International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 08-05-2021, Accepted: 20-05-2021, Published: 01-06-2021
Volume 6, Issue 3, 2021, Page No. 527-533

Characterization of morphological diversity in teak (*Tectona Grandis* L.F.) germplasm in India for preparation and implementation of dus testing (Distinct, Uniform and Stable)

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Abstract

The present study was carried out to identify the phenotypic variability among available *Tectona grandis* (Teak) germplasm in India for generating DUS descriptors phonetically for better scheming of their genetic inheritance and to prove the Distinct, Uniform and Stability (DUS) testing guidelines through Numerical Taxonomy. Grouping and Identification are the two main aims of the Numerical Taxonomy. Among 112 accessions/germplasm of “all India Teak clones” (based on aerial plant parts), a set of morphological descriptors were identified and grouping was done with the aid of statistical tools. ‘Grouping' are those, which are known from experience not to vary, or to vary only to a lesser extent, within a clone, can be used to discriminate the germplasms for DUS testing into different groups to facilitate the examination of Distinctiveness. As per the present study 18 out of 36 characteristics states were dimorphic (Presence of two states), while 13 were Trimorphic and the remaining 6 were polymorphic characters (including both quantitative and qualitative phenotypic traits). The accessions were significantly variable in all traits. The highest variability among accessions was found in leaf traits. It was difficult to identify all the cultivars based on a single morphological trait so a combination of different traits makes the clones distinct among all.

Keywords: characteristic state, teak, the proportion of character representation

Introduction

One of the main strategies for sustainable development is the amalgamation of agro-biodiversity conservation by small farmers with agricultural escalation. In India, the most suitable species for the agroforestry for Timber yielding is Teak (*Tectona grandis* L.f.), which belongs to the family Lamiaceae. 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 [1, 2]. [3] Also used morphological traits in his genetic research. The knowledge of the morphological diversity of the species is essential to define the morphological descriptors for the characterization and management of genetic resources, as well as the use of the genetic variability for genetic improvement with multiple purposes. In Teak, a considerable effort to assessing morphological descriptors was done with 36 morphological traits to described fifty-one accessions. The identification of easily detectable morphological characteristics of the aerial parts would allow for the creation of an identification key to differentiating the Teak clone. The objective of this work was to select an optimal set of morphological descriptors to characterize the phenotypical diversity or to differentiate clones based on their characteristics states to develop the DUS Descriptor. Characterization is the technique used to evaluate phenotypic diversity through agromorphological traits [4].

Teak is already one of the expensive hardwoods in the world timber market. The world’s teak supply from natural and planted forests adds up to a total of 2–2.5 million m^3 annually. In India, the Clonal plantation of Teak was not practised 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 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/m^3 for high-quality logs and 350–750 USD/m3 for small dimensional plantation logs of 20-25 yrs, IFGTB has taken initial steps to prepare and implement the DUS descriptor for Teak germplasm collection (112 clones) with known pedigree details for the present study.

Materials and Methods

Teak clone Genotype/germplasm

The genetic material used in the research for data collection included 102 *Tectona grandis* clones were collected in the locality of ‘National Teak Germplasm bank’ at Lohara in Chandrapur district in Maharashtra and 20 clones collected from ‘walayar germplasm’ situated in Kerala. An experimental design with randomized blocks was adopted, with 3 plants per plot with three replications in a 5.09 Hectare area. The spacing distance between plants was 8 m.

Categorical variables

Categorical variables are an amalgamation of unit character and characteristic states. The major grouping was done based on the plant parts (Unit character) viz young leaf, juvenile leaf, adult leaf, panicle, inflorescence, flower, fruit. Every character was given equal weightage.

Methods and Observations

The experiment was conducted at the Institute of forest genetics and tree breeding, Coimbatore to generate the phenotypic data under field conditions. Observations were recorded as per DUS UPOV 2011, in which three random plants from each line were taken for the recording of data. Quantitative and qualitative characters were examined using measurements from a single plant or its part or groups of plants or their parts, visual assessments from a single plant or its part or groups of plants or their parts, depending on the element used to characterize the accession and analysis carried out as per DUS, UPOV 2011. To assess distinctness(D), uniformity(U) and stability(S), the characteristics and their states as given for the characteristics were used at the optimum plant growth stage.
The assessment of Distinctiveness and Stability of all observations shall be made on 6 plants or parts taken each of 6 plants, which will be equally divided among 3 replications (2 plants per replication), chosen parts were taken and digitalized without further treatment using SLR Canon 750D camera. The assessment of Uniformity of characteristics shall be made in 6 plants per replication, with an acceptable probability of at least 95%. The maximum number of off-type allowed would be 1 in 18 plants. All branch characters shall be observed in the middle of the crown. All observations of a leaf shall be made in mature leaves at the middle of the crown in the middle third of the youngest shoots not showing signs of active growth. Observations on the inflorescences shall be made at the time of full flowering on terminal panicles of typical shoots from the exposed regions of the tree. Individuals are selected and their characters marked out. There is no limitation to the number of characters to be noted. However, the larger the number of characters, the better is the approach for the generality of the taxa. The superlative technique to delimitate taxa is, to utilize the maximum number of characters, with similar weightage given to all of them. For each leaf sample, different morphological attributes (including both qualitative and quantitative traits) were calculated using Leica Q Win V.3.5.0. At last, preparation of data files in the form suitable for analysis. Types of scales of data DUS depends on the level of scales of data, which are recorded for the characteristics. The scale may be quantitative or qualitative. Quantitative scaled data the data that is recorded by measuring or counting is said to be quantitative scaled data. This data can have continuous or discrete distribution. Continuous data results from measurement. Discrete quantitative data result from counting. Example -Continuous: Plant length in cm. – measurement - Discrete: Number of stamens (1, 2, 3, 4 and so on). In discrete quantitative data, there are no real values between two neighbouring units but is allowed to compute an average, which is in between these units. Totally 36 characters were investigated and used in this analysis. Under this minor characterization employing different characteristics states like dimorphic, Trimorphic and polymorphic characters based on the Presence of two states, three states and more than three states respectively were done. And they are represented by the simplest form of coding as 1to 9 for each state of expression for electronic data processing. The clones were significantly variable employing a combination of all traits; to prove this numerical taxonomy (statistical tools –cluster analysis) were used.
All cultivars under study were evaluated for 36 DUS characters at a specified stage of tree growth period when characters understudy had full expression following the guidelines of [5, 6, 7]. All observations on the leaf were recorded on fully developed but not old leaves, preferably between the 5th and 8th node when the plant had at least one fruit set while the observations on the fruit traits were made on 1st or 2nd well developed mature fruit. Observations on the ovary were recorded on the day of the anthesis. All observations on width were recorded at the maximum point of the width of the part concerned.

Table 1: Details of the source of selected Operational Taxonomic Units (OTC) 112 clones and their Abbreviation

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	ORANP 3	ST11	KLK1	TNT1	UPA	WB4
2	AIA	ACI	APKEA2 3	MHALA3	PT41	GUJ8	ORANP 7	ST14	KLK2	TNT10	UPC	
3	AID	AS4	APKEA2 4	MHALA4	PT46		ORANR 2	ST21	KLN2	TNT11	UPD	
4	AIE		APKEA2 5	MHALA6	BLC10		ORANR 3	ST26	KLN4	TNT13	UPG	
5	AIF		APKEC2	MHALA7			ORANR 4	ST27	KLS1	TNT15		
6	AII		APKEN1	MHALA8			ORANR 6	ST33	KLS2	TNT16		
7	AIJ		APMN4	MHALP1			ORANR 7	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			ORPLM 1	ST49		TNT3		
12			APNPL4	MHALP6			ORPUB 11			TNT4		

13			APNPL5	MHALP7			ORPUB 12			TNT5		
14			APNPL6	MHALP9			ORPUB 15			TNT6		
15			APNPL7	MHEMR1			ORPUB 21			TNT7		
16			APNPL8	MHSCA1			ORPUB 5			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									

Results and Discussion

Characterization: The requirement of distinctness, uniformity and stability are assessed based on characteristics. The characteristics are a feature of the whole plant or part of the plant. Such characteristics may be morphological, biochemical, molecular or any other nature. Describing the characteristics of a crop species based on standard descriptors is effective for better utilization and conservation of germplasm [8]. The table of characteristics chosen by experts forms the main part of test guidelines and DUS testing. In Genetic resources, the term ‘characteristic’ is known as descriptors (with descriptor states) and describing a plant-based on such descriptor is known as ‘characterization’.

Numerical taxonomy: It involves two main aspects: (i) classification and identification & (ii) Discrimination. Among these classification and identification of characters are imperative for DUS testing. Therefore “selection and assemblage of the variability present in the morphological traits among 112 clones of teak are presented in this article.

(a) Construction of Taxonomic Groups for classification and identification:

A total of 36 Characters and 112 descriptors/character states were determined through examination of different plant parts characters. Both qualitative and quantitative characters were coded as binary-state or in numerical notes (0 - 9). The characters and their binary states used for numerical taxonomic studies are listed in (Table 1, 2). So, Numerical taxonomy is a classification system in biological systematics which deals with the grouping by numerical methods of taxonomic units based on their character states.

Descriptor

Foliar descriptor: It is one of the commonly available and easy techniques based on foliar characters.

Inflorescence descriptors: Inflorescence traits are important characters, which influence pollination, fruiting, fruit and seed yield.

Fruit Descriptors: Fruit descriptors are more apparent and promising which can be utilized to differentiate cultivars [9, 10, 11]. Results from the present study combine all the morphological variation, therefore it will be helpful in the selection of distinguishable, uniform and stable traits.

It is divided into 7 major morphological group based on the young leaves (2), juvenile leaves (10), adult leaves (8), panicles (3), inflorescence (1), flowers (5) and fruits (4). A subset of 112 descriptor states was used to identify the individual clones were provided for showing distribution percentage with categorical variables (Table 1, 2).

Dimorphic character

In Teak, there are 17 dimorphic characters like Juvenile leaf lateral vein angle, juvenile leaf petiole, juvenile leaf presence of hair in abaxial side, juvenile treat primary branch attitude, adult leaf tip torsion, adult leaf margin undulation, panicle shape, inflorescence second-order angle, panicle compactness, flower style length, flower style pubescence, flower petal hairiness, flower petal apex shape, flower petal position, flower petal aestivation, fruit exocarp compactness and fruit shape (Figure 1-8).

Trimorphic characters

Young leaf anthocyanin colouration, young leaf margin, leaf petiole length, juvenile leaf colour of abaxial side, juvenile tree primary branch thickness, adult leaf length, adult leaf breath, adult leaf adaxial surface structure, adult leaf presence of trichomes in the adaxial side, panicle length, flower stigma position, flower petal colour and fruit width are 13 Trimorphic characters (Figure 1-8).

Polymorphic characters

Young leaf shape, young leaf base, young leaf apex shape, young leaf colour of abaxial side, adult leaf shape, adult leaf apex shape and flower number of petals these seven has Polymorphic characters (Figure 1-8)

Table 2: Unit characters, characteristic states and their Notes for 112 OTU of teak clones for descriptors for DUS testing.

S. No	Unit characters	Characteristic state	Notes	Distribution %
1	Young leaf: Anthocyanin colouration	Absent or weak	3	44
		Medium	5	46

		Strong	7	10
2	Young leaf: Shape	Ovate	1	45
		Elliptic	2	17
		Wide elliptic	3	5
		Narrow Obovate	4	13
		Wide Obovate	5	16
		Sub Orbiculate	7	2
		Deltoid	9	2
3	Juvenile leaf: Base	Truncate	1	3
		Obtuse	2	22
		Cuneate	3	46
		Attenuate	4	16
		Round		13
4	Juvenile leaf: Apex Shape	Subacute	1	50
		Cuspidate	2	14
		Obtuse	3	2
		Acute	4	34
5	Young leaf: margin	Entire	3	87
		Serrate	5	8
		Double serrate	7	5
6	Juvenile leaf: Lateral vein angle	Narrow <50)	1	33
		Wide (>50)	9	67
7	Juvenile leaf: Petiole	Absent (Sessile)	1	10
		Present	9	90
8	Juvenile leaf Petiole: Length	Short (<2 cm)	3	16
		Medium (2-8)	5	76
		Long (>8cm)	7	8
9	Juvenile leaf: Colour of abaxial side	Light yellow	1	9
		White	2	6
		Light Green	3	85
10	Juvenile leaf: Presence of hair in abaxial side	Present	1	63
		Absent	9	37
11	Juvenile tree Primary branch: Attitude	Upright <50)	1	8
		Horizontal (50-80)	9	92
12	Juvenile tree Primary branch: Thickness	Thin (<1/8th of main branch)	3	74
		Medium (1/8th to 1/4th of main branch)	5	16
		Thick (< 1/4th of main branch)	7	10
13	Adult leaf: Shape	Ovate	1	54
		Elliptic	2	14
		Sub Orbiculate	3	5
		Obovate	4	25
		Decurrent	5	1
		Rhombic	6	1
14	Adult leaf: Length	Short (<18 cm)	3	8
		Medium (18-30 cm)	5	36
		Long (>30cm)	7	56
15	Adult leaf: Breadth	Narrow (<12 cm)	3	11
		Medium (12 to 22 cm)	5	41
		Wide (>22 cm)	7	48
16	Adult leaf: Apex Shape	Subacute	1	40
		Cuspidate	2	20
		Obtuse	3	2
		Caudate	4	2
		Acute	5	36
17	Adult leaf Tip: Torsen	Absent	1	76
		Present	9	24
18	Adult leaf margin: Undulation	Present	1	21
		Absent	9	79
19	Adult Leaf adaxial surface: Texture	Glabrous (smooth not hairy)	1	46

		Coriaceous (leather -like)	2	49
		Scabrose (rough like sandpaper)	3	5
20	Adult leaf: Presence of trichomes in adaxial side	Low to absent	3	68
		Medium	5	26
		High	7	6
21	Panicle: Length	Short (<20 cm)	3	15
		Medium (20 to 60 cm)	5	75
		Long (> 60cm)	7	10
22	Panicle: Shape	Conical	1	88
		Spherical	9	12
23	Inflorescence: Second order angle	Upright <60)	1	83
		Horizontal (60-90)	9	17
24	Panicle: compactness	Sparse	1	72
		Dense	9	28
25	Flower stigma: Position	Non-herkogamy	1	64
		Approach Herkogamy	2	25
		Reverse Herkogamy	3	11
26	Flower style: Length	Short (<5mm)	1	86
		Long (>5mm)	9	14
27	Flower style: Pubescence	Present	1	75
		Absent	9	25
28	Flower: No of petals	5 alone	1	9
		6 alone	3	42
		7 alone	5	5
		5-9 mixed		44
29	Flower petal: Colour	White	1	45
		Light yellow	2	37
		Pink	3	18
30	Flower petal: Hairiness	Present	1	13
		Absent	9	87
31	Flower petal apex: Shape	Acuminate	1	58
		Rounded	2	42
32	Flower petal: Position	Horizontal	1	54
		Deflexed	9	46
33	Flower petal: Aestivation type	Valvate	1	98
		Twisted	2	2
34	Fruit exocarp: Compactness	Loose	1	72
		Compact	9	28
35	Fruit: Shape	Globose	1	72
		Subtetragonous	2	28
36	Fruit: Width	Narrow (<1.2 cm)	3	9
		Medium (1.2-2.0cm)	5	59
		Broad (>2.0cm)	7	32

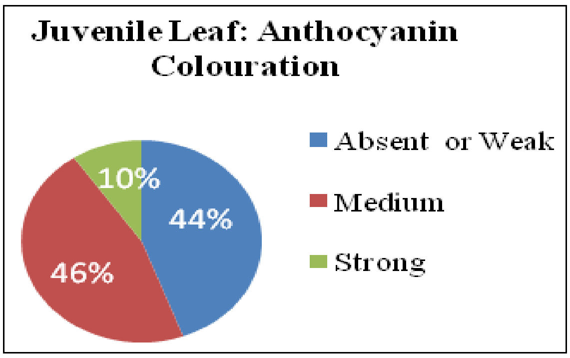

Fig 1: Trimorphic character states of Anthochanin

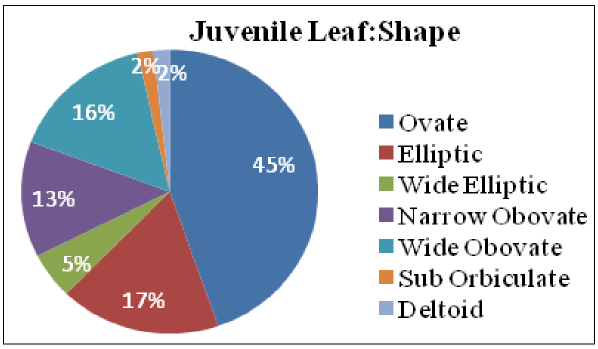

Fig 2: Polymorphic character states of juvenile leaf shape

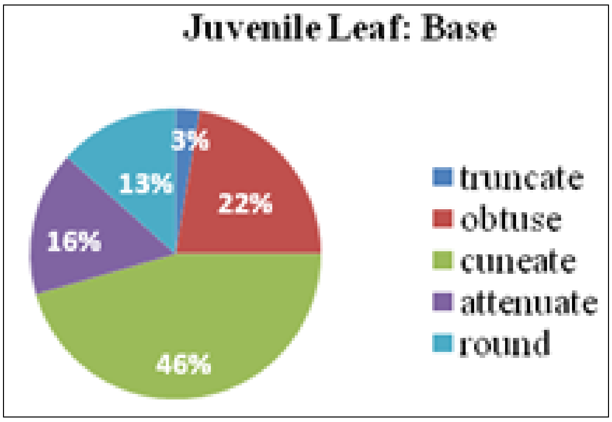

Fig 3: Polymorphic character states of juvenile leaf base shape

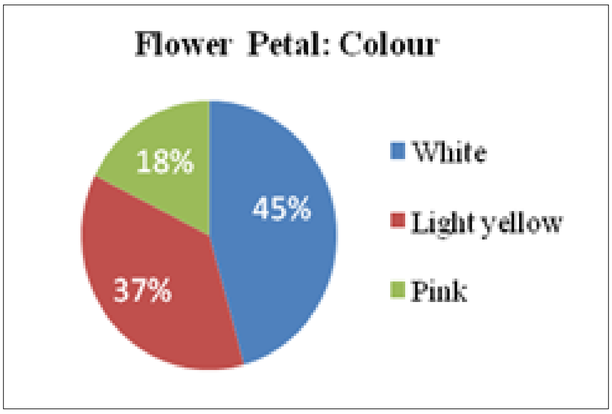

Fig 4: Trimorphic character states of flower petal colour

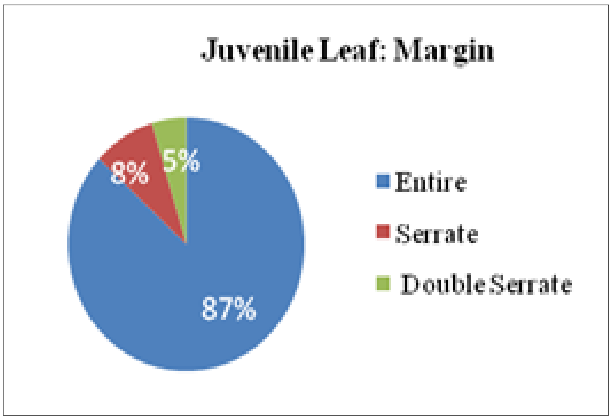

Fig 5: Trimorphic character states of juvenile

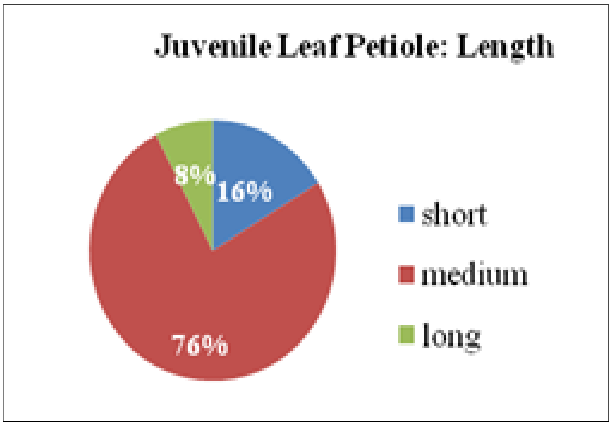

Fig 6: Trimorphic character states of leaf margin juvenile leaf petiole length

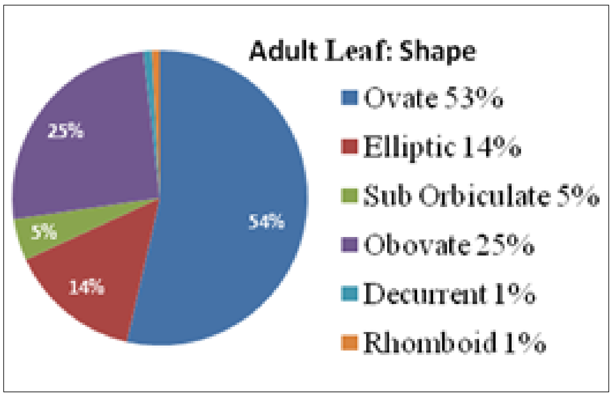

Fig 7: Polymorphic character states of

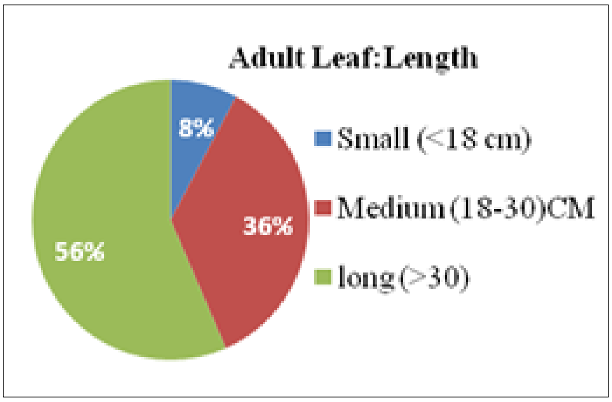

Fig 8: Trimorphic character states of adult leaf adult leaf shape length

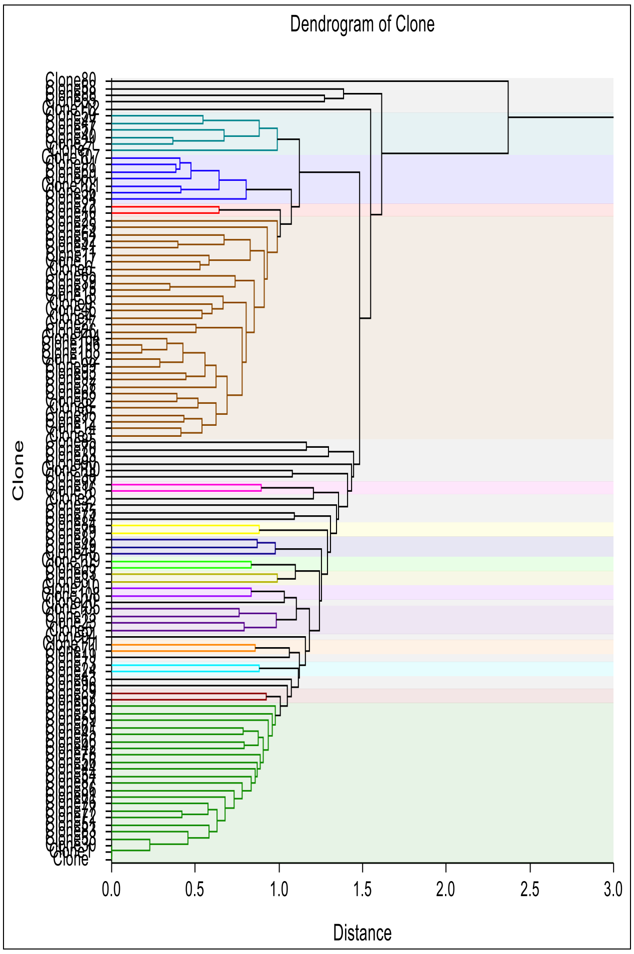

Fig 9: Dendrogram showing clusters of clones

(b) Discrimination of the Taxonomic Groups

When the taxonomic groups were chosen for the study show overlapping of characters, discrimination should be used to select them. Discrimination analysis can be done by various techniques, specially devised for such purposes. Numerical taxonomy is thus, based on certain principles [12] also called neo Adansonian principles. It aims to create a taxonomy using numeric algorithms like cluster analysis rather than using subjective evaluation of their properties.

Statistical analysis

The data matrix was scored using a binary matrix. A dissimilarity matrix was prepared based on the data matrix. Cluster analysis was performed using NCSS 2021, v21.0.1, Clustering Method-Group Average (Unweighted Pair-Group), Distance Type – Euclidean, Scale Type-Standard Deviation and a dendrogram was constructed to show the relationship among the species.

Cluster analysis

Based on the descriptor data were transformed into a binary matrix for statistical analysis (cluster analysis) and graphically displayed through a dendrogram, UPGMA tree derived from cluster analysis reveals 15 major clusters, the first of which consists of two clones, the second cluster comprises seven clones and the third to ninth are composed of two clones each and so on (Figure 9) showing 27 different groups based on the morphometric characters.

Results obtained from the present study is significant.

Acknowledgements

The authors are immensely thankful and acknowledge Dr. Kuhnikannan, the Director of Institute of Forest Genetics and Tree Breeding, Coimbatore. The authors also are grateful to former Director Dr. N. Krishnakumar, IFS, who allowed us to do the research.

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