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# Fibre morphology of mulberry genetic resources

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**Abstract**
Mulberry is the staple food for *Bombyx mori*. L since they have evolved; besides that has been utilized for rearing silkworm. In addition, mulberry leaves, stem, root and fruits are used for various purposes. Mulberry branches are used to make baskets, toys, sports goods and the bark is used for paper production. Several scientists attempted to examine the pulp quality of mulberry. In this regard, mulberry wood was tested for its pulpability. Various genotypes collected from Central Sericultural Germplasm Resource Centre (CSGRC), Hosur were taken to study its anatomical features to check its suitability for paper making. Fibre morphology and its derived indices were observed and evaluated under the image analyzer (Optika). Fibre dimensions for mulberry genotypes are in the range of hardwoods. Among 21 genotypes investigated, two genotypes *viz.,* Thaibeelad and Kalimpong registered better results for average fiber length (799.30 and 805.04µm), fibre diameter (19.46 and 21.25µm), lumen width (12.84 and 13.78µm) and cellwall thickness (3.31 and 3.74µm) which are in the range of hardwoods. Hence, these genotypes can be commercially exploited for pulp and paper making.

**Keywords:** Mulberry genotypes, fibre dimensions, derived indices, paper making, pulpability

## 1. Introduction

Mulberry (Morus; Moraceae) is a fast growing deciduous woody perennial tree with deep root system consisting of more than 20 species and several subspecies or varieties. It has been cultivated widely in Asian countries for a very long time with the sole purpose of feeding the monophagous silkworm *Bombyx mori* L. Being a perennial tree crop, mulberry offers additional benefits such as conservation of soil and water, enhancement of biodiversity by providing shelter to shade loving plants and food to birds and small animals (Vijayan *et al*., 2011) [13].

In addition to the major utilization of mulberry leaves as a feed for silkworm, it is being used for many other purposes, for which it is called as "Kalpavriksha". Each part of mulberry *viz.,* leaves, stem, root, fruits have plentiful medicinal values. The mulberry woods are used to make baskets, toys and sports goods (Sujathamma P *et al*., 2013) [11]. It is used as a firewood in rural areas which have a calorific value of 4850 kcal/kg and less smoky in nature (Sharma *et al*., 2014) [10] and the bark is used for paper production too.

Wood is one of the abundant biomaterials on earth (Salmen, 2015) [8]. As with the expansion of paper industries in the country, the pulp containing plants are being used to an appreciable extent. The world wood fiber is the original source of over 98% of the fibrous component of paper (Walia, 2013) [14]. In this regard, the use of non-wood plants offer several advantages including short growth cycle, moderate irrigation and fertilizer requirements and low lignin content resulting to reduced energy and chemicals use during pulping (Ververis, 2003) [12]. The successful conversion of pulp into a marketable product depends on the original fibre characteristics. The analysis of fibre characteristics such as fibre length, fibre diameter, lumen width, cellwall thickness and their derived morphological factors became important in estimating pulp quality of fibre (Oluwadare and Ashimiyu, 2007) [6].

Some of the scientists were attempted to check the suitability of mulberry for pulp quality because of its rapid juvenile growth; the proleptic and sylleptic shoots show strong woody biomass apart from their major economic commodities (foliage and berries) (Guha and Reddy, 2013) [3]. However such studies were not carried out in mulberry genetic resources. Against this backdrop, the current studies on the fibre morphology of mulberry genotypes were accessed to ensure its pulpability.

## Materials and methods

The well-established mulberry garden with twenty one mulberry genotypes collected from the Central Sericultural Germplasm Resource Centre (CSGRC) is maintained at Forest College & Research Institute (FC&RI), Mettupalayam. The existing Mulberry genotypes are *Morus cathayana*, Thaibeelad, ACC.165, Large black, S-13, Assamabola, Kollegal, UP-8, Kalimpong, UP-23, ERRC-32, Vadapuram, Query pit, L-5, Khakad-3, Badagoan, Anklow, Jalalgavah-3, Hosur-8, Hosur-C15, ME-065 which belongs to several species of mulberry *viz.*, *M. alba, M. indica, M. latifolia and M. laevigata*.

## Sample preparation

The logs from mulberry genotypes were felled and prepared into chips using pilot chipper. The wood samples each of dimension 2x2x2x $cm^3$ were sliced out separately from the mulberry genotypes. From these wood samples thin microscope sections of size 15 to 20 µm were taken using 'Yorco rotater microtome' (lipshaw type). Temporary slides were made by staining these sections with safranin stain and subjected to measurements and photography using image analysis system (Optika). Measurement of various parameters was done using the optika software.

## Maceration

Maceration is done using Jeffrey's method (Lim and Son, 1997). Jeffrey's solution is prepared by mixing equal volumes of 10 percent potassium dichromate and 10 percent nitric acid. Radial chips of wood shavings were taken from the 1 $cm^3$ wood blocks. These chips were boiled in the maceration fluid for 15-20 mins so that the individual fibres were separated. Then these test tubes were kept for 5-10 mins so that the fibres settled 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.

## Fibre length

Fibre length (µm) was measured from macerated wood samples by measuring both end of the fibre through optika image analysis software.

## Fibre diameter

Diameter (µm) of the fibre was measured from macerated wood samples by measuring cross sectional area through optika image analysis software.

## 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.

## 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.

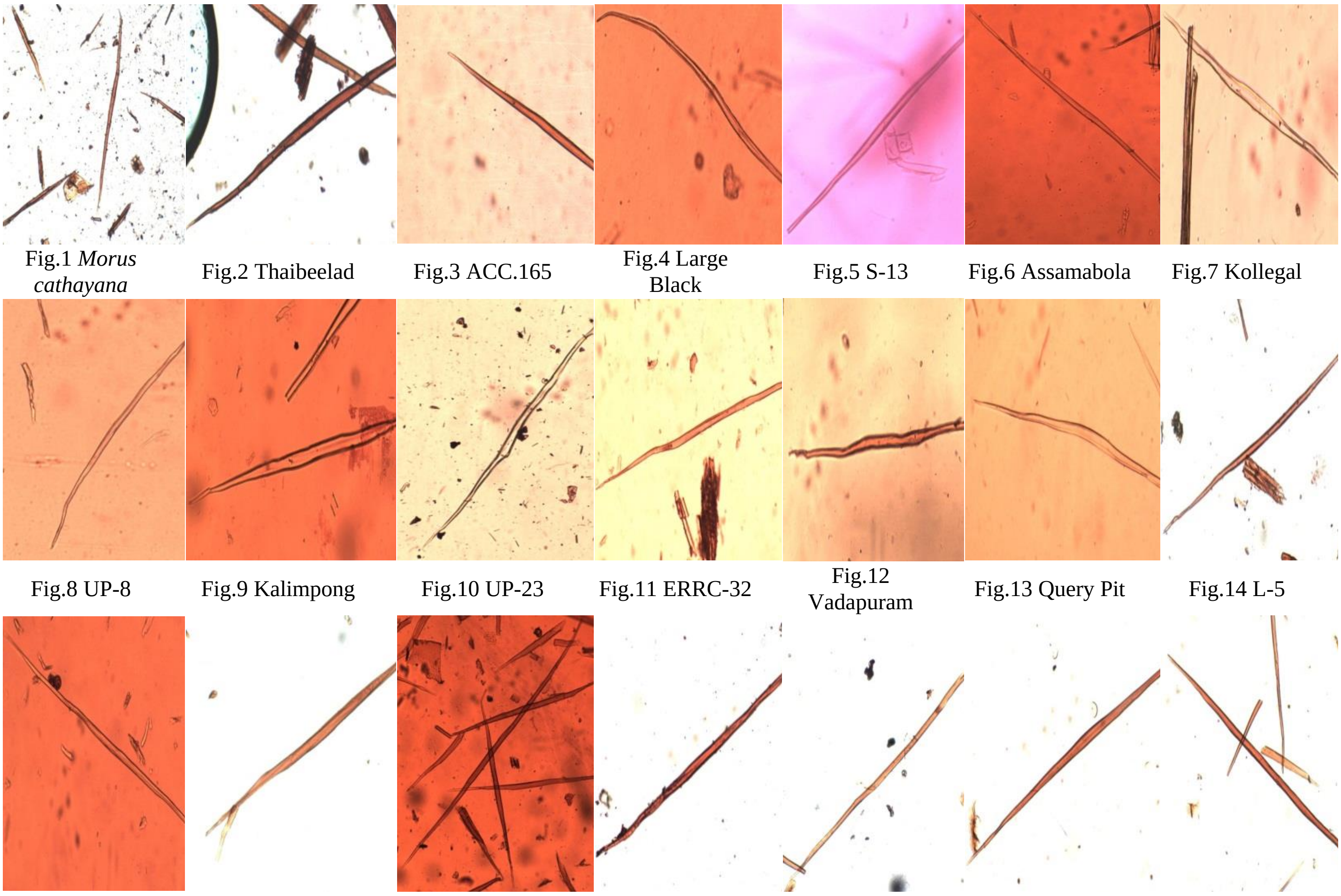

Fig.1 *Morus cathayana* | Fig.2 Thaibeelad | Fig.3 ACC.165 | Fig.4 Large Black | Fig.5 S-13 | Fig.6 Assamabola | Fig.7 Kollegal

Fig.8 UP-8 | Fig.9 Kalimpong | Fig.10 UP-23 | Fig.11 ERRC-32 | Fig.12 Vadapuram | Fig.13 Query Pit | Fig.14 L-5

Fig.15 Khakad-3 | Fig.16 Badagaon | Fig.17 Anklow | Fig.18 Jalalgavah-3 | Fig.19 Hosur-8 | Fig.20 Hosur-C15 | Fig.21 ME-065

**Fig 1-21:** Shows the fiber morphology of different mulberry genotypes observed under image analyzer (Optika)

**Derived values (Indices) from fibre dimensions**

Four derived values were also calculated using fibre dimensions:

$$Slenderness\ ratio = \frac{Fibre\ length}{Fibre\ diameter}$$ (Varghese, 1995)

$$Runkel\ ratio = 2 \times \frac{Fibre\ wall\ thickness}{Fibre\ lumen\ width}$$ (Runkel, 1949)

$$Flexibility\ coefficient(\%) = \frac{Fibre\ lumen\ width}{Fiber\ diameter} X\ 100$$ (Wangard, 1962)'

$$Rigidity\ coefficient = \frac{Fibre\ wall\ thickness}{Fibre\ diameter}$$ (Tamolang and Wangaard, 1961)

**Statistical analysis**

All the data were subjected to analysis of variance (ANOVA) using Completely Randomized Design (CRD).

**Results**

**Fibre morphology**

The fibre morphology *viz.*, average fibre length, fibre diameter, lumen width and cell wall thickness were analyzed for 21 mulberry genotypes and the results are furnished in Table 1.

**Average fibre length (µm)**

The characterization of mulberry genotypes for fibre length indicated significant variability. The average fibre length recorded was 719.29 µm. Among 21 genetic resources, eight genotypes viz., Thaibeelad (799.30µm), Kalimpong (805.04µm), ERRC-32 (817.15 µm), Query pit (807.71µm), Khakad-3 (798.69 µm), Badagaon (785.55 µm), Anklow (808.77 µm) and Hosur-C15 (782.42 µm) exhibited higher fibre length compared to the average value (719.29 µm). The highest fibre length was recorded by the genotype ERRC-32 (817.15 µm) and the lowest fibre length was recorded by the genotype *Morus cathayana* (614.18µm).

**Table 1:** Fibre Morphology of Mulberry Genotypes

| S. No | Genotype | Average fibre length (µm) | Fibre diameter (µm) | Lumen width (µm) | Cellwall thickness (µm) |
|---|---|---|---|---|---|
| 1 | *Morus cathayana* | 614.18 | 17.90 | 10.32* | 3.79 |
| 2 | Thaibeelad | 799.30* | 19.46* | 12.84* | 3.31 |
| 3 | ACC.165 | 625.36 | 13.99 | 7.60 | 3.20 |
| 4 | Large black | 651.26 | 17.50 | 11.12* | 3.19 |
| 5 | S-13 | 732.63 | 16.08 | 8.13 | 3.98 |
| 6 | Assama bola | 743.09 | 16.05 | 8.89 | 3.58 |
| 7 | Kollegal | 619.64 | 17.92 | 7.96 | 4.98* |
| 8 | UP-8 | 676.77 | 14.22 | 7.27 | 3.48 |
| 9 | Kalimpong | 805.04* | 21.25* | 13.78* | 3.74 |
| 10 | UP-23 | 643.99 | 16.59 | 8.12 | 2.82 |
| 11 | ERRC-32 | 817.15* | 17.97 | 9.89* | 4.04 |
| 12 | Vadapuram | 596.62 | 13.92 | 7.08 | 3.42 |
| 13 | Query pit | 807.71* | 16.07 | 8.38 | 3.85 |
| 14 | L-5 | 720.61 | 14.75 | 6.24 | 4.26* |
| 15 | Khakad-3 | 798.69* | 15.11 | 8.18 | 3.47 |
| 16 | Badagaon | 785.55* | 15.75 | 7.09 | 4.33* |
| 17 | Anklow | 808.77* | 17.04 | 7.45 | 4.80* |
| 18 | Jalalgavah-3 | 705.19 | 16.36 | 7.26 | 4.55* |
| 19 | Hosur – 8 | 705.44 | 16.01 | 7.37 | 4.32* |
| 20 | Hosur – C15 | 782.42* | 18.78* | 9.57* | 4.60* |
| 21 | ME-065 | 665.74 | 16.70 | 7.71 | 4.50* |
| Mean | | 719.29 | 16.64 | 8.68 | 3.91 |
| SEd | | 25.92 | 0.70 | 0.37 | 0.16 |
| CD (0.05) | | 52.31 | 1.41 | 0.73 | 0.32 |

(* Significant at 5% level)

**Fibre Diameter (µm)**

The fibre diameter for mulberry genetic resources recorded significant variability. The highest fibre diameter was registered in Kalimpong (21.25µm) and the lowest fibre diameter was registered in Vadapuram (13.92 µm). The average fibre diameter recorded was 16.64 µm.

**Lumen width (µm)**

The anatomical studies of the evaluated genetic resources showed significant variability for lumen width. The mean value recorded for this parameter was 8.68 µm. Among the twenty one genetic resources, Kalimpong recorded highest lumen width (13.78µm) followed by Thaibeelad (12.84 µm) and L-5 recorded the lowest (6.24 µm). Three genetic resources viz., Hosur-C15 (9.57 µm), ERRC-32 (9.89 µm), *Morus cathayana* (10.32 µm) showed significantly higher values when compared to average lumen width (8.68 µm).

**Cellwall thickness (µm)**

The cellwall thickness of mulberry genetic resources exhibited significant variations. Among the genetic resources, Kollegal (4.98 µm) showed highest and UP-23(2.82 µm) showed lowest cellwall thickness. The mean value recorded was about 3.91µm. Four genetic resources viz., Anklow (4.80µm), Jalalgavah-3 (4.55µm), Hosur- C15 (4.60µm) and ME-065 (4.50µm) showed significant higher values compared with average value cellwall thickness (3.91µm).

**Derived values (Indices) from fibre dimensions**

The derived indices from fibre morphology of mulberry genetic resources are derived and tabulated (Table 2).

**Slenderness ratio**

The slenderness ratio of mulberry genetic resources found highest in the Khakad-3 (52.86) and lowest value was recorded in Morus cathayana (34.31). The mean value registered was 43.54. Among the genetic resources, three genotypes viz., Query pit (50.26), Khakad-3 (53.86), Badagaon (49.88) expressed parity for slenderness ratio.

**Runkel ratio**

The runkel ratio differed significantly for the mulberry genetic resources. The genotype L-5 (1.37) recorded highest runkel ratio and Thaibeelad (0.52) recorded lowest runkel ratio. The genetic resources L-5 (1.37), Anklow (1.28) expressed parity on each other. The mean value registered was 0.95.

**Table 2:** Derived Indices from Fibre Dimensions of Mulberry Genotypes

| S. No | Genotype | Slenderness ratio | Runkel ratio | Flexibility coefficient (%) | Rigidity coefficient |
|---|---|---|---|---|---|
| 1 | *Morus cathayana* | 34.31 | 0.73 | 57.65* | 0.21 |
| 2 | Thaibeelad | 41.07 | 0.52 | 65.88 | 0.17 |
| 3 | ACC.165 | 44.70 | 0.84 | 54.32 | 0.23 |
| 4 | Large black | 37.21 | 0.57 | 63.54* | 0.18 |
| 5 | S-13 | 45.56 | 0.98 | 50.56 | 0.25 |
| 6 | Assama bola | 46.3 | 0.81 | 55.38 | 0.22 |
| 7 | Kollegal | 34.58 | 1.25* | 44.42 | 0.28* |
| 8 | UP-8 | 47.59* | 0.96 | 51.13 | 0.24 |
| 9 | Kalimpong | 37.88 | 0.56 | 64.84* | 0.18 |
| 10 | UP-23 | 38.82 | 0.72 | 48.85 | 0.17 |
| 11 | ERRC-32 | 45.47* | 0.82 | 55.04 | 0.22 |
| 12 | Vadapuram | 42.86 | 0.97 | 50.86 | 0.24 |
| 13 | Query pit | 50.26* | 0.92 | 52.15 | 0.23 |
| 14 | L-5 | 48.85* | 1.37* | 42.31 | 0.29* |
| 15 | Khakad-3 | 52.86* | 0.85 | 54.14 | 0.22 |
| 16 | Badagaon | 49.88* | 1.22* | 45.02 | 0.27* |
| 17 | Anklow | 47.46* | 1.28* | 43.72 | 0.28* |
| 18 | Jalalgavah-3 | 43.1 | 1.25* | 44.38 | 0.28* |
| 19 | Hosur - 8 | 44.06 | 1.17* | 46.03 | 0.27* |
| 20 | Hosur – C15 | 41.68 | 0.96 | 50.88 | 0.25 |
| 21 | ME-065 | 39.86 | 1.17* | 46.17 | 0.27* |
| Mean | | 43.54 | 0.95 | 51.77 | 0.24 |
| SEd | | 1.88 | 0.048 | 2.58 | 0.009 |
| CD (0.05) | | 3.80 | 0.096 | 5.20 | 0.019 |

(* Significant at 5% level)

**Flexibility Coefficient (%)**

The genetic resources showed significant variations for flexibility coefficient. The genotype Thaibeelad (65.88%) found to be highest and L-5 (42.31%) found to be lowest. The two genetic resources expressed parity for flexibility coefficient viz., Thaibeelad (65.88%), Kalimpong (64.84%) and Large black (63.54%). The mean value recorded was 51.77%.

**Rigidity coefficient**

The rigidity coefficient was recorded highest for the genotype L-5 (0.29) and Thaibeelad (0.17) found to be lowest. The mean value recorded was about 0.24. Among the twenty one genotypes, L-5 (0.29), Kollegal (0.28), L-5 (0.29), Anklow (0.28), Jalalgavah (0.28) expressed parity with each other.

**Discussion**

Fiber length is recognized as an important parameter for pulp and paper properties. Fiber length influences paper strength, particularly, tear and paper machine runnability (Rahman and Jahan, 2014) [7]. Hence the fibre morphology of the mulberry genotypes deployed in the current study was carried out and the results exhibited significant variations among the genotypes. The fibre length of the genotypes viz., Thaibeelad, Kalimpong, ERRC-32, Query pit, Khakad-3, Badagaon, Anklow and Hosur-C15 registered higher values and expressed parity with each other. The properties of fiber length influence the tensile and bursting strength and slightly affect the folding endurance. It is an observation that greater the fiber length, higher will be the tearing resistance of paper. On the other hand, longer fiber tends to give a more open and less uniform paper sheet structure (Walia, 2013) [14]. The current investigation revealed that the fiber length of mulberry genotypes ranged between 614.18 µm to 817.15 µm which is considered as short fibers. Similarly, the same line of findings was reported in mulberry that the average fiber length was slightly increased from 0.63 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 to 1.5mm) considered as short fibers (Rahman and Jahan, 2014) [7].

Fibre diameter was higher in the genotypes Kalimpong and Thaibeelad which comes under the hardwoods fiber. The fiber width of green *Paulownia* was found as about 34.59 µm which was in normal range when compared to hardwoods fiber (20 to 40µm) (San *et al.*, 2016) [9] which lend support to findings of the current investigation.

Fibre 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 (Walia, 2013) [14]. Thus, in the current study the genotype Kalimpong registered higher lumen width followed by Thaibeelad, Large black, Hosur-C15, *Morus cathayana* which would show better results during beating of pulp.

The thickness of cellwall was important in pulp refining process. The strength properties of cellwall were directly affected by cellwall thickness. The thicker the cellwall, more flexibility of fibers in pulp refining process (San *et al.*, 2016) [9].Thin walled fibres favourably affect the bursting and tensile strengths and folding endurance of paper. In the current investigation, the genotype Kollegal recorded higher cellwall thickness which reveals more flexibility of fibers.

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 short and thin fibers are readily collapsed to double walled ribbons and produce good surface contact and fibre to fibre bonding (Dutt and Tyagi 2011) [2]. A high value of slenderness ratio provides better forming and well-bonded paper. Generally, the acceptable value for slenderness ratio of papermaking is more than 33 (San *et al.*, 2016) [9]. In the current study, majority of the mulberry genotypes registered higher values (>33) for slenderness ratio indicating the suitability of the genotypes for paper production.

Runkel ratio is usually used to determine the suitability of a fibrous material for pulp and paper production. If a wood species has a runkel ratio more than 1, its fiber will be stiff, less flexible and poor bonding ability. Whereas, fibers with low ratio (<1) produce good quality pulp and paper [15]. Jang and seth (1998) [5] reported that materials having a runkel value less than 1 would be suitable for papermaking, because they collapse (become ribbon like) and provide a large surface area for bonding. In the current study, barring the genotypes Hosur-8, Jalalgavah-3, Anklow, Badagaon, L-5, Kollegal, ME-065 the remaining genotypes obtained the runkel ratio value less than 1 which produce thin wall fibres and are more suitable for paper production.

Fibre diameter and wall thickness governs the fibre flexibility. Thin walled fibers favourably affect the bursting and tensile strengths and folding endurance of paper. A decrease in the variables, which are measures of the flexibility and wet plasticity of fibres, results in higher degree of conformability within the sheet, which gives rise to a sheet of a higher density (or) lower bulk. This gives good physical strength properties with more opaque sheet and less porosity (Dutt and Tyagi 2011) [2]. Flexibility gives the bonding strength of individual fiber and by extension the tensile strength and bursting properties. According to flexibility ratio, there are 4 groups of fibers (Bektas *et al*., 1999) [1]:

a) High fibers having elasticity coefficient greater than 75
b) Elastic fibers having elasticity ratio between 50 and 75
c) Rigid fibers having elasticity ratio between 30 and 50
d) Highly rigid fibers having elasticity ratio less than 30

According to this classification, the flexibility coefficient of the mulberry genotypes was categorized between elastic to rigid fibers. Among the twenty one genotypes, Thaibeelad recorded as elastic fibre and L-5 recorded as rigid fibre. Thus, the investigated genotypes showed suitability for paper production with acceptable breaking length.

Dutt & Tyagi (2011) [2] reported that short fibers of E.alba and E-471 are rigid and less flexible because of its higher values of rigidity coefficient (0.50 and 0.59 respectively). Thus, increase in rigidity of fibres results in decrease in fibre bonding [2]. The rigidity coefficient for the evaluated mulberry genotypes ranged between 0.17 and 0.29 which recorded lower values so that the fibers are more flexible for paper making. However, the genotypes produced parallel values on rigidity coefficient. Finally, it is examined that all the genotypes deployed in the current wood characterization are preferred for paper production barring the genotypes L-5, Vadapuram and ACC.165.

**Conclusion**

The wood anatomical characterization of mulberry genotypes gives an idea about the morphology of fiber dimensions. The values of fibre morphology like fiber length, fibre diameter, lumen width, cellwall thickness, runkel ratio, slenderness ratio, flexibility and rigidity coefficient indicates that the mulberry genetic resources are suitable for making paper. It has been verified from the above observed values of fibre morphology that mulberry genotypes are in the normal range for hardwoods. Thus, it can be concluded that mulberry genotypes Thaibeelad and Kalimpong are well suited to produce pulp and paper.

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