



Research Article

GENETIC DIVERSITY OF *MELIA DUBIA* USING ISSR MARKERS FOR NATURAL POPULATIONS AND PLANTATIONS

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Abstract- *Melia dubia* Cav. (Family: Meliaceae) is a deciduous tree species native to India. It is an important plantation species with short rotation and multipurpose uses, highly valued as a pulpwood and plywood. Genetic variation was assessed in eleven natural populations and seven plantations across eight districts of Karnataka comprising 232 samples through 15 ISSR markers. At species level, genetic diversity estimates viz., Percentage polymorphism (94.6), percentage of polymorphic loci (PPL) (98.8), observed number of alleles ($N_a=1.98$), effective number of alleles ($N_e= 1.59$), Nei's gene diversity (H) (0.34 ± 0.15) and Shannon's information index (I) (0.51 ± 0.19) were found to be high. In individual populations H ranged from 0.10 ± 0.19 to 0.32 ± 0.18 and I ranged from 0.15 ± 0.26 to 0.47 ± 0.25 . Among different natural populations, Bhadravati exhibited the highest level of variability while in plantations Hunsur had maximum variability. Analysis of Molecular Variance showed that much of the genetic variation resided within the populations (68%) than among populations. The dendrogram obtained by using Unweighted Pair-Group method with Arithmetic average did not reflect geographical sub clustering of genetic diversity except for few populations. Based on the genetic variability found, superior seed sources can be identified and tree improvement strategies could be developed for conservation and further improvement of the species.

Keywords- *Melia dubia*, ISSR markers, Genetic Diversity, Superior seed sources

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Introduction

Melia dubia Cav. synonym *Melia composita*, (Family – Meliaceae), is a large deciduous, fast growing tree species native to India. Outside India, it is found in Sri Lanka, Malaysia, Java, China and Australia [1]. In Karnataka state, it is predominantly found in Southern parts [2] and commonly known as Malabar neem. The tree reaches 20 to 25 m height with a straight bole of about 9 to 12 m and nearly 1.5 m in girth at breast height. It grows well on variety of soils, however in deep fertile sandy loam soils it shows optimum growth. The timber is used for packing cases, ceiling planks, building purposes, agricultural implements, match boxes and Kattamarans [3]. The tree with the minimum size of 40cm girth is saleable at the minimum rate of Rs 2000 per ton for match, veneer and pulp industry [1]. It is an ideal species for plywood and pulpwood industry apart from being extensively used in afforestation [1]. Considering its fast growing ability and multipurpose uses, it is also accepted as an ideal agroforestry species. Large scale plantations of *M. dubia* have been raised by various state forest departments and private entrepreneurs in Southern India [4]. Fruits (drupes) of *M. dubia* are used for propagation. The trees growing in southern part of India produces fruits with hard endocarp, due to which, the germination is inherently low and varies from 14 -34.5% [5-7]. During the field survey, it was observed that trees in general were found to be scattered. Most of these isolated trees had profuse fruiting which formed the source for raising plantations. The species is reported to be predominantly self-fertilized [8]. We believe that the progenies raised from such seeds may harbour low diversity. Despite its large scale cultivation and its importance, there is no information available on the extent of genetic diversity existing in this species. Documentation of genetic variation in different populations of *M. dubia* would facilitate in carrying out programmes aimed at its improvement,

conservation, sustainable management and utilisation. For this, suitable molecular markers are required which can reliably assess existing genetic variation. In the early 1990's, the 'Inter-Simple sequence repeat (ISSR)' markers belonging to a class of multilocus, dominant genetic markers were independently developed by several research groups [9-12]. The generation of ISSR markers makes use of microsatellite sequences that are highly variable, ubiquitously distributed across the genome, higher reproducibility and costs less in terms of time and money compared to AFLPs. All these properties make ISSR an ideal genetic marker for various genetic studies, most notably on genetic variation/diversity [13, 14], DNA fingerprinting [15] and phylogenetics [16].

Considering its self-pollinating nature, fragmented natural populations and low germination, the present study was aimed to assess the genetic diversity of *M. dubia* existing in natural populations as well as in plantations in Karnataka.

Materials and methods

Extensive survey was carried out in eight districts of Karnataka (which fall under four different agroclimatic zones), 11 natural populations and seven plantations were identified and a total of 232 trees were selected to assess the genetic variability. In the present study, natural populations refers to those trees growing in forest areas, isolated trees along roadside and farmer's fields, which would not have been planted but grown naturally. In plantations, trees were selected randomly at a minimum distance of 50 m. The samples were collected from Bangaluru (IWST), Nelamangala (NEL), Hunsur (HNSR), Periyapatna (PRPT), Kushalnagar (DBRE), Gundlupet (GNDPT), H D Kote (HDK), Ramnagar (RAM), Shimoga (SMG), Bhadravati (BDR), Kollegal (KOL) and Hiriyyur (HIR) areas, distance ranging from 11°51'22.5" N to 14°3'15.37" N [Table-1].

Table-1 Details of 18 *Melia dubia* populations identified across Karnataka

SN	Location	Code	District	Trees selected	Latitude (North)	Longitude (East)	Agroclimatic zone
1	IWST campus, Bangalore	IWST NP	Bangalore Urban	9	13°00'41.3"	77°34'17.6"	Eastern Dry zone
2	Nelamangala	NEL NP	Bangalore Rural	10	13°00'39.9"	77°34'14.9"	Eastern Dry zone
3	Hulyal, Hunsur	HNSR NP	Mysore	7	12°23'0.96"	76°21'38.4"	Southern transition zone
4	Baslapura, Periyapatna	PRPT NP	Mysore	8	12°22'36.0"	76°08'44.6"	Southern transition zone
5	Dubare Elephant Camp, Kushalnagar	DBRE NP	Kodagu	3	12°22'07.7"	75°54'15.2"	Southern transition zone
6	Maddur, Gundlupet	GNDPT NP	Chamrajnagar	8	11°51'22.5"	76°40'24.7"	Southern dry zone
7	Heggadadevana Kote (H D Kote)	HDK NP	Mysore	7	12°07'20.7"	76°16'50.4"	Southern dry zone
8	Ramanagara	RAM NP	Ramanagara	6	12°41'39.64"	77°14'48.27"	Eastern dry zone
9	Shettihalli Wildlife range, Shimoga	SMG NP	Shimoga	11	13°55'36"	75°25'46.2"	Southern dry zone
10	Channagiri Range, Bhadravati	BDR NP	Shimoga	9	13°50'28.7"	75°51'26.2"	Southern transition zone
11	Gundal dam, Wild life Range, Kollegal	KOL NP	Chamrajnagar	10	12°04'30.6"	77°12'20.1"	Southern dry zone
12	Arepalya, Kollegal	KOL P	Chamrajnagar	20	12°04'30.6"	77°12'20.1"	Southern dry zone
13	Chennabasappa's Farm Hulyal, Hunsur	HNSR P	Mysore	21	12°23'0.96"	76°21'38.4"	Southern transition zone
14	Galli Bore Estate, Kamplapura, Periyapatna	PRPT P	Mysore	20	12°23'31.9"	76°10'08.8"	Southern transition zone
15	Kanakapura	KNK P	Ramanagara	20	12°33'30.6"	77°25'30.17"	Eastern dry zone
16	Yeshwantpur Nursery, Nallal, Hoskote	NALL P	Bangalore Rural	25	13°06'8.20"	77°50'44.04"	Eastern dry zone
17	Pooja Farm, Hariyabbe, Hiriyur	PHIR P	Chitradurga	19	14°3'15.37"	76°49'25.99"	Central dry zone
18	Kumar's Farm, Ishweregere, Hiriyur	KHIR P	Chitradurga	19	14°1'22.04"	76°44'20.42"	Central dry zone

Note: NP – Natural populations, P-Plantations

Table-2 Fifteen ISSR marker codes, sequence, Tm, Ta, percent polymorphism (%) and range of amplification

S	Marker code	Sequence (5'-3')	Tm (°C)	Optimum Ta (°C)	Total no. of bands	No. of polymorphic bands	Percent polymorphism (%)	Range of amplification (bp)
1	UBC-809	AGAGAGAGAGAGAGAGG	46.6	50.0	6	6	100	316-386
2	UBC-810	GAGAGAGAGAGAGAGAT	42.9	45.0	19	18	94.7	222-1419
3	UBC-811	GAGAGAGAGAGAGAGAC	43.3	45.0	13	11	84.6	182-1388
4	UBC-813	CTCTCTCTCTCTCTCT	45	50.4	8	8	100	190-1265
5	UBC-823	TCTCTCTCTCTCTCTCC	47.5	50.0	8	7	87.5	222-1792
6	UBC-840	GAGAGAGAGAGAGAGAYT	45.8	47.0	17	16	94.1	182-1388
7	UBC-845	CTCTCTCTCTCTCTCTRG	43.4	47.0	4	4	100	466-1144
8	UBC-847	CACACACACACACACARC	54.2	53.0	9	9	100	262-1357
9	UBC-855	ACACACACACACACACYT	60.2	61.0	10	9	90	167-1361
10	UBC-857	ACACACACACACACACYG	57.1	58.0	7	7	100	271-1894
11	UBC-864	ATGATGATGATGATGATG	51.2	52.0	12	12	100	295-1484
12	UBC-880	GGAGAGGAGAGGAGAGA	49	44.7	16	15	93.7	185-2054
13	UBC-888	BDBCACACACACACACA	52.3	55.4	18	16	88.8	199-1652
14	UBC-890	VHVTGTGTGTGTGTGTG	51.8	52.0	8	8	100	218-673
15	UBC-891	VHVTGTGTGTGTGTGTG	51.8	55.0	11	11	100	214-1668

Note: Tm- melting temperature, Ta- annealing temperature

Table-3 Different diversity parameters analysed in 18 populations (n=232) in *M. dubia*

Parameters	Overall diversity estimates
PPL	98.80%
Observed number of alleles	1.98±0.10
Effective number of alleles	1.59±0.32
H	0.34±0.15
I	0.51±0.19

Table-4 Different diversity parameters, PPL, observed number of alleles, effective number of alleles, H and I analysed for 18 populations of *M. dubia* using ISSR primers

SN	Population	PPL	Observed number of alleles±SD	Effective number of alleles±SD	H±SD	I±SD
1	IWST(NP)	58.43	1.58±0.49	1.46±0.42	0.25±0.22	0.36±0.32
2	NEL(NP)	56.63	1.57±0.50	1.44±0.43	0.24±0.22	0.34±0.32
3	HNSR(NP)	66.27	1.66±0.47	1.51±0.43	0.28±0.22	0.40±0.31
4	PRPT(NP)	51.20	1.51±0.50	1.39±0.41	0.21±0.22	0.31±0.31
5	DBRE(NP)	49.40	1.49±0.50	1.34±0.39	0.20±0.21	0.29±0.30
6	GNDPT(NP)	52.41	1.52±0.50	1.34±0.39	0.19±0.21	0.29±0.30
7	HDK (NP)	36.75	1.37±0.48	1.27±0.40	0.15±0.21	0.22±0.30
8	RAM(NP)	30.12	1.30±0.46	1.23±0.38	0.13±0.20	0.18±0.29
9	SMG(NP)	24.70	1.25±0.43	1.18±0.35	0.10±0.19	0.15±0.26
10	BDR(NP)	69.88	1.70±0.46	1.52±0.40	0.29±0.21	0.42±0.29
11	KOL(NP)	62.65	1.63±0.49	1.48±0.41	0.26±0.22	0.38±0.31
	Mean	50.77	1.51	1.38	0.21	0.30
12	KOL(P)	60.24	1.60±0.49	1.41±0.39	0.23±0.21	0.34±0.30
13	HNSR(P)	83.73	1.84±0.37	1.56±0.36	0.32±0.18	0.47±0.25
14	PRPT(P)	78.92	1.79±0.41	1.52±0.36	0.30±0.19	0.44±0.26
15	KNK(P)	41.57	1.42±0.49	1.28±0.38	0.16±0.20	0.24±0.29
16	NALL(P)	71.69	1.72±0.45	1.48±0.36	0.28±0.19	0.41±0.28
17	PHIR(P)	53.01	1.53±0.50	1.34±0.38	0.20±0.21	0.29±0.29
18	KHIR(P)	55.42	1.55±0.50	1.37±0.38	0.21±0.21	0.31±0.30
	Mean	63.51	1.64	1.42	0.24	0.36

Note: PPL- Percentage of Polymorphic loci, H- Nei's gene diversity, I- Shannon's information index, SD-standard deviation

Table-5 AMOVA for 18 populations, 11 natural populations and seven plantations of *M. dubia* with 15 ISSR markers

Source	Df	SS	MS	Est. Var.	%	P-value
Among 18 Population	17	1987.600	116.918	7.907	32%	0.001
Within Population	214	3540.103	16.543	16.543	68%	
Total	231	5527.703		24.450	100%	
Among 11 natural population	10	712.609	71.261	6.907	29%	0.001
Within natural population	76	1289.827	16.971	16.971	71%	
Total	86	2002.437		23.879	100%	
Among seven plantations	6	1177.794	196.299	8.702	35%	0.001
Within plantations	138	2250.275	16.306	16.306	65%	
Total	144	3428.069		25.008	100%	

Note: Significance test (999 permutations), Df-degrees of freedom, SS-sum of square, MS-mean sum of square, Est. Var.-estimated variance, %-percentage of Variation

Table-6 Nei's (1972) unbiased measure of genetic distance for 18 populations of *M. dubia*

	IWST (NP)	KOL (NP)	NEL (NP)	GNDPT (NP)	HDK (NP)	RAM (NP)	SMG (NP)	BDR (NP)	PRPT (NP)	HNSR (NP)	NALL (P)	PHIR (P)	KHIR (P)	KOL (P)	HNSR (P)	PRPT (P)	KNK (P)	DBRE (NP)
IWST (NP)	****																	
KOL (NP)	0.06	****																
NEL (NP)	0.11	0.11	****															
GNDPT (NP)	0.22	0.23	0.17	****														
HDK (NP)	0.19	0.20	0.14	0.08	****													
RAM (NP)	0.29	0.29	0.22	0.12	0.17	****												
SMG (NP)	0.17	0.14	0.13	0.24	0.16	0.35	****											
BDR (NP)	0.20	0.18	0.15	0.08	0.11	0.16	0.20	****										
PRPT (NP)	0.13	0.12	0.06	0.21	0.16	0.31	0.13	0.17	****									
HNSR (NP)	0.19	0.20	0.15	0.14	0.17	0.18	0.24	0.12	0.14	****								
NALL (P)	0.06	0.06	0.10	0.25	0.19	0.27	0.14	0.20	0.09	0.18	****							
PHIR (P)	0.08	0.07	0.12	0.29	0.21	0.33	0.14	0.23	0.11	0.21	0.05	****						
KHIR (P)	0.12	0.12	0.08	0.20	0.14	0.29	0.08	0.18	0.09	0.18	0.12	0.09	****					
KOL (P)	0.09	0.07	0.12	0.18	0.15	0.26	0.12	0.16	0.11	0.17	0.08	0.06	0.08	****				
HNSR (P)	0.23	0.21	0.20	0.14	0.21	0.15	0.32	0.09	0.24	0.12	0.24	0.26	0.25	0.20	****			
PRPT (P)	0.18	0.17	0.15	0.08	0.12	0.10	0.22	0.08	0.18	0.11	0.19	0.21	0.17	0.14	0.06	****		
KNK (P)	0.28	0.25	0.23	0.14	0.21	0.14	0.37	0.15	0.29	0.21	0.28	0.34	0.31	0.23	0.15	0.09	****	
DBRE (NP)	0.25	0.23	0.20	0.19	0.26	0.22	0.32	0.16	0.23	0.15	0.25	0.31	0.27	0.27	0.16	0.14	0.16	****

Populations of *M. dubia* possessed high level of polymorphism (157 polymorphic bands detected by fifteen primers). At species level, *M. dubia* had high genetic diversity measures viz. PPL was 98.8%, however, using RAPD markers moderate level of diversity was observed in other members of Meliaceae family like *Melia azedarach* (51.5%) [30] and *A. indica* (68.4%) [29]. Nei's gene diversity was 0.34 and Shannon's information index was 0.51 where as in *Swietenia macrophylla* high H (0.45) was reported [31]. In other cross-pollinated species, high level of H (0.36) and I (0.54) were reported in *T. grandis* [26]. However lower level of H (0.29 and 0.28) and I (0.44 and 0.43) were reported in other tropical species such as *G. arborea* and *P. pinnata* [32, 33]. Among 11 natural populations, highest diversity was observed in BDR whereas lowest diversity in SMG population [Table-4]. Though both the populations are geographically nearer, it is of importance to mention that in case of Bhadravati the trees were well dispersed in the natural forest compared to Shimoga, where they were found along the forest road side. In plantations, HNSR (P) had showed high diversity and lowest was found in KNK (P). When compared to natural populations plantations showed higher genetic diversity. This phenomenon occurs when plantations are raised from germplasm collected from diverse populations in its native range. Similarly, genetic diversity was found higher in plantations compared to natural populations in *Neolamarckia cadamba* and *G. arborea* [34, 32]. AMOVA based on UPGMA method revealed 68% of the total variation resided within the natural populations of *M. dubia* whereas 32% variation among populations. Similarly, high variation was observed within (65%), than among (35%) populations in plantations. However, within population variation was more in natural populations compared to plantations. In *M. dubia* it has been reported that one of the major mode of seed dispersal is through herbivores as they feed on the fruits [2]. This might have resulted in high diversity within natural populations. Species whose seeds are dispersed by animal ingestion or by wind maintain high levels of within-population genetic variability [28, 35]. High variation within populations has been reported in other Meliaceae members, *S. macrophylla*, *M. azedarach* [31, 30] and in other tree species like; *Populus tremuloides*, *Abies nephrolepis*, *T. grandis* and *Prosopis cineraria* [20, 36, 26, and 37]. The data for *M. dubia* agrees with general observation that tree species, maintain most of their variation within the populations [38]. Cluster

analysis revealed that the natural populations were not grouped according to the geographical distance except for GNDPT (NP) (11°51'22.5") and HDK (NP) (12°07'20.7"). In plantations, PRPT (12°23'31.9") and HNSR (12°23'0.96") which are located geographically closer also grouped together. Similarly no correlation between genetic distance and geographical distance have been reported in natural populations of number of species viz., *C. latifolia*, *Enterolobium contortisiliquum*, *Theobroma speciosum* and *H. rhamnoides* [22, 39, 40, 28]. However, correlation between genetic distance and geographical distance has been reported in other species like *G. arborea*, *Tectona grandis*, *P. pinnata* and *Acacia senegal* [32, 26, 33, 41]. Plantations of HNSR and PRPT clustered with natural population of HNSR and KNK plantation clustered with natural population of RAM. This could be because the seed source for raising plantation might have been from above natural populations. Such pattern was not observed in other plantations. This may be attributed to variation in source of origin of planting material [34]. The present study is the first attempt to analyze genetic variation in natural populations and plantations of *M. dubia*. It revealed over all high genetic diversity at species level. When compared to natural populations, the diversity estimates were higher in plantations, which may be due to fragmentation of populations and occurrence of isolated trees. It has been reported that *M. dubia* is predominantly self-pollinating species, however, large intra populations variation was observed which was similar to the other tree species that are outbreeding. In natural populations, BDR (NP) showed highest diversity followed by HNSR (NP) and KOL (NP) whereas in plantations HNSR (P) was found to be most diverse followed by PRPT (P) and NALL (P). Being a self-pollinating species, it is suggested to use germplasm from more than one population while raising plantations so that broad genetic base is maintained.

Conclusion

Genetic diversity assessed from eleven natural populations and seven plantations across eight districts of Karnataka revealed over all high genetic diversity at species level. Analysis of Molecular Variance showed that much of the genetic variation resided within the populations (68%) than among populations. Cluster analysis did not reflect geographical sub clustering except for few populations.

Application of research: This study would be helpful in selection of superior seed sources for raising plantations and forming a base for initiating tree improvement programs for this species.

Research Category: Genetic diversity

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