



Original article

Seed and seedling diversity delimitation and differentiation of Indian populations of *Melia dubia* cav.



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ABSTRACT

Melia dubia is one of the most important industrial tree species in the South East Asia. In last few decades, the populations of *M. dubia* has rapidly expanded in the Indian sub-continent, leading to an increase in the genetic diversity of species. However, very less information is available on intra-specific variation in *Melia* under the Indian subcontinent. Therefore, a present investigation was undertaken, to assess the level of diversity in seed and saplings of the *Melia* populations (ecotypes) collected from three agro-ecological regions of India. Results revealed that the seed and saplings of all the ecotypes are significantly different for all the traits, except for number of branches per plant, and the maximum variability was recorded in germination percentage, seed weight, internodal length, and sapling height of the species. The high heritability for seed weight (0.99), length (0.99), and width (0.97), and germination percentage (0.99) indicated that selection and genetic gain for these traits would be effective during the commencement of improvement program. Trait association analysis explained that higher seed weight significantly reduced sapling height, collar diameter, number of leaves per plant, internodal length, petiole length, and germination percentage ($r = -0.86$; $p < 0.001$) that ultimately reduced the seedling vigor in *Melia dubia*. Interestingly, the number of branches per plant were not associated with any of the morphological traits. The first principal component explained 50.09% of the entire variation and all the traits contributed greatly to the variation for this principal component, except for number of branches, leaf width and seed length. The clustering approach assorted geographic variation of *M. dubia* populations into three main sub-clusters i.e. South, North, and North East populations each consisting of five, seven and one populations (including cultivar), respectively. Among different ecotypes, Bahumukhi, Varsha and US Nagar seed sources outperformed all others in seedling vigour (sapling height) and rest of the growth parameters. Overall, findings explained that considerable scope exists for the development of superior planting material of *M. dubia* through exploration of seeds and selection at the early seedling stage.

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1. Introduction

Globally, forest resources are declining at an alarming rate because of the anthropogenic influences and the changing climatic

conditions. The long-term unremitting adverse effect of these factors causes ecosystem degradation and loss of the species biodiversity (MacDougallet al., 2013), which makes some species threaten with the global extinction (Kumar et al., 2016; Fortini and Dye, 2017). For instance, habitat destruction causes loss of valuable genetic resources of multipurpose tree species that reduces the species biological diversity in natural ecosystem (de Oliveira et al., 2015; Kulkarni and De Laender, 2017). Alternatively, genetic diversity assessment and morphological characterization are prerequisite to enhance climate resilience and productivity of trees through the breeding and selection approaches (Raza et al., 2019). Recognizance of suitable seed source has been considered

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as one of the most important strategies for improvement and gene conservation programmes in tree species (O'Brien et al., 2007).

Melia dubia is widely grown in the tropical and subtropical region of India, South East Asia and Oceania (Ram et al., 2014). In India, *M. dubia* is distributed all over the country, except in high altitudes, and is frequently observed along the roadside, farm boundary, pure plantation and in natural forests (Susheela et al., 2008). It can be grown successfully in all soils types and elevations ranging from 600 to 1800 m having the annual rainfall >500 mm, although species potential was also observed in drylands receiving annual rainfall as low as 200 mm (Pradeep, 2015). Being fast growing and multipurpose in nature, the species are grown extensively in the various Agroforestry systems of India (Anusha, 2012), and interestingly, it is slowly expanding in South East Asia, China, and Australian sub-continent (Parthiban et al., 2009). Species are mainly used in pulpwood, plywood, and timber industry (Chinnaraj and Malimuthu, 2011). Furthermore, some parts of plants have important biological properties as; antiviral, antibacterial, and antifungal (Vijayan et al., 2004; Gerige and Ramjaneyulu, 2007).

Evolutionary evidences suggest that the species with diverse ecotypes and broad distribution depicts high levels of genetic diversity (Francesco et al., 2019) which creates better prospects of selection and improvement in the plant species (Hasan and Abdullah, 2015; Maxted et al., 2006). The natural populations of *M. dubia* contain a large amount of genetic variation, although with respect to the morphological variation diminutive information is documented under the Indian conditions (Rawat et al., 2018). Further consequences of the injudicious exploitation of the species were reflected through a limited number of trees, scattered species population, and restriction distribution in Indian conditions (Kumar et al., 2021a). In spite, negligible attention has given to the seed source identification, systematic cultivation, and conservation practices of *M. dubia* in the region. Moreover, shift in population distribution and ecological niche of *M. dubia* is projected with the changing climate in the tropics (Kramer et al., 2010). Under these conditions, genetic diversity assessment could play an important role in successful cultivation and conservation of the species. Previously, studies on seed source variability has been reported in the several economically important tree species, such as *Celtis australis* in India (Kumar et al., 2018) Kumar et al., 2021b), *Magnolia officinalis* in China (Zheng et al., 2009), and *Tamarindus indica* in Bangladesh (Azad et al., 2014) which suggested the role of genetic variation in evolutionary flexibility and enabling a response to the environmental changes (Booth & Grime, 2003). Further, in most tree species, trait associations have been observed between the seed attributes, and ecological and geo-climatic factors (Villeneuve et al., 2016), and such associations assisted to identify the genetic and environmental factors affecting the plant structure and functions. Notably, in tree breeding program, diverse populations are required to develop desired quality planting material for better economic return to the farmers and industries (Porth and El-Kassaby, 2014). Artificial selection in natural ecotypes leads to the development of complex population structures in the subsequent generations, which determine the population diversity and functions (Glaszmann et al., 2010). Consequently, the seed and seedling diversity analysis in tree species are one of the most important pre-requisite consideration for planning and executing the tree breeding programs (Rengefors et al., 2017). In view of scanty information on population diversity in *M. dubia*, the objective of the present investigation was to measure the diversity and association in seed and seedling traits of *M. dubia* ecotypes collected from the different agro-climatic regions of India. We hypothesized that seed and sapling traits are associated with the species performance at an early stage and to achieve these

objectives, the thirteen 13 ecotypes of *Melia dubia* was considered in the present investigation.

2. Material and methods

2.1. Plant material

A total of thirteen populations of *Melia dubia* were considered for the experiments, out of which four improved populations (i.e. Bhaumukhi, Kshitiz, Ritu and Varsha) were obtained from Forest Research Institute, Dehradun, India, while nine natural population (ecotypes) were collected through exploratory survey from natural as well as cultivated habitats of three agro-ecological regions of India. During the survey, the population consisting of more than 1000 species individuals was selected and divided into various sub-groups adopting the stratified sampling. From each sub-group one tree per stratum was identified and marked purposefully for seed collection adopting the plus tree selection methodology based on the height and diameter growth criterion (Bouffier et al., 2008). A total of ten individuals per population was selected following the quota sampling procedure. The seed samples were collected from three agro-ecological regions, representing the North, South, and North East regions of the India. The each seed source was considered as the representative of *M. dubia* plantations located in cultivated field or natural forests in that region. The geographical features and climatic factors of each seed source are mentioned in Table 1. For each identified seed source, approximately 1 kg seeds per tree were collected from ten candidate trees per population considering minimum 50-meter spatial distance between the strata's to avoid inbreeding/relatedness and maximize variation in the seed sources, as suggested by Zheng et al. (2009).

2.2. Experimental site

The experiments on seed germination and seedling growth were conducted during 2018–19 at ICAR-CSSRI, Karnal, India (29°84'30" N and 76°85'80" E) situated at 245 m amsl. Regional climate is considered as semi-arid, characterized by winters from November to March and summers from April to October. A mean (2018 & 2019) monthly maximum (38.6 °C) and minimum temperature (5.8 °C) was recorded during May and January, respectively. The two years mean annual precipitation of the region was 942 mm, with 80% received during the rainy season (July–September).

2.3. Experiment setup and data measurement

Seed variability traits (i.e. length and width in millimeter) were measured using a micrometer (Besto make) in the laboratory (Fig. 1). Five samples per seed source, each containing 10 seeds per replicate were considered in measuring the seed dimensions. Further seed germination and emergence observations were recorded up to forty days as per the procedure described by Kumar et al. (2014). Seed weight (g) was recorded as per ISTA standard using an electronic weighing balance. Further, for recording the seedling traits, 20 seeds per ecotype were grown in polybags sized 23 × 11 cm filled with a mixture containing soil (sandy loam), vermicompost, and sand in 2:1:1 ratio and normal tap water was applied every alternate day for proper growth of the seedlings. Ten six months old *Melia dubia* seedlings of each seed source were tagged to record the various seedling growth parameters, such as seedling height, collar diameter, leaf length, leaf width, internodal and petiole length. Leaf length and width (mm) were measured with the help of the leaf area meter, while collar diameter

Table 1
Ecological and climatic factors of different sites considered for the seed collection of *Melia dubia*.

Ecological Regions	Seed source	Latitude	Longitude	Altitude (m)	Rainfall (mm)	Average annual temperature (°C)
North	Bhaumukhi*	30°20'41"	77°59'55"	665.0	1896.0	21.8
	Kshitiz*					
	Ritu*					
	Varsha*					
	Fatehabad					
	Panipat					
US Nagar	29°28'48"	75°18'45"	223.0	390.0	24.9	
South	Nilgiri	29°21'17"	76°49'45"	235.0	672.0	24.6
	Coimbatore-1	28°58'52"	79°31'29"	215.0	1250.0	24.3
	Coimbatore-2	11°32'18"	76°56'01"	1231.0	1337.0	17.6
	Peechii	29°38'39"	79°38'02"	1216.0	634.0	26.5
	Salem	11°07'37"	76°53'42"	821.0	618.0	26.3
North-East	Hojai	10°31'34"	76°21'37"	84.0	3000.0	27.5
		11°37'46"	78°11'26"	480.0	900.0	27.2
		26°05'24"	92°51'59"	1220.0	1562.0	24.4

*Improved populations of *Melia dubia*.



Fig. 1. Morphological variability in *Melia dubia* in different regions of India.

was measured using a Vernier Caliper. Petiole length (mm) of the longest leaf was measured manually through metric scale. However, internodal length (mm) was measured between the third and fourth fully extended nodes from the apical meristem using a metric scale. Number of branches per plant and number of leaves per branch on each plant were counted manually.

2.4. Statistical analysis

All the recorded data were spread out on the excel sheet and arranged thematically for analysis. A Statistical Tool for Agricultural Research (STAR) software developed by International Rice Research Institute (IRRI), Philippines (IRRI, 2016), was applied for group comparison, analysis of variance and repeated measures. Further, means of seed and seedling parameters were compared through Duncan's multiple range test (DMRT) procedure (Steel and Torrie, 1980) at the 5% level of significance. Variances (phenotypic, genotypic and environmental) and their coefficients of variations were estimated as per procedure elaborated by Burton

(1952). Broad sense heritability ($h^2b = V_G/V_P$), genetic advance (GA), and genetic gain (GG) were computed in Microsoft excel sheet adopting the methodology described by Johnson et al. (1955). Heatmaps along with correlation coefficients and their significance was constructed using R program (R Core Team, 2016) following R package: corrplot (Wei and Simko, 2021). Principal component analysis (PCA) and dendrogram were constructed using Clustvis software (Metsalu and Vilo, 2015). Singular value decomposition (SVD) of the data matrix imputation is used to calculate the principal components; however, the ward aggregation method was applied to construct a dendrogram to graphically represent the variability and to group the similar types among the different seed sources.

3. Results

3.1. Estimates of variability and genetic parameters

Mean seedling height and collar diameter in different seed sources was ranged between 38.0–83.0 cm and 4.89–10.26 mm, respectively. The maximum genotypic and phenotypic variability was observed in 100 seed weight followed by a germination percent (Table 2) as indicated by maximum genotypic and phenotypic variance alternatively least genetic and phenotypic variability was observed in leaf width. Similarly the maximum phenotypic (2495), genotypic (2500) and environmental (162) coefficient of variation was observed for 100 seed weight (Table 3). However, minimum phenotypic (24.23), genotypic (18.69) and environmental CVs (15.42) were recorded for the leaf width. Genotypic variance and genotypic CV for Seed traits (length, width, 100 seed weight, and germination) and seedlings parameters (petiole length, internodal length, leaf length, and width) were observed higher, compared to corresponding environmental variance and environmental CV. It indicated that the genetic component of variance had the greatest contribution to the total variance, and these traits are least influenced by the environment. Alternately, sapling height, collar diameter, number of branches per plant, and number

Table 2
Variability estimates for seed and seedling characteristics.

Parameters	Range	Mean	Standard deviation	Coefficient of variation
Height (cm)	38.00–83.00	61.45	9.87	16.06
Collar diameter (mm)	4.89–10.26	7.09	1.30	18.35
Number of branches (no.)	5.00–16.00	32.87	9.08	27.62
Leaves per branch (no.)	12.00–49.00	12.80	3.31	25.83
Internodal length (cm)	8.00–21.45	2.22	1.43	64.68
Petiole length (mm)	0.70–6.38	4.49	1.13	25.05
Leaf length (cm)	2.20–6.30	1.92	0.33	17.23
Leaf width (cm)	1.40–2.70	1.92	0.33	17.23
Seed length (mm)	4.50–17.26	9.70	3.56	36.73
Seed width (mm)	3.40–15.00	8.28	2.75	33.16
100 Seed weight (g)	47.00–222.80	119.53	59.52	49.80
Germination (%)	5.00–95.00	41.69	33.26	79.78

Table 3
Variances and coefficient of variability for seed and seedling characteristics.

Parameters	V_E	V_G	V_P	ECV	GCV	PCV	h^2b	GA	GG (%)
Height (cm)	85.18	33.82	119.00	118.13	74.44	139.63	0.28	6.39	10.46
Collar diameter (mm)	1.08	0.56	1.64	40.51	29.23	49.96	0.34	0.90	13.73
Number of branches (no.)	6.07	0.60	6.67	86.57	27.29	90.77	0.09	0.48	5.94
Leaves per branch (no.)	56.65	29.57	86.22	138.98	100.41	171.45	0.34	6.56	22.37
Internodal length (cm)	4.99	5.12	10.11	62.41	63.24	88.85	0.51	3.32	25.92
Petiole length (mm)	0.49	1.66	2.15	46.93	86.67	98.55	0.77	2.33	105.61
Leaf length (cm)	0.18	1.12	1.30	19.97	49.98	53.82	0.86	2.03	45.12
Leaf width (cm)	0.05	0.07	0.11	15.42	18.69	24.23	0.59	0.41	21.43
Seed length (mm)	0.16	13.22	13.38	18.16	166.08	167.07	0.99	7.45	155.35
Seed width (mm)	0.24	7.67	7.91	25.82	146.17	148.43	0.97	5.62	156.47
100 Seed weight (g)	15.86	3720.71	3736.57	162.90	2495.10	2500.44	0.99	125.30	2098.05
Germination (%)	7.10	1159.86	1166.96	41.27	527.44	529.05	0.99	69.94	167.76

Note: V_P , Phenotypic variance; V_G , Genotypic variance; V_E , Environment variance; GCV, Genotypic coefficient of variation; PCV, Phenotypic coefficient of variation; ECV, Environment coefficient of variation; h^2b , Broad sense heritability; GA, Genetic Advance; GG, Genetic gain.

of leaves per branch showed a comparatively lower genetic variance indicated that these traits are highly influenced by the environment. Broad sense heritability estimation was varied from 0.09 (number of branches) to 0.99 (100 seed weight). Notable, it was observed that all the seed traits are heritable since the heritability of all the seed traits were >0.97 . In sapling traits, it observed that leaf length was highly heritable followed by petiole length. On the other hand, number of branches per plant was least heritable followed by sapling height, and were highly influenced by the environment. Genetic gain was ranged between 5.94 (number of branches) and 2098% (100 seed weight). Some parameters such as leaf length and petiole length explained the higher heritability (0.86 and 0.77), but depicted the lower genetic gain (Table 3).

3.2. Traits variability in *Melia* ecotypes

Analysis of variance revealed that all the ecotypes were significantly different ($p < 0.07$) for most of the traits, except number of branches (Table 4a, b). Repeated measure analysis indicated that the sapling height, number of leaves per plant, and internodal length largely increased in Bahumukhi and Varsha germplasm, compared to rest of the population. In absolute terms, 42 and 40% higher growth height was recorded in Varsha and Bahumukhi, respectively, compared to the Salem seed source, which recorded the lowest sapling height. Similarly, collar diameter growth was also observed significantly higher in Varsha (53%) followed by Bahumukhi (39%) and US Nagar (37%) seed sources, compared to Coimbatore-1 which attained lowest growth in collar diameter. However, number of branches per plant (11.0) were observed maximum in US Nagar and minimum (5.67) in the Coimbatore-1 seed source. Collectively, highest number of leaves per branch (44.0), inter-nodal (17.87 mm), and petiole length (5.43 mm) was observed in Bahumukhi seed source, while, maximum leaf length

and leaf width were observed in Coimbatore-2 and Kshitiz seed source, respectively. Group comparison analysis revealed that seed sources from North and South region were significantly diverse only for the inter-nodal length, leaf length, and leaf width.

However, seed sources from North and North East region were significantly different for collar diameter and leaf width. Similarly, South and North-East seed source were significantly different from each other in all the recorded traits, except petiole length. Interestingly, variability in sapling growth pattern between the improved and feral seed sources explained that, these were significantly diverse from each other in all the traits except collar diameter and number of branches per plant.

3.3. Seed traits variability in *Melia* ecotypes

Initially, the seed morphological traits were measured in the laboratory. Analysis of variance showed that all the seed sources were significantly different from each other for seed length, seed width, 100 seed weight and germination percentage (Table 5). Maximum seed length (16.95 mm) and breadth (11.77 mm) were recorded in the Hojai and Fatehabad seed sources, respectively, while the lowest seed length (4.63 mm) and seed breadth (3.60 mm) was recorded in the Kshitiz seed source. Seed germination percentage is an important trait for growers and foresters, and in our study, out of 12 seed sources maximum seed germination percentage was observed in Hojai (73%) followed by US Nagar (68%) and Bahumukhi (67%) seed sources. However, the minimum seed germination was observed in Peechi (13%) followed by Nilgiri and Coimbatore-2 seed sources. The variability for most of the seed traits was highly significant in ecotypes from the North, South and North East regions of India. Contrast analysis depicted that seed sources from North and North East regions were similar in seed width and 100 seed weight and the reason may be non-availability of sufficient *Melia* ecotypes in the North-East region.

Table 4
a,b: Mean comparison of growth parameters in saplings of *Melia* ecotypes.

(a)					
Ecological Region	Population Name	Growth Parameters			
		Sapling Height (cm)	Collar Diameter (mm)	Number of Branches	Number of leaves
North	Bhaumukhi	73.3 ± 2.40 ^a	8.40 ± 0.78 ^{ab}	9.67 ± 2.03 ^a	44.33 ± 3.18 ^a
North	Fatehabad	58.0 ± 3.06 ^{ab}	7.23 ± 0.28 ^{bc}	7.33 ± 0.33 ^a	41.33 ± 4.10 ^{ab}
North	Kshitiz	66.3 ± 8.35 ^{ab}	7.20 ± 0.42 ^{bc}	8.00 ± 1.73 ^a	38.67 ± 6.44 ^{abc}
North	Panipat	64.0 ± 9.54 ^{ab}	7.43 ± 1.43 ^{abc}	8.33 ± 0.88 ^a	34.33 ± 5.21 ^{abcd}
North	Ritu	63.7 ± 0.88 ^{ab}	6.90 ± 0.26 ^{bc}	7.67 ± 0.88 ^a	27.00 ± 6.11 ^{bcd}
North	US nagar	64.0 ± 2.08 ^{ab}	8.27 ± 0.77 ^{ab}	11.00 ± 1.53 ^a	32.00 ± 1.15 ^{abcd}
North	Varsha	74.3 ± 2.19 ^a	9.23 ± 0.09 ^a	6.00 ± 0.58 ^a	37.67 ± 2.03 ^{abc}
South	Coimbatore-1	59.7 ± 4.33 ^{ab}	6.03 ± 0.45 ^c	5.67 ± 0.33 ^a	28.67 ± 4.18 ^{bcd}
South	Coimbatore-2	62.3 ± 4.37 ^{ab}	6.37 ± 0.09 ^{bc}	7.00 ± 1.15 ^a	25.33 ± 1.20 ^{cd}
South	Nilgiri	53.8 ± 0.88 ^b	6.17 ± 0.15 ^c	9.67 ± 0.88 ^a	28.33 ± 2.03 ^{bcd}
South	Peechii	53.4 ± 0.32 ^b	6.40 ± 0.10 ^{bc}	10.00 ± 0.58 ^a	27.00 ± 1.53 ^{bcd}
South	Salem	52.3 ± 3.71 ^b	6.13 ± 0.75 ^c	9.00 ± 1.00 ^a	22.67 ± 5.61 ^d
North East	Hojai	53.7 ± 7.88 ^b	6.53 ± 0.61 ^{bc}	9.67 ± 3.28 ^a	40.00 ± 6.03 ^{abc}
Contrast					
Population		*	*	NS	*
North vs South		NS	NS	NS	NS
North vs North East		NS	*	NS	NS
South vs North East		*	**	*	*
Natural vs Improved		*	NS	NS	*
(b)					
Ecological Region	Population Name	Growth Parameters			
		Inter-nodal length (mm)	Peduncle length (mm)	Leaf length (cm)	Leaf width (cm)
North	Bhaumukhi	17.87±1.83 ^a	5.43±0.92 ^a	4.47±0.52 ^c	2.30±0.10 ^{ab}
North	Fatehabad	9.60±0.17 ^d	1.90±0.31 ^{cdef}	3.07±0.43 ^e	1.67±0.13 ^{def}
North	Kshitiz	12.93±0.41 ^{bcd}	2.43±0.55 ^{cd}	3.50±0.29 ^{de}	1.60±0.10 ^{ef}
North	Panipat	10.33±1.20 ^{cd}	3.13±0.28 ^{bc}	3.33±0.17 ^{de}	1.60±0.10 ^{ef}
North	Ritu	14.17±3.23 ^{abc}	2.47±0.20 ^{cd}	4.83±0.44 ^{bc}	2.07±0.23 ^{abcd}
North	US nagar	12.47±2.04 ^{bcd}	2.23±0.34 ^{cde}	4.10±0.21 ^{cd}	2.23±0.23 ^{ab}
North	Varsha	15.80±1.69 ^{ab}	2.63±0.27 ^{bc}	3.70±0.15 ^{de}	1.77±0.03 ^{cdef}
South	Coimbatore-1	10.13±0.58 ^{cd}	0.77±0.03 ^f	5.60±0.12 ^{ab}	1.90±0.15 ^{bcd}
South	Coimbatore-2	8.83±0.15 ^d	0.90±0.06 ^f	6.00±0.17 ^a	2.13±0.09 ^{abc}
South	Nilgiri	9.97±0.09 ^{cd}	0.97±0.03 ^{ef}	5.27±0.09 ^{ab}	1.90±0.06 ^{bcd}
South	Peechii	9.67±0.09 ^{cd}	1.03±0.03 ^{ef}	5.97±0.15 ^a	2.00±0.06 ^{abcde}
South	Salem	12.87±0.41 ^{bcd}	1.23±0.09 ^{def}	5.43±0.12 ^{ab}	2.37±0.09 ^a
North East	Hojai	13.13±1.53 ^{bcd}	3.77±0.71 ^b	3.17±0.17 ^e	1.50±0.00 ^f
Contrast					
Population		**	***	***	***
North vs South		*	NS	***	***
North vs North East		*	NS	NS	***
South vs North East		*	NS	**	*
Natural vs Improved		*	***	*	**

Note: Within each column, means followed by same superscript are not significantly different at 5% level of significance (DMRT test). NS- Non-significant difference. *,** indicating Significant differences at $p < 0.07$, and < 0.01 respectively. Data represented in the form of mean ± standard error.

Note: Within each column, means followed by same superscript are not significantly different at 5% level of significance (DMRT test). NS- Non-significant difference. ***,*** indicating significant differences at $p < 0.07$, < 0.01 , and < 0.001 , respectively. Data represented in the form of mean ± standard error.

Interestingly, natural and improved seed sources were significantly diverse for all the recorded seed traits indicating that seed traits may play an important role in the selection of suitable seed sources.

3.4. Traits associations in *Melia* ecotypes

Karl Pearson's traits association coefficients and their level of significance depict that the seedling height was significantly positively associated with collar diameter ($r = 0.67$; $p < 0.001$), number of leaves per plant ($r = 0.43$; $p < 0.01$), internodal length ($r = 0.44$; $p < 0.01$), and petiole length ($r = 0.40$; $p < 0.05$). Further, it was observed that higher seed weight significantly reduces the sapling height, collar diameter, number of leaves per plant, internodal length, petiole length, and germination percentage ($r = -0.86$; $p < 0.001$) in *Melia dubia* and ultimately reduced the seedling vigor indirectly. Alternatively, seed weight showed a significant positive association with leaf length (0.80 ; $p < 0.001$), leaf width ($r = 0.32$;

$p < 0.05$), and seed width ($r = 0.55$; $p < 0.001$). Accordingly, a significant positive linear association of collar diameter was observed with the sapling height, number of leaves per plant ($r = 0.42$; $p < 0.01$), inter-nodal length ($r = 0.49$; $p < 0.01$), and germination percentage ($r = 0.46$; $p < 0.01$), and the significant negative association was observed with leaf length, seed width, and seed weight (Fig. 2). Interestingly, a good indication was observed in *Melia* ecotypes with respect to the number of branches per plant; as it was not associated with any of the morphological traits. Therefore, negative selection can be applied for selecting single stemmed *Melia* saplings in segregating populations and various seed source progenies to develop improved *Melia dubia* genotypes for growers as well as timber industries.

3.5. Principal component analysis (PCA) and clustering

Principal components (PCs) analysis exploited the total variability among *Melia* ecotypes based on the phenotypic descriptors. The

Table 5
Mean comparison of seed parameters of *Melia* ecotypes.

Ecological Region	Population Name	Seed Parameters			
		Seed length (mm)	Seed width (mm)	100 Seed weight (g)	Germination %
NORTH	Bhaumukhi	6.00 ± 0.06 ^f	5.20 ± 0.15 ^d	88.67 ± 2.40 ^e	67.28 ± 1.39 ^b
NORTH	Fatehabad	14.10 ± 0.20 ^b	12.00 ± 0.34 ^a	112.80 ± 1.10 ^e	36.26 ± 1.04 ^d
NORTH	Kshitiz	4.63 ± 0.07 ^g	3.60 ± 0.15 ^e	50.93 ± 1.21 ^f	38.63 ± 1.23 ^d
NORTH	Panipat	12.70 ± 0.61 ^c	11.00 ± 0.67 ^a	96.20 ± 3.47 ^f	38.43 ± 1.42 ^d
NORTH	Ritu	5.67 ± 0.18 ^f	5.70 ± 0.18 ^d	88.53 ± 1.81 ^e	64.45 ± 1.29 ^b
NORTH	US nagar	11.50 ± 0.35 ^d	9.60 ± 0.67 ^b	51.27 ± 1.19 ^f	67.61 ± 1.88 ^b
NORTH	Varsha	5.33 ± 0.09 ^f	4.00 ± 0.12 ^e	68.93 ± 1.22 ^h	45.76 ± 1.16 ^c
SOUTH	Coimbatore-1	10.10 ± 0.17 ^e	10.00 ± 0.17 ^b	191.00 ± 1.15 ^b	18.42 ± 0.55 ^e
SOUTH	Coimbatore-2	9.90 ± 0.17 ^e	9.90 ± 0.17 ^b	187.00 ± 1.15 ^{bc}	15.32 ± 0.65 ^{ef}
SOUTH	Nilgiri	10.10 ± 0.15 ^e	10.00 ± 0.15 ^b	171.30 ± 1.19 ^d	13.76 ± 0.42 ^f
SOUTH	Peechii	9.70 ± 0.06 ^e	9.70 ± 0.06 ^b	215.20 ± 6.63 ^a	12.92 ± 0.00 ^f
SOUTH	Salem	9.40 ± 0.12 ^e	9.40 ± 0.12 ^b	181.30 ± 2.33 ^c	16.07 ± 0.36 ^{ef}
North East	Hojai	17.00 ± 0.24 ^a	7.20 ± 0.21 ^c	49.00 ± 1.01 ^f	72.79 ± 2.21 ^a
Contrast					
Population		***	***	***	***
North vs South		***	***	*	***
North vs North East		***	NS	NS	***
South vs North East		***	***	***	***
Naturalvs Improved		***	***	***	***

Note: Within each column, means followed by same superscript are not significantly different at 5% level of significance (DMRT test). NS- Non-significant difference. *, **, *** indicating significant differences at $p < 0.07$, <0.01 , and <0.001 , respectively. Data represented in the form of mean ± standard error.

first three PCs together explained 84.02% of the total variation for the seed and seedling growth traits. The first principal component explained 50.09% of the entire variation. All the traits except number of branches, leaf width, and seed length contributed greatly to the variation for this principal component, because the contribution of associated SVD in the first principal component is equal or greater than 0.290 (Table 6 and 7). The second principal component explained 20.85 % of the total variation, and the sapling height, internodal length, leaf length, leaf width, and seed width contributed greatly to this PC. The third principal component explained only 13.08 % variation, and the number of branches, leaf length, and germination percentage contributed to variation in this component. PCA biplot also bi-furcated the *M. dubia* ecotypes in the three distinct groups as per their adaptability in different agro-ecological regions (Fig. 3). Dendrogram was used to graphically represent clustering pattern based on the relationship between 13 populations and their morphological traits. As shown in Fig. 4, clusters highlighting the presence of strong genetic diversity for different traits in *Melia dubia*. Group 1 comprises of four germplasms (Kshitiz, Bhaumukhi, Varsha, Ritu, U S Nagar), while group 2 had only three germplasms (Panipat, Hojai, Fatehabad). Group 3 contains six germplasm (Salem, Coimbatore-1, Coimbatore-2, Peechii, Nilgiri), and it was the largest among the three groups.

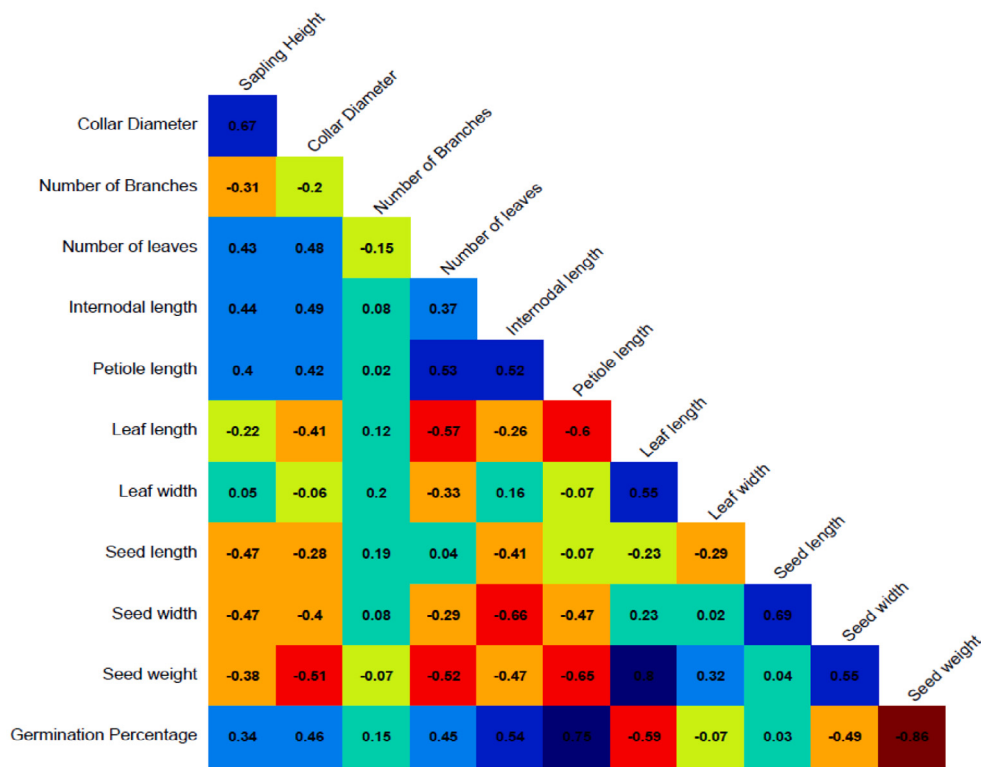
4. Discussion

The present investigation is first and unique study in term of diversity analysis in *Melia dubia* populations collected from different agro-ecological regions of India. The various investigators have conducted studies on molecular genetics approaches, genetic variability analysis, and population structure in *M. dubia* (Rawat et al., 2018; Kumar et al., 2013; Sharma et al., 2019). The RAPD marker analysis shows considerable genetic variation in 24 populations of *Melia* in North-West India ((Johar et al., 2017)). Likewise, ISSR marker explained higher (68%) genetic variability within the eighteen seed sources of *M. dubia* (Rawat et al., 2018). The present study also explained high population genetic diversity in *M. dubia*

species across its geographic distribution range in the Indian sub-continent. Moreover, in most of the plant species, the seed and seedling growth traits are mainly controlled by the genotype rather than the environmental factors (Khan et al., 2012). For example, in *Celtis australis* (Kumar et al., 2021a), *Magnolia officinalis* (Shu et al., 2012), and *Tamarindu sindica* (Azad et al., 2014), seed size is strongly influenced by the genetic factors and therefore, such variability in tree species indicates great potential for selection and improvement in the species. Our research findings further explained that higher genetic variance and genetic gain for the various seedling growth (height and collar diameter) and leaf traits (number, length and width) was observed in the *M. dubia* species, indicating the strong possibility of achieving improvement in these traits through simple selection and conventional tree breeding method. Higher genotypic coefficient of variability with higher genetic advance for seedling height and collar diameter traits could change to a large extent, if superior individuals are considered through selection at the 5% level of selection intensity. Clearly, selection for one or more traits depends on the correlated response with higher heritability, which could be used as a gross indicator for the selection (Prokuda and Roff, 2014).

Overall, our results explained that seed source variation affected the growth of *M. dubia* seedlings. Repeated measures analysis indicated that Bahumukhi, Varsha and US Nagar seed sources outperformed to all other seed sources, in terms of the growth and germination percentage. Eventually, it can be predicted that these seed sources have the better adaptability and growth response, compared to others; once exposed to the regional climatic factors. Broadly, seed sources depicting better early seedling growth are anticipated to be least affected by the regional environmental stresses (Ahanger et al., 2017). Therefore, improvement in the growth traits as a consequence of seed source variation, leads to increased tree biomass production (Whittet et al., 2016). In general, identification of superior seed sources of tree species for climate related adaptation could reduce regional vulnerability to the climate change. In our study, higher variability was observed for seed and seedling characteristics of the *M. dubia* seedlings. Similar observations on seed source variation in a large number of spe-

a



b)

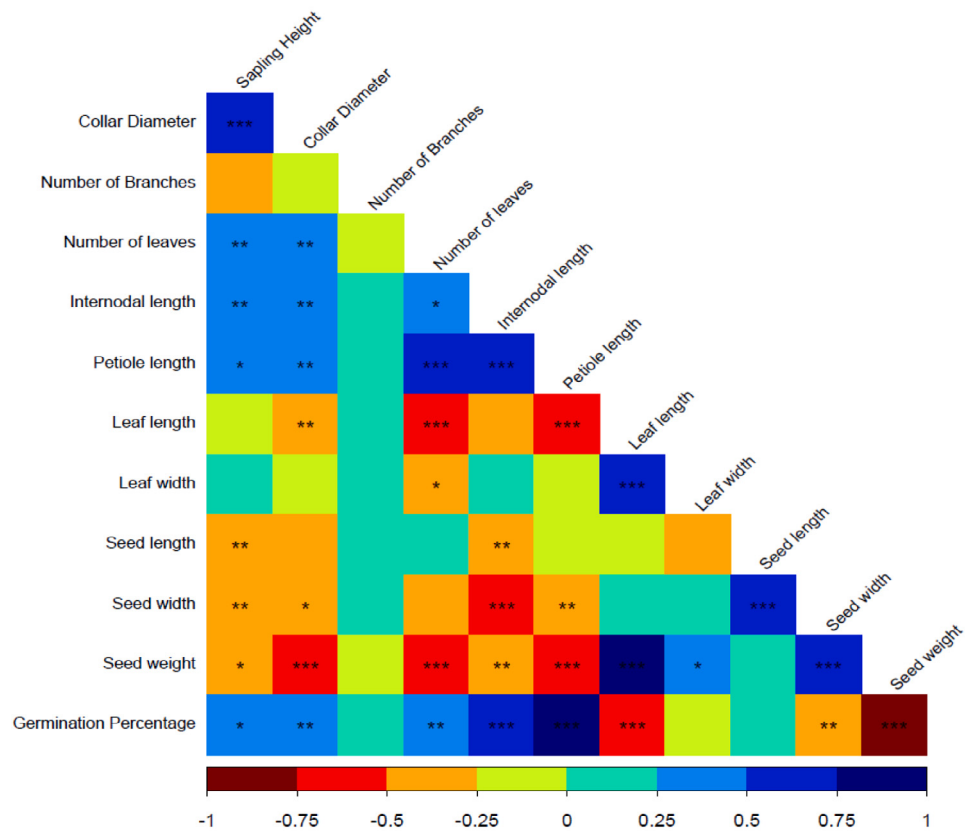


Fig. 2. Traits association in melia populations (a) associations are depicted by colour and values in boxes as Karl Pearson's coefficients (b) Significant associations are depicted by astricks. * means significant at $p < 0.05$, ** means significant at $p < 0.01$ and *** means significant at $p < 0.001$.

Table 6
Proportion of total variability among *Melia* germplasm explained by the principal components analysis.

Principal Components	% Variability	% Accumulated variability
PC1	50.09	50.09
PC2	20.85	70.94
PC3	13.08	84.02
PC4	5.73	89.75
PC5	3.50	93.24
PC6	3.23	96.47
PC7	1.39	97.86
PC8	0.91	98.78
PC9	0.82	99.59
PC10	0.31	99.90
PC11	0.10	100.00
PC12	0.00	100.00

cies were also reported previously by Murali (1997) and Saleem et al. (1994). The genetic variability in populations is responsible for differences in the plant morphological traits (Singh and Pokhriyal, 2000). Further, geographical and environmental factors also determine the genetic constitution of plant species that results in the morphological variation within the same species (FAO, 1985). Accordingly, a particular plant population may exhibit better adaptation under the changing climatic scenario, compared to rest of the populations. Consequently, genetic variability within a species can play significant role in improving the growth and productivity of agroforestry trees under the climate change scenario (Dawson et al., 2011). present result showed a limited magnitude of variation among populations belonging to the same ecological region and high degree of variation within ecotypes belonging to the different regions.

Previous investigation also advocated the similar results on genetic variability in the *M. dubia* (Rawat et al., 2018). Nonetheless, inter-population variability within the same ecological region was responsible for population’s differentiation of all the three ecological regions. The confined variation among populations of each ecological subgroup suggests closeness and relatedness in the species individuals. The multipurpose nature of *Melia* contributes in the sustainable livelihood of the local people; consequently, anthropogenic seed dissemination between different geographical regions could also be accountable for the low variability in *Melia* populations within the each ecological region. In contrast, the low variability among the populations may result from the occurrence of socio-economic and historical disturbances in the *Melia* forests. Group comparison analysis showed that the population differentiation for different traits was more prominent between the geographical regions. In spite of the large distances, some populations were genetically connected to each other in North and

Table 7
Associated SVD- Values in major 6 principal components for different traits of melia.

Growth & Seed Parameters	PC1	PC2	PC3	PC4	PC5	PC6
Sapling height	-0.311	0.273	0.223	-0.402	-0.016	0.019
Collar diameter	-0.338	0.103	0.041	-0.534	-0.254	0.240
Number of branches	-0.019	-0.131	-0.712	0.093	-0.157	0.596
Number of leaves	-0.329	-0.235	0.098	-0.105	0.518	0.284
Inter-nodal length	-0.333	0.259	-0.185	0.181	0.208	-0.178
Petiole length	-0.355	-0.065	-0.219	0.000	0.521	-0.089
Leaf length	0.296	0.384	-0.147	0.026	0.200	-0.084
Leaf width	0.088	0.437	-0.466	-0.333	0.011	-0.238
Seed length	0.115	-0.553	-0.174	-0.177	0.113	-0.347
Seed width	0.291	-0.292	-0.103	-0.583	0.040	-0.089
Seed weight	0.366	0.169	0.018	-0.115	0.481	0.099
Germination	-0.342	-0.112	-0.264	0.070	-0.204	-0.514

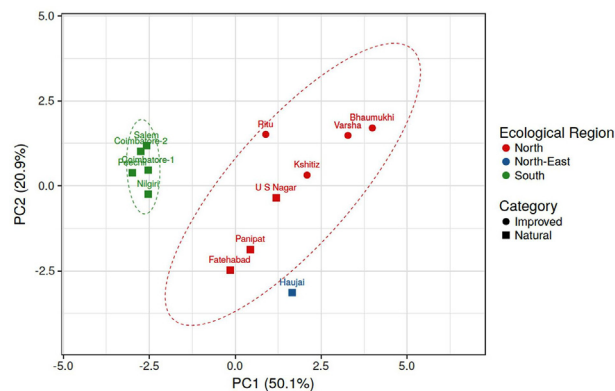


Fig. 3. Clustering pattern of *Melia* populations revealed through PCA biplot.

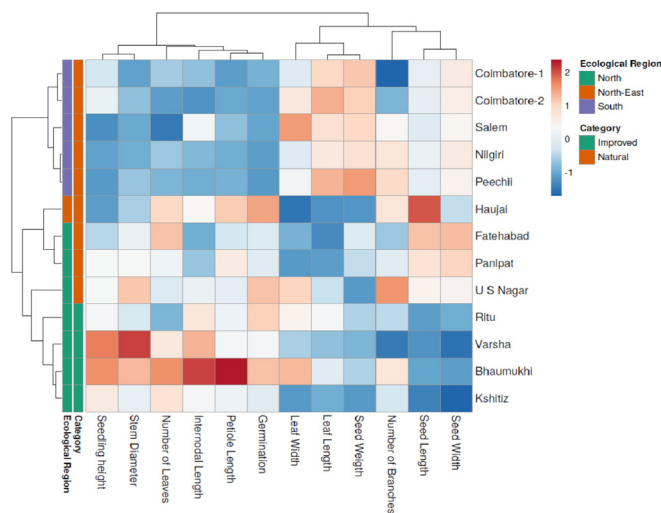


Fig. 4. Clustering of melia populations through dendrogram with heatmap.

South region, indicating that seed dispersal and dissemination by the travelers during the ancient time (Fig. 3).

5. Conclusion

Experimental results exhibited the significant differences in seed and seedling traits among the *M. dubia* populations. The higher genetic variance for some traits suggests that the most of evolutionary processes that shaped the *Melia* populations in all the three agro-ecological regions are responsible for the differenti-

ation of populations into different ecotypes. Principal component analysis and hierarchical clustering grouped *Melia* germplasm into three main clusters, explaining the formation of provenance within the same species. The theory and science of provenance are widely adopted in tree improvement to explain the evolutionary origin of seed sources, and consequent genetic variation in provenance may be the outcome of long-term effect of evolutionary processes in that region. Ecotypes or different seed sources were grouped in three main clusters; indicating that ecotypes evolved in same ecological region have a narrow genetic base or genetic proximity to each other. High heritability for seed length, seed width, 100 seed weight, germination percentage, internodal length, petiole length, leaf length, and leaf width suggests that selection for these traits would be more effective in early segregating generations. Notably, higher heritability with higher genetic gain for a particular trait would be considered as a selection criterion for identifying the best individual seedlings from the natural populations. Understanding the genomic structure, distribution pattern of genes, population polymorphism, and description of evolutionary patterns within the species through molecular approaches are prerequisite in *M. dubia*. Additionally, the development of a high-density molecular map through genotype by sequencing (GBS) for transferring the important traits and to access the genomic estimated breeding value (GEBV) for the selection of suitable candidate tree would be much more effective than conventional breeding, which ultimately enhance the genetic gain per unit time.

Author contribution statement

RK conceptualized and designed the experiment, recorded data and wrote the manuscript. AK prepared the graphics and analyzed the data. RB, AS, MK and RKY design the experiment and wrote the manuscript. SB and SS contributed in writing the manuscript.

Author agreement

All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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