Original Research Article

Genetic divergence between half-sib families under greenhouse environment in Kadamb (*Neolamarckiacadamba*)

ABSTRACT

Neolamarckiacadamba is a widespread medium-sized deciduous forest tree over most of India, Bangladesh, and Sri Lanka. Twenty half sib progenies of kadamb (*Neolamarckiacadamba*) belonging to different geographic regions of Bihar, Rajasthan and Uttar Pradesh were sown in glasshouse condition during 2022 with a view to assess the genetic diversity using non-hierarchical Euclidean cluster analysis. Substantial variability was recorded in morphological and biomass traits of selected plus trees. Significant variations were observed in growth characters of plus tree progenies under greenhouse conditions. The Genetic divergence study reveals that twenty NPTs were grouped into six clusters. The maximum inter cluster distance was observed between cluster VI and V (5.530). The maximum intra cluster distance was shown by cluster (4.258). Hence, the clusters were genetically divergent from each other. Therefore, hybridization involving trees of clusters VI and V is recommended in order to achieve high yielding genotypes in *Neolamarckiacadamba*. Collar diameter contributed maximum to the total divergence and played a predominant role in creating the genetic diversity. On the basis of high cluster mean and wide genetic distance, the superior plus trees of cluster VI (NPT_{18}, NPT_{19}, NPT_{20}) and cluster V (NPT_4, NPT_5, NPT_{11}, NPT_{12}, $NPT_{13,}$ NPT_{17}) may be considered as potential parents for further tree improvement programme in *Neolamarckiacadamba*.

Keywords:*Neolamarckia*Plus Tree (NPTs), genetic divergence, Euclidean cluster, D^2 statistics

INTRODUCTION

Neolamarckiacadamba Syn. A. *indicus,* A. *chinensis* (Lam.), *Anthocephaluscadamba* (Family-Rubiaceae), also known as kadamba, has a prestigious place in Ayurveda, an Indian indigenous system of healing. It is also known as the burflower tree. The Kadamb tree is revered in India as a sacred tree to Lord Krishna and is incorporated in Indian faiths and traditions.This tree is endemic to India, the Indo-Malayan area, Jawa, Sumatra, China, Indonesia, Malaysia, Bangladesh, Sri Lanka, Cambodia, Papua New Guinea, the Philippines, and Singapore **(Bijalwan et al. 2014).**It grows in southern tropical semi-evergreen forests, secondary north Indian tropical wet deciduous forests, and tropical fresh water swamp forests. Except in the desert part of Rajasthan, it is found in semi-evergreen to evergreen woods throughout much of India **(Champion, and Seth, 1968).**

Kadamb (*Neolamarkiacadamba*) is a valuable and rapidly growing tree in South Asia. Kadamb is a medium to big deciduous tree that may grow to be 45 m tall. The bole is straight and cylindrical, frequently branchless for more than 25 m, up to 100 (-160) cm in diameter but usually less, occasionally with tiny buttresses up to 2 m high and reaching up to 60 cm from the stem **(Luna 1996).**Kadamb grows quickly and is ideal for regeneration in watersheds and degraded regions, as well as windbreaks in agroforestry systems. It's also a great shade tree for dipterocarp line planting. It is a lightweight hardwood with a short lifespan. It is mostly used to make pulp for low- and medium-quality paper. Because it is perishable when in touch with the earth, the wood may only be utilized for minor building work indoors **(Pandey et al., 2016).** The bark is used to treat fevers, while the leaf extract is used to gargle **(Kapil et al. 1995)**.According to research, practically all components of the kadam plant are useful in the treatment of numerous ailments. A decoction of the leaves is used to treat aphthae or stomatitis, ulcers, and wounds **(Dubey et al, 2011).**

The species has widespread distribution all throughout India on the slopes of evergreen forests up to 500 m in elevation with enormous variability in its growth, form, vigour, fruit and seed yield. This widespread geographical distribution of the species shows that there should be a wide range of genetic variability in *Neolamarckiacadamba* that needs to be identified and analyzed. Therefore, a significant genetic gain can be achieved by identification and selection of plus trees with desirable traits. The knowledge of genetic divergence in tree species is of paramount importance for any breeding programme. The assessment of genetic diversity is vital for tree improvement (**Zobel, 1971**). The major goal of genetic variation study is to classify different population into similar groups for the conservation purpose or to be used in future breeding and improvement programmes. D^2 analysis is the most appropriate statistical tool to assess the genetic divergence quantitatively between biological populations. In the present investigation, an attempt was made to ascertain the magnitude of genetic diversity among the plus tree species of *Neolamarckiacadamba* using D^2 analysis and to identify the stable and diverse genotypes for *Neolamarckiacadamba* tree improvement programme.

MATERIALS AND METHODS

The investigation was carried out in Nursery of College of Forestry, SHUATS, Prayagraj, U.P. during 2021-22in greenhouse condition.More than ten mature fruits were collected from different parts of the crown of an individual plus tree selected from twenty different regions of Bihar, Rajasthan and Uttar Pradesh on the basis of morphological characters. Observations on fruit characters were taken and after taking observations, seeds were extracted from fruit and kept plus tree and replication wise for taking observations on seed characters in the month of December- January, 2021. The details of the plus trees are given in Table 1.

The seeds were sown in a Completely Randomized Design in three replications in poly bags of size 15 x25 cm filled with amixture of sand, soil and FYM (1 :1 :1) at a depth of 2 cm under greenhouse condition. Regular irrigation, weeding and hoeing wasdone as and when required. The data was recorded immediately after the seeds emerged outsidethe soil i.e., after germination. The morphological and biomass data was recorded. Fifteen seedlings of each plus tree progenies were randomly selected and tagged and the following characters were recorded.

Table 1: Details of twenty plus trees of *Neolamarckiacadamba* collected from twenty different locations.

Seed source	Locality	State	Latitude	Longitude	Tree height (m)	Tree girth DBH (m)	Fruit size (mm)	100- Seed weight (mg)
NPT$_1$	Chajjubagh,Patna,	Bihar	25.6160^{0}N	85.1371^{0}E	17.38	1.02	38.72	17
NPT$_2$	Sohsarai, Bihar Sharif	Bihar	25.2271^{0}N	85.5161^{0}E	10.50	0.40	38.27	15
NPT$_3$	Kalyanbigah	Bihar	25.3831^{0}N	85.5070^{0}E	6.70	0.50	38.61	11
NPT$_4$	Bedhna road, Barh	Bihar	25.4619^{0}N	85.7097^{0}E	12.92	0.31	37.56	12
NPT$_5$	Near Nursery, Chandi	Bihar	25.3192^{0}N	85.4070^{0}E	18.28	1.19	42.71	23
NPT$_6$	Narauli, Karauta	Bihar	25.4651^{0}N	85.4410^{0}E	11.86	0.63	39.16	17
NPT$_7$	Mohmadpur, Samastipur	Bihar	25.9839^{0}N	85.6656^{0}E	6.09	0.76	39.67	20
NPT$_8$	Dumri, Begusarai	Bihar	25.4057^{0}N	86.1202^{0}E	17.00	0.83	38.34	18
NPT$_9$	Patna (Fatuhamarg)	Bihar	25.5712^{0}N	85.2451^{0}E	12.00	0.64	38.27	17
NPT$_{10}$	Sharifganj, Katihar	Bihar	25.5224^{0}N	87.5646^{0}E	5.53	0.584	37.07	11
NPT$_{11}$	Sudari	Bihar	25.3920^{0}N	85.4419^{0}E	14.32	0.45	38.72	22
NPT$_{12}$	NayatolaMadhopur, Bakhtiarpur	Bihar	25.4559^{0}N	85.5361^{0}E	18.00	0.82	34.12	16
NPT$_{13}$	Rahui, Nalanda	Bihar	25.1304^{0}N	85.4475^{0}E	15.24	0.14	40.28	10
NPT$_{14}$	Chandi mod, Harnaut	Bihar	25.3653^{0}N	85.5294^{0}E	16.55	0.74	43.88	21
NPT$_{15}$	Hanuman temple, Sangam	Prayagraj	25.4313^{0}N	81.8813^{0}E	16.76	0.14	41.52	19
NPT$_{16}$	Khan chauraha, Naini	Prayagraj	25.4087^{0}N	81.8405^{0}E	18.93	0.12	36.52	13
NPT$_{17}$	GTB Nagar, Kareli	Prayagraj	25.4255^{0}N	81.8186^{0}E	19.00	1.20	42.55	16
NPT$_{18}$	Subhashchauraha, Civil lines	Prayagraj	25.4500^{0}N	81.8345^{0}E	20.11	0.424	32.19	23
NPT$_{19}$	Saipau road, Dholpur,	Rajasthan	26.7015^{0}N	77.8698^{0}E	13.65	0.96	28.01	19

NPT$_{20}$	Chadi, Jodhpur	Rajasthan	25.3426^0N	75.9606^0E	5.79	0.58	30.90	21

After three months growth fifteen seedlings of each families of plus trees/replication were randomly selected and tagged excluding the border ones during last week of June. The data were recorded on eight morphological and biomass traits viz., seedling height, collar diameter, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, shoot/root ratio, and seedling biomass. Genetic divergence was calculated by using non-hierarchical Euclidean cluster analysis **(Spark, 1973)**and genotypes were grouped into different clusters following Tocher's method as described by **Rao (1952).**

RESULTS AND DISCUSSION

The availability of statistical tool to quantitatively measure the genetic divergence between two or more populations and the relative contribution of individual character to the total divergence has permitted the tracing of evolutionary patterns in some annual species such as rice and tobacco in specific choosing parents for hybridization and general for many crop plants and a few forest tree species **(Rao, 1952, Burley and Burrows, 1972)**. In addition to it, the basic premise of the genetic divergence is to cluster the large number of selected phenotypically superior batches into several small homogeneous groups, so as to include representative plants from each group to reduce the total number of entries in a provenance/progeny trial and seed orchard and to find out the magnitude of divergence among groups, so that the groups having wider genetic variance could be hybridized (crossed) to produce greater genetic diversity at a later period for selection.

The analysis of variance revealed the existence of significant difference among 20 plus tree progenies for all the traits, indicating the existence of huge genetic variability.Twenty plus tree progenies were grouped into six clusters on the basis of morphologicaland biomass traits under greenhouse condition during 2021-22 (Table 2).Cluster V and III exhibited the highest number of 6 and 5 plus trees (NPT_4, NPT_5, NPT_{11}, NPT_{12}, $NPT_{13,}$ NPT_{17}) and (NPT_1, NPT_3, NPT_9, $NPT_{10,}$ NPT_{15}) respectively. Cluster VI comprehended three (NPT_{18}, NPT_{19}, NPT_{20}). Cluster IV exhibited two plus tree (NPT_7, NPT_{14}). Cluster I exhibited two plus trees (NPT_6, NPT_{15}) and Cluster II exhibited two plus trees (NPT_8, NPT_{16}).

Table 2: Distribution of twenty plus trees in different clusters (Greenhouse condition)

Cluster	No. of seed sources	Notation of Cluster
I	2	6, 15
II	2	8,16
III	5	1, 3, 9 , 10, 15
IV	2	7, 14
V	6	4, 5, 11, 12, 13, 17
VI	3	18, 19, 20

Table 3: Inter and intra cluster distance of twenty plus tree progenies of *Neolamarckiacadamba* (Greenhouse condition)

Cluster	I	II	III	IV	V	VI
I	**0.762**	1.079	3.462	2.010	4.096	3.592
II		**0.967**	3.706	1.663	4.339	3.729
III			**4.042**	3.918	4.412	4.865
IV				**1.775**	4.085	4.405
V					**4.258**	5.530
VI						**3.386**

Table 4: Mean cluster for various traits in different cluster for twentyplus tree progenies of *Neolamarckiacadamba* (Greenhouse condition)

Traits Clusters	Seedling height	Collar Diameter	Shoot fresh Weight	Root fresh Weight	Shoot Dry Weight	Root Dry Weight	Shoot Root ratio	Seedling Biomass
I	24.94	2.66	1.05	0.76	0.56	0.40	1.37	0.96
II	23.35	2.70	0.96	0.52	0.47	0.28	1.67	0.75
III	42.30	3.09	2.65	1.35	1.29	0.82	1.69	2.10
IV	30.99	3.32	1.51	0.71	0.56	0.35	1.67	0.91
V	32.45	3.33	2.64	1.49	1.00	0.79	1.39	1.79
VI	16.10	1.16	0.88	0.53	0.21	0.17	1.68	0.38
Percent contribution	8.95	23.68	14.74	7.37	11.05	21.05	10.00	3.16

The distribution of progenies in different clusters indicates that, even though the genotypes were selected from different eco-geographic areas the genetic makeup along with breeding system heterogeneity, genetic drift, natural and unidirectional selection pressure must be the cause of genetic diversity among different progenies besides geographic variations, to some extent the cluster pattern proved that geographical diversity need not necessarily be related to genetic diversity. Therefore, selection of genotypes for hybridization may be made on the basis of genetic diversity rather than geographic diversity. However, due attention needs to be paid to geographic diversity, to accommodate sufficient genetic diversity in the germplasm. The present result supports the findings of **Yadav and Murthy (2022)**in *Balanitesroxburghii*, **Paw et al. (2020)**in *Curcuma caesia*,**Wani and Chauhan (2007)**in *Bauhinia variagata*, **Anand (2003)** in *Bauhinia variegata* and **Parthiban et al. (2019)**in *Neolamarckiacadamba*half-sib progeny, **Gupta, *et. al.*,(2020)**in*Acacia nilotica.*

Table 3 revealed that generalized inter-cluster distance (5.530) was observed highest between cluster VI and V which was followed by cluster VI and III (4.865).The minimum inter-cluster distance was observed between cluster II and I (1.079).The maximum intra cluster was found in cluster V and V (4.258) and minimum was found in cluster I and I (0.762). The inter-cluster distance was as high as 5.530 (between cluster VI and V) which indicated presence of higher order of divergence among the progeny of plus trees.The variation under greenhouse condition for total number of clusters and different cluster containing different characters could be attributed mainly due to environmental differences as the range of day and night temperature and humidity are wide in greenhouse condition.Mean values for morphological and biomass traits under greenhouse condition revealed that maximum was observed by cluster III for six traits, followed by cluster V for two traits (Table 4). Highest mean values for different traits under greenhouse condition was shown by cluster III for seedling height (42.30), shoot fresh weight (2.65), shoot dry weight (1.29), root dry weight (0.82), shoot root ratio (1.69) and seedling biomass (2.10) (Table 4) whereas, collar diameter (3.33) and root fresh weight (1.49) were observed in maximum in cluster V. Contribution of different characters to total divergence is illustrated in Table 4. Collar diameter (23.68%) was the main contribution for genetic divergence followed by root dry weight (21.05%) and shoot fresh weight (14.74%). Minimum contribution was observed for seedling biomass (3.16%) under greenhouse conditions. Further, the result of this study suggest that classification of a number of plus tree progeny using

D^2statistics provide not only a set of groups from which parent can be chosen for further breeding progamme but also indicates that such grouping remains unstable over environments that means the material is vulnerable to the variable environmental conditions. The present study result substantiates, the findings of **Hooda, et al. (2009)**in *Pongamiapinnata* and **Kumar and Gurumurthi (2000)** in*Casuarinaequisetifolia***.**

CONCLUSION

On the basis of present investigation, we could conclude that, the selection of genotypes from the cluster-VI (NPT_{18}, NPT_{19}, NPT_{20}), cluster-V (NPT_4, NPT_5, NPT_{11}, NPT_{12}, $NPT_{13,}$ NPT_{17}) exhibit maximum inter cluster distance and can be utilized as potential parents and crossing between these genotypes resulting in high heterotic expression for yield and its components. Mean values for morphological and biomass traits under greenhouse condition revealed that maximum was observed by cluster III for six traits, followed by cluster V for two traits. Collar diameter was the main contribution for genetic divergence. The genotypes from above clusters may be included in hybridization programme for obtaining superior and desirable recombinants in tree improvement programs.

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