



# International Journal of Research in Agronomy

E-ISSN: 2618-0618

P-ISSN: 2618-060X

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2024; SP-7(8): 360-366

Received: 08-06-2024

Accepted: 12-07-2024

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## Characterization of anatomical properties for different mulberry clones

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**DOI:** <https://doi.org/10.33545/2618060X.2024.v7.i8Se.1269>

### Abstract

India is a leading producer and consumer of wood products in the Asian and Pacific regions, with a significant demand driven by population growth and urbanization. Despite this demand, the Forest Conservation Act of 1980 and Supreme Court directives have restricted wood availability from natural forests, highlighting the need for alternative sources. Mulberry (*Morus* spp.) has emerged as a promising species for agroforestry due to its fast growth and multiple industrial applications, including sericulture, handicrafts, sports goods, and paper production. This study aimed to identify superior mulberry clones through detailed wood anatomical analysis, focusing on vessel and fiber morphology. Vessel length and diameter, fiber length, fiber diameter, lumen size, cell wall thickness, and derived indices such as slenderness ratio, runkel ratio, flexibility, and rigidity coefficient were examined. The findings indicated significant variations among clones, with several clones demonstrating characteristics favorable for industrial applications, particularly in paper production. The study underscores the potential of mulberry as an alternative raw material for the wood-based industry, contributing to the sustainable development of agroforestry systems in India.

**Keywords:** Wood anatomical analysis, mulberry clones, agroforestry potential, fiber morphology, vessel characteristics

### Introduction

India is a leading producer and consumer of wood products in the Asian and Pacific regions. The growing population and urbanization, along with changes in policies and legal frameworks, have significantly increased the demand for wood and wood products (Parthiban and Fernandez, 2017) <sup>[19]</sup>. However, this rise in demand has not been matched by an increase in organized plantation development. The Forest Conservation Act of 1980 and directives from the Honourable Supreme Court, which include a complete ban on felling in natural forests, have restricted or halted the availability of wood from these sources. This mismatch between the growing demand and restricted supply from natural forests has created significant challenges (Parthiban and Seenivasan, 2017) <sup>[20]</sup>.

It is estimated that the country will need over 152 million cubic meters of wood to meet the raw material requirements of the wood-based industry (FAO, 2020) <sup>[8]</sup>, with an equal amount needed for the unorganized wood-based sector. Additionally, more than 380 million tonnes of wood are necessary to meet the energy requirements of both domestic and industrial sectors. This high demand for wood underscores the necessity for the development and promotion of organized plantation and agroforestry systems. To address these issues, the Government of India introduced a National Agroforestry Policy in 2014, providing guidelines and strategies to promote agroforestry (GOI, 2014) <sup>[10]</sup>. The policy emphasizes identifying new and alternative species suitable for agroforestry and plantation forestry programs.

Mulberry has emerged as a promising alternative species for agroforestry due to its multiple uses. This study aims to identify superior clones of mulberry suitable for various industrial applications. Mulberry is a fast-growing, deciduous, perennial, and highly heterozygous plant believed to have originated in the Northern Hemisphere and spread to the tropics of the Southern Hemisphere (Benavides *et al.*, 1994) <sup>[4]</sup>. It is the primary food source for silkworms (*Bombyx mori*), and the availability of high-quality leaves significantly impacts the sustainability and

profitability of the sericulture industry (Vijayan, 2010) [32]. Mulberry wood is highly valued in Indian handicrafts, cabinet work, and as a raw material for sports goods like hockey sticks and tennis rackets (Sanchez, 2000) [26]. It is also suitable for paper production (Walia, 2013) [34].

Currently, the Indian paper industry primarily relies on eucalyptus and casuarina to meet its raw material needs. This reliance has its advantages and disadvantages, prompting the industry to seek alternative materials with high-yielding, short-rotation characteristics (Parthiban *et al.*, 2020) [12]. Among the various tree species promoted across the country, mulberry has shown significant potential (Rahman and Jahan, 2014) [22]. The *Morus* genus, including several mulberry species, is favored in India due to its fast growth and multiple industrial uses, such as raw material for pulp and paper production, energy generation, and timber for sports goods (Sujathamma *et al.*, 2013) [27]. Mulberry wood is characterized by moderate cellulose, lignin, and pentosan content (Rahman and Jahan, 2014) [22].

In China and Bangladesh, mulberry is a suitable source for pulping. Mulberry pulp can be produced by the kraft process and blended with jute fiber pulp up to 50%, which can be used as kraft liner. Reinforcing jute pulp with mulberry pulp up to 50% enhances tensile, tear, and burst indexes (Rahman and Jahan, 2014) [22]. However, in India, mulberry has not been utilized as a raw material by the pulp and paper industries due to the lack of suitable varieties with improved wood properties. Therefore, this study aims to conduct a detailed wood anatomical analysis of mulberry to evaluate its potential for various industrial applications.

## Materials and Methods

The experiment was conducted in Agroforestry laboratory of Forest College and Research Institute, Mettupalayam, Tamil Nadu during the year 2020-2021. The experimental material for the present study consisted of twenty one mulberry germplasm (genetic resources) obtained from Central Sericultural Germplasm Resource Centre, Hosur, Tamil Nadu and these germplasms are registered with CSGRC (<http://csgrc.res.in/>) which were raised in germplasm garden and maintained from 2020 onwards at Department of Sericulture, Forest College and Research Institute, Mettupalayam, Tamil Nadu. Experiment was carried out in Randomized Block Design (RBD) with five replications per variety. Mulberry wood from two years six month old plants were used to wood anatomical properties analysis.

### Sample preparation and Maceration of mulberry wood sample

The wood samples each of dimension 2x2x2cm<sup>3</sup> were sliced out separately from the mulberry clones and those wood samples were taken thin microscopic sections of size 15 to 20 µm using 'Yorcorotater microtome' (lipshaw type).

Maceration was done by Jeffrey's method (Lim and Son, 1997) [15]. Jeffrey's solution was prepared by mixing equal volumes of 10 percent potassium dichromate and 10 percent nitric acid. Radial chips of wood shavings were taken in the test tubes and boiled in the maceration fluid for 15-20 mins so that the individual fibers were separated, then the test tubes were kept for 5-10 mins so as to make the fibers settles at the bottom. The solution was discarded and the resultant material was thoroughly washed in distilled water until traces of acid were removed. The samples were stained using safranin and mounted on temporary slides using glycerin as the mountant subjected to measurements and photography was taken using image analysis system

(Optika). Measurement of anatomical parameters was done using the Optika software.

### Vessel morphology

**a) Vessel length:** Length (µm) of the vessels were measured on the transverse sections (TS) using the optika software .

**b) Vessel diameter:** Tangential diameter (µm) of the vessels were measured on the transverse sections (TS) using the Optika Image Analysis software.

### Fibre morphology

**a) Fibre length:** Fibre length (µm) was measured from macerated wood samples by measuring both end of the fibre through Optika Image Analysis software.

**b) Fibre diameter:** Diameter (µm) of the fibre was measured from macerated wood samples by measuring cross sectional area through Optika Image Analysis Software.

**c) Fibre wall thickness:** Wall thickness (µm) of the fibre was measured from macerated wood samples by measuring thickness of the fibre wall cross sectional area through Optika Image Analysis Software.

**d) Fibre lumen width:** Lumen width (µm) of the fibre was measured from macerated wood samples by measuring width of the lumen at cross sectional area through Optika Image Analysis Software.

### Derived values (Indices) from fibre dimensions

$$\text{Slenderness ratio} = \frac{\text{Fibre length}}{\text{Fibre diameter}} \quad (\text{Varghese, 1995})^{[30]}$$

$$\text{Flexibility Coefficient} = \frac{\text{Fibre lumen width}}{\text{Fibre diameter}} \times 100 \quad (\text{Wangaard, 1962})^{[35]}$$

$$\text{Runkel ratio} = 2 \times \frac{\text{Fibre wall thickness}}{\text{Fibre lumen width}} \quad (\text{Runkel, 1949})^{[23]}$$

$$\text{Rigidity Coefficient} = \frac{\text{Fibre wall thickness}}{\text{Fibre diameter}} \quad (\text{Tamolang and Wangaard, 1961})^{[29]}$$

## Results and Discussion

### Vessel morphology of mulberry clones

Vessels are responsible for long distance water transport in majority of the plants and contribute towards growth and development. The vessel length is the key trait that determines plant hydraulic efficiency and safety (Parthiban and Seenivasan, 2017) [20]. The vessel element length is not so important for conduction purposes, but its diameter is very important, because the vessel area is quadratically related to its diameter. On the other hand, the relationship between length and diameter of each vessel cell determines important characteristics of these elements for using these woods for pulp and paper manufacturing *i.e.* the process of wood conversion into cellulose pulp. Their presence in the hardwoods favors the chip impregnation process by the cooking liquor (Foelkel, 2007) [9].

On the contrary, large vessel elements cause a vessel-picking problem in papermaking when hardwoods are used and the vessel picking problems are affected by vessel width, length and number of unit weight. (Panula-Ontto, 2007 and Karjalainen *et al.*, 2012) [18, 13]. Hence the wood samples of all the clones deployed in the current study were analysed for vessel morphology *viz.*, Vessel length and Vessel diameter. The study indicated that the clones differed significantly for vessel length and diameter and it ranged from 33.63  $\mu\text{m}$  to 102.25  $\mu\text{m}$  and 10.50  $\mu\text{m}$  to 27.50  $\mu\text{m}$  respectively (Table 1 & Fig 1). The clone MI-0536 (102.25  $\mu\text{m}$ ) exhibited higher vessel length and MI-0013 (27.50  $\mu\text{m}$ ) registered higher vessel diameter. Narrow vessel elements (diameter 10  $\mu\text{m}$  to 50  $\mu\text{m}$ ) can be entangled and

form a network with fibrous material and are not harmful in papermaking *i.e.* narrow vessels does not encourage vessel picking problem in paper (Karjalainen *et al.*, 2012) [13]. In current investigation almost all the clones observed narrower vessel elements. Similar findings were reported in wheat straw pulp (Karjalainen *et al.*, 2012) [13] and they reported the vessel length and diameter differed from 100  $\mu\text{m}$  to 1.4 mm and 10  $\mu\text{m}$  to 20  $\mu\text{m}$  respectively. Alipon *et al.*, (2021) reported the highest vessel length and width in 7-yr-old trees with an average of 0.479 mm and 0.321 mm, respectively in *Falcataria moluccana*. In eucalyptus, vessel elements differed from 150  $\mu\text{m}$  to 600  $\mu\text{m}$  (length) and 60  $\mu\text{m}$  to 250  $\mu\text{m}$  (diameter) (Foelkel, 2007) [9] which thus lend support to the current findings.

**Table 1:** Vessel length and Diameter of different mulberry clones

Clones	Vessel length ( $\mu\text{m}$ )	Vessel diameter ( $\mu\text{m}$ )
ME-0025	41.25	11.50
MI-0211	90.13**	22.00
ME-0001	74.88	24.50**
ME-0109	78.63	23.25**
MI-0013	90.50**	27.50**
MI-0349	76.00	25.38**
MI-0395	46.00	24.50**
MI-0536	102.25**	22.38
MI-0615	96.88**	19.63
MI-0718	82.38*	18.13
MI-0768	95.50**	20.50
MI-0034	33.63	10.50
MI-0663	79.88	26.00**
MI-0685	66.63	23.38**
MI-0017	58.50	24.00**
ME-0006	98.13**	23.00*
MI-0807	86.50**	24.50**
MI-0845	86.13**	23.25**
ME-0220	71.00	21.38
ME-0095	93.25**	24.25**
MI-0308	86.63**	22.38
Mean	77.84	21.99
SEd	1.83	0.46
CD(0.05)	3.64	0.91
CD (0.01)	4.82	1.21

\*\* Significant at 1% level

\* Significant at 5% level

### Fibre morphology of mulberry clones

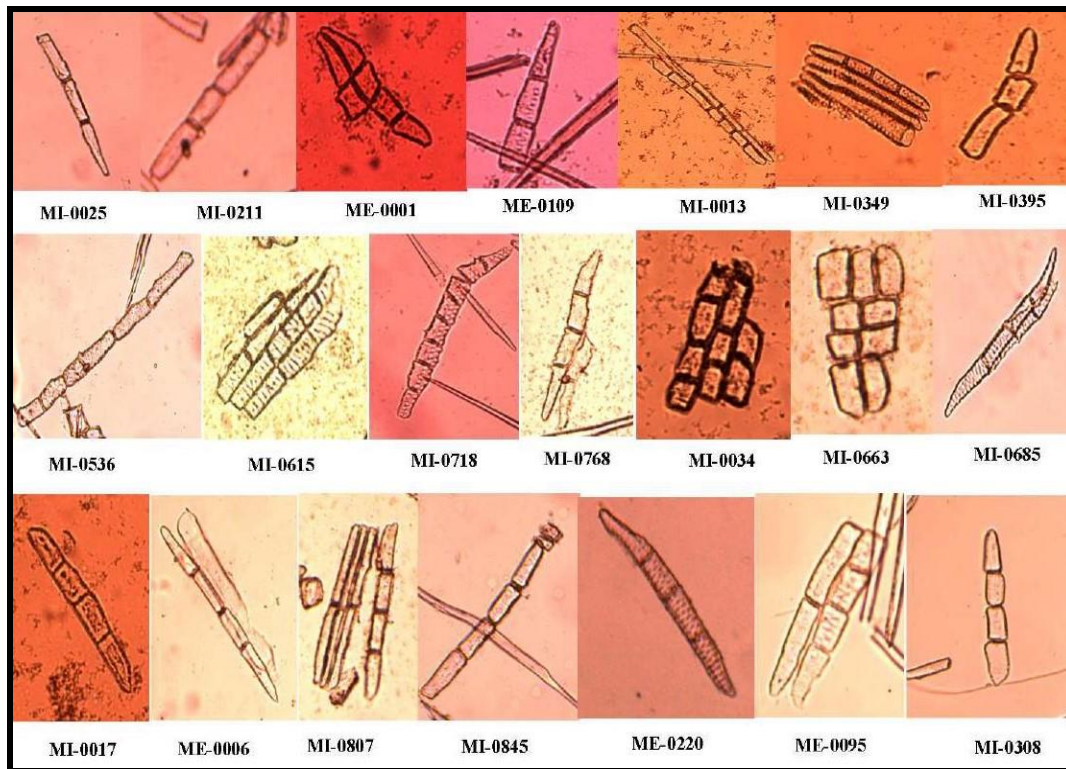
The current study revealed that the fibre length of mulberry clones varied from 692.63  $\mu\text{m}$  to 961.38  $\mu\text{m}$  which is considered as short fibres. Similarly, the same line of findings was reported in mulberry that the average fibre length was slightly increased from 0.63 mm to 0.73 mm with the increase of tree age from 8 to 12 months. It was similar to shorter range of tropical hardwoods (0.7 mm to 1.5mm) which are considered as short fibres (Rahman and Jahan, 2014) [22]. Short fibre contributes to the properties of pulp blends, especially opacity, printability and stiffness as well as good printability (Sadiku *et al.*, 2019) [24]. Similarly, the fibres of the species are shorter, relatively thinner and flexible. The fibres will be able to pack tightly together and thus produce smooth and dense paper (McDougall *et al.*, 1993) [16]. According to Walia (2013) [34] and Monga *et al.*, (2017) [17], Fibre length affects the tearing strength of paper and the greater the length of fibre, higher will be the tearing resistance of paper. On the contrary, longer fibre tends to give a more open and less uniform paper sheet structure. Hence it is important to

understand relationship between fibre length and paper strength properties.

Fibre diameter was higher in the clone MI-0663 (20.20  $\mu\text{m}$ ) which comes under the hardwood fiber. The fibre width of green *Paulownia* was found as about 34.59  $\mu\text{m}$  which was in normal range when compared to hardwood fiber (20 to 40  $\mu\text{m}$ ) (San *et al.*, 2016) [25] which lend support to the findings in the current investigation. Greater value of fibre diameter increases void volume and forms a coarse surfaced paper sheet (Kaur and Dutt, 2013) [14]

Lumen size is important for fibre dimensions because it will effect on the rigidity and strength properties of paper (Akpakpan *et al.*, 2012) [1]. In the current investigation, the mulberry clone MI-0663 registered higher lumen width followed by ME-0006, MI-0308, MI-0807, MI-0013, MI-0211 and MI-0845 which would show better results during beating of pulp. According to Walia (2013) [34], lumen width affects the beating of pulp. Larger the fiber lumen width better will be the beating of pulp, because of penetration of liquid into empty space between the fibers.





**Fig 1:** Vessel morphology of different mulberry clone

The thickness of cell wall was important in pulp refining process. Thick walled fibres affect the bursting strength, tensile strength and double fold number of paper negatively (Monga *et al.*, 2017) <sup>[17]</sup>. In the current study, the cell wall thickness varied from 3.13  $\mu\text{m}$  (ME-0220) to 5.33  $\mu\text{m}$  (ME-0025). According to Monga *et al.*, (2017) <sup>[17]</sup>, the paper made from thick walled fibres will be porous, more opaque, bulky and coarse surface with a large void volume. On contrary, the sheet made from thin walled fibres will be less opaque, less porous, denser and well formed. Fibres with large lumen and thin walls tend to flatten to ribbons during pulping and paper making, giving good contact between the fibres and consequently having good strength characteristics (Sadiku and Abdulkareem, 2019) <sup>[24]</sup>. Current findings are in concurrence with the studies of Anusuya (2019) <sup>[3]</sup> and it is reported that the cell wall thickness ranged from 3.19  $\mu\text{m}$  to 4.98  $\mu\text{m}$  in different mulberry genetic resources. In *Morus nigra* cell wall thickness was 3.6  $\mu\text{m}$  (Walia, 2013) <sup>[34]</sup>, in poulownia wood it ranged from 2.17  $\mu\text{m}$  to 6.33  $\mu\text{m}$  (San *et al.*, 2016) <sup>[25]</sup>. Dutt and Tyagi (2011) <sup>[5]</sup> recorded cell wall thickness from 2.80  $\mu\text{m}$  to 5.90  $\mu\text{m}$  in 11 different eucalyptus species. Dutt *et al.*, (2004) <sup>[6]</sup> reported that cell wall thickness of some Indian non woody fibrous raw materials ranged from 3.54  $\mu\text{m}$  to 7  $\mu\text{m}$  which lend support to the findings of current study.

#### Derived Indices of fibre dimensions from mulberry clones

The derived indices calculated from the fibre dimensions were slenderness ratio, runkel ratio, flexibility and rigidity coefficient. The short and thin fibres produce a good slenderness ratio, which is related to paper sheet density and to pulp digestibility and in turn, increase tearing resistance. This is partly because of

short and thin fibres readily collapse to become double walled ribbons and produce good surface contact and increased fibre to fibre bonding (Dutt and Tyagi, 2011) <sup>[5]</sup>. A high slenderness ratio provides better forming and well-bonded paper. Generally, the acceptable value for slenderness ratio of paper making is more than 33 (San *et al.*, 2016 and Syed *et al.*, 2016) <sup>[25, 28]</sup>. In the current study, all the clones registered higher values (>33) for slenderness ratio indicating suitability of the mulberry clones for paper production.

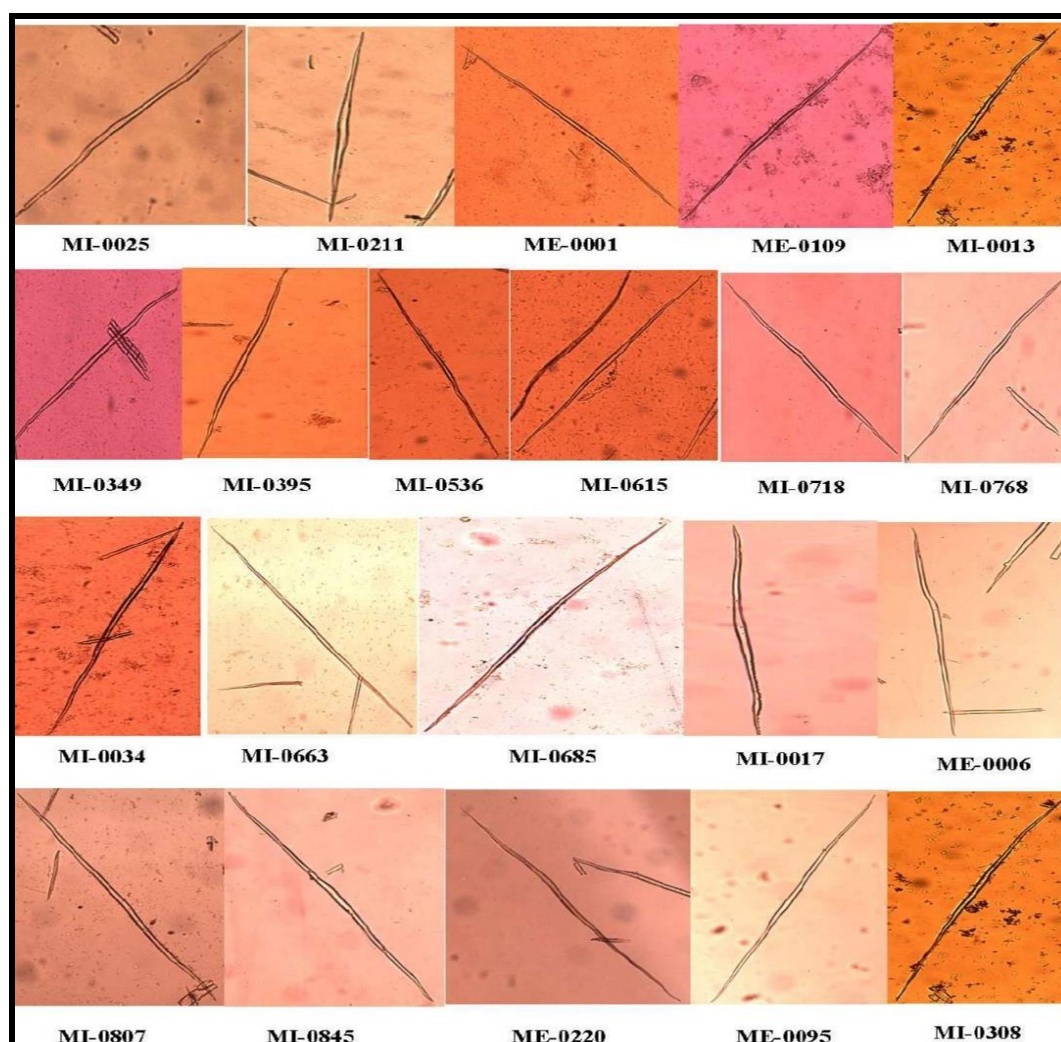
Runkel ratio is the most important and primary parameters needed to find the suitability of any raw material for pulp and paper. In a wood species with a runkel ratio of more than one, its fibre will be stiff, less flexible and poor bonding ability whereas, fibres with a ratio less than one considered as thin walled fibres and produce good quality pulp and paper (Dutt and Tyagi, 2011 and Monga *et al.*, 2017) <sup>[5, 17]</sup>. According to the Jang and Seth (1998), runkel value less than 1 would be suitable for paper making, as they collapse (become ribbon like) and provide large surface area for bonding. In the current study, barring the clones *viz.*, ME-0025, ME-0109, MI-0349, MI-0395, MI-0768, MI-0034, MI-0685 and MI-0017 the remaining clones recorded the runkel ratio of less than one which indicates that these 13 clones produce thin wall fibres and are more suitable for paper production. A superfluity of workers reported in various tree species like *Grewia tiliaefolia* (Kapadi *et al.*, 2020) <sup>[12]</sup>; *Melia dubia* (Vishnu and Revathy, 2019) <sup>[33]</sup> and *Morus nigra* (Walia, 2013) <sup>[34]</sup> with low runkel ratio and deployed as alternate pulpwood species which lend support to the current findings in mulberry.

**Table 2:** Fibre morphology of different mulberry clones

Clones	Average fiber length ( $\mu\text{m}$ )	Average fiber diameter ( $\mu\text{m}$ )	Average lumen width ( $\mu\text{m}$ )	Average cell wall thickness ( $\mu\text{m}$ )
ME-0025	865.98	19.28**	8.62	5.33**
MI-0211	896.25**	18.68**	9.52**	4.44
ME-0001	961.38**	16.75	8.75	4.00
ME-0109	826.50	18.00	7.38	5.31**
MI-0013	692.63	16.38	9.75**	3.31
MI-0349	713.38	17.75	8.38	4.69**
MI-0395	725.65	18.03	8.67	4.68*
MI-0536	810.71	16.86	8.71	4.07
MI-0615	830.13	16.00	8.63	3.69
MI-0718	836.00	16.38	8.25	4.06
MI-0768	839.88	16.75	7.75	4.50
MI-0034	838.00	17.32	8.23	4.55
MI-0663	753.00	20.20**	10.80**	4.70**
MI-0685	736.13	18.75**	8.13	5.31**
MI-0017	892.75**	19.13**	8.50	5.31**
ME-0006	894.38**	17.75	10.13**	3.81
MI-0807	886.25*	19.38	9.88**	4.25
MI-0845	953.00**	18.69	9.50**	4.06
ME-0220	775.50	14.88	8.63	3.13
ME-0095	957.38**	18.25	9.25	4.50
MI-0308	846.88	18.00	10.00**	4.00
Mean	832.94	17.61	8.88	4.37
SEd	20.24	0.41	0.21	0.12
CD(0.05)	40.27	0.81	0.41	0.24
CD (0.01)	53.44	1.07	0.54	0.32

\*\* Significant at 1% level

\* Significant at 5% level

**Fig 2:** Fibre morphology of different mulberry clones



The flexibility coefficient is directly governed by fiber diameter and inversely affected by lumen width. The fiber flexibility enhances the area for bonding among the fibers due to better fiber collapsing and improves the bursting index, tensile index and double fold number (Monga *et al.*, 2017) [17]. The higher value of fiber length to width ratio increased the fiber flexibility and change of forming well bonded paper (Sadiku and Abdulkareem, 2019) [24]. This gives good physical strength properties with more opaque sheet and less porosity (Dutt and Tyagi, 2011) [5]. Depending on the elasticity rate, the flexibility coefficients of fibers are grouped into four groups (Istas *et al.*, 1954 and Bektas *et al.*, 1999) [11, 7].

- High fibres having elasticity coefficient greater than 75
- Elastic fibres having elasticity ratio between 50 and 75
- Rigid fibres having elasticity ratio between 30 and 50
- Highly rigid fibres having elasticity ratio less than 30

According to this classification, the flexibility coefficient of the mulberry genotypes was categorized between elastic and rigid fibres. Among the twenty one mulberry clones studied, MI-0013 recorded as elastic fibre and ME-0109 recorded as rigid fibre. Thus, the investigated clones exhibited suitability for paper production with acceptable breaking length. Similar results were also observed by Ververis *et al.*, (2004) [31] in olive tree (41 to 75) wherein it registered as elastic fibre which attests the findings of current study.

Dutt and Tyagi (2011) [5] reported that *Eucalyptus alba* and E-471 recorded short fibre with rigid and flexible because of its higher values of rigidity coefficient (0.50 and 0.59 respectively) resulting in an increased rigidity of fibre and decreased fibre bonding. In the present study, the rigidity coefficient of evaluated mulberry clones ranged from 0.20 and 0.30 which recorded less rigidity coefficient value and revealed that the mulberry fibres are more flexible for paper making.

**Table 3:** Derived Indices of fibre dimensions from different mulberry tree species

Clones	Slenderness ratio	Runkle Ratio	Flexibility coefficient	Rigidity coefficient
ME-0025	44.92	1.24**	44.71	0.28**
MI-0211	49.28	0.93	50.96	0.24
ME-0001	57.40**	0.91	52.24	0.24
ME-0109	45.92	1.44**	40.97	0.30**
MI-0013	42.30	0.68	59.54**	0.20
MI-0349	40.19	1.12**	47.18	0.26*
MI-0395	40.25	1.08**	48.09	0.26*
MI-0536	48.09	0.93	51.69	0.24
MI-0615	51.88**	0.86	53.91**	0.23
MI-0718	51.05**	0.98	50.38	0.25
MI-0768	50.14*	1.16**	46.27	0.27**
MI-0034	48.38	1.10**	47.52	0.26*
MI-0663	37.28	0.87	53.47*	0.23
MI-0685	39.26	1.31**	43.33	0.28**
MI-0017	46.68	1.25**	44.44	0.28**
ME-0006	50.39*	0.75	57.04**	0.21
MI-0807	48.23	0.86	50.98	0.23
MI-0845	54.07**	0.86	50.83	0.23
ME-0220	52.13**	0.72	57.98**	0.21
ME-0095	52.46**	0.97	50.68	0.25
MI-0308	47.05	0.80	55.56**	0.22
Mean	47.49	1.00	50.55	0.25
SEd	1.18	0.03	1.26	0.006
CD(0.05)	2.35	0.05	2.51	0.012
CD (0.01)	3.11	0.07	3.32	0.016

\*\* Significant at 1% level

\* Significant at 5% level

## Conclusion

The detailed wood anatomical analysis of different mulberry clones revealed significant variations in vessel and fiber morphology, which are crucial for determining their suitability for various industrial applications. The study found that mulberry clones exhibited vessel lengths ranging from 33.63  $\mu\text{m}$  to 102.25  $\mu\text{m}$  and diameters from 10.50  $\mu\text{m}$  to 27.50  $\mu\text{m}$ , with the clone MI-0536 showing the highest vessel length and MI-0013 the highest vessel diameter. Fiber length varied from 692.63  $\mu\text{m}$  to 961.38  $\mu\text{m}$ , placing them in the short fiber category, which is advantageous for pulp and paper production due to better packing and bonding properties. Fiber diameter and lumen size also showed significant differences, with the clone MI-0663 registering higher values, indicating better pulp beating properties. The derived indices, including slenderness ratio, runkel ratio, flexibility coefficient, and rigidity coefficient, further supported the suitability of mulberry fibers for paper making, with most clones exhibiting favorable characteristics. The findings highlight the potential of mulberry as an alternative raw material for the wood-based industry, addressing the growing demand for wood while promoting sustainable agroforestry practices.

## Acknowledgement

The authors gratefully acknowledge the Central Sericulture Germplasm Resources Centre, Hosur, for providing mulberry genetic resources for this research. We also extend our thanks to the Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, for their valuable assistance and support throughout this study.

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