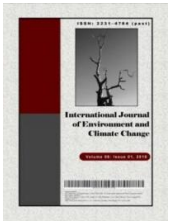

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Genetic Divergence Studies in *Ailanthus excelsa* Using D2 Analysis

S. Umesh Kanna[1*], N. Krishnakumar[2] and K. Md. Mohideen Abdul Kather Jailani[3]

[1]Department of Forest Biology and Tree Improvement, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, India.
[2]Department of Agroforestry, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, India.
[3]Department of Forest, Government of Tamil Nadu, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author SUK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KMMAKJ and NK managed the analyses of the study. Authors NK and KMMAKJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To estimate the genetic diversity studies among the biometric attributes of 30 progenies in *Ailanthus excelsa* Roxb.
Place and Duration of Study: The study has conducted at Forest College and Research Institute, TNAU, Mettupalayam during 2015-2018.
Methodology: The D^2 statistics was adopted for the estimation of genetic divergence. Using D^2 statistical results, the clustering of progenies was done. The progenies were grouped into different clusters using 'GENERES' statistical package on the basis of D^2 values according to Tocher's method as suggested by Rao.

**Corresponding author: E-mail: umeshforestry@gmail.com;*

Results: The 30 progeny of *Ailanthus excelsa* has grouped into nine clusters and among the nine clusters, the cluster IV has ten progenies. The maximum intra cluster distance was exhibited by the cluster VIII followed by cluster IV. The maximum inter cluster distance was in cluster III which indicated the presence of wider genetic distance between *Ailanthus excelsa* progenies. Among the growth attributes, volume index contributed maximum percentage towards genetic divergence.
Conclusion: The results of 30 progeny of *Ailanthus excels* showed the presence of wider genetic distance between *Ailanthus excelsa* progenies.

Keywords: Ailanthus excels; biometric attributes; genetic resources; diversity; genetic distance; D2 clustering.

1. INTRODUCTION

Ailanthus excelsa Roxb. is a tree belonging to family Simaroubaceae, indigenous to Central and Southern India and commonly it is known as Tree of Heaven. It is a large deciduous tree and will be growing 18-25 m tall with straight trunk and 60 to 80 cm in diameter. It is mainly used for making plywood as well as match splint production [1]. Due to the demand of both plywood and match wood this study has conceived. Rapid socio-economic changes are having profound impacts on all sectors including forestry. Societal transformations are changing people's perceptions of forests, while growing and often conflicting demands for forest-derived goods and services have increased the complexity of forest management. Concerns over climate change, escalating energy prices and deepening water deficits have moved forestry into the spotlight of global and national development. Currently, the forest area in the country is around 23.81 per cent and in the state of Tamil Nadu it is around 17.59 per cent which is much low against the demanded requirement of 33.0 per cent. The productivity in terms of MAI is also one of the lowest comparing to the global average [2]. The annual estimated production of wood from forest is estimated to be 3.173 million m^3 and the annual potential production of wood from outside the forests is estimated to be 42.77 million m^3 [2]. The country's timber imports value I growing at 12 per cent per annum and is likely to increase in years ahead. The liberalization of imports has benefited the domestic timber market, otherwise faced paucity of the desired wood in the required quantity and quality. However, there is a potential to increase the domestic production of industrial wood through tree planting, afforestation and reforestation programmes [3].

Hence shrinking forest area associated with low productivity established a total mismatch between the demand and supply of both domestic and industrial wood requirement besides creating environmental disequilibrium [4]. The current supply of raw materials for industries like match wood, pulpwood, plywood, furniture and biomass energy in India particularly in Tamil Nadu is far behind the demand. Hence, to meet the growing raw material demand and also to meet the National Forest Policy (1988). Guidelines, the industries must expand sharply its plantation programme. There are over 400 small-scale sector Splints and Veneer Industry involved in the manufacturing of veneers and splints in southern India of which 75% are located in Kerala [5]. Per capita consumption of matches in India increased steadily from 2.45 sticks per capita in 1970 to 8.35 in 2013 .There are wide fluctuations in the annual growth rate in the consumption of matches varying from as low as 3 per cent (before 1970) to as high as 28 per cent. The rising levels of income, growing urbanization, swelling numbers of smokers, and changes in fuel consumption patterns indicates that the future rate of growth could be higher than the 6 per cent as supported by past trends [6].

The major raw materials used in the production of safety matches are soft woods. Safety matches manufactured in India are of the standard type with wooden veneer or cardboard boxes and wooden splints. Historically the Indian match industry depended on imported wood including Aspen (*Populus tremula*) from Sweden, Canada, America, and Russia; Cotton Wood (*Populus deltoides*) from Canada; Balsam Poplar (*Populus balsamifera*) from Manchuria; and Linden (*Tilia japonica*) from Japan. But the government quickly moved to encourage the use of indigenous woods by restricting the import. Even though there are number of alternative match wood species are available to replace the imported wood, *Ailanthus excelsa* occupies predominant position because of its suitability for the production quality match splints. However there is no systematic evaluation or improvement programme in order to utilize the existing genetic variation among broader genetic base population

which warrants a systematic tree improvement programme in *Ailanthus excelsa* which will also address the shortage of suitable raw material to the match industries.

2. MATERIALS AND METHODS

2.1 Materials

The species *Ailanthus excelsa* was chosen as the experimental material for the present study which consists of 30 progenies established as a progeny evaluation trial.

2.2 Methods

Estimation of morphometric attributes:

Source of progenies

The predominant eleven *Ailanthus excelsa* distributed districts of Tamil Nadu *viz*., Coimbatore, Tirupur, Erode, Salem, Theni, Dindugal, Viruthunagar, Darmapuri, Krishnagiri, Villupuram, and Karur were surveyed and a total number of 30 candidate plus trees were selected. These selected Candidate Plus Trees (CPTs) were given with the accession number as FCRI AE. The details on the actual locations of the 30 selected candidate plus trees were presented in Table 1.

Determination of genetic diversity

The data recorded at 6 MAP in *Ailanthus excelsa* progeny evaluation trial were used for diversity analysis.

Determination of genetic divergence

The D^2 statistics was adopted for the estimation of genetic divergence [7]. Using D^2 statistical results, the clustering of progenies was done.

Table 1. Details of *Ailanthus excelsa* genetic resources and their location

Sl. no.	District	Sources	Name of sources	Latitude	Longitude
1	Coimbatore	Akkarai sengapalli	FCRI AE 1	11°19'28"N	77°04'53"E
2	Coimbatore	S. Pungampalayam	FCRI AE 2	11°03'24"N	77°19'51"E
3	Coimbatore	Cherannagar – 1	FCRI AE 3	11°03'05"N	76°56'32"E
4	Coimbatore	Cherannagar – 2	FCRI AE 4	11°03'05"N	76°56'32"E
5	Coimbatore	Teachers colony	FCRI AE 5	11°09'37"N	76°56'33"E
6	Coimbatore	Annur – 1	FCRI AE 6	11°14'03"N	77°06'19"E
7	Coimbatore	Annur – 2	FCRI AE 7	11°14'03"N	77°06'19"E
8	Coimbatore	Alamelu mangapuram	FCRI AE 8	11°02'45"N	76°58'40"E
9	Coimbatore	Vaikalpalam	FCRI AE 9	10°58'53"N	76°55'17"E
10	Tirupur	Pogalur	FCRI AE 10	11°15'25"N	77°02'26"E
11	Tirupur	Samundipuram	FCRI AE 11	11°07'28"N	77°18'60"E
12	Tirupur	Kulathu thottam	FCRI AE 12	11°03'33"N	77°15'56"E
13	Tirupur	Salakkudi	FCRI AE 13	10°41'04"N	77°36'22"E
14	Tirupur	Chettipalayam	FCRI AE 14	11°08'38"N	77°20'28"E
15	Erode	Appachimar madam	FCRI AE 15	11°19'51"N	77°28'47"E
16	Erode	Perundurai	FCRI AE 16	11°16'26"N	77°35'18"E
17	Salem	Pethanayakkanpalayam	FCRI AE 17	11°38'51"N	78°30'20"E
18	Salem	Idapadi	FCRI AE 18	11°35'05"N	77°50'20"E
19	Theni	Uthamapalayam	FCRI AE 19	9°48'20"N	77°19'40"E
20	Theni	Thevaram	FCRI AE 20	9°53'44"N	77°16'31"E
21	Theni	Bodi	FCRI AE 21	10°01'00"N	77°21'00"E
22	Dindugal	Kallimandayam	FCRI AE 22	10°35'28"N	77°44'11"E
23	Viruthunagar	Srivilliputhur	FCRI AE 23	9°30'44"N	77°38'03"E
24	Darmapuri	Harur	FCRI AE 24	12°03'05"N	78°28'49"E
25	Darmapuri	Papparettipatti	FCRI AE 25	11°54'49"N	78°21'57"E
26	Krishnagiri	Oothangarai	FCRI AE 26	12°15'57"N	78°32'07"E
27	Villupuram	Thiruvakkarai	FCRI AE 27	12°01'34"N	79°39'06"E
28	Villupuram	Mathangadipattu	FCRI AE 28	11°57'59"N	78°45'28"E
29	Villupuram	Pudupattu	FCRI AE 29	11°58'21"N	78°53'52"E
30	Karur	Salikaraipatti	FCRI AE 30	10°45'04"N	78°10'70"E

D^2 statistics

The D^2 statistics was carried out using the traits *viz*., plant height, diameter at breast height and volume. The mean squares and the mean products were estimated between groups and within components by one-way analysis of variance, covariance and the significance were tested at progeny level. A variance – covariance was formed from the above and subjected to pivotal condensation to obtain the linear function for transformation of character mean values (x) to a set of independent variables (uncorrected mean) value (y).

The difference between any two mean values for each pair of progeny was squared and added to give the D^2 values. For each character Cumulative D^2 values in all the possible combination of progeny were estimated.

$$y_1 = x_1$$
$$y_2 = x_2 - a_2 x_1$$
$$y_3 = x_3 - a_{32} y_2 - a_{31} y_1$$
$$yp = yp - a_{pp-1} y_{p-1} \ldots\ldots ap_1 y_1$$

where,

x1 = normalized variables

$$a_{ij} = b_{ij}/v(y_j)\ S < -1$$
$$v(y_j) = \lambda \sum a_{(ij)}\, b_{ij} - b_{ij} = \lambda_{ij} - 1/atbt$$
$$\lambda_{ij} = \text{Covariance of i and } j^t = j^i$$

All possible $\frac{n(n-1)}{2} D^2$ values were calculated by taking sum of difference between pair of corresponding 'y' values taking two progenies at a time.

Determination of clusters or grouping

The progenies were grouped into different clusters using 'GENERES' statistical package on the basis of D^2 values according to Tocher's method as suggested by Rao [8].

Tocher's method

All the $\frac{n(n-1)}{2} D^2$ values were clustered by using Tocher's method [8].

Average intra and inter cluster distances

On completion of clustering, the intra and inter cluster relationships were studied and the mutual relationship between clusters and their distances were represented. The average intra cluster distances were measured using the formula:

$$D^2 = D^2/n$$

Where D^2 was the sum of distances between all possible combinations of the progeny included in a cluster whereas the average inter cluster divergences were arrived at by taking into consideration of all the component D^2 values possible among the numbers of the two clusters. Then the genetic distance 'D' between the clusters were obtained from square root of the average D^2 values.

3. RESULTS AND DISCUSSION

Observations on morphometric traits *viz.,* survival percentage, plant height, basal diameter, number of branches and volume index and biochemical attributes *viz.,* chlorophyll 'a' , chlorophyll 'b', total chlorophyll and chlorophyll a / b ratio were recorded in 30 progenies of *Ailanthus excelsa*. The morphometric traits were measured at four growth periods *viz.,* initial, 2 MAP, 4 MAP and 6 MAP whereas biochemical attributes were recorded only 6 MAP. The data were subjected to genetic diversity analysis and the results were presented below.

3.1 Genetic Divergence

The genetic divergence was analyzed using multivariate analysis among the 30 progenies with computer based "GENRES" statistical package. The D^2 were computed for all positive pairs. The morphometric traits *viz.,* plant height, basal diameter, number of branches and volume index were used for divergence and clustering analysis. The 30 progenies of *Ailanthus excelsa* were resolved into nine genetically distinct clusters.

3.2 Intra and Inter Cluster Average Distance

The average intra and inter cluster values among the nine clusters are presented in Table 2. The progenies resolved within the intra cluster VIII has high genetic distance of 13.78, while the least genetic distance of 0.21 was observed in the cluster III. The highest inter cluster genetic distance was recorded between the cluster III and IX (80.88).The minimum inter cluster genetic distance was recorded between the cluster I and V (4.56).

Table 2. Inter (diagonal) and intra cluster estimates of *Ailanthus excelsa* progenies based on morphometric attributes

Cluster	1	2	3	4	5	6	7	8	9
I	1.12	15.94	63.18	5.90	4.56	8.89	6.17	9.02	11.72
	(1.06)	(3.99)	(7.94)	(2.43)	(2.13)	(2.98)	(2.48)	(3.00)	(3.42)
II		7.67	32.96	15.70	6.48	33.99	11.30	13.45	42.83
		(2.77)	(5.74)	(3.96)	(2.54)	(5.83)	(3.36)	(3.66)	(6.54)
III			0.21	59.09	44.69	77.24	50.85	51.88	80.88
			(0.46)	(7.68)	(6.68)	(8.78)	(7.13)	(7.20)	(8.99)
IV				10.87	6.55	16.60	9.46	11.08	20.74
				(3.29)	(2.55)	(4.07)	(3.07)	(3.33)	(4.55)
V					0.60	18.29	4.38	4.91	25.72
					(0.77)	(4.27)	(2.09)	(2.21)	(5.07)
VI						8.83	13.56	22.61	8.72
						(2.97)	(3.68)	(4.75)	(2.95)
VII							2.56	11.58	25.63
							(1.60)	(3.40)	(5.06)
VIII								13.78	28.19
								(3.71)	(5.31)
IX									0.00
									(0.00)

Plant diversity is a variety and variability of a plant in an ecosystem [9]. Most forest trees are long lived, out breeding and generally highly heterozygous, which have developed a number of natural mechanisms to maintain hetero-zygosity and *intra* specific variations. These genetic mechanisms combined with the often variable environment, in which forest trees occur, have contributed to the fact that, with a few exceptions, forest trees seem to be among the most genetically variable of all organisms studied to date [10]. The extent and pattern of genetic diversity in forest trees are influenced by their native system and the movement of genes between dispersed populations of the same species. Measuring genetic diversity in trees has typically been done by either provenance testing [9] or electrophoresis analysis of the enzymes [11] and [12] and also by DNA based molecular techniques [13,14]. In the current study, genetic diversity existed among the 30 selected genotypes of *Ailanthus excelsa* had been assessed through D^2 analysis which resolved the 30 progenies into nine clusters.

3.3 Cluster Components

The multivariate analysis grouped 30 progenies into nine clusters. The cluster members and number of progenies constituting each cluster are furnished in Table 3. Among the nine clusters, the cluster IV resolved with ten progenies *viz*., FCRI AE 7, FCRI AE 8, FCRI AE 11, FCRI AE 12, FCRI AE 13, FCRI AE 15, FCRI AE 17, FCRI AE 18, FCRI AE 19, and FCRIAE 20. Whereas, Cluster II had five progenies *viz.,* FCRI AE 2, FCRI AE 3, FCRI AE 4, FCRI AE 10and FCRI AE 14 and Cluster I and VI constituted only three progenies each (FCRI AE 1, FCRI AE 4, FCRI AE 9and FCRI AE 21, FCRI AE 22, FCRI AE 24).The cluster III, cluster VII and cluster VIII had two progenies *viz*., FCRI AE 6, and FCRI AE 16; FCRI AE 23 and FCRIAE 29 and FCRIAE 26 and FCRI AE 28 respectively. The cluster IX consisted only one progeny (FCRI AE 25).

The cluster mean for different biometric traits was estimated and furnished in the Table 4.The maximum cluster mean for plant height (69.73 cm) was observed in cluster III, whereas, the least cluster mean for plant height (38.67 cm) was exhibited by the cluster IX. The highest performance in basal diameter was exhibited by the cluster III which accounts 3.88 cm followed by cluster VIII (3.17 cm) whereas, the minimum was observed for the cluster IX (1.65 cm) and in no. of branches the maximum was observed in cluster VI (0.85) and minimum found in cluster VIII (0.00). In case of volume index, the cluster mean was highest for cluster III (1056.78) and the lowest was exhibited by the cluster IX (105.68).

Table 3. Clustering pattern of *Ailanthus excelsa* progenies for morphometric attributes

Cluster No	Number of progenies	Members
I	3	FCRI AE 1, FCRI AE 4, FCRI AE 9
II	5	FCRI AE 2, FCRI AE 3, FCRI AE 5, FCRI AE 10, FCRI AE 14
III	2	FCRI AE 6, FCRI AE 16
IV	10	FCRI AE 7, FCRI AE 8, FCRI AE 11, FCRI AE 12, FCRI AE 13, FCRI AE 15, FCRI AE 17, FCRI AE 18, FCRI AE 19, FCRI AE 20
V	2	FCRI AE 27, FCRI AE 30
VI	3	FCRI AE 21, FCRI AE 22, FCRI AE 24
VII	2	FCRI AE 23, FCRI AE 29
VIII	2	FCRI AE 26, FCRI AE 28
IX	1	FCRI AE 25

Table 4. Cluster mean values of *Ailanthus excelsa* progenies for morphometric attributes

Cluster	Plant height (cm)	Basal diameter(cm)	No. of branches	Volume index (cm^3)
I	45.30	2.27	0.22	238.06
II	58.45	3.05	0.02	561.59
III	69.73	3.88	0.33	1056.78
IV	47.52	2.51	0.14	320.80
V	50.31	2.92	0.11	454.30
VI	40.42	2.10	0.85	183.56
VII	50.79	2.69	0.66	377.56
VIII	46.22	3.17	0.00	503.58
IX	38.67	1.65	0.44	105.68

Clustering methods have the goal of separating a pool of observations in many subgroups to obtain homogeneity within and between the formed subgroups. D^2 statistics is an important tool in plant breeding for estimating genetic divergence [15]. The exploitation of heterosis and success in getting desirable segregates in a breeding programme largely depends on the degree of divergence in a chosen population [16]. Genetic diversity is essential to meet the diversified goals of tree breeding such as breeding for cultivation, increasing yield, wider adaptation, desirable quality, pest and disease resistance. The genetic divergence analysis estimates the extent of diversity existed among selected genotypes [17].

The application of D^2 clustering technique in *Ailanthus excelsa* resolved the thirty genotypes into nine clusters. Among the nine clusters, the clusters IV were the biggest with ten progenies. Similarly, earlier studies in *Ailanthus excelsa*, 30 progenies were grouped into eight clusters, of which group A formed the largest cluster containing ten progenies followed by group B with five progenies [18]. In *Acacia nilotica* also by D^2 clustering technique, 27 seed sources were grouped into five clusters (A, B, C, D and E) which showed that group A was the largest in size and possessed 21 seed sources. Group B and C included two seed sources each and Group D and E included only one seed source each [19]. Similarly, 80 batches of teak had been grouped into eight clusters, of which group A formed the largest cluster containing 46 batches [20].

In the present investigation, it could be seen that the progenies from different locations got clubbed together to form a single major cluster as evident in cluster IV and therefore the pattern of divergence was not depend on the geographic locations. The above findings also confirmed the earlier report of Bagchi [20] in Teak; *Eucalyptus* [21]; Leucaena *leucocephala* [22] and *Melia dubia* [18] and [23]. The inclusion of geographically divergent provenances in the same cluster may be attributed to the fact that the factors other than geographic distribution might be responsible for their genetic

similarity [24]. Hence the divergent progenies used in the current project and grouped under one cluster might be due to the factor other than the geographical distribution as The intra and intercluster analysis indicated that the cluster IX showed that there is no intra cluster generalized distance since it contained only one progeny. The maximum intra cluster distance was shown by the cluster VIII. The maximum inter cluster distance was recorded between cluster III and II which indicated the presence of wider genetic distance between *A. excelsa* progenies. Such inter and intra cluster distance among *Pinus gerardiana* genotypes was also evidenced which support the current conclusion [27].

3.4 Contribution of Characters towards Genetic Divergence

The number of times each character ranking first was counted and percentage contribution towards divergence was calculated and presented in Table 5. Volume index contributed maximum percentage towards divergence (50.34 %) followed by plant height (30.11 %) and number of branches (10.34 %). The minimum percentage contribution towards divergence was recorded by basal diameter (9.19 %).

Table 5. Percentage contributions of morphometric traits of *Ailanthus excelsa* progenies to genetic divergence

S.no	Character	No. of first rank	% Contribution
1.	Plant height	131	30.11
2.	Basal diameter	40	9.19
3.	No. of branches	45	10.34
4.	Volume index	219	50.34
Total		**435**	**100.00**

Volume index contributed maximum towards genetic divergence followed by plant height and the minimum by basal diameter. Paramathma [28] reported similar results in six Eucalyptus species and twelve interspecific hybrids; Bagchi [20] in *Tectona grandis*; Manga and Sen [29] in *Prosopis cineraria*; Tewari et al. [30] in *Dalbergia sissoo*; Chauhan and Sehgal [31] in *P. roxburghii* and Vennila [21] in Eucalyptus also reported evidenced in *Santalum album* [25] and *Prunus armeniaca* [26] 'which supported the results of this work.

contribution of volume index along with other morphometric traits towards genetic divergence among the genotypes tested which might be due to the existence of broader genetic base. Kumar [18] also reported similar results in *Ailanthus excelsa* genetic resources. Based on the past work and present finding, the contributions of volume for genetic divergence indicated that this factor could be used as an index for *Ailanthus excelsa tree* improvement programme.

4. CONCLUSION

The multivariate analysis grouped 30 progeny of *Ailanthus excelsa* genetic resources into nine clusters. Among the nine clusters, the cluster IV has ten progenies. The maximum intra cluster distance was exhibited by the cluster VIII followed by cluster IV. The maximum inter cluster distance was in cluster III which indicated the presence of wider genetic distance between *Ailanthus excelsa* progenies. Among the growth attributes, volume index contributed maximum percentage towards genetic divergence followed by plant height and number of branches while the basal diameter recorded minimal contribution to the divergence.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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