



# Genetic Diversity in *Toona ciliata* M. Roem., progenies using D<sup>2</sup> Analysis

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**Abstract:** Genetic diversity studies were carried out for morphometric attributes in sixteen progenies of *Toona ciliata*. Among the genetic resources of *Toona ciliata* a multivariate analysis was carried out using D<sup>2</sup> clustering technique and classified sixteen progenies into five clusters. Cluster II revealed the maximum intra-cluster distance. The maximum inter cluster distance was found between clusters III and IV which exhibits a higher genetic distance between *Toona ciliata* progenies. Volume index (81.66 percent) contributed the highest genetic divergence among the growth characters, followed by basal diameter and plant height.

**Keywords:** Toona, Growth attributes, Genetic divergence, D<sup>2</sup> clustering

*Toona ciliata* comes under the family is called Meliaceae, which is native to India, Australia, Indo-china peninsula, Pacific Islands and southern china. It is commonly known as Australian red cedar or Chinese mahogany (Yang et al 2020). It is a shade bearer and tree attains 30m height with well spreading crown. The wood of *Toona* is more valued because of its colour, durability and timber quality (Zhan et al 2019). The current forest and tree cover of the country is 21.67 per cent (71.22 million ha) and 2.89 per cent (9.51 million ha), respectively. The annual harvest of the timber from the forest declined from 10 million m<sup>3</sup> in the 1970's to 4 million m<sup>3</sup> in 1990 and currently it is 3 million m<sup>3</sup>. The annual timber production of TOF is 85 million m<sup>3</sup> in 2020. The total wood demand of India is 153 million m<sup>3</sup> in 2020 and the productivity of wood is only 60 million m<sup>3</sup> (Shrivastava and Saxena 2017). To meet the demand of large sized wood, the policy of importing wood has been liberalized since the 1990s. Total import of round wood and sawn wood logs was 6 million cubic meters from 2014-15 to 2019-20. India imports all type of wood except pulp, paper and news prints (Rai and Chakrabarti 2001). The importing of wood increases day by day so to meet out the wood demand we need to identify and screen alternate fast growing multipurpose trees. *Toona ciliata* is the one among the best fast growing and alternative species which could be used as raw material for wood based industries.

The study of genetic diversity becomes more valuable in tree improvement programme because it increases the chances of recovering superior genotypes (Zobel and Talbert 1984). To assess the genetic divergence among the

genotypes D<sup>2</sup> statistics has been considered as one of the most powerful technique. D<sup>2</sup> analysis, the superior group or individual may legitimately be used to raise profitable plantations to meet industrial wood demand. None of the studies have indicated about genetic diversity in *Toona ciliata* against this backdrop the current investigation have been carried out.

## MATERIAL AND METHODS

Progenies evaluation trail was established at Forest College and Research Institute, Mettupalayam with 16 which were collected from elected Candidate Plus trees (CPTs) from 3 different states viz., Tamil Nadu, Karnataka and Punjab. *Toona ciliata* progenies collected form CPTs were planted in a Randomized Block Design (RBD) with three replications at an espacement of 4m x 4m. The details of the actual locations of the sixteen chosen candidate plus trees were presented in the Table 1.

**Plant height:** The plant height was measured in centimeters with the help of measuring tape from ground level to the tip of the stem.

**Basal diameter:** The Basal diameter was measured in centimeters with the help of digital caliper at the base of the stem (near the ground level).

**Volume index:** After estimating the plant height and basal diameter, the volume index was calculated using the formula below.

$$V.I. = (\text{Basal diameter})^2 \times \text{Height (cm)} \quad (\text{Hatchell 1985, Manavalan 1990})$$

**Estimation of genetic diversity:** The data were resolved 2

months interval after planting in *Toona ciliata* progenies for evaluation of genetic diversity.

**Determination of genetic divergence:** Genetic divergence was calculated using  $D^2$  statistics design. The progenies were clustered using  $D^2$  statistical results. The parameters used in the  $D^2$  statistics were plant height, collar diameter and volume. The mean squares and mean products between groups and within components at the progeny level were estimated using one way analysis of variance, covariance and level of significance. A variance – covariance was calculated and essential condensation was performed to obtain the linear function for transforming character mean values (x) to set of independent variables (uncorrected mean) value (y).

To calculate the  $D^2$  square values the variation between any two mean values for any pair of progenies was squared and added. Collective  $D^2$  square values in all possible combinations of progeny were determined for each character.

$$y_1 = x_1$$

$$y_2 = x_2 - a_{21}x_1$$

$$y_3 = x_3 - a_{32}y_2 - a_{31}y_1$$

$$y_p = y_p - a_{pp-1}y_{p-1} \dots \dots a_{p1}y_1$$

Where,

X1 = Normalized variables

$$a_{ij} = b_{ij}/v(y_j) \quad S < - 1$$

$$v(y_j) = \lambda \sum a_{(ij)} b_{ij} - b_{ij} = \lambda_{ij} - 1/atbt$$

$$\lambda_{ij} = \text{Covariance of } i \text{ and } j, i - j_i$$

All probable  $n(n-1)/2$   $D^2$  values were calculated by

adding the sum of the differences between two corresponding 'y' values for two progenies at a time.

**Estimation of clusters or grouping:** The genotypes were classified into different clusters using the "GENERES" statistical packages based on the root of  $D^2$  values, by using Tocher's method as recommended by Rao (1952).

**Tocher's method:** Tocher's method used to cluster all values. Rao (1952)

$$\frac{n(n-1)}{2} D^2$$

**Estimation of Average intra and inter cluster distance:**

Once clustering was achieved, the intra and inter cluster associations were arranged, and the communal associations among clusters and their distances were represented. The following formula was used to calculate the average intra cluster distances.

$$D = D^2/n$$

The average inter cluster divergences were calculated by taking into account all possible  $D^2$  values between the records of the two clusters, where  $D^2$  was the sum of distances between all possible combinations of progeny included in a cluster. Following that, the genetic distance 'D' between the clusters was calculated by taking the square root of the average  $D^2$  values.

**Statistical analysis:** GENERES statistical package new version was used to determine the genetic diversity analysis Kanna et al (2019).

**Table 1.** Details of *Toona ciliata* progenies and their location

Sources	District	State	Name of sources	Latitude	Longitude
Katteri, Nilgiri hills	Nilgiri	Tamil Nadu	FCRI TC 1	11°33'31"N	076°79'7"E
Aravenu, Kotagiri stretch	Nilgiri	Tamil Nadu	FCRI TC 2	11°32'43"N	076°95'58"E
Nadugani, Gudalur division TC1	Nilgiri	Tamil Nadu	FCRI TC 3	11°47'21"N	076°41'31"E
Nadugani, Gudalur division TC2	Nilgiri	Tamil Nadu	FCRI TC 4	11°44'59"N	076°41'27"E
Pandalur, Gudalur division	Nilgiri	Tamil Nadu	FCRI TC 5	11°33'71"N	076°93'66"E
Devala, Gudular division	Nilgiri	Tamil Nadu	FCRI TC 6	11°33'52"N	076°92'50"E
Choondi, Gudalur division	Nilgiri	Tamil Nadu	FCRI TC 7	11°32'40"N	076°92'60"E
Thadiyankudisai TC4	Dindigul	Tamil Nadu	FCRI TC 8	10°29'44"N	077°70'50"E
Thadiyankudisai TC3	Dindigul	Tamil Nadu	FCRI TC 9	10°29'27"N	077°70'19"E
Pongalamedu, Thadiyankudisai beat	Dindigul	Tamil Nadu	FCRI TC 10	10°31'98"N	077°65'40"E
Sampallioothu, Thadiyankudisai beat	Dindigul	Tamil Nadu	FCRI TC 11	10°31'03"N	077°65'29"E
Kodagu	Kodagu	Karnataka	FCRI TC 12	12°14'43"N	075°93'95"E
Ponampet	Kodagu	Karnataka	FCRI TC 13	12°14'47"N	075°94'51"E
Ludhiana TC5	Ludhiana	Punjab	FCRI TC 14	30°90'10"N	075°80'71"E
Ludhiana TC6	Ludhiana	Punjab	FCRI TC 15	30°90'10"N	075°80'71"E
TC 02	Ludhiana	Punjab	FCRI TC 16	30°90'10"N	075°80'71"E

## RESULTS AND DISCUSSION

The biometric parameters viz., plant height, collar diameter, and volume index were recorded at four different growth phases viz., Initial, 60 DAP (Days after Planting), 120 DAP and 180 DAP in sixteen progenies of *Toona ciliata*. The data were examined for genetic diversity, and the results are shown below.

**Genetic divergence:** For all positive pairs,  $D^2$  values were computed. The biometric characteristics of plant height, collar diameter and volume index were used to examine divergence and clustering. The sixteen *Toona ciliata* progenies were divided into 5 genetically divergent clusters.

**Intra and inter cluster average distance:** Table 2 depicts the average intra and inters cluster values for each of the five clusters. Cluster II revealed the highest average intra cluster value (1.546), while Cluster IV and V had the lowest (0.00). Cluster IV and III showed the highest inter-cluster value (2.941). The lowest inter-cluster value was found between clusters I and II (1.693).

According to Tewari and Singhania, (1994), the plant diversity refers to the variety and variability within an environment. To improve all kind of tree species, breeders are constantly engaged in the effective selection of desirable parents with high genetic variability so that the individuals with desirable character combinations can be selected achieved. The majority of plant species especially forestry species have very long rotation period, out crossing mechanism and huge heterozygosity which evolved a amount of natural mechanisms to keep up heterozygosity and intra specific variations. Such genetic mechanisms, combined with natural environment variations, appear to have contributed to forest species particularly in trees being among the most genetically variable organisms studied to date (Libby WJ, 1987). Traditionally the genetic diversity in trees has been done through provenance testing, enzyme electrophoresis and molecular techniques using DNA (Kertadikara and Prat 1995, Mandal and Gupta 1996, Williams et al 1990).

**Cluster components:**  $D^2$  analysis classified sixteen progenies into five clusters. Table 3 exhibits the cluster members and the number of progenies in each cluster. In the five clusters, the cluster II showed highest number of progenies which is resolved with nine progenies, namely FCRI TC 3, FCRI TC 4, FCRI TC 5, FCRI TC 7, FCRI TC 8, FCRI TC 9, FCRI TC 10, FCRI TC 11, and FCRI TC 12. Cluster I had three progenies, namely FCRI TC 1, FCRI TC 2, and FCRI TC 6, Whereas Cluster III had only two progenies, namely FCRI TC 14, and FCRI TC 16. Clusters IV and V each had only one progeny, FCRI TC 13 and FCRI TC 15, respectively.

Cluster mean for each morphometric character was calculated and it is shown in table 4. Cluster IV had the highest cluster mean for plant height (556.667cm), while cluster II had the lowest cluster mean for plant height (145.620 cm). Cluster V performed best in terms of collar diameter (4.898 cm), followed by cluster I (4.794 cm), and cluster II exhibited the worst performance (3.822 cm). In terms of volume index, cluster I had the highest cluster mean value (4876.568 cm<sup>3</sup>) and cluster II had the lowest (2410.375 cm<sup>3</sup>). The main goal of the clustering method is to divide the pool of observations into many subgroups in order to achieve homogeneity within and between the subgroups. Genetic divergence is an important tool in the field of plant breeding for achieving desirable qualities in trees and plants. The genetic diversity estimates the degree of diversity that existed between the genotypes that were chosen.

The  $D^2$  clustering method was used in *Toona ciliata* to segregate the sixteen progenies into five clusters; cluster II

**Table 2.** Intra and Inter cluster values of *Toona ciliata* progenies based on biometric attributes

Cluster	1	2	3	4	5
I	1.693	2.092	2.005	2.563	1.278
II		1.546	1.704	2.941	1.777
III			0.679	2.546	1.050
IV				0.000	2.271
V					0.000

**Table 3.** *Toona ciliata* cluster pattern details based biometric attributes

Cluster No	No of progenies	Members
I	3	FCRI TC 1, FCRI TC 2, FCRI TC 6
II	9	FCRI TC 3, FCRI TC 4, FCRI TC 5, FCRI TC 7, FCRI TC 8, FCRI TC9, FCRI TC 10, FCRI TC 11, FCRI TC 12
III	2	FCRI TC 14, FCRI TC 16
IV	1	FCRI TC 13
V	1	FCRI TC 15

**Table 4.** *Toona ciliata* cluster mean values based on biometric characters

Cluster	Plant height (cm)	Basal diameter (cm)	Volume index (cm <sup>3</sup> )
I	197.056	4.754	4876.568
II	145.620	3.822	2410.375
III	154.917	4.585	3388.913
IV	556.667	4.657	4085.778
V	181.417	4.898	4626.987

exhibited the most progenies (nine). The results stand similar with the findings of Kanna et al (2019) classified thirty *Ailanthus excelsa* progenies into nine clusters. Cluster IV had most progenies (10 progenies) of the nine clusters. In *Neolamarckia cadamba* 20 progenies were divided into five clusters. Cluster II had demonstrated the greatest intra-cluster distance. The greatest inter-cluster distance was measured between clusters III and V (Parthiban et al 2019). According to Singhdoha et al. (2017), sixty CPTs of *Acacia nilotica* were distributed among seven different clusters, with cluster I had the most (14 CPTs), followed by cluster V. The average intra-cluster distance was found to be greatest in cluster I, followed by cluster IV and cluster II, while the inter-cluster distance ranged from 2.681 to 5.734, with cluster I and VII having the greatest distance. The genetic diversity studies classified twenty *Neolamarckia cadamba* progenies into five clusters, with cluster III having the greatest intra-cluster distance. Clusters I and IV had the greatest inter-cluster distance (Shree et al 2018). In groundnut (*Arachis hypogaea*) 93 genotypes were divided into eight clusters. Cluster VI has the most members (18), followed by cluster VII and I. cluster V had the highest intra cluster values, followed by cluster IV Dhakar et al (2017). In sorghum (*Sorghum bicolor* L.) 20 genotypes were classified into four distinct clusters. The maximum (6) and lowest (4) number of genotypes were found in clusters I and IV respectively. Clusters II and IV (150.99) and cluster III and IV (150.99) had the greatest and smallest inter cluster distances respectively Navya et al (2021).

In the present study, it was observed that progenies from various locations were grouped together to form a single major cluster, as seen in cluster II, and thus the degree of divergence was not dependent on geographic location. The above-mentioned findings also confirmed an earlier report of Kanna et al (2019), Parthiban et al (2019), Shree et al (2018), Singhdoha et al (2017), Sangram and Keerthika (2013). The presence of divergent provenances in the same cluster in nature may be attributed to factors other than geographic distribution being responsible for their genetic relationship. The intra and inter cluster recorded values indicated that IV and V had no intra cluster because they only had one progeny.

#### Contribution of Characters towards Genetic Divergence

Table 5 explained the percentage and number of times each character ranked first, along with their genetic divergence. The volume index contributed the most to divergence (81.667%), followed by the collar diameter (15.833%). Contribution of plant height was found to have the lowest to the genetic divergence (2.500%). The findings are in corroboration with the findings of Singhdoha et al (2017) in

**Table 5.** Percentage wise contribution of each character towards genetic divergence in *Toona ciliata* progenies based on biometric attributes

Character	No. of first rank	Contribution (%)
Plant height	3	2.5000
Basal diameter	19	15.8333
Volume index	98	81.6667
Total	120	100

*Acacia nilotica*, Parthiban et al. (2019) in *Neolamarckia cadamba*; Kanna et al (2019) in *Ailanthus excelsa*. Volume index, along with other biometric characteristics, contributed to genetic divergence between the progenies tested, which could be due to the existence of broad genetic base (Kanna et al. 2019). Based on previous research and current findings, the Volume index for genetic divergence could be used as an index for the *Toona ciliata* tree improvement programme.

#### CONCLUSION

The D<sup>2</sup> analysis classified sixteen *Toona ciliata* genetic resources into five clusters. Cluster II had the most progenies among the five clusters. Cluster II determined the greatest intra-cluster distance. Cluster IV had the greatest inter-cluster distance, indicating a greater genetic distance between *Toona ciliata* progenies. Among the growth characters, volume index contributed the greatest percentage to genetic divergence, followed by collar diameter and plant height played the smallest role in the divergence.

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