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Progeny testing and genetic diversity of *Melia dubia* plus trees in Kerala

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ABSTRACT: *Melia dubia Cav., a fast-growing multipurpose tree species, holds substantial value for timber production and medicinal applications. This study investigates progeny testing and genetic diversity in 25 half-sib plus tree progenies from diverse regions across Kerala. Morphological and physiological traits were assessed, and genetic variation was analyzed using 15 Inter-Simple Sequence Repeat (ISSR) markers. Significant variability was observed in growth traits, with progeny FCV-MD-03 (Tholpetty, Wayanad) exhibiting superior performance. High heritability estimates for collar girth and transpiration rate highlight their potential as selection criteria. ISSR marker analysis revealed 68% polymorphism, Nei's gene diversity at 31%, and cluster analysis identified two genetic clusters. These findings provide valuable insights for tree breeding and conservation efforts.*

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1. INTRODUCTION

Melia dubia Cav., commonly known as Malabar neem, is a fast-growing, tropical, moist-deciduous species within the Meliaceae family. This species is naturally distributed across regions including Sikkim, the Himalayas, northeastern India, Khasi Hills, Odisha, the Deccan Plateau, and the Western Ghats, typically thriving at altitudes of 1400–1900 m (Shrivastava *et al.*, 2017). Due to its high strength and excellent pulp recovery, M. dubia is highly valued as a raw material in wood-based industries, including plywood, matchwood, and paper production. Economically, it outperforms many other fast-growing timber species, offering a higher rate of return in both pulp yield and profitability. Beyond its industrial value, M. dubia is also known for its medicinal and pesticidal properties, with every part of the plant contributing to traditional herbal medicine preparations for treating conditions such as leprosy, eczema, asthma, malaria, fever, and venereal diseases (Govindachari, 1992).

The genetic diversity of M. dubia, reflected in its wide range of morphological and physiological traits, presents valuable opportunities for tree breeding and improvement programs. Understanding this diversity is essential for selecting superior genotypes suited to various industrial and medicinal applications. Previous progeny studies conducted under nursery conditions have documented substantial growth and physiological variation among seed progenies from natural populations of M. dubia across Kerala (Binu and Santhoshkumar, 2023). Despite its economic significance, a critical gap exists in understanding its genetic diversity and field performance. While previous studies have focused on progeny evaluations under controlled conditions, limited research has assessed their adaptability and growth in natural environments. This study aims to bridge this gap by evaluating progeny performance through an integrated analysis of morphological, physiological, and molecular traits, identifying superior genotypes that can contribute to tree improvement and conservation programs.

2. MATERIAL AND METHODS

The study was conducted at the College of Forestry, Kerala Agricultural University, Vellanikkara, Thrissur, from 2020 to 2021. Situated 40 m above sea level at 10º31'N latitude and 76º26'E longitude, the progeny testing field lies within the college campus and experiences a warm, humid climate with a distinct rainy season, receiving less than 3000 mm of annual rainfall. The soil in this area is porous and well-drained. The experimental material comprised samples from a two-year-old M. dubia gene bank established at the College of Forestry, with 25 plus tree populations representing the natural distribution of M. dubia across Kerala (Table 1). The gene bank includes 150 plus tree seed progenies arranged in a compact family block design with two replications of 25

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Table 1. Details of the 25 selected Melia dubia plus tree mother plants for progeny evaluation.

Sl. No.	Location	Accession No.	Location
1	Thirunelly	FCV-MD-01	11°54'16.9" 75.59°'59.77"
2		FCV-MD-02	11°54'37.68" 76°00'02.40"
3	Tholpetty	FCV-MD-03	11°47'46.90" 76°05'04.10"
4		FCV-MD-04	11°53'13.20" 76°04'34.70"
5	Dasanakara	FCV-MD-05	11°52'53.10" 76°04'39.20"
6	Neykavala	FCV-MD-06	11°25'32.10" 76°06'02.30"
7	Dhoni	FCV-MD-07	10°27'02.10" 76°12'24.30"
8	Pothundy	FCV-MD-08	10°31'00.92" 76°37'00.28"
9	Attappady	FCV-MD-09	11°05'30.50" 76°43'23.50"
10	Thiruvazhiyadu	FCV-MD-10	10°31'15.20" 76°36'18.70"
11		FCV-MD-11	10°31'01.90" 76°36'30.40"
12	Walayar	FCV-MD-12	10°51'35.90" 76°37'15.70"
13		FCV-MD-13	10°51'33.50" 76°37'27.10"
14	Parambikulam	FCV-MD-14	10°22'56.90" 76°45'45.01"
15		FCV-MD-15	10°26'41.3" 76°49'35.90"
16		FCV-MD-16	10°24'40.40" 76°49'35.30"
17	Chinnar	FCV-MD-17	10°21'16.20" 77°11'32.20"
18		FCV-MD-18	10°21'16.20" 77°11'29.60"
19		FCV-MD-19	10°21'13.50" 77°11'38.20"
20	Peechi Thrissur	FCV-MD-20	10°31'56.20" 76°22'26.20"
21		FCV-MD-21	10°29'01.00" 76°22'00.01"
22	Akamala	FCV-MD-22	10°40'46.10" 76°18'10.30"
23	Kulathupuzha	FCV-MD-23	08°50'36.80" 77°02'53.2”
24	Aryankavu	FCV-MD-24	08°51'22.3” 77°08'48.60”
25		FCV-MD-25	08°55'55.40” 77°08'38.70"

accessions, each containing three seedlings (25 x 3 x 2). Regular morphological and physiological observations were conducted on these progenies, and leaf samples from the 25 accessions were collected for genetic and biochemical analysis.

Evaluation of the seed progenies

Biometric data for Plant height, Collar girth, Volume, Absolute growth rate, and Crown width were studied at intervals of three months for one year, *i.e.* 24 Month After Planting (MAP), 27 MAP, 30 MAP, 33 MAP, 36 MAP, along with Physiological Observations like Chlorophyll Content (using SPAD 502, Minolta), Photosynthetic Rate, Transpiration Rate, Leaf Temperature, Stomatal Conductance (using IRGA), Relative Water Content for two periods at 27 MAP and 33 MAP. These traits were subjected to variability Studies *viz.*, Genotypic Variance, Phenotypic Variance (Johnson *et al.* 1955), PCV, GCV, ECV (Burton and De-Vane 1953), heritability (Lush 1940) and genetic advance (Johnson *et al.* 1955)

Analysis of genetic variation

Genomic DNA is extracted using a modified CTAB method (Rawat *et al.*, 2016). The quantity and quality of DNA extracted were estimated using the Nanodrop spectrophotometer and gel electrophoresis. The genetic diversity was analyzed using Fifteen ISSR (Inter-Simple Sequence Repeats) primers (Rawat *et al.*, 2018) (Supplementary material Table 1) in PCR. The gel was profiled under the U.V. Transilluminator and documented using a molecular imager gel Doc^{TM} XR+ imaging system (BIO-RAD). Scoring of bands in agarose gel was carried out based on the presence and absence of the band. The bands were encoded using the binary method of Wendel and Weeden (1989) for the presence (1) or absence (0) of bands, respectively. Based on this, hierarchical cluster analysis was done. Similarity coefficients were computed as Jaccard's coefficient through the SIMQUAL routine, and clustering was done using the SAHN routine of the package. A dendrogram was constructed based on Jaccard's similarity coefficient and Nei's gene diversity coefficient generated using UPGMA (Unweighted Pair Group Method with Arithmetic mean) in NTSYSpc ver. 2 software. Association between the accessions was found from the dendrogram.

3. RESULTS

Evaluation of the seed progenies

Morphological and Physiological traits

Significant differences were observed among the Melia dubia seed progeny accessions in morphological characteristics, including height, girth, volume, and crown diameter, measured at three-month intervals. Notably, progenies FCV-MD-02

(Thirunelly) and FCV-MD-03 (Tholpetty) consistently exhibited superior height, girth, and volume across all growth stages, whereas FCV-MD-25 demonstrated the highest crown diameter throughout the study (Supplementary Tables 2 and 3).

Regarding physiological traits, including chlorophyll content, photosynthetic rate, stomatal conductance, transpiration rate, and relative water content—significant variations were observed among the accessions at 27 MAP and 36 MAP (Supplementary Table 4). Except for chlorophyll and relative water content, all physiological parameters recorded higher mean values at 36 MAP than at 27 MAP, indicating enhanced physiological adaptability over time. Among these, progeny FCV-MD-03 from Tholpetty consistently exhibited the highest values across multiple physiological parameters, reinforcing its potential as an elite genotype for future breeding and genetic improvement programs.

Genetic diversity analysis

The phenotypic and genotypic coefficients of variation (PCV and GCV) were highest for volume (66.8 and 53.9, respectively), followed by crown diameter (44.82, 35.51), collar girth (37.9, 33.1), and height (27.55, 23.7), indicating substantial variability among progenies. The elevated PCV and GCV values underscore these traits' potential for genetic improvement (Table 2).

Among physiological traits, the highest PCV and GCV values were recorded for stomatal conductance (65.28, 58.50), followed by photosynthetic rate (43.74, 37.53), transpiration rate (31.41, 30.03), chlorophyll content (9.01, 8.67), and relative water content (5.06, 4.52). The consistently higher PCV values compared to GCV across physiological traits suggest a notable environmental influence on their expression (Table 2).

Broad-sense heritability estimates ranged from moderate to high for key morphological traits, including collar girth (0.75), height (0.68), volume (0.65), and crown diameter (0.62). The high heritability of collar girth (0.75) indicates a strong additive genetic component, making it a crucial selection criterion for breeding programs.

For physiological traits, heritability estimates were also moderately high, with transpiration (0.91), photosynthesis (0.75), chlorophyll content (0.82), stomatal conductance (0.82), and relative water content (0.79) all exceeding 50%, reflecting strong genetic control. The highest genetic advance (GA) was recorded for volume, followed by crown diameter (73%), collar girth (50%), and height (37%), suggesting substantial scope for genetic improvement in these traits (Table 2).

Path analysis and correlation

Path analysis revealed that morphological traits had the most substantial impact on volume, with girth showing the highest direct effect (0.63), followed by height (0.43). Girth also exerted the strongest indirect influence on volume through its effect on height. In contrast, physiological traits had a minimal impact on volume, with stomatal conductance contributing only marginally (0.01). The low residual value (0.0082) indicates a well-fitted model, affirming the strong genetic influence of morphological traits on tree growth and volume accumulation (Fig. 1).

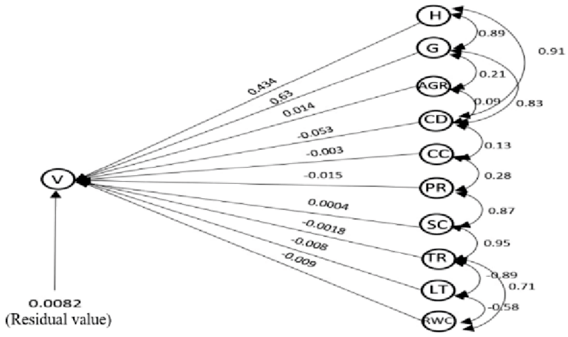

Fig 1. Path diagram

Table 2. Estimated genetic parameters of morphological and physiological traits

Parameters	P.V.	G.V.	H2	PCV	GCV	ECV	GA (%) of mean
Height	1.1	0.8	0.68	27.5	23.7	15	37
Girth	0.01	0.01	0.75	37.9	33.1	18	50
Crown diameter	1.5	0.94	0.62	44.8	35.5	27	73
Volume	0.2	0.18	0.65	66.8	53.9	39	78
Chlorophyll content	60	52	0.86	9	8.67	2.4	18%
Photosynthetic rate	89	67	0.75	43	37.5	21.9	33
Stomatal conductance	84	69	0.82	65	58.5	26.7	13%
Transpiration	1.0	0.9	0.91	31	30.0	9.1	20%
RWC	16	13	0.79	5	4.5	2.2	8%

* **PV**: Phenotypic variation, **GV**: Genotypic variation, **H^2**: Heritability, **PCV**: Phenotypic coefficient of variation, **GCV**: Genotypic coefficient of variation, **GA**: Genetic Advance

Correlation analysis demonstrated strong positive associations among structural traits, including height, girth, crown diameter, and volume (r = 0.83–0.98**), reinforcing their interdependence in growth performance. Moderate to strong correlations were also observed within physiological traits, particularly among photosynthetic rate, stomatal conductance, and transpiration rate (r = 0.61–0.95*). In contrast, the absolute growth rate exhibited weak correlations with other traits, suggesting that structural attributes predominantly influence the overall growth performance of plus tree progenies (Table 3).

Analysis of genetic variation using ISSR molecular markers

The extracted DNA exhibited an OD260/280 ratio between 1.8 and 2.0, with concentrations ranging from 141.94 µg/mL to 1126 µg/mL. For genetic diversity analysis, 15 ISSR primers (Table 4) were selected, generating 164 reproducible amplicons, of which 113 (68%) were polymorphic and 51 were monomorphic. Primers containing dinucleotide repeats such as CA, CT, AG, GA, GT, TC, and AC, along with the trinucleotide repeat ATG, exhibited high polymorphism, suggesting the prevalence of these sequences within the M. dubia genome. The ISSR primers produced a variable number of reproducible bands, ranging from seven (UBC 847) to fifteen (L9), with polymorphic band counts varying between three and thirteen. The highest polymorphism was observed in UBC 847 (86.6%), followed by UBC 809 (85.7%), whereas L4 exhibited the lowest polymorphism (24.2%) (Table 4).

Certain primers generated accession- and population-specific bands, including primer 891 for Chinnar (17) and primers 890, 891, and L9 for populations from Walayar, Thiruvazhiyadu, and Thirunelly, respectively, indicating their potential for reliably identifying specific M. dubia plus tree progenies. The polymorphism information content (PIC) values ranged from 0.05 to 0.30 (Figure 2), with the lowest recorded for primer L4 and the highest for UBC 813, highlighting UBC 813 as particularly effective for polymorphism studies in M. dubia.

Genetic clustering and diversity analysis using DARwin software grouped the 25-plus tree progenies into four clusters based on genetic similarity and geographical distribution (Figure 3). The Jaccard similarity coefficient ranged from 0.604 to 1.000, with the highest similarity observed among accessions from Wayanad (FCV-MD-01 to 06) and the lowest (0.604) between Parambikulam (FCV-MD-14) and Thirunelly (FCV-MD-1). A hierarchical dendrogram from these similarity coefficients revealed two primary clusters (Figure 4). Cluster I consisted of accessions from the Wayanad region, while Cluster II encompassed populations from Palghat, Thrissur, Idukki, Kollam, and Thiruvananthapuram, with further sub-clustering within each major cluster. Populations from regions south of the Palghat Gap (FCV-MD-10 to 13) and those north (FCV-MD-7 to 9) formed distinct sub-clusters. A separate cluster comprised populations from south of the Western Ghats, which further divided into two sub-clusters, one containing the Kulathupuzha (FCV-MD-23) population and the other comprising two accessions from Aryankavu pass in Kollam (FCV-MD-22 and 23). The overall similarity between Clusters I and II was 64%.

Based on Nei's gene diversity coefficient, a second dendrogram revealed an overall genetic diversity of

Table 3. Correlation study of the morphological and physiological characters of plus tree seed progeny

	H	G	AGR	V	CD	CC	PR	SC	TR	LT	RWC
H	1	-	-	-	-	-	-	-	-	-	-
G	.893**	1	-	-	-	-	-	-	-	-	-
AGR	.218	.214	1	-	-	-	-	-	-	-	-
V	.954**	.980**	.232	1	-	-	-	-	-	-	-
CD	.912**	.827**	.095	.868**	1	-	-	-	-	-	-
CC	.271	.147	.370	.177	.129	1	-	-	-	-	-
P.R.	.183	.221	.041	.213	.144	.275	1	-	-	-	-
S.C.	.343	.405*	.089	.395	.245	.373	.874**	1	-	-	-
T.R.	.369	.411*	-.053	.402*	.287	.422*	.840**	.946**	1	-	-
L.T.	-.185	-.231	.103	-.207	-.107	-.399*	-.802**	-.856**	-.895**	1	-
RWC	.304	.314	.058	.311	.306	.397*	.609**	.674**	.708**	-.587**	1

*H: Height, G: Girth, AGR: Absolute growth rate, V: Volume, CD: Crown diameter

CC: Chlorophyll content, PR: Photosynthetic rate, SC: Stomatal conductance,

T.R.: Transpiration rate, L.T.: Leaf temperature, RWC: Relative water content

31% among the 25 progenies, as assessed using the 15 ISSR markers (Figure 5). These findings highlight moderate genetic variation within the M. dubia populations, providing valuable insights for conservation and breeding strategies.

Discussion

Progeny trials conducted on Melia dubia using a compact family block design (CFBD) demonstrated substantial genetic variation among the 25 evaluated progenies, reinforcing the efficiency of this design in minimizing environmental noise and emphasizing genetic determinants of growth. CFBD plays a crucial role in limiting environmental variations, ensuring that observed differences in progeny performance are primarily due to genetic factors rather than external influences. By grouping related progenies into smaller, controlled units, CFBD enhances the precision of genetic evaluations, facilitating a clearer understanding of genotype–phenotype relationships. This allows breeders to make more accurate selections based on heritable traits rather than traits influenced by microenvironmental fluctuations.

The study uniquely integrates field-based progeny evaluations with molecular diversity analysis, addressing a significant gap in previous research primarily focused on nursery-based assessments (Binu and Santhoshkumar, 2023). While nursery trials offer controlled conditions, they often fail to capture the full spectrum of environmental interactions that influence phenotypic expression in field conditions. CFBD bridges this gap, allowing researchers to evaluate progeny under real-world conditions while maintaining control over environmental variability.

The CFBD approach, as advocated by Zobel (1971), has been widely applied in other tree species, particularly for breeding programs aimed at improving commercially important traits. However, its utility in M. dubia remains underexplored. This study establishes its effectiveness in field progeny trials, facilitating highly controlled genetic differentiation among accessions and identifying progenies FCV-MD-02 (Thirunelly) and FCV-MD-03 (Tholpetty) as elite candidates for breeding. The successful use of CFBD in this study highlights its value in tree improvement programs, ensuring that selected progenies exhibit genetic superiority rather than environmentally induced advantages, thus strengthening the genetic gains achieved through selection. Path analysis revealed collar girth and plant height as the strongest determinants of tree volume, with indirect contributions from physiological traits such as stomatal conductance and photosynthetic rate, which function through correlated trait networks. These findings challenge assumptions that physiological traits have negligible direct effects on biomass accumulation (Parthiban *et al.*, 2017), instead underscoring stomatal efficiency as a critical selection criterion. This novel insight aligns with emerging studies on drought-resilient forestry species (Sharma and Bakshi, 2014; Gago *et al.*, 2016) and highlights the potential of M. dubia for climate-adaptive breeding.

Genetic diversity analysis revealed high heritability

Table 4. Total number of bands and polymorphism exhibited by different primers in *M. dubia* plus tree seed progenies

Primer	Total bands	Polymorphic bands	Monomorphic bands	Percentage polymorphism
UBC 809	7	6	1	85.7%
UBC 810	8	6	2	75.0%
UBC 813	10	8	2	80.0%
UBC 816	11	9	2	81.2%
UBC 823	9	6	3	66.6%
UBC 847	15	13	2	86.6 %
UBC 864	12	10	2	83.3%
UBC 884	10	6	4	60.0%
UBC 888	14	6	8	42.0%
UBC 890	10	7	3	70.0%
UBC 891	12	9	3	75.0%
L1	8	6	2	75.0%
L3	12	8	4	66.6%
L4	11	3	8	27.2%
L9	15	10	5	66.6%
Total	164	113	51	

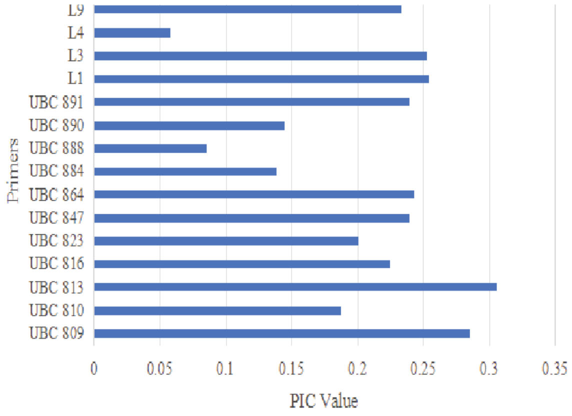

Fig. 2. PIC value of 15 ISSR primer

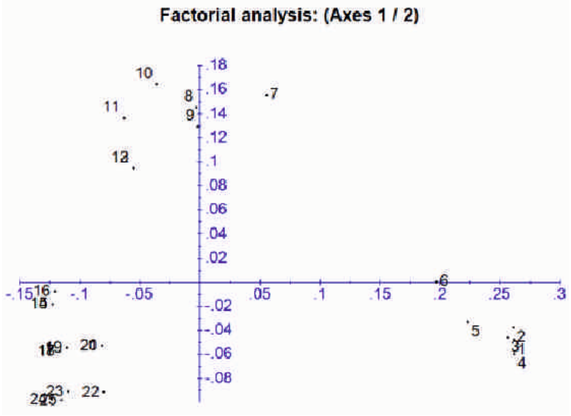

Fig. 3. Factorial analysis based on ISSR primer amplicons.

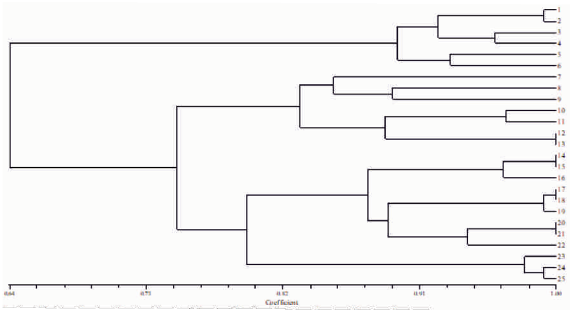

Fig. 4. Hierarchical dendrogram of 25 plus tree progenies based on Jaccard's similarity coefficient.

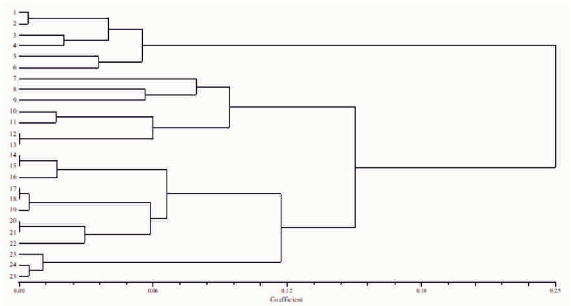

Fig. 5. Hierarchical dendrogram of 25 plus tree progenies based on Nei's gene

polymorphism (68%) validating strong genetic variation within Kerala populations. Notably, this is the first study to assess the genetic diversity of Melia dubia populations in the Western Ghats of Kerala using molecular markers, filling a crucial knowledge gap in the species' genetic resources. The ISSR markers proved to be an efficient and rapid tool for genetic diversity assessment, offering several advantages over other molecular markers such as RAPD, SSR, and AFLP. Unlike SSR markers, which require prior sequence information, or AFLP, which is labor-intensive, ISSR markers are cost-effective, do not require prior genomic data, and generate highly reproducible results in a short time frame, making them ideal for tree breeding and conservation studies. Their ability to detect high levels of polymorphism in non-model species further supports their application in marker-assisted selection (MAS) and genetic fingerprinting.

Cluster analysis using ISSR markers revealed distinct genetic differentiation influenced by geographic barriers, particularly the Palghat Gap, which separated M. dubia progenies into two major genetic clusters. This pattern of genetic structuring across physical barriers is well-documented in other tree species (Woo *et al.*, 2008; Sharma *et al.*, 2011) and underscores the role of topography in shaping gene flow within forest tree populations. Importantly, primer UBC 813 exhibited the highest polymorphic information content (PIC), making it a valuable marker for distinguishing genetic lineages and facilitating population-specific progeny identification. This first-time molecular characterization of M. dubia in Kerala's Western Ghats provides an essential baseline for conservation strategies, ensuring the preservation of unique genetic reservoirs adapted to diverse ecological conditions.

The findings reinforce previous observations on genetic variability (Subramanian *et al.*, 1995; Rawat *et al.*, 2018) and introduce a novel integrative approach by linking morphological, physiological, and molecular analyses into a unified breeding strategy. Identifying genetically superior plus trees validated through morphological traits and ISSR markers lays the groundwork for elite germplasm selection for commercial forestry and breeding programs. Furthermore, this study emphasizes the need for region-specific breeding strategies, particularly for Wayanad populations, which exhibit distinct genetic clustering and suggest unique adaptive traits.

By demonstrating the efficiency of ISSR markers in rapid genetic diversity assessment, this study advances the use of molecular tools in forestry breeding programs, ensuring the selection of high-yield, stress-resilient M. dubia progenies for sustainable plantation management and timber production. The ability to integrate morpho-physiological performance with molecular diversity analysis is a major step forward in the genetic improvement of M. dubia, ensuring enhanced productivity, adaptability, and resilience in this economically and ecologically valuable species.

SUMMARY

The progeny evaluation of Melia dubia populations across Kerala revealed substantial variation in both morphological and physiological traits, underscoring the genetic diversity within this species. Progenies FCV-MD-02 (Thirunelly) and FCV-MD-03 (Tholpetty) demonstrated superior biometric performance, highlighting their potential as valuable candidates for future tree improvement and breeding programs. Variability analysis identified volume (a morphological trait) and stomatal conductance (a physiological trait) as the most variable traits, exhibiting the highest phenotypic and genotypic coefficients of variation (PCV and GCV), indicating strong potential for selection. Heritability estimates were moderate to high, with collar girth and transpiration rate showing the highest heritability in their respective categories, reinforcing their suitability as selection criteria.

The positive correlation between morphological and physiological traits further supports integrated trait selection for genetic improvement. Additionally, ISSR marker analysis revealed 68% polymorphism, confirming substantial genetic diversity among M. dubia progenies. The clustering pattern based on ISSR markers aligned with geographic barriers such as the Palghat Gap, suggesting the influence of natural topography on genetic differentiation. This high level of diversity provides a robust foundation for selective breeding and domestication efforts, emphasizing the need to harness these genetic resources to enhance resilience, productivity, and adaptability in M. dubia populations.

By integrating morphological, physiological, and molecular analyses, this study provides critical insights into the genetic structure of M. dubia and offers a strategic framework for breeding programs. These findings are expected to play a crucial role in conservation efforts, genetic improvement strategies, and the sustainable management of this economically and ecologically valuable species.

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