P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2023.v36i01.12

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Characterization and Evaluation of Mulberry Genetic Resources for the Identification of Promising Accessions

Mallegowdanakoppalu C. Thriveni*, Sampathkumar Deepa, Ganesan Thanavendan, G. Ravikumar and B. T. Sreenivasa

Abstract

Mulberry is a dioecious species but, is usually cultivated as low bush or dwarf tree form by repeated pruning. Apart from its importance in silkworm rearing, it is also valued for its delicious fruits, medicinal properties in infusions, and ornamental shade tree. The conservation of mulberry genetic resources for future use and their subsequent usage in plant breeding are two critical areas of action in any genetic resource management programme. The current study used morphological, reproductive, anatomical, biochemical, propagation, growth, and yield variables to know the potential value of 22 mulberry accessions. Based on multiple trait analysis, the accessions MI-0946, MI-0945, MI-0953, MI-0948, MI-0935, MI-0948, MI-0936 and MI-0952 were identified as top performers for anatomy, propagation, biochemical, growth and yield parameters. These accessions may serve as potential parents for the future breeding program.

Keywords: Mulberry germplasm, Characterization, Evaluation, Conservation.

Central Sericultural Germplasm Resources Centre, Central Silk Board, Govt. of India, Ministry of Textiles, Thally road, Hosur-635109, Tamil Nadu, India.

***Author for correspondence:**

thrivenimc@gmail.com

Received: 01/02/2022 **Revised:** 30/06/2022

Accepted: 22/07/2022

How to cite this article: Thriveni, M.C., Deepa, S., Thanavendan, G., Ravikumar, G., Sreenivasa, B.T. (2023). Characterization and Evaluation of Mulberry Genetic Resources for the Identification of Promising Accessions. Indian J. Plant Genetic Resources. 36(1), 85-95. **DOI:** 10.61949/0976-1926.2023.v36i01.12

Introduction

Mulberry is a highly adaptable plant with numerous uses, the most notable of which is as the sole food source for the monophagous silkworm *Bombyx mori*. Plant genetic resources play an important role in crop improvement (Khurana and Checker, 2011). Mulberry genetic resources are becoming more widely acknowledged as a critical component for sustainable silk production in the face of changing environmental conditions. The current scenario of the sericulture industry necessitates the development of new varieties that are suitable for various agro-climatic conditions. From a huge number of germplasm collections, suitable parent material must be selected (Sharma *et al*., 2000). Several studies have highlighted the variability of mulberry germplasm (Thangavelu *et al*., 2000; Tikader and Rao, 2002).

The key to understanding germplasm's potential and actual value is to characterize and evaluate it. This will facilitate the utilization of germplasm in crop improvement programme. Thus, characterization and evaluation is an important activity and maintaining the germplasm without losing its genetic integrity is also important in plant genetic resources management. The clonal repositories, field gene banks and herbal gardens are strategies for the maintenance of germplasm of vegetatively propagated species. Characterization and evaluation of germplasm is gaining importance all over the world due to the utilization of plant genetic resources in crop improvement programme. Such activities are also consistent with the Convention on Biological Diversity under which countries agreed to conserve, sustainable use and benefit sharing from plant genetic resources. Therefore, characterization and

evaluation of germplasm is essential for conservation and utilization of plant genetic resources (Sharma *et al*., 2000).

But, the genetic improvement of mulberry depends on the availability of diverse germplasm. Selection of suitable genotypes from gene pool requires a thorough knowledge on availability and distribution of traits of economic importance for utilization in hybridization programmes (Tikader and Kamble, 2008). Realizing the need for enhanced use of mulberry germplasm resources for crop improvement programmes, Central Sericultural Germplasm Resources Centre (CSGRC) has taken up characterization and evaluation of entire germplasm collection of the centre. A total of 1317 mulberry accessions (Indigenous-1032 and Exotic-285) are being conserved in the form of ex situ field gene bank. This germplasm are systematically characterized in phased manner and recently sixth volume of catalogue with data pertaining to 52 additional mulberry accessions was published (Thriveni *et al*., 2021). In order to know the potential value of the mulberry germplasm, the characterization and evaluation is prerequisite. Studies have shown that the identification of trait-specific potential genotype(s) from a broad genetic base and their subsequent utilization in targeted breeding work are desirable. However, reports on mulberry genetic resources evaluation through multivariate analysis is still very limited (Banerjee *et al*., 2011).

In this context, the present study was conducted to know the performance of mulberry germplasm accessions for different parameters for effective utilization in future breeding program. The characterization includes morphology, anatomy, and reproductive parameters. The evaluation parameters comprise propagation, growth and yield and biochemical traits.

Materials and Methods

Stem cuttings of 22 mulberry accessions with 12 to 15 cm length and 1.0 to 1.5 cm diameter from 6 to 8 months mature shoots with 2 to 3 healthy buds are planted in nursery beds. The characterization and evaluation parameters are observed after 70 to 90 days of planting. The methodology for recording the characterization data is given in Table 1.

Methodology for Characterization

Morphology

- Branching nature

The branching nature of plants were recorded based on visual observations from 3 plants/accession after 90 days of pruning and grouped into erect, semi-erect, and spreading.

- Curve or straightness of branch

Curve or straightness of the branches were scored based on visual observations from 3 plants/accessions and grouped as curved, slightly curved and straight depending on plant habit.

- Color of young shoot

Young shoot color were recorded on visual observations from 3 plants/accession after 30 days of pruning and scored as brown, green and purple.

- Color of mature shoot

Mature shoot color on visual observations from 3 plants/accession from the mature shoot after 90 days of pruning and grouped into brown, green, greenish brown, greenish grey and purple brown.

- Phyllotaxy

Phyllotaxy is the mode of arrangement of leaves on the stem. The phyllotaxy is called 1/2 rank (distichous) followed by 1/3 and 2/5. Different combinations of all (1/2, 1/3 and 2/5) are termed as mixed type.

- Stipule nature

Stipules are outgrowths from leaf base, which usually protects the young axillary buds- depending on hanging arrangement of stipules nature, grouped into foliaceous, free-lateral and bud scale.

- Stipule duration

The attachment of stipules with leaf base was observed visually and depending on attachment duration, it was grouped into caducous and persistent.

- Leaf lobation type

The leaf lobation was observed visually and grouped primarily into unlobed and lobed. Under lobed group it was again grouped into deeply lobed, medium lobed and shallow lobed.

- Leaf lobation number

The lobation number on a leaf was counted and scored as No lobation (0), lobation with (1–5) and lobation with (6–10).

- Leaf nature

The leaf nature was recorded based on unlobed, lobed and grouped into homophyllous (All the leaves in the plant are of similar nature either entire or lobed), heterophyllous (when some leaves are lobed and some are unlobed either in the same branch or in different branches of the same plant).

- Leaf color

The mature leaf color was observed from each accession on 3 plants and scored visually as green (G) and dark green (DG).

- Leaf surface

The leaf surface was grouped based on feeling by touch on it and grouped into rough, slightly rough and smooth.

- Leaf texture

The leaf texture was grouped based on feeling method and grouped into a) chartaceous, opaque and like writing paper and b) coriaceous, leathery, thick and stiff.

- Leaf apex

It is the portion of leaf bounded by approximately the upper 25% of the leaf margin and based on observation, the leaf apex grouped into a) Acute (pointed and narrow), b) Acuminate (the apex is drawn out into a short tapering tail) and c) Caudate (when the apex shows long tapering tail).

Table 1: List of descriptors for characterization and evaluation

S. No.	*Descriptors*	*Days after pruning (DAP)*
1	Morphology	70–90 days
2	Reproductive behavior	Flowering season (April-May and Sept-Oct.)
3	Leaf anatomy	Leaf samples after 60–70 days
4	Growth and yield	70 days
5	Biochemical	60 days
6	Propagation	90 days

- Leaf margin

The margin of the lamina was grouped into a) crenate (margin toothed and teeth are rounded without a pointed apex) b) Serrate (serrations are pointed with their axes inclined to the trend of margin), c) Dentate (if the margin is tooth-like edges), and d) Repand (when the margins form a smooth line or arc without noticeable projections).

- Leaf base

It is the portion of the leaf bounded by approximately lower 25% of the margin. The observations were recorded into a) Cordate, leaf base embayed in a sinus whose sides are straight or convex, b) Truncate, leaf base terminating abruptly as if cut, margin perpendicular to the mid vein and nearly so, c) Lobate, leaf base small to large rounded projections whose inner margins towards the petiole and are in partly concave shape.

- Leaf shape

The leaf shape is ovate in nature which represents the greatest width intersecting the leaf axis basal to the mid point of the later axis and based on (L/W) ratio of leaf it was grouped as Narrow Ovate (2:1), Ovate (1.5:1), Wide Ovate (1.2:1) and Cordate (<1:1).

Leaf Anatomy

- *Stomata size*

The mature leaf at 5th to 7th position from the longest branch was collected from the shoots of 3 plants and was preserved in FAA (5 mL formalin, 5 mL glacial acetic acid and 90 mL 70% absolute alcohol). Stomatal impression was made by quick fix method from the lower surface of the leaf and observed under microscope. Nine observations were recorded and measured both length and width of stomata in µm.

- Stomatal frequency

Stomatal frequency was recorded in a unit area under microscope in nine observation fields. The number counted in an unit area was expressed in mm^2.

- Idioblast length

The FAA fixed leaf material was taken and free hand section was made and observed under light microscope. The idioblast projection length protrude from the epidermis was measured. A total of nine observations were recorded in µm.

- Idioblast width

A total of nine observations were made as described in idioblast length.

- Idioblast Frequency

The fixed leaf peeling was made by quick fix method to get the impression of idioblast in the upper surface of leaf. The idioblast frequency was counted in an unit area under microscope in nine microscopic fields and converted as sq. mm.

- Palisade thickness

The ground tissue system, called mesophyll tissues, is often differentiated into columnar palisade parenchyma on the adaxial side of the leaf. A thin free hand section of leaf was made and thickness of the palisade tissues was measured under a microscope. Total 9 observations taken in µm.

- Spongy thickness

The mesophyll tissues consist of irregular or isodiametric spongy parenchyma on the abaxial surface of leaf. A thin free hand section of leaf was made and thickness of spongy tissue was measured under microscope. A total of 9 observations in µm recorded.

- Palisade-Spongy ratio

It is calculated as the value of the palisade/spongy ratio.

- Upper cuticle layer

Epidermal cells have unevenly thickened walls, the outer and radial walls being much thicker than the inner walls. The walls are much cutinised with fatty substance cutin, which forms the cuticle occurring all over the outer wall of the epidermal cells. A thin free hand section of leaf was made and thickness of upper cuticular layer was measured under microscope. A total of 9 observations in µm recorded.

- Lower cuticle layer

The outer layer of the leaf's lower surface (abaxial) is cutinised with fatty substance - cutin, which covers the outer layer of epidermal cells. Thin free hand section of leaf was made and layer's thickness was measured under microscope. A total of 9 observations in µm recorded.

- Upper epidermis

Epidermal tissue system consist of epidermal layers occurring on the leaf's adaxial surface (upper). After making thin free hand section it was observed under microscope and thickness measured in µm for 9 observations.

- Lower epidermis

Epidermal tissue system consists of epidermal layers occurring on the leaf's abaxial surface (lower). After making hand section of preserved leaf, it was observed under microscope and thickness measured in µm for 9 observations.

- Leaf thickness

Total leaf thickness is the addition of palisade, spongy, upper cuticle, lower cuticle, upper epidermis and lower epidermis layers.

- Chloroplast number/stomata

Chloroplast is the most important plastid responsible for carrying out the important functions of photosynthesis. In mulberry the chloroplast number indirectly indicates the ploidy status. Young leaves from 3rd to 5th position leaves from a branch were collected in a polythene bag and preserved in distilled water. The fresh peel from lower leaf surface was cut into pieces, avoiding veinlets and observed under microscope after stained with 2% Potassium Iodide (KI) solution. The chloroplast absorbs the KI solution, becomes dark black in color, and were counted under microscope. A total 9 observations/slide were recorded.

Biochemical

- Chlorophyll-a

The fresh leaves from 7 to 9th position were collected after 60 days of pruning for chlorophyll estimation. The leaf samples were processed following Hiscox and Israelstam (1979) method. The absorbance at 663 and 645 nm was recorded in a spectrophotometer. The amount of chlorophyll-a was calculated as per Arnon (1949).

- Chlorophyll-b

The amount of chlorophyll-b was calculated as per Arnon (1949).

- Total chlorophyll

Total chlorophyll was calculated as per Arnon (1949).

- Chlorophyll- a/b

It is a ratio of chlorophyll a and chlorophyll b.

- Protein content (%)

Nine leaves each from three plants/ accessions at 7th- 9th position were collected after 60 days of pruning. Dried the leaves at 60°C for 72 hours and take the dry weight. Weighed 100 mg of the sample, added 10 mL of 5% TCA, and centrifuged at 10000 rpm for 10 minutes. Discarded supernatant, added 1N NaoH to the pellet, and incubated for 12 hours. Centrifuged at 10000 rpm for 10 minutes and collected the supernatant. Taken 0.2 mL of the aliquot and estimated protein content as per Lowry *et al.* (1951). Read absorbance at 700 nm. Protein content on fresh weight basis was calculated based on moisture content of the leaf and protein content on dry weight basis.

- Carbohydrate content (%)

Prepared the leaf dry powder per the method described similar to protein and took 100 as leaf dry powder. Added 10 mL of 80% hot ethanol, taken the extract, evaporated in hot water bath and diluted to 10 mL with distilled water. Estimated carbohydrates as per Plumer (1971). Taken 0.2 mL of extract and added 4 mL of 0.2% freshly prepared anthrone reagent. Mixed the solution thoroughly and incubated for 10 minutes in boiling water bath and cool rapidly in water. Read the absorbance at 620 nm. Carbohydrated content on fresh weight basis was calculated based on moisture content of the leaf and carbohydrate content on dry weight basis.

Results

Morphological Characterization of Mulberry Genetic Resources

The variability and frequency distribution of 22 indigenous mulberry accessions for different morphological characters are presented in Tables 2 (a, b) 3. Out of 22 indigenous accessions studied, 14 accessions are erect (63.64%), 5 semi-erect (22.73%), and 3 spreading (13.64%) in nature of growth. The leaf lobation is mostly unlobed (81.82%) followed by medium lobation (13.64%) and shallow lobed (4.55%). All the studied accessions are with chartaceous (thin papery) leaf texture, while only one accession has persistent stipules.

Reproductive Characterization of Mulberry Genetic Resources

The reproductive characters were recorded for 22 accessions during the flowering season (Sept. to Oct. and Feb.-March). The sex expression of the accessions studied indicated preponderance of unisexuality with dioecious nature. Among 22 accessions, 14 are female, 6 male and 2 had both male and female inflorescences Table 4).

Highest variability (CV=97.54%) was recorded in female inflorescence followed by fruit weight (CV=78.38%) for the reproductive parameters among the mulberry accessions studied. The number of male flowers varied from 20–50 and female flowers from 15-101/ catkin. The range, mean and variability for different reproductive parameters are presented in the Table 5.

Leaf Anatomical Characterization of Mulberry Genetic Resources

Total 22 mulberry accessions were characterized for 15 anatomical characters following the method described by Metcalf and Chalk, (1972). Leaves were selected from 9th position and preserved in FAA solution till further study. For counting the number of stomata epidermal peelings of fresh young leaves was used. The variability in respect of leaf anatomical characters is given in Table 6.

The top performing five mulberry accessions for different anatomical parameter and promising accessions based on anatomical parameters are presented in Tables 7 and 8.

Evaluation of Mulberry Genetic Resources for Propagation Traits

Total 22 mulberry accessions were evaluated for propagation traits in RBD with three replications and two check variety. Maximum survival percentage was given in Table 9.

Table 2A: Details of morphological characterization of mulberry genetic resources

S. No.	*Acc. No.*	*Branching nature*	*Curve or straightness of the branch*	*Lobation type*	*Leaf color*	*Leaf surface*
1.	MI-0933	Erect	Straight	Unlobed	Green	Smooth
2.	MI-0934	Erect	Straight	Unlobed	Green	Smooth
3.	MI-0935	Erect	Slightly curved	Unlobed	Green	Smooth
4.	MI-0936	Erect	Straight	Unlobed	Green	Smooth
5.	MI-0937	Erect	Straight	Unlobed	Green	Smooth
6.	MI-0938	Erect	Straight	Medium lobed	Green	Smooth
7.	MI-0939	Erect	Slightly curved	Unlobed	Deep green	Slightly rough
8.	MI-0940	Erect	Straight	Unlobed	Green	Smooth
9.	MI-0941	Semi erect	Slightly curved	Unlobed	Green	Smooth
10.	MI-0942	Spreading	Slightly curved	Unlobed	Green	Smooth
11.	MI-0943	Erect	Straight	Unlobed	Green	Smooth
12.	MI-0944	Spreading	Straight	Unlobed	Green	Smooth
13.	MI-0945	Erect	Straight	Unlobed	Green	Smooth
14.	MI-0946	Erect	Straight	Unlobed	Green	Smooth
15.	MI-0947	Spreading	Straight	Medium lobed	Green	Smooth
16.	MI-0948	Erect	Slightly curved	Shallow lobed	Green	Smooth
17.	MI-0949	Semi erect	Slightly curved	Unlobed	Green	Smooth
18.	MI-0950	Semi erect	Curved	Unlobed	Green	Smooth
19.	MI-0951	Semi erect	Slightly curved	Unlobed	Green	Smooth
20.	MI-0952	Semi erect	Straight	Unlobed	Green	Smooth
21.	MI-0953	Erect	Straight	Unlobed	Green	Smooth
22.	MI-0954	Erect	Straight	Medium lobed	Green	Smooth

Table 2B: Contd…

S. No.	*Acc. No.*	*Phyllotaxy*	*Leaf color*	*No. of lobes*	*Leaf nature*	*Leaf apex*
1.	MI-0933	1/3	Green	0	Homophyllous	Acute
2.	MI-0934	1/2, 2/5, 1/3	Green	0	Homophyllous	Acute
3.	MI-0935	1/3, 1/4	Green	0	Homophyllous	Acuminate
4.	MI-0936	1/2, 1/4	Green	0-4	Homophyllous	Acute
5.	MI-0937	1/2, 1/4	Green	0	Homophyllous	Acuminate
6.	MI-0938	1/2, 1/3	Deep green	0	Homophyllous	Acuminate
7.	MI-0939	1/2, 1/4, 2/5	Green	0	Homophyllous	Acuminate
8.	MI-0940	1/3, 2/5	Green	0	Homophyllous	Acuminate
9.	MI-0941	1/2, 1/4, 1/5	Green	0	Homophyllous	Acuminate
10.	MI-0942	1/3, 1/5, 2/3	Green	0	Homophyllous	Acuminate
11.	MI-0943	1/3, 1/4	Green	0	Homophyllous	Acuminate
12.	MI-0944	1/3, 1/5	Green	0	Homophyllous	Acuminate
13.	MI-0945	1/3, 1/5	Green	0-3	Heterophyllous	Acuminate
14.	MI-0946	1/3	Green	0-2	Heterophyllous	Acuminate
15.	MI-0947	1/3	Green	0	Homophyllous	Acuminate
16.	MI-0948	1/2, 1/3	Green	0	Homophyllous	Acuminate
17.	MI-0949	2/4	Green	0	Homophyllous	Acuminate
18.	MI-0950	1/5, 1/3, 2/4	Green	0	Homophyllous	Acuminate
19.	MI-0951	1/2, 1/3	Green	0-4	Homophyllous	Acuminate
20.	MI-0952	1/3	Green	0	Heterophyllous	Acuminate
21.	MI-0953	2/3	Green	0	Homophyllous	Acute
22.	MI-0954	1/2	Green	0	Homophyllous	Acuminate

Table 3: Frequency table for qualitative descriptors

Characters	*Frequency*	*Percentage*	*Characters*	*Frequency*	*Percentage*
Growth nature			*Number of lobation*		
Erect	14	63.64	0	18	81.82
Semi erect	5	22.73	0-2	1	4.55
Spreading	3	13.64	0-3	1	4.55
Total	22	100.00	0-4	2	9.09
Branching nature			*Total*	*22*	*100.00*
Curved	1	4.55	Leaf nature		
Slightly curved	7	31.82	Heterophyllous	4	18.18
Straight	14	63.64	Homophyllous	18	81.82
Total	22	100.00	Total	22	100.00
Color of young shoot			*Leaf color*		
Green	20	90.91	Deep green	1	4.55
Light green	2	9.09	Green	21	95.45
Total	22	100.00	Total	22	100.00
Color of mature stem			*Leaf texture*		
Greenish brown	7	31.82	Chartaceous	22	100.00
Greenish grey	2	9.09	Total	22	100.00
Grey green	13	59.09	Leaf apex		
Total	22	100.00	Acuminate	19	86.36
Stipule duration			*Acute*	*3*	*13.64*
Caducous	21	95.45	Total	22	100.00
Persistent	1	4.55	Leaf base		
Total	22	100.00	Cordate	20	90.91
Stipule nature			Truncate	2	9.09
Foliaceous	1	4.55	Total	22	100.00
Free-lateral	21	95.45	Leaf margin		
Total	22	100.00	Serrate	22	100.00
Lobation type			Total	22	100.00
Medium lobed	3	13.64			
Shallow lobed	1	4.55			
Unlobed	18	81.82			
Total	22	100.00			

Table 4: Details of reproductive characterization of mulberry genetic resources

S. No.	*Acc. No.*	*Sex*	*Inflorescence length (cm)*		*Inflorescence width (cm)*		*No. of flowers*		*Style length*	*Stigma length*
			♂	♀	♂	♀	♂	♀		
	MI-0933	MLFL	2.30	1.64	0.62	0.54	32.40	54.60	1.12	2.00
	MI-0934	FEML	--	1.73	--	0.45	--	45.00	0.23	3.65
	MI-0935	FEML	--	1.98	--	0.68	--	64.00	0.68	4.16
	MI-0936	FEML	--	1.80	--	0.56	--	41.40	0.74	4.48
	MI-0937	FEML	--	1.58	--	0.50	--	60.80	0.36	3.62
	MI-0938	FEML	--	9.66	--	0.48	--	101.20	0.72	3.22
	MI-0939	FEML	--	1.36	--	0.52	--	26.00	0.50	4.90
	MI-0940	FEML	--	1.50	--	0.60	--	24.40	1.20	4.84
	MI-0941	FEML	--	1.06	--	0.40	--	15.40	1.42	4.28

MI-0942	FEML	--	1.66	--	0.52	--	37.00	1.06	4.64
MI-0943	FEML	--	2.14	--	0.56	--	44.40	1.30	4.50
MI-0944	MALE	1.74	--	0.46	--	19.60	--	--	--
MI-0945	MALE	2.73	--	0.58	--	37.20	--	--	--
MI-0946	MLFL	3.38	1.88	0.56	0.56	49.80	62.40	0.50	3.80
MI-0947	FEML	--	1.18	--	0.56	--	42.60	0.24	2.48
MI-0948	MALE	2.26	--	0.48	--	33.80	--	--	--
MI-0949	MALE	3.18	--	0.48	--	40.60	--	--	--
MI-0950	FEML	--	1.98	--	0.70	--	90.20	0.66	3.06
MI-0951	MALE	3.44	--	0.72	--	48.80	--	--	--
MI-0952	MALE	2.10	--	0.42	--	22.80	--	--	--
MI-0953	FEML	--	1.34	--	0.48	--	33.00	0.42	2.76
MI-0954	FEML	--	1.04	--	0.46	--	17.00	1.08	4.82

Table 5: Variability study in mulberry genetic resources for reproductive parameters

Parameters	*Min.*	*Max.*	*Mean ± SE*	*CV%*
Female inflorescence length (cm)	1.04	9.66	2.09 ± 0.53	97.56
Female inflorescence breadth (cm)	0.40	0.70	0.54 ± 0.02	14.76
Male inflorescence length (cm)	1.74	3.44	2.65 ± 0.24	24.14
Male inflorescence breadth (cm)	0.42	0.72	0.54 ± 0.04	18.76
No. of flowers/catkin (Female)	19.60	49.80	35.63 ± 4.13	30.69
No. of flowers/catkin (Male)	15.40	101.20	47.46 ± 6.25	50.98
Style length (mm)	0.23	1.42	0.76 ± 0.10	50.45
Stigma length (mm)	2.00	4.90	3.83 ± 0.23	23.71
Fruit length (cm)	1.02	1.20	2.11 ± 0.26	42.33
Fruit diameter (cm)	0.40	1.00	0.68 ± 0.07	33.92
Fruit peduncle length (cm)	0.20	1.49	0.51 ± 0.08	54.71
Fruit weight (g)	0.13	4.90	0.54 ± 0.12	78.38

Table 6: Details of anatomical characterization of mulberry genetic resources

Parameters	*Min.*	*Max.*	*Mean*	*CV%*
Stomatal size (sq.µm)	204.30	315.87	256.60	12.84
Stomatal frequency/sq.mm	418.04	1102.30	779.76	27.26
No. of chloroplasts/stomata	8.00	17.20	12.02	21.06
Idioblast length (µm)	15.17	23.45	18.33	12.16
Idioblast diameter (µm)	17.24	26.90	21.09	13.41
Idioblast frequency/sq.mm	8.35	31.23	15.54	39.11
Cystolith length (µm)	37.24	80.29	57.64	20.16
Cystolith breadth (µm)	44.83	74.48	59.65	13.98
Upper cuticle thickness (µm)	6.21	12.41	8.28	23.03
Lower cuticle thickness (µm)	3.45	6.90	4.02	27.19
Upper epidermis thickness (µm)	17.24	24.83	20.86	10.65
Lower epidermis thickness (µm)	6.21	11.03	7.87	20.95
Palisade thickness (µm)	58.62	88.96	68.05	15.07
Spongy thickness (µm)	64.14	87.58	71.32	10.94
Leaf thickness (µm)	164.82	217.24	180.40	9.26
Palisade spongy ratio	0.81	1.32	0.97	13.36

The mean values for stomatal size ranged from 204.30–315.87 sq. µm. the stomatal frequency ranged from 418.04–1102.30 per sq.mm and leaf thickness from 164.82–217.24 µm. maximum variability was observed in idioblast frequency/ sq. µm (CV= 39.11%) followed by stomatal frequency 27.26%), whereas minimum variability was observed for stomatal width (7.05%), length (7.33%) and leaf thickness (9.26%) among different anatomical characters recorded in the 22 mulberry accessions.

Table 7: Top performing accessions for anatomical characters

Parameters	*Range*	*Top five accessions*
Stomatal size (µm)	174.46–224.96	MI-0933, MI-0954, MI-0953, MI-0940, MI-0941
Stomatal frequency (mm^2)	345.25–519.95	MI-0955, MI-0946, MI-0949, MI-0946, MI-0947
Idioblast length (µm)	15.17–16.55	MI-0949, MI-0952, MI-0948, MI-0951, MI-0953
Idioblast frequency/sq.mm	8.35–12.23	MI-0950, MI-0955, MI-0933, MI-0937, MI-0940
Palisade tissue thickness (µm)	68.96–88.96	MI-0946, MI-0948, MI-0951, MI-0953, MI-0942
Spongy tissue thickness (µm)	73.10–87.58	MI-0948, MI-0935, MI-0951, MI-0953, MI-0954
Palisade spongy ratio	1.02–1.32	MI-0946, MI-0947, MI-0956, MI-0942, MI-0954
Upper cuticle thickness (µm)	10.35–18.62	MI-0947, MI-0935, MI-0942, MI-0952, MI-0950
Lower cuticle thickness (µm)	4.83–6.90	MI-0945, MI-0952, MI-0937, MI-0953, MI-0933
Upper epidermis thickness (µm)	24.83–42.76	MI-0935, MI-0946, MI-0940, MI-0952, MI-950
Lower epidermis thickness (µm)	8.28–11.04	MI-0946, MI-0951, MI-0953, MI-0948, MI-0938
Leaf thickness (µm)	193.79–217.24	MI-0946, MI-0951, MI-0953, MI-0947, MI-0939
No. of chloroplasts/stomata	13.60–17.2	MI-0951, MI-0953, MI-0945, MI-0940, MI-0935

Table 8: Top performing accessions for anatomical characters

Acc. No.	*No. of traits*	*Trait number (value)*
MI-0945	8	2 (418.04), 3 (15.17), 4 (8.34), 6 (73.10), 8 (11.03), 10 (24.82), 11 (8.27), 13 (17.20)
MI-0946	7	2 (345.24), 3 (15.17), 8 (18.62), 9 (6.89), 10 (42.75), 11 (11.03), 12 (202.06)
MI-0952	5	1 (219.45), 2 (476.27), 5 (69.65), 6 (82.06), 12 (193.78)
MI-0951	4	5 (88.96), 7 (1.32), 11 (11.03), 12 (202.44)
MI-0936	4	5 (75.17), 6 (83.44), 12 (195.85), 13 (14.40)
MI-0950	4	8 (10.34), 2 (519.95), 7 (1.02), 10 (24.82)
MI-0948	4	1 (204.30), 7 (1.06), 8 (11.03), 10 (31.72)

1. Stomatal size (µm), 2. Stomatal frequency (mm^2), 3. Idioblast length (µm), 4. Idioblast frequency (mm^2), 5. Palisade tissue thickness (µm), 6. Spongy tissue thickness (µm), 7. Palisade:Spongy ratio (µm), 8. Upper cuticle thickness (µm), 9. Lower cuticle thickness (µm), 10. Upper epidermis thickness (µm), 11. Lower epidermis thickness (µm), 12. Leaf thickness (µm), 13. Number of chloroplasts/stomata.

Among the characters studied, highest coefficient of variation (CV%) was observed in root weight (37.76%) followed by root shoot ratio and percent survival (Table 10). The top performing mulberry accessions for different propagation traits and based on the multiple propagation traits are presented in Table 11. The accessions MI-0953 and MI-0948 proved to be promising for propagation parameters.

Evaluation of Mulberry Genetic Resources for Growth and Yield Parameters

A twenty two mulberry accessions were studied for growth and yield parameters and maximum variability was observed in biomass (62.75%) and leaf yield (62.09%), while lowest variability was observed for moisture content of the leaves (Table 12).

The top performing mulberry accessions for individual growth and yield traits and multiple growth and yield traits are presented in Tables 13 and 14, respectively. The accessions MI-0952 and MI-0948 proved to be promising for growth and yield parameters.

Evaluation of Mulberry Genetic Resources for Biochemical Parameters

Leaf anatomical status plays an important role in growth and development of silkworm larvae and in turn cocoons production per unit area. Total 22 mulberry accessions were evaluated for important biochemical parameters that contribute to leaf quality and variability for different parameters which is presented in Table 15. The results indicate that the variability was medium for different biochemical parameters among the 22 accessions evaluated. The parameter-wise and multiple trait based top performing accessions are indicated in Tables 16 and 17. Among the mulberry accessions studied MI-0935, MI-0948 and MI-0938 are promising based on biochemical analysis.

Discussion

Characterization and evaluation of any crop is a continuous process to evolve new varieties suitable for commercial utilization. Studies have indicated that the variation of important aboveground and underground agronomic traits

Table 9: Survival percentage of mulberry accessions

Survival (%)	*No. of Accns*	*Accessions*
> 90	3	MI-0953, MI-0948, MI-0938
80–90	4	MI-0951, MI-0947, MI-0952, MI-0936
< 60	8	MI-0940, MI-0939, MI-0941, MI-0933, MI-0954, MI-0950, MI-0946, MI-0935
No survival	7	MI-0934, MI-0937, MI-0945, MI-0943, MI-0949, MI-0942, MI-0944
Total	22	

Table 10: Variability for propagation parameters

Parameters	*Min.*	*Max.*	*Mean*	*SE*	*CV%*
Survival %	20.00	96.67	76.67	8.27	32.34
Shoot length (cm)	30.89	52.56	42.66	2.65	18.65
Number of roots	2	4	3	0.2	20.00
Longest root length (cm)	17.00	25.11	20.52	0.97	14.11
Dry root weight (g)	0.40	1.25	0.80	0.1	37.76
Root volume (ml)	0.22	0.49	0.33	0.02	22.00
Root shoot ratio	0.10	0.27	0.15	0.02	33.20

Table 11: Top performing mulberry accessions based on multiple traits

Accessions	Traits	Trait number (value)
MI-0953	6	1 (91.67), 2 (3), 3 (25.11), 5 (0.99), 6 (0.16)
MI-0948	5	2 (3.44), 3 (24.67), 4 (1.25), 5 (0.50), 6 (0.27)
MI-0938	4	3 (21.22), 4 (1.15), 5 (0.33), 6 (0.27)
MI-0951	4	1 (88.33), 2 (4.22), 4 (0.99), 6 (0.19)
MI-0947	3	2 (3.56(, 3 (21.24), 6 (0.34)

1. Survival %; 2. Number of roots; 3. Longest root length (cm); 4. Dry root weight (g); 5. Root volume (mL); 6. Root shoot ratio.

Table 12: Variability for growth and yield parameters

Parameters	Min.	Max.	Mean	SE	CV%
Number of shoots	8.33	28.67	15.94	1.83	38.14
Length of longest shoot (cm)	140.00	239.33	185.75	8.72	15.57
Length of lamina (cm)	10.67	24.67	15.58	1.12	23.75
Width of lamina (cm)	7.00	16.00	10.40	0.73	23.32
Weight of lamina (g)	0.92	4.97	2.40	0.34	46.82
Total shoot length (cm)	1075.33	4086.33	2065.28	280.94	45.12
Leaf yield/plant (kg)	0.45	2.20	0.92	0.17	62.09
Inter-nodal distance (cm)	3.26	8.28	4.84	0.37	25.21
Weight of 100 leaves (g)	106.07	614.50	270.97	42.94	52.56
Moisture content of leaves (%)	61.50	74.20	66.03	1.03	5.15
Moisture content after 6 hrs (%)	51.14	65.92	56.17	1.48	8.71
Moisture retention capacity (%)	54.14	78.17	66.21	2.09	10.47
Biomass/plant (kg)	0.92	4.10	2.28	0.43	62.75

Table 13: Top performing mulberry accessions for growth and yield traits

Traits	*Range*	*Accessions*
Number of shoots	17.33–28.67	MI-0936, MI-0941, MI-0948, MI-0951, MI-0952
Length of longest shoot (cm)	184.33–239.33	MI-0936, MI-0938, MI-0952, MI-0942, MI-0954
Length of lamina (cm)	17.67–24.67	MI-0951, MI-0952, MI-0936, MI-0943, MI-0950
Width of lamina (cm)	11.83–16.00	MI-0933, MI-0940, MI-0948, MI-0952, MI-0938

Total shoot length (cm)	2154–4086	MI-0949, MI-0936, MI-0952, MI-0944, MI-0933
Leaf yield/plant (kg)	0.97–2.2	MI-0944, MI-0936, MI-0949, MI-0942, MI-0936
Inter-nodal distance (cm)	3.19–4.05	MI-0952, MI-0938, MI-0950, MI-0949, MI-0944
Weight of 100 leaves (g)	43.00–61.5	MI-0933, MI-0949, MI-0953, MI-0950, MI-0938
Moisture content of leaves (%)	68.16–74.20	MI-0949, MI-0944, MI-0948, MI-0933, MI-0953
Moisture content after 6 hrs (%)	60.37–65.92	MI-0951, MI-0953, MI-0944, MI-0947, MI-0950
Moisture retention capacity (%)	72.16–78.17	MI-0949, MI-0950, MI-0938, MI-0950, MI-0954
Biomass/plant (kg)	2.3–4.40	MI-0941, MI-0948, MI-0933, MI-0954, MI-0938

Table 14: Top performing mulberry accessions based on multiple traits

Acc. No.	*No. of Traits*	*Traits (Value)*
MI-0936	6	1 (22), 2 (239.33), 4 (11.83), 6 (3633.67), 6 (1.29), 12 (4.31)
MI-0952	5	1 (28.67), 2 (206), 5 (4086.33), 6 (0.967), 12 (3.61)
MI-0933	5	2 (196.67), 5 (217.33), 6 (1.79), 7 (3,26), 12 (4.12)
MI-0944	5	1 (21.33), 5 (2248.66), 9 (68.16), 10(60.84), 11 (72.65)
MI-0949	5	2 (184.33), 3 (18), 9 (68.77), 10 (63.26), 11 (78.17)

1. Number of shoots; 2. Longest root length (cm); 3. Length of lamina (cm); 4. Width of lamina (cm); 5. Total shoot length (cm); 6. Leaf yield/plant (kg); 7. Inter-nodal distance (cm); 8. Weight of 100 leaves (g); 9. Moisture content of leaves (%); 10. Moisture content after 6 hrs (%); 11. Moisture retention capacity (%); 12. Biomass/plant (kg).

Table 15: Variability of mulberry accessions for biochemical parameters

Parameter	*Mean*	*Min.*	*Max.*	*SE*	*CV %*
Chlorophyll a (mg/g fr. wt.)	1.64	1.08	2.00	0.06	17.33
Chlorophyll b (mg/fr. wt.)	0.27	0.17	0.36	0.01	21.95
Total chlorophyll (mg/fr. wt.)	1.91	1.27	2.35	0.07	16.66
Chlorophyll a/b ratio	6.4	4.17	9.32	0.29	21.71
Protein (% dry wt.)	8.43	5.52	12.34	0.35	19.75
Water soluble carbohydrates (% dry wt.)	13.15	8.96	17.94	0.54	19.24

Table 16: Top performing mulberry accessions for biochemical parameters

Parameter	*Range*	*Accessions*
Chlorophyll a (mg/g fr. wt.)	1.90–2.00	MI-0935, MI-0950, MI-0948, MI-0944, MI-0938
Chlorophyll b (mg/fr. wt.)	0.34–0.36	MI-0950, MI-0949, MI-0948, MI-0952, MI-0955
Total chlorophyll (mg/fr. wt.)	2.18–2.35	MI-0950, MI-0948, MI-0944, MI-0935, MI-0951
Chlorophyll a/b ratio	7.96–9.32	MI-0940, MI-0936, MI-0935, MI-0937, MI-0938
Protein (% dry wt.)	9.47–12.34	MI-0935, MI-0948, MI-0938, MI-0952, MI-0954
Water soluble carbohydrates (% dry wt.)	15.65–17.94	MI-0951, MI-0947, MI-0952, MI-0938, MI-0935

Table 17: Top performing mulberry accessions based on multiple traits

Acc. No.	*No. of Traits*	*Traits (Value)*
MI-0935	5	1 (200), 3 (2.24), 4 (8.29), 5 (12.34), 6 (15.65)
MI-0948	4	1 (1.94), 2 (0.35), 3 (2.29), 5 (11.88)
MI-0938	4	1 (1.90), 4 (7.96), 5 (11.38), 6 (16.22)

1. Chlorophyll a (mg/ g fr. wt.); 2. Chlorophyll b (mg/ g fr. wt.); 3. Total chlorophyll (mg/ g fr. wt.); 4. Chlorophyll a/b ratio; 5. Protein (% dry wt.); 6. Water soluble carbohydrates (% dry wt.).

in mulberry is largely continuous in nature (Vijayan 2008). Ananda Rao *et al*. (2011) have studied the variation among different mulberry genetic resources for morpho-reproductive characters. They identified promising drought tolerant lines and potential areas based on the variation and abundance of mulberry genetic resources for *in-situ* conservation. Jayeoba *et al*. (2014) characterized eight mulberry varieties using morphological and chemical parameters and recommended K2, S34, S30 and S54 as promising varieties. The exotic mulberry accessions were evaluated for growth and yield traits by Tikader and Kamble (2009). The study showed maximum coefficient

of variation in leaf yield per plant followed by total shoot length and leaf moisture content. Similarly in the present study highest coefficient of variation was observed in leaf yield per plant (62.09%). Suresh *et al*. (2018) conducted a multivariate analysis of indigenous and exotic mulberry germplasm and identified diverse genotypes as promising donors in improving foliage production. Tikader and Kamble (2008) have studied the variation in indigenous mulberry germplasm on growth and yield and identified better performing accessions. Our study also highlighted the variation of indigenous accessions among various parameters.

Conclusion

Germplasm is a valuable natural resource that provides knowledge about the genetic composition of a particular species and is crucial for conserving plant diversity. Mulberry breeders will require much genetic diversity to select and recombine favorable traits through cross breeding. In this context the study was conducted and the performances of indigenous mulberry accessions were evaluated statistically. The accessions MI-0946, MI-0945, MI-0953, MI-0948, MI-0935, MI-0948, MI-0936 and MI-0952 were identified as top performers based on multiple trait analysis. These accessions can serve as promising donors for future breeding program for the development of improved varieties in mulberry.

Acknowledgment

The authors are thankful to CSGRC and Central Silk Board for providing the facility to conduct the study.

References

Arnon, D. (1949). Copper enzymes isolated chloroplasts, polyphenoloxidase in Beta vulgaris. Plant Physiology, 24, 1-15.

Banerjee, R., Roychoudhury Sau, S., Das, B., Saha, A.K., Saratchandra, B., & Bajpai, A.K. (2011). Phenotypic divergence in mulberry (Morus spp.) germplasm collections. Journal of Crop Improvement, 25(5), 459-471.

Hiscox, J.D., & Israelstam, G.F. (1979). A method for extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany, 57, 1332-1334.

Jayeoba, F.M., Ogunbanjo, O.R., & Awotoye, O.O. (2014). Characterization of mulberry (Morus alba L.) varieties in South Western Nigeria. Continental Journal of Agricultural Science, 8(1), 13-19.

Khurana, P., & Checker, V. (2011). The advent of genomics in mulberry and perspectives for productivity enhancement. Plant Cell Reports, 30, 825-838.

Lowry, O.H., Rosebrough, N.J., Lewis Farr, A., & Randall, R.J. (1951). Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193(1), 256-275.

Plumer, D.T. (1971). An introduction to practical biochemistry. Tata McGraw-Hill Publications Co. Ltd., New Delhi.

Sharma, A., Sharma, R., & Machii, H. (2000). Assessment of genetic diversity in a Morus germplasm collection using fluorescence-based AFLP markers. Theoretical and Applied Genetics, 101, 1049-1055.

Suresh, K., Ghosh, M.K., Chakravarty, B.R., & Trivedy, K. (2018). Multivariate analysis of indigenous and exotic mulberry (Morus spp.) germplasm for identifying diverse genotypes under humid subtropical region. International Journal of Pure and Applied Bioscience, 6(1), 618-627.

Thangavelu, K., Tikader, A., Ramesh, S.R., Rao, A.A., Naik, V.S., Sekar, S., & Deole, A.K. (2000). Catalogue on mulberry (Morus spp.) germplasm CSGRC, Hosur, India 2, pp 1-225.

Thriveni, M.C., Thanavendan, G., Raju Mondal, Jhansilakshmi, K., Saraswathi, P., Ravikumar, G., & Sekar, S. (2021). Catalogue on Mulberry (Morus spp.) germplasm, vol. 6, CSGRC, Hosur.

Tikader, A., & Rao, A.A. (2002). Phenotypic variation in mulberry (Morus spp.) germplasm. Sericologia, 42, 221-233.

Tikader, A., & Kamble, C.K. (2008). Mulberry wild species in India and their use in crop improvement: a review. Australian Journal of Crop Science, 2(2), 64-72.