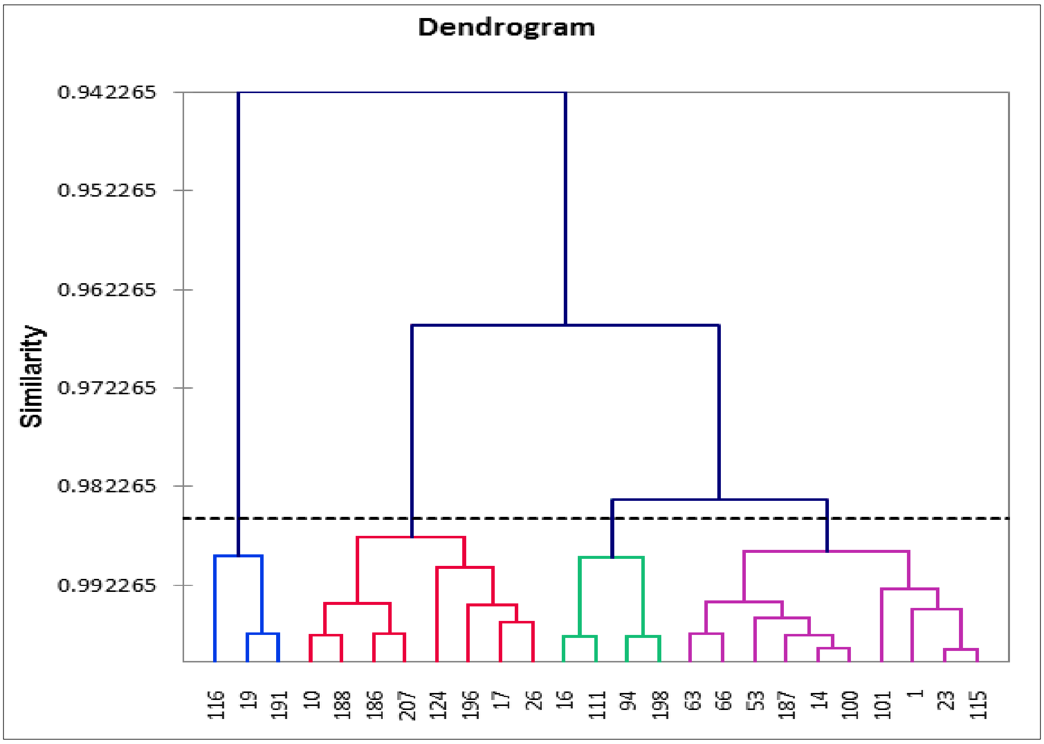

Figure 4. Dendrogram showing the grouping based on the similarity based on Juvenile leaf trait.

In mature leaf traits of Eucalyptus clones the dendrogram drawn depicted three distinct clusters based on the similarity using Pearson correlation coefficient method . The Cluster I comprised of eight groups with 9 clones (Clone 1, 23, 26, 53, 63, 101, 124, 191, 196), Cluster II consists of seven subgroups with 8 clones (Clone 10, 14, 16, 19, 116, 186, 188, 207), Cluster III showed seven sub groups with 8 clones (Clone 17, 66, 94, 100, 111, 115, 187, 198) (Table 3; Fig. 5). The discrimination analysis revealed the distinct nature of the *Eucalyptus* clones in large number of different clusters in the dendrogram showed greater diversity, which could be exploited in proving the weightage of high yielding tree species that combine desirable leaf traits. Our study provides evidence that quantitative leaf characteristics determined by image analysis techniques can be used for taxonomic differentiation based on Numerical Taxonomy in *Eucalyptus* clones for DUS testing.

Table 3. Results of Cluster analysis using Agglomeration method based on Similarity using Pearson correlation coefficient

		Clusters		
		1	**2**	**3**
Objects		9	8	8
Sum of weights		9	8	8
Within-class variance		272.96	94.56	308.94
Minimum distance to centroid		3.38	2.43	1.93
Average distance to centroid		12.75	6.95	13.32
Number of clones recorded in	C1	C10	C 17	
each clusters based on the	C23	C 14	C 66	
similarity using Pearson	C26	C 16	C 94	
correlation coefficient method	C53	C 19	C 100	
	C63	C 116	C 111	
	C101	C 186	C 115	
	C124	C 188	C 187	
	C191	C 207	C 198	
	C196			

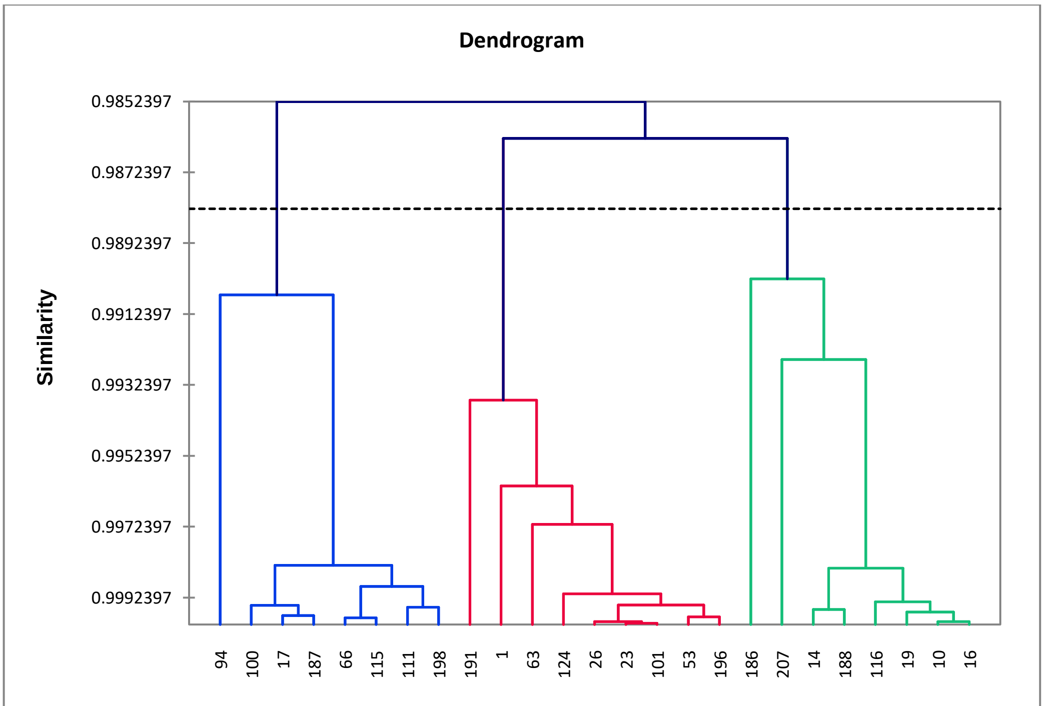

Figure 5. Dendrogram showing the grouping based on the similarity based on mature leaf trait.

The study of Agyeno *et al.* (2014b) and Raj *et al.* (2011) also highlighted that qualitative character of leaf type, and quantitative characters, such as the pod length, seed number and petiole length, are phylogenetically important. Findings of this study also prove that morphological characters are effective in showing similarities among the species within the studied 25 *Eucalyptus* clones. This study considered some characters, such as petiole length, intramarginal width, vein angles etc. which were not reported earlier, and these characters were found to be effective in morphometric analysis of the clones.

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Supporting information

Appendix I: DUS descriptors of Eucalyptus clones (OUT) based on Qualitative and Pseudo-qualitative characteristic traits and their attribute state (in number / notes).

Appendix II: Mean data of quantitative traits of Juvenile leaf architectural traits of *Eucalyptus* germplasm/clones.

Appendix III: Mean data of quantitative traits of Mature leaves architectural traits of selected 25 Eucalyptus clones.

Appendix 1: DUS descriptors of Eucalyptus clones (OUT) based on Qualitative and Pseudo-qualitative characteristic traits and their attribute state (in number / notes).

OTU	TCBH	CS	BST	BSS	BSP	PBSP	BT	BA	BAT	BAP	BAPT	FBC	DBC	RBC	JSTW	JSAC	JLS	JLM	JLBS	JLAS	JLAC	MLS	MLM	MLBSY	MLBS	MLAS	OS
Clone 1	5	2	9	1	1	9	7	1	9	9	2	2	2	1	1	1	1	1	5	2	1	3	9	1	3	1	1
Clone 10	7	1	9	1	4	9	5	2	9	9	2	2	4	2	1	3	2	1	5	1	9	3	1	9	1	3	1
Clone 14	7	1	9	1	4	9	5	1	9	9	1	2	2	1	1	1	2	1	1	2	1	3	9	1	3	1	1
Clone 16	5	1	9	1	4	9	3	3	9	9	2	2	1	1	1	1	2	9	1	1	9	1	1	9	1	3	1
Clone 17	5	1	9	2	3	9	7	1	9	9	2	2	2	2	1	1	1	1	5	2	1	1	1	9	1	3	1
Clone 19	7	1	9	2	4	9	5	1	9	9	2	2	3	1	9	3	1	9	5	1	9	1	1	9	1	3	3
Clone 23	5	3	9	1	4	9	5	1	9	9	2	2	4	1	1	1	2	1	1	1	1	3	9	1	3	1	1
Clone 26	5	3	1	2	4	9	7	1	9	9	2	2	4	1	1	1	1	1	5	2	1	1	1	9	1	3	1
Clone 53	5	1	9	2	4	9	7	2	9	9	2	2	1	1	1	1	2	9	3	1	1	3	9	1	3	1	1
Clone 63	7	2	1	2	1	9	5	1	9	9	2	2	4	1	1	1	2	9	5	1	9	3	9	1	3	1	1
Clone 66	7	1	9	1	4	9	5	1	9	9	2	2	1	3	9	3	2	9	5	1	9	1	1	9	1	3	1
Clone 94	7	1	9	2	2	9	5	1	9	9	2	2	4	2	1	3	2	1	5	2	9	1	1	9	1	1	3
Clone 100	5	2	9	2	1	9	5	2	9	9	2	2	1	1	9	1	2	1	5	1	9	1	1	1	1	3	1
Clone 101	7	2	9	2	3	9	5	1	9	9	2	2	4	1	1	1	1	1	5	1	9	3	9	1	3	3	3
Clone 111	5	3	9	2	3	9	3	2	9	9	2	2	4	1	9	5	2	1	5	1	9	2	1	1	1	1	2
Clone 115	7	2	1	1	1	9	5	2	9	9	2	2	2	3	1	3	1	1	5	2	1	1	1	9	1	3	2
Clone 116	7	3	9	2	4	9	7	1	9	9	2	2	1	1	9	5	2	1	5	1	9	1	1	9	1	3	1
Clone 124	7	3	9	2	4	9	5	2	9	9	2	3	4	1	9	1	2	1	5	1	9	3	9	1	3	1	2
Clone 186	7	2	1	1	1	9	7	3	9	9	2	2	4	3	1	1	2	9	5	1	1	1	1	1	1	1	1
Clone 187	7	2	9	2	2	1	5	1	9	9	2	2	4	1	9	3	2	1	1	2	9	1	1	9	1	3	1
Clone 188	7	2	9	1	1	9	5	3	9	9	2	2	4	1	9	3	2	9	5	1	9	1	1	1	1	1	3
Clone 191	5	3	9	2	3	9	7	1	9	9	2	2	3	2	9	3	2	1	5	1	9	3	9	1	3	1	3
Clone 196	7	2	9	2	4	9	5	1	9	9	2	2	4	1	1	1	1	1	5	1	1	3	9	1	3	1	1
Clone 198	5	3	1	2	4	9	5	3	9	9	3	2	4	1	9	1	1	9	5	2	9	1	1	9	1	3	3
Clone 207	5	3	9	2	3	9	5	1	9	9	2	2	4	1	1	1	1	2	9	5	2	1	2	9	1	3	3

Character attributes and their state of expression with notes for DUS Testing as Numerical Taxonomic approach

Tree Clear Bole Heght- TCBH	**Crown Shape-CS**	**Branch Scar Type-BST**	**Branch Scar Shape -BSS**	**Branch Scar Periphery Projection – BSP**	**Primary Branch Self Pruning - PBSP**	**Branch Thickness – BT**	**Branch Attitude - BA**	**Bark Texture - BAT**	**Annual Peeling - BAP**	**Peeling Type-BAPT**	**Fresh Bark Colour-FBC**	**Dried Bark Colour-DBC**	**Rhytidome Bark Colour - RBC**
Within middle 1/3rd height -5 Above top 1/3rd heigh-7	Lanceolate - 1 Conical - 2 Columnar - 3	Open - 1 Close - 9	Inverted V –1 Spherical - 2	Downward - 1 Horizontal - 2 Flat - 3 Depressed - 4	Absent - 1 Present - 9	Small (<1/8th of main stem) - 3 Medium (1/8th -1/4th of main stem) - 5 Thick (>1/4th of main stem) - 7	Upward - 1 Horizontal - 2 Drooping - 3	Rough - 1 Smooth - 9	Absent - 1 Present - 9	Strip - 1 Flakes - 2 Mixed - 3	Light brown - 1 Light green - 2 Light grey - 3	Light green - 1 Light brown - 2 Grey-3	Light brown - 1 Dark brown - 2 Grey - 3

Juvenile Stem tip waxy-glaucousness - JSTW	**Juvenile Stem anthocyanin coloration - JSAC**	**Juvenile Leaf Shape - JLS**	**Juvenile Leaf Margin - JLM**	**Juvenile Leaf base Shape - JLBS**	**Juvenile Leaf apex Shape - JLAS**	**Juvenile Leaf Anthocynin - JLAC**	**Mature leaf Shape- MLS**	**Mature Leaf Margin-MLM**	**Mature Leaf base symmetry - MLBSY**	**Mature Leaf base shape – MLBS**	**Mature Leaf apex shape – MLAP**	**Operculum shape - OS**
Present – 1 Absent – 9	Absent – 1 Medium – 3 Strong - 5	Ovate - 1 Lanceolate - 2	Entire - 1 Slightly wavy - 9	Acute - 1 Convex - 3 Rounded - 5	Acute - 1 Rounded - 2	Absent or weak - 1 Present - 9	Narrowly lanceolte - 1 Lanceolate - 2 Ovate - 3	Entire - 1 Wavy - 9	Symmetric - 1 Asymmetric - 9	Obtuse - 1 Cuneate - 3	Acute - 1 Obtuse - 3	Hemispherical apiculate - 1 Elongated - 2 Conical - 3

Appendix II: Mean data of quantitative traits of Juvenile leaf architectural traits of *Eucalyptus* germplasm/clones.

Clone	Area (cm^2)	Length (cm)	Breadth (cm)	Perimeter (cm)	Convx Perim (cm)	Aspect Ratio	Curve Length (cm)	Curve Width (cm)	Convex Area (cm^2)	Full Ratio	Mid width (cm)	Widest width (cm)	Vein Angle (I°)	Vein Angle (II°)	Base Angle (I°)	Base Angle (II°)	Intra marginal width (cm)
1	33.17	9.44	5.37	29.58	23.42	1.74	11.54	3.54	43.59	0.93	5.16	5.41	51.94	51.23	45.30	43.56	0.15
10	24.55	8.61	4.23	22.92	20.51	2.05	8.89	3.20	32.78	0.92	4.12	4.19	58.64	58.48	44.31	47.32	0.16
14	27.96	10.24	3.84	25.97	22.81	2.68	10.27	2.72	38.26	0.86	3.55	3.73	57.09	59.56	55.22	53.93	0.15
16	23.33	11.21	3.06	26.46	23.92	3.73	11.17	2.07	39.31	0.77	2.74	2.93	51.53	52.77	41.09	39.44	0.10
17	19.39	7.83	3.53	19.93	18.10	2.24	7.30	2.66	24.25	0.90	2.76	2.79	51.63	57.91	40.10	37.88	0.09
19	31.87	12.01	3.65	29.41	27.09	3.31	11.97	2.74	50.83	0.80	3.33	3.50	47.15	46.15	39.03	36.96	0.11
23	33.24	12.20	3.88	28.17	26.59	3.17	11.00	3.08	49.61	0.83	3.56	3.78	55.37	55.96	57.52	51.22	0.11
26	16.99	8.33	2.87	19.31	18.29	2.93	7.39	2.00	23.98	0.84	2.75	2.79	53.71	51.99	41.49	37.51	0.13
53	28.28	11.74	3.50	27.48	25.42	3.39	11.27	2.44	45.17	0.79	3.04	3.31	54.95	56.82	55.08	55.27	0.08
63	32.05	10.02	4.43	26.61	23.27	2.29	10.03	3.20	40.50	0.89	4.13	4.26	56.45	58.11	50.42	46.00	0.14
66	29.96	9.52	4.71	24.13	22.41	2.02	8.26	3.76	38.36	0.91	4.41	4.69	53.05	53.00	47.90	49.00	0.17
94	31.71	9.57	4.64	24.60	22.61	2.10	8.49	3.79	38.90	0.91	4.49	4.55	60.21	60.33	43.35	44.82	0.14
100	27.85	10.42	4.18	27.17	23.59	2.52	10.78	2.80	41.32	0.86	3.79	4.12	56.97	57.33	57.88	55.83	0.12
101	37.52	9.52	5.75	26.51	24.11	1.63	9.14	4.14	45.52	0.95	5.51	5.74	51.63	53.78	53.30	53.52	0.13
111	19.96	11.68	2.62	26.47	24.53	4.33	11.52	1.71	40.30	0.72	2.45	2.56	51.37	48.24	39.44	38.78	0.11
115	30.51	11.31	4.22	28.04	25.16	2.73	11.14	2.88	46.55	0.84	3.78	4.12	51.40	52.20	50.27	45.50	0.15
116	30.54	11.81	4.25	28.34	25.81	2.81	11.53	2.64	47.71	0.80	4.06	4.18	49.27	48.99	32.06	30.34	0.13
124	12.72	7.13	2.58	17.27	15.85	2.77	6.78	1.85	18.29	0.84	2.38	2.52	57.08	59.01	47.37	43.53	0.11
186	19.07	8.72	3.12	23.45	19.42	2.81	9.69	2.04	27.02	0.84	2.81	3.02	55.73	56.27	49.38	46.79	0.10
187	26.75	9.05	4.30	23.65	21.15	2.16	8.76	3.07	33.59	0.89	3.92	4.24	52.19	54.01	48.25	53.00	0.07
188	24.03	9.11	3.58	21.85	20.59	2.56	7.89	3.03	31.02	0.88	3.50	3.53	57.78	57.80	49.17	50.60	0.15
191	37.53	13.36	4.53	31.35	29.26	2.99	12.35	3.32	58.38	0.83	4.21	4.48	57.74	56.93	49.13	44.92	0.16
196	14.33	7.08	2.78	16.85	15.89	2.62	6.18	2.25	18.77	0.87	2.72	2.77	53.62	53.42	35.64	35.84	0.13
198	31.09	11.04	4.82	28.03	24.07	2.27	10.16	3.55	43.76	0.90	4.54	4.67	60.94	58.62	44.88	45.16	0.14
207	25.25	8.65	3.93	24.65	20.24	2.21	9.67	2.66	30.70	0.91	3.79	3.85	67.51	65.77	61.61	59.61	0.11

Appendix III: Mean data of quantitative traits of Mature leaves architectural traits of selected 25 Eucalyptus clones.

Clone	Area (cm^2)	Length (cm)	Breadth (cm)	Perimeter (cm)	Convex Perimeter (cm)	Roundness	Aspect Ratio	Curve Length (cm)	Curve Width (cm)	Convex Area (cm^2)	Full Ratio	Petiole length (cm)
1	37.12	21	3.65	47.56	43.98	4.78	6.12	22.08	1.7	124.26	0.55	2.27
10	24.84	13.31	3.01	30.62	28.37	3.28	4.92	13.33	1.98	55.39	0.69	1.72
14	22.28	14.19	2.68	31.33	29.79	3.36	5.48	14.03	1.64	58.63	0.64	1.85
16	25.13	13.88	2.96	31.18	29.26	3.01	4.89	13.71	1.88	57.41	0.68	1.31
17	35.94	14.96	3.97	34.97	32.2	2.65	4.02	14.89	2.62	73.29	0.74	2.09
19	26.78	13.8	3.06	31.47	29.16	2.87	4.67	13.79	1.94	57.85	0.7	2.23
23	28.35	16.85	3.13	37.86	35.54	3.87	5.58	17.29	1.65	82.22	0.6	1.74
26	28.25	17.33	3.03	38.15	36.33	3.95	5.93	17.47	1.61	84.55	0.58	0.95
53	27.43	17.62	2.81	39.32	36.61	4.42	6.58	18.18	1.48	85.58	0.57	1.78
63	35.46	17.89	3.42	39.82	37.66	3.46	5.46	17.91	2	94.07	0.63	1.34
66	35.05	15.91	3.86	36.03	34.07	2.91	4.27	15.79	2.22	79.93	0.67	1.2
94	34.27	12.63	4.27	29.68	27.98	2.05	3.2	11.7	3.13	57.19	0.81	1.58
100	40.45	15.95	4.15	36.52	34.54	2.54	3.97	15.63	2.63	82.93	0.71	1.62
101	25.77	15.47	3.06	34.58	32.71	3.56	5.12	15.67	1.62	75.6	0.62	1.13
111	45.96	18.52	4.23	43.14	39.28	3.07	4.44	19.17	2.4	102.02	0.67	1.86
115	38.89	17.45	3.85	39.06	37.02	2.99	4.61	17.28	2.25	90.29	0.66	1.38
116	24.99	13.14	3.12	29.78	28.11	2.83	4.31	13.01	1.88	57.89	0.69	0.89
124	28.47	18.61	2.98	41.08	38.82	4.53	6.53	19.05	1.48	95.76	0.55	1.05
186	16.76	10.48	2.57	23.7	22.38	2.72	4.34	10.17	1.68	35.13	0.71	1.17
187	37.93	16.39	3.63	36.35	34.6	2.64	4.58	15.74	2.44	79.51	0.7	1.33
188	24.1	14.13	2.91	31.55	29.92	3.26	5.11	14.02	1.76	59.17	0.65	1.55
191	23.78	19.5	3.33	43.67	40.9	6.09	6.31	20.7	1.14	107.29	0.47	0.91
196	26.81	18.11	2.48	39.19	37.35	4.51	7.55	18.14	1.46	86.76	0.56	0.91
198	45.14	18.89	4	42.2	39.95	3.16	5.03	18.67	2.43	106.02	0.66	0.88
207	17.81	13.82	2.2	30.2	28.76	3.99	6.47	13.85	1.25	52.39	0.58	1.5