Academia Journal of Biotechnology 7(6): 107-115, June 2019
DOI: 10.15413/ajb.2018.0143
ISSN 2315-7747
©2018 Academia Publishing

Academia Publishing

Research Paper

Phenotyping using pilodyn as a tool, selective genotyping and preliminary genetic diversity estimation for wood basic density in *Melia dubia*

Accepted 30th May, 2019

ABSTRACT

Melia dubia Cav. (Meliaceae), a native species to India, is rapidly emerging as a commercially important tree species due to its fast growth and multiple uses. The wood has potential for plywood, pulping and high value solid wood products. Wood basic density is one of the favoured traits for selection as it influences many of mechanical and anatomical properties. Pilodyn Penetration (PP) which is an indirect measure of wood basic density was measured in a plantation (n=513) and it ranged from 14 to 25 mm in trees with ≥ 37.5 cm Girth at Breast Height (230 trees). An attempt was made to select phenotypes for wood basic density based on the extreme values of PP. Among the 230 trees, available trees for low PP (14 to 17 mm) were 16 (6.95%) and high PP (23 to 25 mm) were 19 (8.26%). These 35 phenotypes were used for selective genotyping and estimating of the genetic diversity. For genetic diversity estimation, bulks of low and high PP were screened with 63 markers and 12 markers showing traits related polymorphism were selected. Diversity estimates like number of observed alleles, heterozygosity, specific alleles and distinct alleles varied between two groups. Distinct alleles were observed in MSSR 2 for high basic density and MSSR 51 and MSSR 54 for low basic density. Cluster analysis resulted in three major clusters of which 51% of genotypes were distinctly segregated into two clusters having either low or high basic density. The study reconfirms that pilodyn can effectively be used for indirect estimation of basic density and these phenotypes can be used for selective genotyping for development of trait specific markers. Identifying more genotypes and markers of above type may facilitate future marker assisted selection programme for wood basic density in *M. dubia*.

Geeta Joshi, Shakti Singh Chauhan, Arunkumar, A. N, Ajay, T. L, Sreedevi, C. N, Aparna Rajan, Annapurna Dhavala*

Institute of Wood Science and Technology, PO 18th Cross, Malleswaram, Bengaluru, Karnataka, India – 560 003

* Corresponding Email: uannapurna@gmail.com

Key words: Pilodyn, *M. dubia*, selective genotyping, wood basic density.

INTRODUCTION

Wood Properties (WPs) have become mandatory traits in tree breeding programmes for the development of improved genotypes/clones. They vary greatly between species, within species and within a tree (Plomion et al., 2001; Chauhan and Walker, 2006) and change with age (Raymond, 2002). Conventional approaches of measuring wood properties are not practical for large scale assessments as they require destructive sampling, preparation of samples of specific dimensions and time consuming processes in laboratory. For efficient selection of superior trees based on wood properties, phenotyping in large number of standing trees quickly and reliably is essential. Silviscan (Evans 1994), Pilodyn tool (Greaves et al., 1996), acoustics (Wang et al., 2000) and near infra red spectroscopy (Schimleck et al., 2001) are novel non-destructive tools which effectively can be used for *in situ* assessment of wood quality traits like wood density, modulus of elasticity, chemical constituents, etc.

Wood density is one of the most favoured traits as it influences other mechanical and anatomical properties, has high heritability and easier to measure by simple methods. Pilodyn tool is widely used for segregating trees /logs with different wood density classes. The tool is originally developed in Switzerland for determining the

degree of soft rot in wooded telephone poles (Raymond and MacDonald, 1998; Hansen, 2000). Pilodyn is attractive in that it is rapid, does not require the use of an increment borer (destructive sampling), and is, in principle, free of operator bias (Cown, 1978; Hansen, 2000).

Pilodyn Penetration (PP), an indirect method for determining wood basic density, has been effective in assessing large number of trees in eucalyptus (Kien et al., 2008; Raymond and MacDonald, 1998; Macdonald et al., 1997) and other species (Ishiguri et al., 2008; Pliura et al., 2007). Correlations between basic density and pilodyn penetration is strong (r= -0.77) with a small standard error (Raymond et al., 2009). In *M. dubia,* pilodyn penetration exhibited a strong negative association with basic density (r = -0.76), (Chauhan and Arun, 2014).

Genetic correlations between growth traits and density have been studied in many species and it was reported that there is little association between the two kinds of traits (White et al., 2007). Genetic correlations between growth and various wood properties were not statistically significant in *E. globulus*, (Apiolaza et al., 2005), *E. nitens* (Hamilton and Potts, 2008), *Melia dubia* (Chauhan and Arun, 2014). In the absence of correlation between morphological and wood traits, it becomes difficult to select superior phenotypes by conventional tree improvement program.

The long generation time along with poor juvenile-mature trait correlations in trees promoted interest in Marker Assisted Selection (MAS) (Grattapaglia et al., 2004). One of the two types of MAS, selective genotyping, using progeny from the extremes of the phenotypic distribution of a trait has been suggested to overcome the high cost of genotyping all individuals (Lander and Botstein, 1989; Darvasi and Soller, 1994; Muranty and Goffinet, 1997). Thereafter, association was inferred by finding allelic frequency differences between groups (Rajib et al., 2014).

Earlier, phenotypic data on wood basic density for carrying out genomics studies was obtained by gravimetric method in eucalyptus (Grattapaglia et al., 1996; Devey et al., 2004) or pilodyn as a tool in *E. globulus* (Bundock et al., 2008) or using Silviscan in *Eucalyptus* spp (Thumma et al., 2005) and *Pinus radiata* (Dillon et al., 2010). However, in *E. globulus,* for the first time, high throughput phenotyping was used to detect quantitative trait loci (QTLs) for fibre length, cellulose, pulp yield and microfibril angle (Thamarus et al., 2004).

Melia dubia Cav. (Meliaceae) is a fast growing multipurpose deciduous tree species native to India. It is a large tree, attaining a height almost of 20 m with a spreading crown and a cylindrical straight bole of 9 to 10 m length and 1.2 to 1.5 m. girth. The sapwood and heartwood is pale yellow and brown coloured, and the wood density is generally low. The wood was traditionally used for fuelwood, musical instruments, agricultural implements, cigar boxes, match sticks, splints, packaging, and catamarans, etc. (Swaminathan et al., 2012). The wood is now recognized to have tremendous potential for plywood, pulping and high value solid wood products (Parthiban et al., 2009; Saravanan et al., 2013).

The aim of the study was to use pilodyn penetration as a tool to assess the variability in wood basic density parameter in a plantation, identify sub-populations having extreme phenotypes for basic density of *M. dubia* and subject them to selective genotyping method for estimation of diversity between the sub-groups of population with different basic densities.

MATERIALS AND METHODS

Phenotyping

Study site

Morphological variability was measured in an eight year old *M. dubia* plantation raised by Karnataka Forest Department near Yeshwantpura (Kolar Range), 50 km from Bangalore, India (Latitude 13°7′56.91″ N and 77°55′53.46″ E). The plantation was mainly rain-fed and the spacing was 5 × 5 m. The planting material for raising this plantation was obtained from the seeds collected from a plantation raised by KFD in 1998 with an objective to select superior genotypes for initiating tree improvement programmes in *M. dubia*. The plantation was mainly rain-fed with normal management practices.

Phenotypic measurement

Girth at Breast Height (GBH), bark thickness and Pilodyn penetration were recorded for 513 trees. Bark thickness was measured using a bark guage (Barktax, Haglof). A 6 j Pilodyn (PILODYN 6 J FOREST) tool was used to measure pilodyn pin penetration at the breast height on two opposite sides (north and south) of each tree, that is, the depth of penetration of the flat-nosed pin (2.5 mm diameter steel pin) into the wood was recorded with an accuracy of 1 mm. The average of two readings was taken as the pilodyn measurement of the tree.

Selective genotyping

For selective genotyping, 35 individuals (16 and 19 individuals representing low and high pilodyn reading, respectively) were identified based on 1.7 σp (phenotypic standard deviation) above and below the mean for pilodyn penetration.

DNA extraction

Total genomic DNA of 35 selected extreme phenotypes

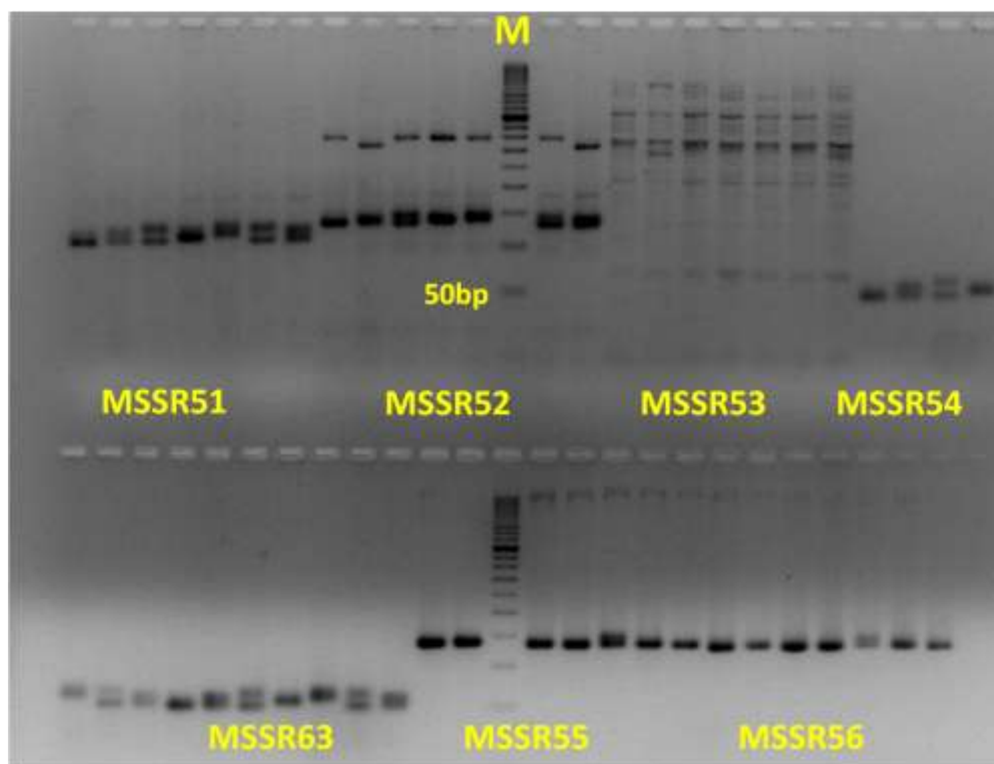

Figure 1: Amplification profile of 7 selected genotypes with MSSR 51-57 SSR markers in *M. dubia*.

was extracted from dry matured leaves of *M. dubia* using the standardized protocol (Rawat et al., 2016).

Primer information

SSR markers were developed through next generation sequence analysis methodology (Genotypic Technology [P] Ltd., India). Microsatellites within candidate genes were identified using BLAST2GO based on Gene Ontology (GO) of *Eucalyptus* and *Populus* species and they were referred as genic markers. Total of 63 primers comprising fifty Random Markers (RM) and thirteen Genic Molecular Markers (GMMs) related to wood properties were tried in *M. dubia* for screening.

For bulk segregant analysis, two bulks were made by mixing equimolar amounts of genomic DNA from 16 and 19 individuals having low and high pilodyn readings, respectively. Among 50 RMs and 13 GMMs used for screening with bulks, ten random and two genic markers showed polymorphism. To confirm this, four genotypes from low pilodyn group and three genotypes from high pilodyn group were again used to screen the markers to find the polymorphism (Figure 1). For the diversity estimates, a total of 10 random and two genic markers showing intact bands and highest trait related polymorphism with bulks and individual genotypes were selected which were further run with all 35 genotypes.

For running SSR-PCR, a reaction volume of 13 µl was used with 2.5 µl (30 ng/µl) of template DNA, 2.5 mM $MgCl_2$, 200 µM of each dNTPs, SSR primer (10 pm/µl) at 1.25 µl (0.625 µl for each forward and reverse primer) and 1 U of Taq DNA polymerase. The forward primer sequence of twelve markers was modified with either of FAM, HEX or TAMRAM dyes. The PCR amplifications were performed (Figure 2). PCR was run using an Eppendorf Master cycler (Eppendorf AG) with standardized conditions (Rawat et al., 2016). The sizing of PCR products was done by capillary electrophoresis using an ABI3730xl Genetic Analyzer (Applied Biosystems). The allele data were then analyzed using GeneMarker 2.2.0 (SoftGenetics, State College, Pennsylvania).

Data analysis

Data analysis for genetic diversity was done using software *GENAlEx* ver. 6.5 (Peakall and Smouse, 2006, 2012). Genetic diversity estimates were carried out by considering 35 genotypes selected from both tails, that is, low pilodyn (16 genotypes) and high pilodyn (19 genotypes) as two groups (Table 1). Genetic diversity of each group was characterized by estimating number of alleles per locus (Na), observed heterozygosity (Ho), expected heterozygosity (He) (Nei, 1978) and effective number of alleles (Ne) estimated by reciprocal of homozygosity (Kimura and Crow, 1964) and Wright's fixation index (Wright, 1978). Clustering of genotypes was

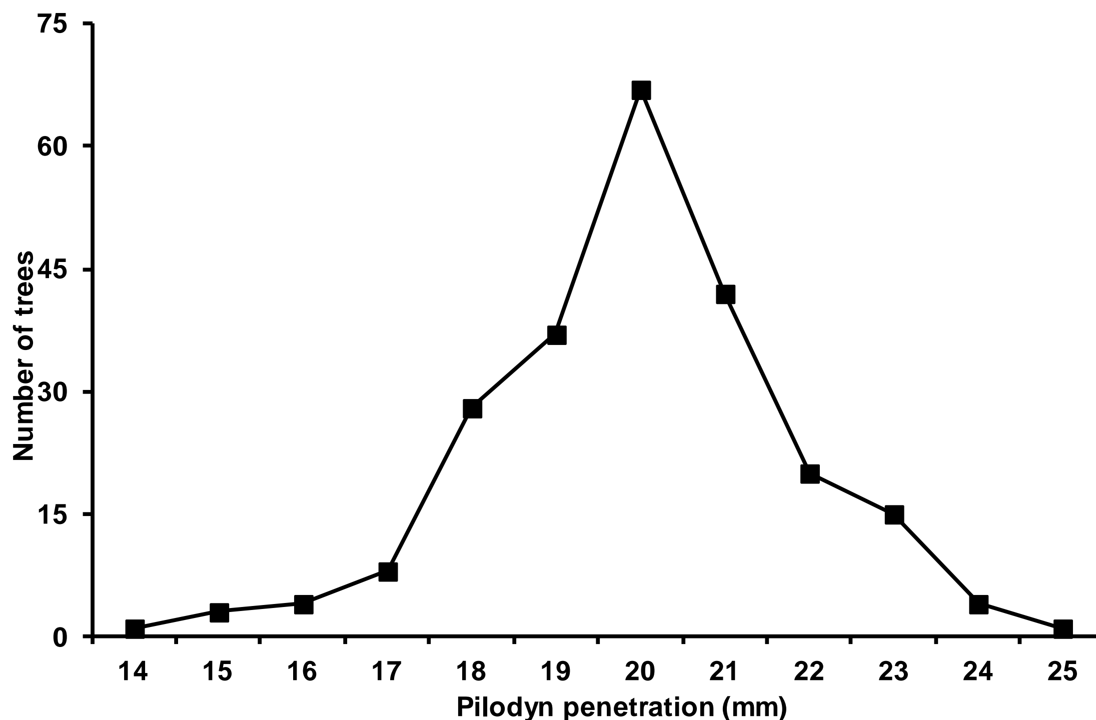

Figure 2: Frequency distribution for Pilodyn penetration mm for trees (n=230) with GBH ≥37.5 cm.

Table 1: Morphological data on GBH and pilodyn penetration of 47 selected genotypes in *M. dubia*.

S/No	Low pilodyn penetration (Group I)			S/No	High pilodyn penetration (Group II)		
	Tree ID	GBH (cm)	Pilodyn penetration (mm)		Tree ID	GBH	Pilodyn penetration (mm)
1	G_1 1	39.5	14.0	1	G_2 1	37.6	23
2	G_1 2	41.0	15.0	2	G_2 2	39.0	23
3	G_1 3	45.5	15.0	3	G_2 3	40.0	23
4	G_1 4	46.3	15.5	4	G_2 4	42.0	23
5	G_1 5	39.0	16.0	5	G_2 5	43.0	23
6	G_1 6	39.5	16.0	6	G_2 6	44.5	23
7	G_1 7	46.5	16.0	7	G_2 7	45.0	23
8	G_1 8	49.0	16.0	8	G_2 8	45.0	23
9	G_1 9	40.0	17.0	9	G_2 9	45.5	23
10	G_1 10	41.8	17.0	10	G_2 10	47.1	23
11	G_1 11	43.5	17.0	11	G_2 11	49.5	23
12	G_1 12	44.0	17.0	12	G_2 12	51.3	23
13	G_1 13	44.5	17.0	13	G_2 13	54.5	23
14	G_1 14	46.2	17.0	14	G_2 14	56.0	23
15	G_1 15	47.0	17.0	15	G_2 15	63.0	23
16	G_1 16	53.0	17.0	16	G_2 16	38.5	24
17	-	-	-	17	G_2 17	46.0	24
18	-	-	-	18	G_2 18	67.0	24
19	-	-	-	19	G_2 19	40.4	25
Range			14-17.0	Range			23-25

done by Neighbour Joining method using Darwin software (version 6).

We also estimated the number of specific alleles (present in either of two groups). Dissimilarity when observed in 3 or more than 3 alleles continuously was considered as distinct alleles.

Table 2: Variability for GBH and pilodyn penetration (mm) in *M. dubia* plantation located at Yeswanthpura (Kolar Range).

Parameter	N=513			N=230 (considering gbh>37.5 cm)		
	Range	Mean	SD	Range	Mean	SD
GBH (cm)	16.0-79.5	37.38	9.25	37.6-79.5	45.41	6.69
Pilodyn penetration (mm)	12.0-25.0	19.69	1.83	14.0 - 25.0	19.97	1.78
Bark thickness (cm)	1.00- 1.40	0.79	0.15	1.0-1.2	0.79	0.14

Table 3: Pearson's correlation coefficient in *M. dubia* plantation.

Parameters	GBH	Pilodyn	Bark thickness
GBH	1		
Pilodyn	**0.22**	1	
Bark thickness	0.59	0.23	1

Letters in bold- significant at 0.05 level.

RESULTS

Phenotyping

In this population, highest variability was observed for GBH and pilodyn penetration. Out of 513 trees, GBH ranged from 16.0 to 79.5 cm with a mean of 37.38 cm. In the entire population, pilodyn penetration ranged from 12 to 25 mm with mean of 20 mm and SD of 1.83 (Table 1). Among 513 trees, 230 trees (44.83%) had GBH >37.5 cm. Considering trees with GBH >37.5 cm, the pilodyn penetration ranged from 14 to 25 mm.

Therefore, the high variability in pilodyn penetration provided an opportunity to select the extreme individuals. Among 230 trees, 6.95% of trees (16 numbers) had low pilodyn readings (14 to 17 mm), while 8.26% (19) trees had high pilodyn readings (23 to 25 mm).

Tree girth was positively related to depth of pilodyn penetration (Table 2) indicating a negative correlation of tree girth with wood density.

However, the strength of association was very moderate to arrive at any specific conclusions. As expected, GBH was strongly related to bark thickness. Pilodyn penetration was also related positively with bark thickness.

Generally, in tree improvement programs, trees for superior wood properties without compromising on growth were selected. Out of 513 trees, 230 trees (44.83%) had GBH ≥37.50 cm (Table 1).

Figure 1 shows trees with GBH ≥ 37.5 cm and frequency distribution curve for pilodyn penetration.

Approximately, 15% of the sub-population from both the tails of frequency distribution was selected for selective genotyping.

Pilodyn penetration ranged from 14 to 17 mm for low pilodyn group and 23 to 25 mm for high pilodyn penetration group (Table 3).

Selective genotyping and genetic diversity estimation

Phenotyping 513 trees for pilodyn penetration in this plantation showed 16 individual trees with low pilodyn (14 to 17 mm) and 19 with high pilodyn (23 to 25 mm) readings. Among twelve primers tried, five had tri-nucleotide repeats, while seven had di-nucleotide repeats with a product range of 90 to 217 (Table 4). In the case of analysis of data with low pilodyn (16 genotypes) and high pilodyn (19 genotypes) as two separate groups, diversity estimates in terms of observed alleles were higher in high pilodyn group (22.0, 18.0 and 17.0 and 11.0) with MSSR 34, MSSR 54, MSSR 15 and MSSR 51 primers, respectively. The observed heterozygosity was high (Mean Ho=0.67) in low pilodyn group than high pilodyn group (Mean Ho=0.61).

Among the 12 markers, MSSR 2, MSSR 7 and MSSR 45 showed negative fixation index for both groups. We also estimated the number of specific alleles (present in either of two groups). Number of specific alleles observed in either of groups was higher in MSSR 2 and MSSR 34. Dissimilarity when observed in 3 or more than 3 alleles continuously was considered as distinct. Among the 10 random markers, only one marker MSSR 2 showed three distinct alleles in low pilodyn group whereas two genic markers, MSSR 51 and MSSR 54 showed distinct alleles in high pilodyn group.

Allelic data of 35 genotypes with 12 markers resulted in 3 major clusters (Figure 3). Cluster 1 contained 14 genotypes out of which 10 genotypes were from high pilodyn group, while the remaining four were from low pilodyn group with pilodyn value of 17 mm. Second cluster which had 12 genotypes comprised eight from low pilodyn group and four from high pilodyn group. The third cluster had 9 genotypes of which 4 were from low pilodyn and 5 from high pilodyn group.

Table 4: Details of 12 SSR primers, motif type, product size and genetic diversity parameters for low (n=16) and high (n=19) pilodyn penetration with 35 genotypes of *M. dubia*.

S/No	Primers	Motif type	Product range	Group I					Group II				
				Low pilodyn (n=16)					High pilodyn (n=19)				
				Na	Ne	Ho	He	F	Na	Ne	Ho	He	F
1	MSSR 2	(TTA)12	91-142	13.0	7.5	1.00	0.87	-0.15	14.0	5.4	1.00	0.81	-0.23
2	MSSR 3	(ATA)12	163-178	8.0	4.8	0.75	0.79	0.052	7.0	3.6	0.58	0.72	0.20
3	MSSR 7	(TAA)15	143-171	12.0	4.7	0.88	0.79	-0.11	11.0	4.1	0.84	0.76	-0.11
4	MSSR 10	(ATC)14	97-124	8.0	4.0	0.50	0.75	0.33	10.0	4.0	0.58	0.75	0.23
5	MSSR 11	(GA)15	145-152	7.0	3.7	0.12	0.73	0.83	6.0	4.5	0.16	0.78	0.80
6	MSSR 12	(TA)17	161-193	9.0	5.9	0.88	0.83	-0.05	7.0	2.0	0.42	0.51	0.17
7	MSSR 15	(AT) 16	146-173	10.0	5.0	0.69	0.80	0.14	17.0	4.7	0.84	0.79	-0.07
8	MSSR 18	(AT)17	105-143	10.0	6.0	0.81	0.83	0.03	10.0	5.1	0.53	0.81	0.35
9	MSSR 34	(ATC) 10	91-122	15.0	11.38	0.94	0.91	-0.03	22.0	7.9	0.84	0.87	0.04
10	MSSR 45		217	2.0	1.0	0.06	0.06	-0.32	6.0	1.0	0.05	0.05	-0.03
11	MSSR 51	(TA) 12	160-172	5.0	3.3	0.63	0.69	0.01	11.0	1.9	0.58	0.80	0.27
12	MSSR 54	(TA10)	97-117	11.0	5.3	0.75	0.81	0.08	18.0	9.1	0.84	0.89	0.05
	Mean			9.2	5.2	0.67	0.74	0.10	9.3	4.7	0.61	0.71	0.14

Na= Number of observed alleles; Ne= Number of effective alleles; Ho=Observed heterozygosity; He=Expected heterozygosity; F=Fixation index; I=Shannon information index.

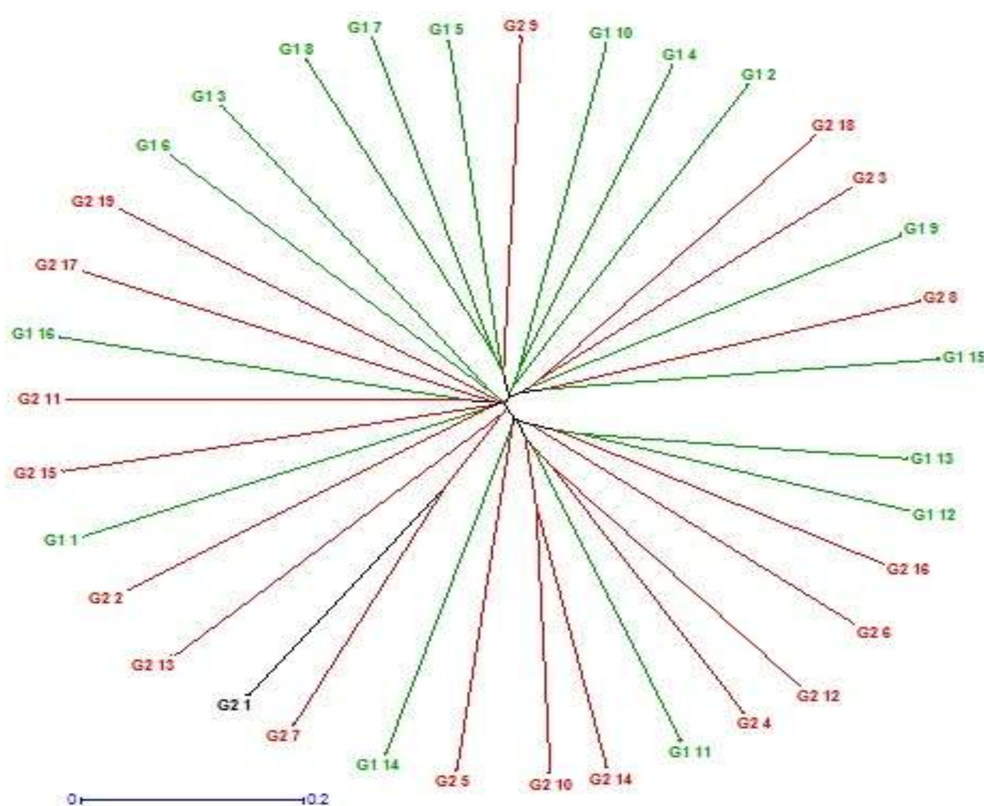

Figure 3: Cluster analysis of 35 selected genotypes for low (n=16) and high (n=19) pilodyn penetration with 12 SSR markers in *M. dubia*.

DISCUSSION

Measurement of wood density by conventional method is expensive and time consuming and also create varying degrees of damage to experimental materials, and that has restricted the number and accuracy of the studies

Table 5: Details of dissimilarity of alleles in high and low stress wave velocity groups with 12 microsatellite loci in *M. dubia*.

S/No	Marker	Number of observed alleles (Na)			Number of specific alleles			Number of distinct alleles and range	
		n=35	Group I Low pilodyn (n=16)	Group II High pilodyn (n=19)	(n=35)	Low pilodyn (n=16)	High pilodyn (n=19)	Low pilodyn (n=16)	High pilodyn (n=19)
1	MSSR 2	17	13.0	14.0	10	5	5	3(131-135)	Absent
2	MSSR 3	7	8.0	7.0	1	1	0	-	-
3	MSSR 7	12	12.0	11.0	5	4	1	-	-
4	MSSR 10	9	8.0	10.0	3	1	2	-	-
5	MSSR 11	7	7.0	6.0	3	2	1	-	-
6	MSSR 12	9	9.0	7.0	3	2	1	-	-
7	MSSR 15	13	10.0	17.0	6	3	3	-	-
8	MSSR 18	13	10.0	10.0	8	4	4	-	-
9	MSSR 34	20	15.0	22.0	9	4	5	-	-
10	MSSR 45	1	2.0	6.0	0	0	0	-	-
11	MSSR 51	9	5.0	11.0	5	1	4	Absent	3(169-172)
12	MSSR 54	17	11.0	18.0	8	2	6	Absent	3(106-109

published (Hansen, 2000). However, pilodyn sampling is faster, cheaper, and not destructive, thus, resulting in overall higher expected gains for selection of trees in comparison with the more destructive direct assessment of density (Greaves et al., 1996). The suitability of pilodyn in ranking trees according to their wood density was demonstrated by a number of researchers on both hardwoods and softwoods. A significant negative relationship of pilodyn penetration with wood basic density in this species was reported earlier (Chauhan and Arun, 2014). The broad range in pilodyn penetration (14 to 25 mm) observed in the Yeswanthpura plantation provides an opportunity for selecting extreme phenotype for selective genotyping (Table 3).

In the study, through selective genotyping with SSR markers, DNA samples from low and high pilodyn group showed distinct polymorphism with three SSR markers. In *Pinus radiata*, selective genotyping was used as a method to find associations for density and diameter (Devey et al., 2004). In marker-assisted selection (MAS) breeding, simple sequence repeat (SSR) markers are ideal because they are hypervariable, codominant and highly informative (Varshney et al., 2007).

Earlier study on *M. dubia* for estimation of diversity for 17 populations with six SSR markers showed lower number of observed alleles (5.03) and observed heterozygosity (0.49) (Swathi, 2016). In this study, diversity estimates were high in terms of observed alleles and observed heterozygosity. This might due to trait specific selection of individuals based on extreme values of pilodyn penetration. Cluster analysis showed only 51% of genotypes was distinctly segregated into two clusters having either low or high basic density character. This might be as a result of high observed heterozygosity (Table 5). Though the number of observed alleles was high in 12 markers, distinct alleles were found in three markers only. Among the 10 random markers, only one marker (MSSR 2) showed three distinct alleles in low pilodyn group whereas two genic markers showed distinct alleles in high pilodyn group. Above markers may prove to be potential markers for selection of trees based on basic density in future MAS programme.

It is expected that diversity in wood quality will be dependent on variability in numerous genes involved in different molecular categories such as lignin biosynthesis, cellulose synthesis, cell wall structure, cell expansion and abiotic stress (Dillon et al., 2010). In the present study, the genic markers MSSR 51 and MSSR 54 with CesA6 gene showed distinct dissimilarity. In plant breeding, GMMs are superior to RGMs for selection of parental materials and provide more direct estimate of functional diversity (Varshney et al., 2007).

In *P. tomentosa*, associations were observed between 15 cellulose synthase genes (Pto CesA) and traits including growth and wood properties (micro fibril angle, holocellulose, α cellulose, and lignin content) (Du et al., 2013). In *E. urophylla*, CesA5 gene showed significant trait association for density (-0.48 at P 0.023) (Quang et al., 2012).

For the first time, these studies successfully show the utility of pilodyn as a tool for identifying phenotypes for wood basic density and use these for selective genotyping. These phenotypes expressed distinct polymorphism for wood basic density with one RM and two GMMs. Identification of more genic molecular markers and validation of these markers in larger populations can lead to efficient early selection procedure for wood basic density in *M. dubia* tree improvement program.

Statement on data availability

SSR markers were developed through next generation sequence analysis methodology (Genotypic Technology [P] Ltd., India). The data is available at National Centre for Biotechnology information (NCBI) with accession number SRX2834475. The details of markers used in this study can be provided if required.

REFERENCES

Apiolaza LA, Raymond CA, Yeo BJ (2005) Genetic variation of physical and chemical wood properties of Eucalyptus globulus. Silvae Genet. 54(4-5): 160-166

Bundock PC, Potts BM, Vaillencourt R (2008) Detection and stability of quantitative trait loci (QTL) in Eucalyptus globulus. Tree Genet. Genomes. 4:85-95

Chauhan S, Arun Kumar AN (2014) Assesment of variability in morphological and wood quality traits in Melia dubia Cav. for selection of superior trees. J. Indian Aca. Wood Sci. DOI 10 1007/s 13196-014-0113 3

Chauhan SS, Walker JCF (2006) Variation in acoustic and density with age, and their interrelationships in radiation pine. For. Ecol. Manag. 229:388-394

Cown DJ (1978) Comparison of the Pilodyn and torsiometer methods for the rapid assessment of wood density in living trees. N Z J For Sci: 384 – 3911

Darvasi A, Soller M (1994) Selective DNA pooling for determination of linkage between a molecular marker and a quantitative trait. Genetics. 138:1365–1373

Devey ME, Carson SD, Nolan MF, Matheson AC, Te Riini C, Hohepa J (2004) QTL associations for density and diameter in Pinus radiata and the potential for marker aided selection. Theor. Appl. Genet. 108:516-524

Dillon SK, Nolan M, Li W, Bell C, Wu HX, Southerton SG (2010) Allelic variation in cell wall candidate genes a solid wood properties in natural populations and land races of Pinus radiata. Genetics. 185: 1477–1487

Du Q, Wei Pan, Baohua Xu, Bailian Li, Deqiang Z (2013). Polymorphic simple sequence repeat (SSR) loci within cellulose synthase (PtoCesA) genes are associated with growth and wood properties in Populus tomentosa. New Phytologist. 197: 763–776

Evans R (1994). Rapid measurement of the transverse dimensions of tracheids in radial wood sections from Pinus radiata. Holzforschung 48(2):168–172

Grattapaglia D, Ribeiro VJ, Rezende GDSP (2004). Retrospective selection of elite parent trees using paternity testing with microsatellite markers: an alternative short term breeding tactic for Eucalyptus. Theor. Appl. Genet. 109: 192–199

Greaves B, Borralho NMG, Raymond CA, Farrington A (1996). Use of Pilodyn for the indirect selection of basic density in Eucalyptus nitens. Can. J. For. Res. 26: 1643–1650

Hamilton MG, Potts BM (2008). Review of Eucalyptus nitens genetic parameters. N Z J For Sci 38:102–119

Hansen CP (2000). Application of the Pilodyn in forest tree improvement. DFSC Series of Technical Notes. TN55. Danida Forest Seed Centre, Humlebaek, Denmark.

Ishiguri F, Matsui R, Lizuka K, Yokota S, Yoshizawa N (2008). Prediction of the mechanical properties of lumber by stress-wave velocity and Pilodyn penetration of 36-year-old Japanese larch trees. Oiginal Arbelten Originals 66: 275–280

Kien N D, Jansson G, Harwood C, Almqvist C, Thinh HH (2008). Genetic variation in wood basic density and pilodyn penetration and their relationships with growth, stem straightness and branch

Kimura M, Crow JF (1964). The number of alleles that can be maintained in a finite population. Genetics. 49(4):725-738

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics. 121: 185-199

Macdonald AC, Borralho NMG, Potts BM (1997). Genetic variation for growth and wood density in Eucalyptus globulus in Tasmania (Australia). Silva Genetica. 46 (4):236-241

Muranty H, Goffinet B (1997). Selective genotyping for location and estimation of the effect of a quantitative trait locus. Biomtrics. 53:629-643

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Parthiban KT, Bharathi AK, Seenivasan R, Kamala K, Rao MG (2009). Integrating Melia dubia in agroforestry farms as an alternate pulpwood species. International Journal of Life Sciences 1(3):59-63

Peakall R, Smouse PE (2006). Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288–295

Peakall R., Smouse PE (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. Bioinformatics (Oxford, England). 28: 2537–2539.

Pliura A, Zhang SY, Mackay J, Bousquet (2007) .Genotypic variation in wood density and growth traits of poplar hybrids at four clonal trials. Forest Ecology and Management 238: 92-106

Plomion C, Leprovost G, Stokes A (2001). Wood Formation in Trees. Plant Physiol. 127(4):1513-1523

Quang TH, Hallingback H, Gyllenstrand N, von Arnold SClapham D (2012). Expression of genes of cellulose and lignin synthesis in Eucalyptus urophylla and its relation to some economic traits. Trees. 26: 893-901

Rajib R, Abdelmoumen T, Khalid RH, Mohamed RAG, Jagatpati T (2014) Molecular marker-assisted technologies for Crop Improvement. In:*Roychowdhury R. (ed) Crop Improvement in the era of Climate Change, I.K. International Publication House Pvt. Ltd.

Rawat S, Joshi G, Annapurna D, Arunkumar AN, Karaba NN (2016). Standardization of DNA extraction method from Mmature dried leaves and ISSR-PCR conditions for Melia dubia Cav. —a fast growing multipurpose tree species. American Journal of Plant Sciences. 7: 437-445.

Raymond C, Henson M, Shepherd M, Sexton T (2009). Quantitative and molecular genetic control of wood properties and chemistry in Eucalyptus pilularis. In: Proceedings on Revisting Eucalyptus, Apiolaza L, Chauhan S, walker J (eds) University of Canterbury, New Zealand. pp. 13-28

Raymond CA (2002). Genetics of Eucalyptus wood properties. Ann. For. Sci. 59: 525–531

Raymond CA, MacDonald AC (1998). Where to shoot your pilodyn: within tree variation in basic density in plantation Eucalyptus globulus and E. nitens in Tasmania. New For. 15: 205–221

Saravanan V, Parthiban KT, Kumar P, Marimuthu P (2013) Wood characterization studies on Melia dubia cav. for pulp and paper industry at different age gradation. Res. J. Recent Sci. 2:183–188

Schimleck L, Evans R, Ilic J (2001) Application of near infrared spectroscopy to a diverse range of species demonstrating wide density and stiffness variation. IAWA J 22(4):415–429

Swaminathan C, Rao RV, Shashikala S (2012). Preliminary evaluation of variations in anatomical properties of Melia dubia Cav. Wood Int Res J Bio Sci 1(4):1–6

Swathi R (2016). Assessment of morphological and genetic diversity in populations of Melia dubia in Karnataka. Ph.D. Thesis Forest Research Institute, Dehradun, India.

Varshney RK, Mahendar T, Aggarwal RK, Borner A (2007). Genic molecular markers in plants: development and application. In: Varshney RK,

Tuberosa R (eds) Genomics-assisted crop improvement, vol.1: Genomic approaches & platforms, 13-29

Wang X, Ross RJ, Mcclellan M, Barbour RJ, Erickson JR, Forsman JW, Mcgin GD (2000). Strength and stiffness assessment of standing trees using a 37 nondestructive stress wave technique. Res. Pap. FPL-RP-585. U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, Madison, WI.

White JW, Pedro Andrade-Sanchez , Gorea MA, Bronsona KF, Coffelt TA, Conley MM, Feldmann KA, French AN, Heun JT, Hunsaker DJ, Jenks MA, Kimball BA, Roth RL, Strand R J, Thorp KR, Wall GW, Guangyao Wang (2012). Field based phenomics for plant genetic research. Field Crops Research 133:101-112

White TL, Adams WT, Neale D (2007). Forest genetics, CABI publishing. Cambridge MA USA. pp. 682

Wright S (1978). Evolution and the genetics of populations, Vol. IV. Variability within and among natural populations. University of Chicago Press, Chicago

Cite this article as:

Joshi G, Chauhan SS, Arunkumar AN, Ajay TL, Sreedevi CN, Rajan A, Dhavala A (2019). Phenotyping using pilodyn as a tool, selective genotyping and preliminary genetic diversity estimation for wood basic density in *Melia dubia*. Acad. J. Biotechnol. 7(6): 107-115.

Submit your manuscript at
http://www.academiapublishing.org/ajb