

[Report and Opinion]

Biodiversity, Geographical Distribution, Utilization and Conservation of Wild Mulberry *Morus serrata* Roxb.

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ABSTRACT

Exploitation of wild relatives of crop plants to a large extent depends on the efficient use of germplasm resources available in natural habitat and the centre of diversity. The mulberry, sole food for silkworm is cultivated for the production and development of silkworm industry. Four species of mulberry viz., *M. indica* L., *M. alba* L., *M. laevigata* Wall., and *M. serrata* Roxb., are reported in India. Among these species, *M. serrata* is endemic to North Western Himalayan belt and growing in the higher altitude ranging from 560 - 2200m above mean sea level. As a part of survey and exploration, 54 samples of *M. serrata* were collected from three states i.e., Uttaranchal (45), Himachal Pradesh (07) and Jammu and Kashmir (02). During collection, morphological variability, details of habitat viz., natural abode of plant and other related data were recorded. The collected materials were established in the Ex-situ field gene bank of Central Sericultural Germplasm Resources Centre (CSGRC) for further study. The morphological, anatomical, reproductive and growth traits showed variation among the different collections of *M. serrata*. This paper deals with biodiversity, geographical distribution, utilization and conservation of *M. serrata* for posterity.

Keywords: Biodiversity, Conservation, Geographical distribution, *M. serrata*, India

INTRODUCTION

Biodiversity is the combination of genetic variation, species richness, ecosystem and landscape diversity. Biodiversity at all levels is currently being lost at an unprecedented rate. The highest levels of biodiversity are in the tropics, particularly in tropical rain forests. India is one of the 12 mega biodiversity centers of the world. The Global Biodiversity Assessment in 1995 by the United Nation Environment Programme (UNEP) estimates that 13- 14 million species might exist in our planet.

Morus is an important genus of the family Moraceae and distributed widely in China-Japan region, which represents East China, Korea and Japan. In India, *Morus* is represented by four species *M. indica* L., *M. alba* L., *M. laevigata* Wall., and *M. serrata* Roxb. (Brandis, 1906; Hooker, 1885). *M. serrata* is

available in North Western Himalayan part of India. The perennial vegetation and associated fauna in the Himalayas provide an interesting field of investigation and scope for collection of diverse species of flora and fauna. The diversity and uniqueness of the plant component in various habitats of *M. serrata* add to the sound and aesthetic environment of the Himalayas. However, in recent years, excessive exploitation of vegetation, unplanned land use, natural disasters and several other developmental processes have resulted in deterioration of biodiversity of the Himalayas. In such a situation, *M. serrata* is the abode of Himalaya also gets disturbed and destroyed and requires sustainable conservation. In-situ conservation of flora and fauna is better but it is always not practicable and hence the most practical way is ex-situ conservation of plant genetic

resources in ex-situ field gene bank (Tikader *et al.*, 1999b). Chauhan and Thakur (1995) highlighted the conservation of plant genetic resources of Himachal Pradesh to maintain its ecological balance.

The mulberry germplasm collection from natural sources was systematically started from 1993 after the establishment of CSGRC, Hosur, Tamil Nadu, India (Tikader *et al.*, 2002; Tikader and Thangavelu, 2003). As a part of survey and exploration, the present study was undertaken to collect the mulberry germplasm resources from North West India particularly from Uttaranchal, Himachal Pradesh and Jammu and Kashmir. The biodiversity, geographical distribution, conservation and its utilization have been highlighted in this paper.

MATERIALS AND METHODS

As a regular process, survey and exploration was conducted in North West India to identify as well as to collect mulberry germplasm resources from natural and cultivated farms. Before starting the survey, adequate information was gathered from different sources particularly from Botanical Survey of India (BSI), Forest Research Institute (FRI), State Forest Research Institute (SFRI), Universities and local residents of that area. Random and biased sampling procedure was followed for collection of samples depending on the population density. At least 10% of the population was selected and among them collection was made. Herbarium specimen was prepared from the collected samples. Simultaneously photograph was also taken of the tree. During collection of vegetative cutting from the selected plant, pre-cautionary measures were adopted to avoid the disease, damage and infected plant materials. The other required information was collected as per the standard collection format including passport data. A total of 54 samples of *M. serrata* were collected from three states namely Uttaranchal (45 samples), Himachal Pradesh (07 samples) and Jammu and Kashmir (02 samples). After collection, initially the materials were established in the nursery and after one year of establishment transplanted in main ex-situ field gene bank of CSGRC, Hosur. The germplasm is being maintained at CSGRC, Hosur (Latitude 12.45° N and Longitude 77.51° E, altitude

942m above mean sea level under tropical climatic condition). The average rainfall ranges from 700- 1000mm per annum with maximum temperature 34°C and minimum 12°C. The germplasm plantation was maintained under 2.44 x 2.44m with 4 plants per accession as small tree and randomly planted with control plants in Augmented Design. The maintenance of the germplasm followed as per the recommended cultural practices (Tikader and Rao, 2001). When the plantation attained two years of establishment the plants were pruned at 1.5m crown height following two pruning per year for data recording.

After pruning, the morphological data were recorded visually (ordinal scale) from 4 plants and compared with the field data. When the plants attain three months of growth after pruning, fully expanded leaves (5th- 7th position) in descending order from the top of a shoot was collected in the morning at 10- 11 am. A small rectangular piece were taken out from the middle portion of the leaf blade, avoiding vein and veinlets (Metcalf and Chalk, 1979), preserved in FAA (Formalin 5ml, Glacial acetic acid 5ml and 70% Ethanol 90ml) solution. Stomatal studies were conducted by applying a thin layer of Wimbley's quick fix on the abaxial (lower) leaf surface, dried and peeled out the thin layer of that with the impression of stomata. The thin layer was fixed on a glass slide, the quick fix covered with the cover slip and observed under Leica Leitz, DMRB Wetzlar microscope. The stomatal size (length and width) was measured with the help of ocular micrometer attached to the microscope and value was converted by conversion factor. The number of stomata per mm² of leaf was also counted. To observe total leaf thickness, hand section was made and observed under microscope after staining with 1% safranin and mounted in 50% glycerin. Chloroplast number per stomata was counted of 3rd- 5th leaf of a twig in descending order from the fully open leaf at top using ventral surface peelings of freshly collected leaf samples. The peels were stained and mounted on a slide with 2% potassium iodide iodine (KI+ I) solution following the method of Chaudhuri and Barrow (1975). For cytological study, the leaf tips were examined using aceto-orecin haematoxylin staining squash programme, which largely improve the quality of

chromosome staining (Agaev, 1978). The metaphase plates made for counting and ascertaining the ploidy status.

Observations on sex expression was carried out in normal flowering season (spring) as well as after pruning of plants from January through March- May. Sex expression was recorded as male, female. The male flower composed of stamen, anther and pollen. The stamen and anther length was observed under microscope and measured in millimeter. In each flower nine observations were made and such 5 flowers per replication and in 3 replications all together 15 flowers were recorded. To estimate pollen viability, which is an indication of pollen stainability, male catkin from field grown plants were dehiscid over a glass slide stained in a drop of 2% aceto-carmin and covered with a cover slip. After 5 minutes, the percentage of plump fully stained grain was determined (Hussain and Williams, 1997; Tikader, 1999; Tikader and Rao, 2001). Other flower data were recorded from catkin itself. The female flower was composed of floral organs or carpel. A typical carpel has 3 parts, ovary, style and stigma. The style and stigma length was observed under microscope and measured in millimeter. Sorosis characters were recorded after ripening of fruits i.e., fruit length, width, weight, taste and colour. Data on growth parameters were recorded after 90 days of pruning. Leaf moisture content and leaf moisture retention in harvested leaf was calculated following the procedure as reported earlier (Vijayan *et al.*,

1997; Tikader, 2001). The young leaves from 90 days old plants were collected and stored at -80°C and used for DNA extraction. DNA quantification was made by electrophoresis on 0.8% agarose gel (1x TBE) stained with ethidium bromide (0.5 $\mu\text{g}/\text{ml}$) and using uncut λ DNA (10ng/ μl) as standard. All the DNA samples per population were used for PCR amplification separately and those bands that appeared consistently were used for statistical analysis. The data were recorded for two crops per year for two years and mean data presented. All the recorded data were subject to general statistical analysis following the SPSS statistical packages.

RESULTS AND DISCUSSION

The materials collected from different states are presented in Table 1. Maximum collections obtained from Uttaranchal (45) followed by Himachal Pradesh (07) and Jammu and Kashmir (02).

Geographical distribution of *M. serrata*

M. serrata, the Himalayan mulberry was found in natural habitats. The survey and exploration indicates that *M. serrata* is endemic to North Western Himalaya at higher altitudes. Roxburgh (1832) also published similar reports. The state wise distribution of mulberry species is as follows.

Uttaranchal State: The sacred mulberry at Joshimath is the oldest tree and more than 1200 years old (Rau, 1967; Tikader *et al.*, 1999a). The plant girth is 21.33m. The trees of this species were found distributed in natural habitats in different elevations and places. The main places includes Salna (700m), Urgam valley (2000m), Chakrata (2100m), Mussoorie (1900m), Pandukeshar (2100m), Hanumanchetty (2200m), Ranachetti (2200m), Uttarkashi (700m), Dunda (900m), Almora (1900m), Pithoragarh (1850m), Nainital (2100m), Bhimtal (800m), Gangotri (2200m), Yamunotri (2200m), Gangnani (2100m), Barkote (900m) and other places.

Himachal Pradesh State: *M. serrata* is available at higher altitude of hilly areas in Himachal Pradesh. The places are like Solan (1900 m), Joharjii (1350 m), Nainitkar (1100 m), Simla (2000 m), Sirmour (1300 m),

Table 1. Detailed information on distribution of *M. serrata* Roxb.

State	District	Total collections	% of collection
Uttaranchal	Dehradun	11	20.37
	Pauri Garhwal	02	3.70
	Tehri Garhwal	01	1.85
	Rudraprayag	01	1.85
	Uttarkashi	05	9.26
	Chamoli	14	25.93
	Nainital	03	5.56
	Almora	06	11.12
	Pithoragarh	02	3.70
Himachal Pradesh	Sirmour	03	5.56
	Solan	02	3.70
	Simla	02	3.70
Jammu & Kashmir	Rajouri	02	3.70
Total		54	100.00

Chamba (2200 m), Kangra (1700 m), Kullu (2100 m) and other places.

Jammu and Kashmir State: The distribution of *M. serrata* is available in Rajouri (1900m), Sunderbani (1200m), Poonch (1900m) and Batote (2200m) region of Jammu and Kashmir. Dandin *et al.*, (1993) reported the availability of *M. serrata* in this region. Watt (1981) reported this species at higher elevation between 1200 to 2750m in the North Western Himalaya of India. *M. serrata* is associated with Oak, Conifers and Pines which is the abode of higher altitudes

Biodiversity of *M. serrata*.

M. serrata, the natural Himalayan mulberry is confined to North West India. Depending on the agro-climatic distribution, the morphological parameters showed wide variation (Table 2). The bark colour varied from red, brown to dark brown and blackish brown. Leaf varied from unlobed to multilobed, homophyllous to heterophyllous, chartaceous to coriaceous, rough thick, tomentose and velvety. Phyllotaxy is mixed type i. e., 1/2, 1/3, 2/5 and mixed. Internodal distance varied from 3.5 to 7.5cm. Male and female flowers in separate plant, dioecious. Fruit colour varied from black, creamish and pink. The fruit is very sweet to taste after ripening. Mucilage juice is the special character of the species (Tikader and Thangavelu, 2003).

Ploidy status of *M. serrata*

The chromosome count of the collected samples indicates that *M. serrata* is available in polyploid form in nature. Mulberry in general is diploid ($2n=2x=28$). But in nature, the species is found in diploid ($2n=2x=28$), triploid ($2n=3x=42$), tetraploid ($2n=4x=56$)

Table 2. Morphological variability of *M. serrata*.

Morphological trait	Variability of different traits
Bark colour	Brown, Dark brown, Blackish brown
Leaf lobation	Unlobed, Lobed, Mixed
Leaf texture	Coriaceous, Leathery, Charataceous
Leaf shape	Ovate, Wide ovate, Narrow ovate
Leaf margin	Serrate, Dentate
Leaf surface	Smooth, Slightly rough, Rough
Leaf size (cm ²)	150- 400
Sex	Male, Female
Phyllotaxy	1/2, 1/3, 2/5, Mixed
Fruit length (cm)	3.50- 4.50
Fruit colour	Black, White, Pink
Altitude (m)	560- 2200

and hexaploid ($2n=6x=84$). Ploidy level has been used to trace the genesis of plant endemism. The palaeo endemics have high chromosome number and are assumed to be ancient polyploids and grow in higher altitude (Dhar Uppendra, 2002). The similar case also observed for *M. serrata*.

Reproductive parameters in *M. serrata*

The inflorescence length is higher in *M. serrata* Roxb. than other cultivated species in India. The maximum collections of *M. serrata* possess male flower. The analysis of data indicates variation among the accessions (Table 4). The coefficient of variation ranges from 3.99- 35.33% in different reproductive parameters. Maximum CV observed in flower/catkin followed by stamen length and least in pollen viability. If the chromosome number increases, the pollen size also increases in *M. serrata*. The pollen study indicates that the diameter of the pollen spores varies from 16.00- 25.70, 21.00- 28.00 and 23.00- 30.50, 25.00- 33.00 μm in diploid, triploid, tetraploid and hexaploids, respectively. The number and diameter of the

Table 3. Variation in leaf anatomical parameters of *M. serrata* Roxb.

Characters	Mean	Maximum	Minimum	CV%
Stomata length (μm)	30.35	36.47	22.22	15.92
Stomata width (μm)	20.07	25.06	11.60	17.43
Stomata frequency (mm ²)	338.71	416.30	255.82	14.84
Idioblast length (μm)	43.45	83.47	7.88	64.64
Idioblast width (μm)	41.75	65.12	18.07	35.15
Idioblast frequency mm ²)	23.98	35.86	13.90	24.05
Palisade layer thickness (μm)	75.78	107.48	42.26	23.19
Spongy layer thickness (μm)	104.50	173.50	59.90	26.55
Palisade spongy ratio	0.75	1.24	0.52	25.31
Leaf thickness (μm)	242.80	340.87	141.48	21.34
Chloroplast/stomata	17.31	32.88	12.83	27.44

Table 4. Variability in male reproductive characters of *M. serrata* Roxb .

Characters	Mean	Maximum	Minimum	CV%
Inflorescence length (cm)	3.26	4.60	2.40	24.19
Inflorescence width (cm)	0.80	1.03	0.47	22.46
Flower / catkin	44.70	70.33	23.00	35.33
Stamen length (mm)	3.50	5.47	1.70	34.67
Anther length (mm)	1.12	1.55	0.83	22.40
Pollen diameter (μm)	21.86	26.47	18.00	10.56
Pollen viability %	90.82	95.45	83.87	3.99

pollen pores showed 2- 5 porate pollen grain (Tikader *et al.*, 2000). Likewise female inflorescence also showed variation among the accessions in different parameters. Maximum CV was observed in style length and minimum in fruit width (Table 5).

Leaf histological parameters in *M. serrata*

Wide range of variability observed in different leaf anatomical parameters. The stomata frequency ranges from 255.82-416.30 mm^2 whereas idioblast frequency 13.90- 35.86 mm^2 . The palisade and spongy layer thickness plays a vital role for keeping moisture in leaf during adverse climatic condition. Total leaf thickness indicates the quality of leaf. *M. serrata* possesses coarse, hairy leaf suitable to adjust in adverse condition i.e. cold, drought etc. The chloroplast number in guard cells of stomata is the indicator of ploidy level, which ranges from 12.83- 32.88. The chloroplast number per stomata is the indirect way to group mulberry accessions in different ploidy levels (Tikader *et al.*, 1999; Tikader and Rao, 2001).

In *M. serrata*, the chloroplast number per stomata varies from 10- 20 in triploid, 10- 26 in tetraploid and 12- 30 in hexaploid. The variation in chromosome number, chloroplast number per stomata and pollen diameter are useful for grouping the germplasm accessions and also in mulberry crop improvement programme (Tikader and Rao, 2001).

Growth performance of *M. serrata* in ex-situ field genebank

The maintenance and methodology of data recording has indicated in material and methods. The growth behaviour is important for effective utilization of mulberry germplasm for sericultural purposes. The preliminary growth behavior of some *M. serrata* was recorded (Table 3). Wide range of variation was observed among the different

growth traits. Among the parameters, the moisture content (68.10- 80.22%) and moisture retention (55.13- 75.00%) showed consistent and higher value than the commercial varieties. But the coefficient of variation (CV) is 3.08- 8.17%, respectively. The wide CV indicates the variation among the traits and useful for utilization in crop improvement programme (Tikader and Dandin, 2005).

Genetic variability of *M. serrata*

The wild populations of *M. serrata* after collection from natural sources were established in ex-situ field gene bank. The preliminary evaluation highlighted the variability and was subjected to molecular evaluation. The result indicates significant amount of genetic diversity among the collections of *M. serrata* the 17 ISSR primer generated a total of 95 DNA markers, 51 of which were polymorphic revealing 67% polymorphism among the populations (Vijayan *et al.*, 2004). Based on the morphological traits and genetic variability the collections of *M. serrata* may be selected for crop improvement and conservation programme.

Breeding performance of *M. serrata*.

In order to broaden the genetic base, new gene pools have to be incorporated into the gene pool of cultivated cultivars. Earlier the crossability among the different *Morus* species and its inheritance pattern were studied (Dandin *et al.*, 1987; Tikader and Dandin, 2001). *M. serrata* possess several agronomically important traits such as higher leaf thickness, greater leaf moisture content, moisture retention and resistance to abiotic and biotic stresses. Among the abiotic factors, the species is resistance to drought and frost (Tikader and Thangavelu, 2003). Initial breeding performance of *M. serrata* at inter-specific level with *M. indica* found

Table 5. Variability in female reproductive characters of *M. serrata* Roxb.

Characters	Mean	Maximum	Minimum	CV%
Inflorescence length (cm)	1.95	2.77	1.30	32.39
Inflorescence width (cm)	0.81	0.95	0.74	11.88
Flower / catkin	38.17	51.33	23.33	32.15
Style length (mm)	0.53	0.90	0.29	52.21
Stigma length (mm)	3.34	3.85	2.70	17.80
Fruit length (cm)	1.85	2.18	1.22	24.66
Fruit width (cm)	0.87	0.95	0.81	6.79
Fruit weight (cm)	0.90	1.18	0.65	25.44

suitable for crop improvement. More number of crosses at inter-specific and intra-specific level may be tried with *M. laevigata* and *M. serrata* through introgression breeding. F1 hybrid thus developed of *M. indica* and *M. laevigata*, *M. indica* and *M. serrata* should be studied for further utilization (Tikader and Thangavelu, 2005). *M. serrata* provides scope for target oriented selective breeding to incorporate the characters of drought, frost, and disease resistance into cultivated cultivars.

Specific characters of *M. serrata*

M. serrata is known as Himalayan mulberry due to its origin (Roxburg, 1832). The wild species possesses drought tolerant characters like leaf rolling, abundant xylem, less stomata per unit area (255.82– 416.30 mm²) and slow growth in response to moisture stress which are useful in breeding to develop stress tolerant varieties. The information gathered during collection of materials (Tikader and Thangavelu, 2003).

Non - sericultural use of *M. serrata*

Mulberry is a multipurpose tree and has high potential economic value other than sericulture. Besides sericulture use, mulberry can also be exploited for several other biological and industrial purposes. Apart

from sericulture, mulberry serves some of the important requirements viz., food, fodder, fuel, fruit medicine, timber, religious sanctity etc. (Tikader *et al.*, 2002)

Fodder use: *M. serrata* is used as fodder plant in hill areas where the plant grows. The information gathered from local inhabitants during collection of materials. The leaf analysis of mulberry indicates rich protein (28.00%) and carbohydrate contents (22.50%) and can be used as good fodder (Suryanarayana, 2002).

Fuel use: *M. serrata* is a deciduous tree and fast growing produces high biomass along with twigs. The twigs and leaves can be used as fuel for cooking.

Fruit use: *M. serrata* provides delicious fruits and is very popular in Northern part of India. *M. serrata* is having black, white and pink fruit. Mulberry fruit contain high carbohydrates besides vitamin and minerals (Dwivedi *et al.*, 2005). The fruits are used for jam, jelly, juices and other products. Mulberry fruit juice helps to prevent high fever, dyspepsia and melancholia.

Medicinal use: Mulberry is called “Kalpa-bruksha”. The fruit extract is good laxative.

Table 6. Mean growth performance of *M. serrata* Roxb.

Characters	Minimum	Maximum	Range	CV%
Shoot number	5.17	21.33	5.17 - 21.33	29.34
Longest shoot length (cm)	57.50	175.00	57.50 - 175.00	20.02
Total shoot length (cm)	197.50	2411.67	197.50 - 2411.67	38.45
Internodal distance (cm)	3.97	7.63	3.97 - 7.63	10.45
100 leaf weight (g)	298.97	829.38	298.97 - 829.38	23.30
Leaf moisture (%)	68.10	80.22	68.10 - 80.22	3.08
Leaf moisture retention (%)	55.13	75.00	55.13 - 75.00	8.17
Leaf yield / plant (Kg)	0.33	2.22	0.33 - 2.22	39.33

The leaf extract is used to cure throat inflammation, the bark is used as purgative and vermifuge, and the root has antihelmintic and astringent properties. Thus the different parts of the mulberry i.e., root, stem, leaf, fruits and bark can be used as home medicine. The information gathered while collection of materials and the local inhabitants are maintaining as indigenous knowledge.

Timber value: Mulberry is well known for the manufacture of sport goods and toys. The hard wood of *M. serrata* is used for manufacture of tennis racket and cricket bats for the fine grain and polishing. The wood of *M. serrata* is used as pole, plank, doors and windows in local made houses.

Aforestation purposes: *M. serrata* grows in higher altitude in hilly areas, which are generally falls under arid, semi-arid and dry cold zone. The mulberry plant grows in forest area, which helps for conservation of soil, microorganism, increase of organic matter in soil. *M. serrata* is drought tolerant and good for afforestation in different climatic zones. In Himalayan belt *M. serrata* is balancing the ecosystem by its spontaneous growth forming thick forest and conserving the microorganism of the soil.

Conservation of *M. serrata*

Conservation of plant genetic resources and their wild relatives in their natural habitats is of utmost importance and deserve top priority. The people have been using local biodiversity for their lives and livelihoods. Urban and elite lifestyle is leading to biodiversity loss through over exploitation of raw materials and destruction of natural habitats. The Himalayan belt, which is the "Green Gold" and this treasure is to be protected at all costs. More attention should be paid to prevent loss of habitat of endemic plants of *M. serrata* for posterity (Tikader and Thangavelu, 2003; Tikader *et al.*, 2002).

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