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Wood Fibre Properties and Genetic Divergence in *Haldina cordifolia* (Roxb.) Ridsdale

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Haldina cordifolia is a multipurpose tree species. It is one of the best Indian timbers suitable for flooring, panelling and for railway carriages. It is also suitable for pulp and paper, construction, window frames, furniture, bobbins, piano keys and rulers. Wood is also used for pencil manufacturing. Though many phyto-chemicals and pharmacological compounds have been identified from this tree species, the species has not been domesticated so far. ICFRE-Institute of Forest Genetics and Tree Breeding, Coimbatore has initiated a tree improvement programme in this tree species since 2018 and plus trees numbering 141 have been selected from the state of Kerala. *Eucalyptus* spp., *Casuarina* spp., and Poplars are the major sources of wood pulp used by the paper industries in India. Efforts are being undertaken by various research institutions in India to find alternate indigenous tree species suitable for pulp. Therefore, a study was undertaken to understand the wood fibre properties of select Plus Trees of *H. cordifolia*. Wood samples collected

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from 30 select plus trees have been subjected to wood fibre analyses and genetic divergence studies. Wood specific gravity varied from 0.62 to 0.71. Various wood fibre ratios were also studied. Among the 30 Plus Trees studied, HC 01, HC 03, HC 04, HC 05, HC 18, HC 26 and HC 27 have registered ideal values for using them for paper making. The maximum value for broad sense heritability was recorded by Felting coefficient (0.75). Coefficient of fibre flexibility, Isenberg coefficient, Runkel's ratio and Lumen diameter also registered reasonably higher values for H^2. Application of Mahalanobis' D^2 statistics and Tocher's clustering method resolved 30 Plus Trees into 6 clusters. The cluster strength varied from one in cluster 6 to 21 in cluster 1. The remaining 4 clusters contained two trees each. Genetic divergence studies have shown that Plus Trees namely, HC 22, HC 23, HC24, HC 27, HC 28, HC 29 and HC 30 could be utilized for further tree breeding programmes.

Keywords: Haldu; wood properties; wood fibre ratios; pulping traits; plus trees.

1. INTRODUCTION

"*Haldina cordifolia* (Roxb.) Ridsdale (Synonym: *Adina cordifolia* Benth. & Hook. f.) belongs to the family Rubiaceae. It is a South East Asian species. It is distributed throughout India, Burma, Sri Lanka, Bangladesh, Nepal, Thailand, South China, Bhutan, Vietnam, Myanmar and Malaysia. It is found scattered in deciduous forests throughout the greater part of India, ascending to an altitude of 900 m in the sub-Himalayan tract. It is also common in the forests of South India" [1]. "It grows well under 300-1000m altitude and prefers well-drained soil. Suitable soil pH range is between 5.5 to 6.5. The annual temperature requirement is within the range of 25^0C-35^0C and prefers a mean annual rainfall between 1000-2000mm" [2]. "It is not frost tolerant. The tree grows in various geological formations such as granite, gneiss, schist, quartzite, trap and laterite up to an elevation of 1000 MSL" [3].

"*Haldina* is largely used for structural work. It is one of the best Indian timbers suitable for flooring, panelling and for railway carriages" [4]. "It is also suitable for pulp and paper, construction, window frames, furniture, bobbins, piano keys and rulers" [5]. "Wood is also used for pencil manufacturing" [6]. Other uses are canoes and dugouts, planking of river boats, packing cases, cigar boxes, grain- measures, sieve frames, snuff boxes, furniture, yokes combs toys gunstocks, carving and turnery work, brush-backs and drums. Though many phyto-chemicals and pharmacological compounds have been identified from this tree species, the species has not been domesticated so far.

Considering the importance of the species preference by the tree growers, a tree improvement programme was initiated at the Institute of Forest Genetics and Tree Breeding, Coimbatore, India in 2018. *Eucalyptus* spp., *Casuarina* spp., and Poplars are the major sources of wood pulp used by the paper industries in India. Efforts are being undertaken by various research institutions in India to find alternate indigenous tree species suitable for pulp. Therefore, a study was undertaken to understand the wood fibre properties and pulping traits of select Plus Trees of *H. cordifolia*.

2. MATERIALS AND METHODS

Extensive field surveys were undertaken in the southern State of Kerala and selected 141 Candidate Plus Trees (CPT) of *H. cordifolia*. From these CPTs, 30 Plus Trees have been shortlisted based on the biometric and qualitative parameters. The basic experimental materials for this study comprised of wood samples collected from these 30 Plus Trees. The wood core samples were extracted at breast height level (1.37 m from the ground level) in the north-south direction using increment borer. These samples were cut into small pieces with a razor blade and placed in test tubes containing Hydrogen peroxide (H_2O_2), water and glacial acetic acid in the ratio of 1:4:5 followed by maceration [7]. "Subsequently, the 20 (µm) transverse sections of the core samples were put into a test tube and kept in a hot water bath at 70ºC in 12-18 hours. Macerated samples were prepared with sliding and then stained with safranin, dehydrated in a graded ethanol series and mounted on glass slides. Photomicrographs taken with a digital live camera mounted on a microscope were used for measuring fibre length, fibre diameter, lumen diameter and fibre wall thickness measured for 30 fibres per Plus Tree using NIS elements software. Pulp and paper qualities were studied using ratios viz., Felting coefficient, Runkel's ratio, Isenberg coefficient and Coefficient of fibre flexibility" [7].

The experiment was laid out in Completely Randomized Design (CRD) with five replications. All the wood fibre parameters, specific gravity and wood fibre ratios were subjected to analysis of variance (ANOVA) technique for CRD [8]. The significance of difference among treatment means was tested by 'F' test and the means were compared using Duncan's Multiple Range Test (DMRT).

Mahalanobis' D^2 technique [9] was used to study the genetic divergence. The estimation of D^2 values is very complicated especially when the number of characters being studied becomes large because it needs inversion of matrix of higher order. The computation is very much simplified when the characters under study are independent and are expressed in terms of their respective standard errors. Therefore, the correlated variables were transformed into uncorrelated ones by using pivotal condensation method. The significance of the D^2 values was tested against the tabulated value of chi-square for 'p' degrees of freedom where, 'p' is the number of characters considered.

Plus Trees were grouped into different clusters using the Tocher's method [10]. "The first step for grouping was to arrange the trees in order of their relative distances from each other based on D^2 values. Two trees having smallest distance from each other were considered first to which a third tree having smallest average D^2 value from the first two trees was added. The nearest fourth tree was chosen next and the process was continued up to a stage where there was abrupt increase in the average D^2 after adding a particular tree. Similarly, other clusters were formed omitting the trees, which had already been included. The process was continued till all the trees were included into one or other cluster" [10].

After forming the clusters, the intra and inter cluster relationships were studied. The average intra cluster distances were measured using the formula $\sum Di^2$ / n where, $\sum Di^2$ is the sum of distances between all possible combinations (n) of the trees included in a cluster. The average inter cluster distances were worked out by taking into consideration all the component D^2 values possible among the members of the two clusters considered. The square root of the average D^2 values gave the genetic distance 'D' between or within clusters. The analysis was performed using the software SPAR (Statistical Package for Agricultural Research developed by the Indian Agricultural Statistics Research Institute, New Delhi, India).

Phenotypic variance, genotypic variance, phenotypic coefficient of variation, genotypic coefficient of variation, broad-sense heritability and genetic advance were estimated for all the parameters. Analysis of variance method was used for estimating the variability parameters.

Chart 1. Analysis of Variance (ANOVA)

Source	Degrees of Freedom	Mean Square	Expected Mean Square
Replication	(r-1)		
Plus Trees	(t-1)	M1	$\sigma^2e + \sigma^2g$
Error	(r-1) (t-1)	M2	σ^2e
Total	(rt-1)		

Where,
r =Number of replications
t =Number of Plus Trees
M1 =Mean sum of squares for Plus Trees
M2 =Mean sum of squares for error
σ2e =Variance due to error
σ2g =Variance due to Plus Trees

The Genotypic variance (GV) and Phenotypic variance (PV) were worked out based on the method described by Johnson et al. (1955) [11].

Genotypic variance (GV) (σ2g) = (M1-M2) / r

Phenotypic variance (PV) (σ2p) = σ2g + σ2e

Phenotypic coefficient of variation (PCV) and Genotypic coefficient of variation (GCV) were estimated following Burton (1952) [12].

PCV = ((Phenotypic variance)$^{1/2}$ / Grand mean) x 100

GCV = ((Genotypic variance)$^{1/2}$ / Grand mean) x 100

Broad-sense heritability (H^2) was calculated according to Lush [13].

Broad-sense heritability (H^2) = σ2g / σ2p

Genetic advance (GA) was worked out as per Johnson et al. [11].

Genetic advance = (Genotypic variance / (Phenotypic variance)$^{1/2}$) x K

Where, K = selection differential (K = 2.06 at 5 per cent selection intensity, Cotterill and Dean 1990) [14].

3. RESULTS AND DISCUSSION

3.1 Fibre Parameters and Specific Gravity

Mean performance of all the fibre parameters viz., fibre length, fibre diameter, lumen diameter, fibre wall thickness and specific gravity are presented in Table 1. All the parameters except specific gravity were found significant at 5% level of significance.

3.1.1 Fibre length

Fibre length when analysed for the 30 select Plus Trees varied from 629.81 µm to 1212.81 µm with a mean of 933.01 µm and standard deviation of 123.62 µm. The Plus Tree, HC 08 recorded the minimum value and the Plus Tree HC 28 registered the maximum value. Seven Plus Trees namely, HC 06, HC 13, HC 01, HC 27, HC 16, HC 29, and HC 23 were found on par with the top ranking Plus Tree (Table 1).

Table 1. Mean values for various wood fibre parameters and specific gravity in Plus Trees of *Haldina cordifolia*

Plus Tree No.	Fibre Length (µm)	Fibre Diameter (µm)	Lumen Diameter (µm)	Fibre Wall Thickness (µm)	Double Wall Thickness (µm)	Specific Gravity (NS)
HC 01	1096.15 a-d*	21.85 i-n*	15.85 d-g*	2.65 g-j*	5.30 g-j*	0.68
HC 02	962.82 b-h	23.41 g-l	16.74 c-f	3.59 b-h	7.17 b-h	0.67
HC 03	954.73 b-h	18.73 n-o	11.24 h	3.96 b-e	7.93 b-e	0.66
HC 04	1005.41 b-h	21.33 j-o	14.61 f-g	3.40 c-j	6.80 c-j	0.67
HC 05	982.50 b-h	20.14 k-o	13.23 g-h	3.70 b-f	7.40 b-f	0.65
HC 06	1122.24 ab	28.69 bc	20.56 b	3.75 b-f	7.50 b-f	0.70
HC 07	978.93 b-h	27.55 b-d	21.13 b	2.85 f-j	5.70 f-j	0.71
HC 08	629.81 i	24.68 d-i	15.28 f-g	3.89 b-e	7.78 b-e	0.69
HC 09	910.42 f-h	26.97 b-e	18.99 b-d	3.50 b-h	6.99 b-h	0.68
HC 10	999.18 b-h	27.42 b-e	19.63 b-c	3.46 b-i	6.92 b-i	0.66
HC 11	985.22 b-h	18.39 o	11.39 h	3.54 b-h	7.08 b-h	0.68
HC 12	874.63 g-h	20.75 k-o	15.65 e-g	2.41 j	4.83 j	0.67
HC 13	1114.21 a-c	25.96 b-g	20.55 b	2.59 h-j	5.19 h-j	0.70
HC 14	991.32 b-h	21.70 i-o	15.86 d-g	2.85 f-j	5.70 f-j	0.69
HC 15	925.51 e-h	20.37 k-o	14.46 f-g	2.58 h-j	5.16 h-j	0.68
HC 16	1091.26 a-e	26.86 b-f	18.47 b-e	3.67 b-g	7.33 b-g	0.65
HC 17	858.23 g-h	20.37 k-o	15.77 e-g	2.45 i-g	4.90 i-g	0.62
HC 18	929.56 d-h	19.85 m-o	12.69 g-h	3.45 b-i	6.90 b-i	0.63
HC 19	959.40 b-h	22.36 h-m	15.64 e-g	3.19 d-j	6.38 d-j	0.68
HC 20	858.79 g-h	24.12 e-j	14.53 f-g	3.13 e-j	6.25 e-j	0.63
HC 21	1026.87 b-g	28.05 b-d	19.57 b-c	4.44 a-b	8.89 a-b	0.71
HC 22	855.36 h	25.26 d-h	18.97 b-d	3.19 d-j	6.38 d-j	0.70
HC 23	1064.17 a-f	29.06 b	19.05 b-c	4.89 a	9.77 a	0.70
HC 24	981.62 b-h	27.46 b-e	19.20 b-c	4.17 a-d	8.34 a-d	0.68
HC 25	1024.39 b-h	26.24 b-g	18.64 b-e	4.37 a-c	8.74 a-c	0.66
HC 26	946.17 c-h	20.12 k-o	15.23 f-g	2.85 f-j	5.70 f-j	0.69
HC 27	1095.27 a-e	23.56 f-k	16.55 c-f	3.45 b-i	6.89 b-i	0.70
HC 28	1212.81 a	32.99 a	25.04 a	3.66 b-j	7.32 b-j	0.68
HC 29	1084.29 a-e	25.56 c-h	18.94 b-d	3.32 d-j	6.63 d-j	0.67
HC 30	1045.43 b-f	20.15 l-o	17.13 c-f	3.29 d-j	6.57 d-j	0.66
Mean	933.01	25.19	16.95	3.39	6.77	0.75
SD	123.62	5.23	5.00	0.99	1.98	0.82
SEM	10.09	0.43	0.41	0.08	0.16	0.07

** Means with the same letter in a column do not differ significantly at 5% level of significance as per Duncan's Multiple Range Test (DMRT).*
NS: Non-significant

Fibre length is a wood quality character of central importance for the pulp industry [15]. Fibre length has a marked effect in product quality and the use of wood. Generally, conifers are used for making paper pulp since they contain long fibres. The major problem with hardwoods is reduced fibre length. With improved technologies, it is possible to overcome fibre length limitations. For example, some Eucalyptus spp. are now being used to make good grades of papers formerly considered possible only from long fibered conifer woods [16].

3.1.2 Fibre diameter

The Plus Tree HC 28 ranked first with reference to fibre diameter (32.99 µm) and HC 11 recorded the minimum value (18.39 µm). No other trees were found on par with the top-ranking genotype. The mean and SD were 25.19 µm and 5.23 µm (Table 1).

3.1.3 Lumen diameter

Lumen diameter ranged between 11.24 µm (HC 03) and 25.04 µm (HC 28) with a mean and standard deviation of 16.95 µm and 5.00 µm respectively. Thirteen trees registered superior values for this parameter (Table 1).

Fibre diameter and lumen diameter also affect the quality of the end products. Woods of spruce are generally better than some pines due to their relatively long and narrow fibres. According to Artuz-Seigel [17] fibre width had been little emphasized within species, although choices among species were often influenced by the width and lumen size of the fibres.

3.1.4 Fibre wall thickness

Plus Trees namely, HC 21, HC 25 and HC 24 recorded superior values at par with the top ranking tree HC 23 (4.89 µm). HC 12 registered the minimum value (2.41 µm). The mean and standard deviation were 3.39 µm and 0.99 µm respectively (Table 1).

"Wall thickness is of prime importance in wood and is related to specific gravity. Wood with thick cell walls tends to produce paper with a poor printing surface and poor mullen (burst) strength" [18]. The thick-walled fibres do not bend easily and do not collapse upon pulping, which inhibits chemical bonding. The surface of paper made from thick-walled fibres does not smoothen and ink tends to spread rather than to produce sharply defined printed letters. Thinner walled fibres collapse upon pulping, bond well chemically to produce a smooth paper surface. Wall thickness also has a major effect on the bending, tear and tensile strengths of paper. The wall thickness of fibres in the hard wood can vary greatly, even within a species.

3.1.5 Specific gravity

Wood specific gravity when analysed varied from 0.62 to 0.71. HC 17 recorded the minimum value where HC 07 registered the maximum value. The mean and SD were 0.75 and 0.82 respectively. Five trees recorded a specific gravity of above or equal to 0.70.

3.1.6 Double wall thickness

Double wall thickness varied from 4.83 µm in HC 12 to 9.77 µm in HC 23. It showed a direct relationship with wood specific gravity and affects several qualities of paper and pulp. Generally high dense wood produces more quantity of pulp but the quality of the pulp would be poor due to the stiffness of fibres.

3.2 Wood Fibre Ratios

Results obtained for the derived wood fibre ratios namely, Felting Coefficient, Runkel's Ratio, Isenberg Coefficient and Coefficient of Fibre Flexibility are presented in Table 2. All these ratios were found significant at 5% level of significance.

3.2.1 Felting coefficient

Ratio of fibre length to fibre diameter known as Felting coefficient, affects the qualities of paper like ease of sheet formation, sheet smoothness and opacity. Higher the ratio, better the quality of paper. This ratio when studied for 30 Plus Trees ranged between 25.32 (HC 08) and 53.58 (HC 11) with a mean of 39.33 and SD of 10.59. Eleven Plus Trees namely, HC 11, HC 30, HC 03, HC 01, HC 05, HC 04, HC 26, HC 18, HC 27, HC 14 and HC 15 registered higher values for Felting coefficient (Table 2). They are suitable for better quality pulp production.

3.2.2 Runkel's ratio

Double wall thickness to lumen diameter, known as Runkel's ratio, affects the quality of paper and pulp. Thick-walled fibres are usually stiff, resistant to beating and retain their rounded shape during sheet formation. This inhibits inter fibre bonding and the resulting fibres would be of low quality with poor printing surface. On the other hand, thin-walled fibres bend easily and collapse upon pulping and provide a large area for inter fibre bonding. Lower the ratio, better will be the quality of pulp.

Table 2. Mean values for various wood fibre ratios in Plus Trees of *Haldina cordifolia*

Plus Tree No.	Felting Coefficient	Runkel's Ratio	Isenberg Coefficient	Coefficient of Fibre Flexibility
HC 01	50.16 a-c*	0.34 e-g*	0.24 f-k*	0.73 b-f*
HC 02	41.13 c-h	0.43 b-g	0.31 b-i	0.72 b-g
HC 03	50.98 a-c	0.71 a	0.42 a	0.60 h
HC 04	47.10 a-e	0.47 b-f	0.32 b-g	0.69 c-h
HC 05	48.78 a-d	0.56 a-c	0.37 a-c	0.66 d-h
HC 06	39.13 d-h	0.37 c-g	0.26 e-k	0.72 b-f
HC 07	35.53 g-h	0.27 f-g	0.21 j-k	0.77 a-c
HC 08	25.52 i	0.51 b-e	0.32 b-h	0.62 g-h
HC 09	33.75 h-i	0.37 c-g	0.26 e-k	0.70 b-g
HC 10	36.44 f-h	0.35 c-g	0.25 e-k	0.72 b-f
HC 11	53.58 a	0.62 a-b	0.39 a-b	0.62 g-h
HC 12	42.14 b-h	0.31 e-g	0.23 h-k	0.75 b-d
HC 13	42.92 b-h	0.25 g	0.20 k	0.79 a-b
HC 14	45.68 a-g	0.36 c-g	0.26 e-k	0.73 b-f
HC 15	45.44 a-g	0.36 c-g	0.25 e-k	0.71 b-g
HC 16	40.59 c-h	0.40 c-g	0.27 d-k	0.69 c-h
HC 17	42.13 b-h	0.31 e-g	0.24 g-k	0.77 a-c
HC 18	46.82 a-f	0.54 a-d	0.35 b-d	0.64 f-h
HC 19	42.90 b-h	0.41 c-g	0.29 c-j	0.70 b-g
HC 20	35.60 g-h	0.43 b-g	0.26 e-k	0.60 h
HC 21	36.56 f-h	0.45 b-g	0.32 b-h	0.70 b-g
HC 22	33.87 h-i	0.34 d-g	0.25 e-k	0.75 b-e
HC 23	36.61 f-h	0.51 b-e	0.34 b-e	0.66 e-h
HC 24	35.75 g-h	0.44 b-g	0.30 b-i	0.70 b-g
HC 25	39.01 d-h	0.47 b-f	0.33 b-e	0.71 b-g
HC 26	47.01 a-e	0.37 c-g	0.28 d-k	0.76 b-c
HC 27	46.48 a-f	0.42 c-g	0.29 c-i	0.70 b-g
HC 28	36.74 e-h	0.29 f-g	0.22 i-k	0.76 b-c
HC 29	42.43 b-h	0.35 d-g	0.26 e-k	0.74 b-e
HC 30	51.88 a-b	0.38 c-g	0.33 b-f	0.85 a
Mean	39.33	0.44	0.28	0.67
SD	10.59	0.21	0.09	0.13
SEM	1.11	0.02	0.01	0.01

** Means with the same letter in a column do not differ significantly at 5% level of significance as per Duncan's Multiple Range Test (DMRT)*

The Plus Tree HC 13 recorded the lowest value for Runkel's ratio (0.25) and eight other trees registered lower values for this ratio. They were HC 07, HC 28, HC 12, HC 17, HC 01, HC 22 and HC 29 indicating their suitability for good quality pulp production. The genotype HC 03 registered the maximum value for this ratio (0.71). The mean and SD were 0.44 and 0.21 respectively (Table 2).

3.2.3 Isenberg coefficient

The ratio of double wall thickness to fibre diameter, known as Isenberg coefficient, influences the pulping qualities in several ways. It determines the degree of flexibility and collapse of fibres, both of which control the degree of conformability within the paper sheet and thus the numbers of inter fibre bonds. Generally, lower the ratio, better the quality of paper.

Fourteen Plus Trees exhibited lower values for Isenberg coefficient (HC 13, HC 07, HC 28, HC 12, HC 17, HC 01, HC 10, HC 22, HC 15, HC 20, HC 09, HC 29, HC 06 and HC 14). They are better suited for paper manufacture. The minimum value for this ratio was registered by HC 13 (0.20) and the maximum value was recorded by HC 03 (0.42) with a mean and standard deviation of 0.28 and 0.09 respectively.

3.2.4 Coefficient of fibre flexibility

The ratio of lumen diameter to fibre diameter, known as coefficient of fibre flexibility, ranged from 0.60 in HC 03 to 0.85 in HC 30. This ratio affects the flexibility of fibres. Generally, a higher ratio indicates a better flexibility of fibres. Nine trees namely, HC 30, HC 13, HC 17, HC 07, HC 28, HC 26, HC 12, HC 22 and HC 29 estimated higher values for this ratio showing their superiority over other trees for paper making.

Trees with high Felting coefficient and Coefficient of fibre flexibility but low Runkel's ratio and Isenberg coefficient are ideal for pulp. Among the 30 Plus Trees studied, the following trees namely, HC 01, HC 03, HC 04, HC 05, HC 18, HC 26 and HC 27 have registered ideal values for using them for paper making.

3.3 Genetic Parameters

Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) broad sense heritability (H2) and genetic advance (GA) worked out for various wood fibre parameters and wood fibre ratios are presented in Table 3.

Runkel's ratio registered the maximum value for GCV and PCV and Genetic advance. Fibre length registered the minimum values for these genetic parameters. Phenotypic coefficient of variation was found to be higher than the genotypic coefficient of variation for all the parameters indicating that these traits are influenced by the environment. In *Eucalyptus viminalis*, Otegbeye and Kellison (1980) [19] found a heritability of 0.42 for fibre length. However, in the present study fibre length recorded a heritability of 0.01 only. The maximum value for broad sense heritability was recorded by Felting coefficient (0.75). Coefficient of fibre flexibility, Isenberg coefficient, Runkel's ratio and Lumen diameter also registered reasonably higher values for H^2. There are reports that fibre width and lumen diameter are genetically controlled [20]. In this study also reasonably higher values for GCV and PCV and H^2 were obtained for these parameters. Otegbeye and Kellison [19] found a heritability of 0.82 for fibre diameter and 0.94 for wall thickness in young trees of *E. viminalis*. Mergen and Furnival [21] demonstrated genetic control of tracheid wall thickness in the hybrid between *Pinus thunbergii* and *Pinus densiflora*.

3.4 Genetic Divergence

Genetic divergence analysis is essential to estimate the genetic diversity existing among the various provenances / genotypes of a species. Divergence analysis is generally performed to identify the diverse genotypes for hybridization purposes [22]. Among the various methods available, Mahalanobis' D^2 analysis technique is the most appropriate measure of multivariate relationships when the data are normally distributed and homocedastic. Also known as generalized distance method, it can be useful even if the above assumption is not quite true.

The results of genetic divergence studies carried out in Plus Trees of *H. cordifolia* are presented in Tables 4 to 8.

3.4.1 Clustering

Application of Mahalanobis' D^2 statistics and Tocher's clustering method resolved 30 Plus Trees into 6 clusters. The cluster strength varied from one in cluster 6 to 21 in cluster 1. The remaining 4 clusters contained two trees each (Table 4).

Table 3. Genetic parameters for wood fibre properties of *Haldina cordifolia*

	GCV	PCV	ECV	Broad Sense Heritability	Genetic Advance
Fibre length	1.01	14.94	14.90	0.01	0.14
Fibre diameter	13.45	20.32	15.23	0.44	18.35
Lumen diameter	21.99	29.12	19.09	0.57	34.21
Fibre wall thickness	17.27	29.84	24.34	0.34	20.60
Double wall thickness	22.43	30.88	21.21	0.53	33.58
Specific gravity	1.34	8.99	8.89	0.02	0.41
Felting coefficient	30.89	35.71	17.92	0.75	55.04
Runkel's ratio	37.36	48.98	31.67	0.58	58.70
Isenberg coefficient	28.16	34.70	20.28	0.66	47.06
Coefficient of fibre flexibility	17.68	20.90	11.14	0.72	30.82

Table 4. Clustering of plus trees of *Haldina cordifolia*

Cluster No.	Plus Trees Grouped
1	HC 01, HC 02, HC 03, HC 04, HC 05, HC 06, HC 07, HC 08, HC 09, HC 10, HC 11, HC 12, HC 13, HC 14, HC 15, HC 16, HC 17, HC 18, HC 19, HC 20, HC 21
2	HC 24, HC 29
3	HC 25, HC 26
4	HC 27, HC 28
5	HC 22, HC 23
6	HC 30

3.4.2 Intra and inter cluster distances

The average intra and inter cluster D^2 and D values for the 6 clusters obtained are presented in Tables 5 and 6. The largest intra-cluster distance was registered in cluster 5 (6.27) followed by cluster 4 (2.07). Cluster 2 recorded the smallest intra-cluster distance (1.50) followed by cluster 3 (1.60). Intra cluster distance for cluster 6 zero, since it included only one tree.

Inter-cluster distance revealed that maximum divergence existed between clusters 2 and 5 (11.43). The minimum inter-cluster distance was observed between clusters 2 and 3 (2.03). Higher values for inter-cluster distance were also recorded between clusters 4 and 5 (11.41) and 5 and 6 (10.25).

3.4.3 Cluster means of parameters

Cluster means computed for all the ten parameters are given in Table 7. Cluster 4 showed maximum mean value for fibre length (979.06) and fibre diameter (29.99). Cluster 2 recorded the maximum values for lumen diameter (23.28) and coefficient of fibre flexibility (0.82). For fibre wall thickness, cluster 1 showed the maximum value (3.47). Cluster 5 had the maximum values for specific gravity (0.70), felting coefficient (79.18), Runkel's ratio (0.80) and Isenberg coefficient (0.43) (Table 7).

Cluster 2 exhibited the minimum values for fibre length (848.83) and felting coefficient (30.86). Minimum values for fibre diameter (15.37) and lumen diameter (8.67) were registered by cluster 5. For fibre wall thickness, specific gravity, Isenberg coefficient and coefficient of fibre flexibility cluster 4 recorded the minimum values (2.38 and 0.67, 0.16 and 0.40 respectively). Cluster 2 registered the minimum values for felting coefficient (30.86) and Runkel's ratio (0.23).

3.4.4 Contribution of each character to genetic divergence

Relative contribution of each parameter towards genetic divergence is presented in Table 8. Coefficient of fibre flexibility contributed the maximum towards genetic divergence (44.83%) followed by Isenberg coefficient (15.86%), lumen diameter (12.41%) and felting coefficient (9.66%). The minimum percentage contribution towards genetic divergence was registered by fibre diameter (1.84%, Table 8).

Table 5. Inter and intra cluster D-square values

Clusters	1	2	3	4	5	6
1	13.10	17.72	12.01	47.15	96.56	21.23
2		2.25	4.125	58.20	130.59	29.13
3			2.57	51.64	112.37	23.95
4				4.29	130.20	8.60
5					39.35	104.97
6						0.00

Table 6. Inter and intra cluster distances

Clusters	1	2	3	4	5	6
1	3.62	4.21	3.47	6.87	9.83	4.61
2		1.50	2.03	7.63	11.43	5.40
3			1.60	7.19	10.60	4.89
4				2.07	11.41	2.93
5					6.27	10.25
6						0.00

Table 7. Cluster means

Cluster	FL	FD	LD	FWT	DWT	SG	FC	RR	IC	CFF
1	941.49	25.77	17.01	3.47	7.29	0.68	37.64	0.44	0.29	0.68
2	908.74	29.21	23.28	2.73	5.50	0.69	30.86	0.23	0.19	0.82
3	848.83	26.92	19.54	3.03	6.12	0.67	31.60	0.31	0.23	0.78
4	979.06	29.99	12.03	2.38	4.75	0.67	32.67	0.40	0.16	0.40
5	934.07	15.37	8.67	2.63	5.22	0.70	79.18	0.80	0.43	0.58
6	925.96	27.47	14.14	2.79	5.59	0.69	33.70	0.40	0.20	0.52

Table 8. Contribution of each character to genetic divergence

Character	No. of First Rank	% Contribution
Fibre Length	14	3.22
Fibre Diameter	8	1.84
Lumen Diameter	54	12.41
Fibre Wall Thickness	15	3.45
Double Wall Thickness	18	4.14
Specific Gravity	11	2.53
Felting Coefficient	42	9.66
Runkel's Ratio	9	2.07
Isenberg Coefficient	69	15.86
Coefficient of Fibre Flexibility	195	44.83

"When large number of phenotypically superior genotypes of a species are available due to initial selections, the D^2 statistics and clustering technique help to form genetically homogeneous groups and representative samples from such groups will reduce the number of entries of genetically similar selections / provenances for inclusion in the establishment of provenances trials, progeny trials and seed orchards" [23]. Reduced entries will reduce the total area required for planting and in turn reduce the cost of the tree improvement programme.

According to Singh and Chaudhary [22], "three important points which need to be essentially considered while selecting genotypes include (a) choice of the particular cluster from which genotypes are to be used as parents (b) selection of a particular genotype from the selected clusters and (c) relative contribution of characters to total divergence. The results obtained in this study suggest that selected trees included in clusters 2, 4, 5 and 6 in general can be used for hybridization programmes to create variability and exploit hybrid vigour". "As the intra-cluster distances among clusters 1, 4 and 5 were high, trees within a cluster are also adequately divergent for tree improvement programme through hybridization. In selecting genotypes from the already chosen groups, other important characteristics like disease resistance quality or even performance of a particular character should also be considered" [24].

Cluster 2 recorded the maximum cluster mean value for coefficient of fibre flexibility which had contributed maximum towards genetic divergence. The maximum divergence existed between clusters 2 and 5. Higher values for inter-cluster distance were also recorded between clusters 4 and 5 and 5 and 6. Therefore, the Plus Trees namely, HC 22, HC 23, HC24, HC 27, HC 28, HC 29 and HC 30 could be utilized for further tree breeding programmes.

4. CONCLUSION

Based on the fibre ratios namely, Felting coefficient, Runkel's ratio, Isenberg coefficient and Coefficient of fibre flexibility, Plus Trees namely, HC 01, HC 03, HC 04, HC 05, HC 18, HC 26 and HC 27 could be used for paper making. The maximum value for broad sense heritability was recorded by Felting coefficient (0.75). Coefficient of fibre flexibility, Isenberg coefficient, Runkel's ratio and Lumen diameter also registered reasonably higher values for H^2. Wood specific gravity varied from 0.62 to 0.71. HC 17 recorded the minimum value where HC 07 registered the maximum value. Application of Mahalanobis' D^2 statistics and Tocher's clustering method resolved 30 Plus Trees into 6 clusters. The cluster strength varied from one in cluster 6 to 21 in cluster 1. According to Singh and Chaudhary (1985), three important points which need to be essentially considered while selecting genotypes include (a) choice of the particular cluster from which genotypes are to be used as parents (b) selection of a particular genotype from the selected clusters and (c) relative contribution of characters to total divergence. The results obtained in this study suggest that Plus Trees included in clusters 2, 4, 5 and 6 in general can be used for hybridization programmes to create variability and exploit hybrid vigour. Therefore, the Plus Trees namely, HC 22, HC 23, HC24, HC 27, HC 28, HC 29 and HC 30 could be utilized for further tree breeding programmes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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