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Karthik Kumar Manjunath
Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

Harshitha BS
Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

Anusha NM
Division of Fruit Science and Horticulture Technology, Indian Agricultural Research Institute, New Delhi, India

Bhargavi HA
Scientist, Division of Crop Improvement, ICAR- Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh

Corresponding Author:
Harshitha BS
Division of Genetics, Indian Agricultural Research Institute, New Delhi, India

Breeding for mulberry improvement: A review

Karthik Kumar Manjunath, Harshitha BS, Anusha NM and Bhargavi HA

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Abstract
Sericulture, the art of raising silkworms, plays a great role for livelihood support in parts of southern Karnataka, particularly in the districts of Chikkaballapura, Kolar, Mysore, and Ramanagaram, as well as in the states of Andhra Pradesh, Telangana, Tamil Nadu, West Bengal, and Assam. Among all the species of silkworm, *Bombyx mori* is the most commercially raised silkworm for raw silk production which depends on mulberry as a host plant. Mulberry is a perennial plant that grows quickly with deep root system and produces a lot of biomasses. In addition to its usage in sericulture, the leaf extract is recognized to have significant therapeutic effects. Yield of mulberry leaves is contributed by three key elements such as superior variety, package of practices and integrated pest control. Although many mulberry genotypes can be grown commercially, there is a need to produce mulberry varieties that are suitable for a given region and for both rainfed and irrigated environments**.** The development of genotypes with inherent resistance to pests and diseases is essential, as chemical control is not practicable. The genetic improvement methods include conventional breeding through hybridization and selection, mutagenesis, molecular approaches including markers, and genetic engineering. Development of improved varieties of mulberry is the key to support sericulture farmers.

Keywords: Sericulture, mulberry, silkworm, victory-1, varieties

Introduction
The production of raw silk through the rearing of silkworms is called sericulture. Sericulture in rural areas generates employment, earns better returns, and has a regular and stable income at fixed intervals. The nature of the occupation is eco-friendly and women friendly. Further, the utilization and export of silk and silk goods at domestic and international levels contribute to the economy. The silk industry is one of the significant employment-generating sectors of the country; the foreign exchange generated through exports is 211 million US dollars (Minister of Textiles, 2022). India ranks second in the production of raw silk. In India, five different silkworms are generally reared commercially, of which the mulberry silkworm is the most important. The other non-mulberry silkworms include tropical tsar, temperate tsar, ere, and mega silkworms.

Mulberry silk accounts for 75% of all silk production in India (Kaviraj *et al*., 2021) [13]. Mulberry (*Morus* L.) is a perennial tree that belongs to the family Morceau, which is fed to the silkworm *Bombyx mori* (mulberry silkworm) (Dhanyalakshmi & Nataraja, 2018) [9]. It originated in the foothills of the Himalayas (Koidzumi, 1917) [15]. The plant's economic part is its leaves, which are used as feed for silkworms. The fruits of the mulberry tree are edible and a good source of vitamin C and iron, which are known to reduce blood sugar and cholesterol levels and improve digestion (Khalifa *et al*., 2018) [14]. Mulberry leaf extract also possesses anti-diabetic, anti-tumour, anti-oxidative, anti-inflammatory, and anti-microbial properties (Ben Bakrim *et al*., 2018) [4]. The remnants of the mulberry plant can be used as fodder, fuel, and fertilizer.

It is well known that the mulberry silkworm is the most reared and feeds on mulberry leaves. Mulberry is a perennial tree cultivated in paired or single rows to obtain a higher leaf yield. About 15,000 to 25000 Kg of mulberry leaf per year from one acre can be harvested depending upon varieties and method of cultivation. The amount of leaf required for rearing 100 DFLs of the popular crossbreed PM X NB 4 D2 is 1000 kg. So, it is crucial to improve the yield of mulberry to increase silk production indirectly (Vijayan *et al*., 2012) [25]. Hence, varieties with higher leaf yield and good adaptability are the need of the hour. Mulberry plants can be propagated through seed and vegetative parts such as stem cuttings, the latter being more commonly used. This nature of the mulberry is better exploited in breeding programs because desired hereditary characteristics can be maintained without change in genotype, and many plants can be reared quickly and economically.

Pest and disease-resistant clones can be directly released as a variety, and varieties adapted to specific localities can be developed.Breeding for improved mulberry with higher nutritive value, resistant to pests and diseases is important because the chemical control of insect pests is not possible as well as the quality of the mulberry leaves has a direct impact on the normal growth and development of the larvae and hence on the quality of the cocoon (Adolkar *et al*., 2007; Legay, 1958) [1,17]. It is crucial to have appropriate knowledge of the genetic architecture of the plant, plant type, and plant breeding methodologies to make genetic improvement in the mulberry. The present review details the economic botany, breeding methods to develop superior varieties and the list of available varieties for cultivation.

Economic botany

In temperate climates, trees are often deciduous. However, trees cultivated in tropical areas may be evergreen. It is a 10–20 m tall, small to medium-sized mulberry tree that grows quickly (33–66 ft). The leaves on young, active stems can grow to be up to 30 cm (12 in) long, are serrated and occasionally lobed, and are placed alternately along the stems. Each fruit, referred to as a multiple, derives from an entire flower cluster. It bears sweet edible berries, which can be eaten when it is ripe. The fruit's colour cannot determine the mulberry species. For instance, white mulberries might yield white, lavender, or black fruit. The best varieties of red mulberry have fruits that are often deep red, almost black. Black mulberry fruits are large, juicy, and delicious, with an excellent combination of sweetness and acidity.

The trees can be dioecious or monoecious; the flowers are held on short, green, pendulous catkins that develop in the axils of the current season's growth and on spurs on older wood. Intense catkin clusters contain minute flowers. Flowers are yellowish green with chromosome number 2n = 28 (Tahara, 1910) [22]. The wind pollinates them, and some cultivars will produce fruit without being pollinated. Female catkins are ovoid and stalked, whereas male catkins are cylindrical and broad. Female spikes catkins are also shorter than male spikes. Clusters of small fruits from mulberries are placed longitudinally along the center axis. The Mulberry ovary is unicellular and has bifid stigmas. Fruits have many drupes enclosed in a fleshy perianth, up to 5 cm long, sub-globose or ovoid. A bifid stigma is present in the unicellular mulberry ovary. This genus has diverse ploidy levels; *Morus* species with 14, 42, 56, 84, 140, and even 308 chromosomes (Diploid to Decupled) with ploidy levels x to 22x (Osawa, 1920; Janaki-Ammal, 1948) [19, 11] are found.

Mulberries are native to temperate Asia and north America but are widely distributed across Europe, southern Africa, South America, and south Asia. It is a deciduous tree of the genus *Morus* of the flowering plant family Morceau. There are about 68 species of the genus *Morus*, the majority of which are found in Asia. The most widely recognized species of the genus *Morus* are the white mulberry (*Morus alba*), black mulberry (*Morus nigra*), and red mulberry (*Morus rubra*) (Jan *et al*., 2021) [10]. White mulberry (*Morus alba*) has a wide distribution range in Asia, Europe, Africa, and the Americas. The origins of most cultivated mulberry varieties are believed to be in the China/Japan area and the Himalayan foothills. It is one of the main species fed to silkworms. It is a short-lived, fast-growing, small to medium-sized tree that reaches heights of 10 to 20 meters.

The leaves are typically smooth, glossy, and dark green, though they can sometimes be yellowish green. The weeping mulberry (*M. alba* 'Pendula') is often used as a lawn tree. Black Mulberry is the most common species; it is native to west Asia. Before the Roman Empire, it was introduced to Europe, where it has since been grown for its fruits. It is primarily grown for its enormous, juicy, purple-black fruits, which are more flavorful than red mulberries. Red Mulberry (*Morus rubra*) is the largest genus native to eastern North America. It is a medium-sized deciduous tree that grows up to 21 meters in height. The leaves are alternate and more extensive than white mulberry leaves. It bears dark purple edible fruits and leaves that might be two, three, or unlobed. The optimum time to plant white mulberry seeds is right after fruiting. Germination rates can be raised by cold stratification for four to sixteen weeks. Layering is also said to be effective.

Objectives of mulberry breeding

The primary breeding objectives in India are high leaf yield per unit area, good leaf quality for successful silkworm rearing, resistance to one or many local adverse conditions, i.e., alkalinity, drought, disease, early sprouting and late hardening of leaves, adaptability to a wide range of soil and climate, and good rooting ability.

Breeding Strategies in Mulberry

Origin, distribution and genetic resources of mulberry

A wide diversity of germplasm is essential for any breeding program. Mulberry basically originated in the Himalayan foothills but distributed to continents like Asia, Europe, North and South America, and Africa (Yokoyama, 1962; Machii *et al*., 1999) [26, 18] and presently, mulberry is under cultivation in almost all Asian countries, including India (Vijayan *et al*., 2011) [20]. Taxonomical studies reveal that mulberry belongs to the genus *Morus* L. And more than 68 species have been recorded in this genus (Vijayan, 2010) [23].

In China, India, Japan, Korea, and Vietnam, there are several germplasm accessions available. More than 1120 germplasm accessions are reported from India (www.silkgermplasm.com). The genetic resources of mulberry are conserved in India through four different techniques: DNA banks, in vitro conservation, ex-situ conservation, and in situ conservation. The genetic divergence of 25 mulberry (*Morus* spp.) genotypes from various agroclimatic environments in India was examined using 14 morphometric characteristics. (Banerjee *et al*., 2007) [3].

Conventional breeding- Selection and Hybridization

A highly precise process is followed by the traditional breeding method that has been utilized to improve the genetics of mulberries. (Vijayan, 2010) [23]. Prior to parental selection, morphological, biochemical, and physiological traits such as stem cuttings' capacity to root, leaf yield, leaf moisture, protein and sugar contents, photosynthetic efficiency, physiological water usage efficiency, and others are used to characterize germplasm accessions. Parents with the required features are chosen by controlled hybridization, and statistical analysis is carried out. For harvesting of seeds for breeding, ripe fruits from both controlled and natural hybridization of chosen mother plants are gathered. In progeny row trials (PRT), seedlings cultivated in nurseries are transplanted to the field for first screening based on chosen features like growth, branching, leaf texture, and disease

susceptibility. Traditional breeding techniques primarily rely on creating F1 hybrids because nearly all mulberry accessions are highly heterozygous and have a long gestation time (Das, 1984). The progeny row trial identifies hybrids with desired features, further assessed in the primary yield trial (PYT) for important agronomic, biochemical, and silkworm-feeding properties. The top 5-10% hybrids from the PYT are chosen for detailed evaluation in the final yield experiment (FYT), which uses 3-5 replications and 25-49 plants per replication.
Further, the plants' leaf yield, leaf quality, adaptation, susceptibility to pests and diseases, rooting ability, responsiveness to agronomic methods, and silkworm-feeding attributes are thoroughly evaluated. The best hybrid is selected and multiplied vegetatively for additional testing in other locations (MLT). In most cases, regional multi-location studies employ 8–9 hybrids. The All India Coordinated Experiments on Mulberry (AICEM) tests hybrids from all across India under various agro-climatic conditions for at least four years. The hybrids that consistently perform well across all seasons, locations and years are chosen and tested further. The AICEM's top performers are made available for commercial usage. This has led to developing and releasing several high-yielding mulberry cultivars for use in India's commercial agriculture. (Saratchandra *et al*., 2011) [20].

Biotechnological tools

Molecular markers and their application in mulberry breeding

There are several native mulberry species in India, just a few of which are commonly grown. Using inter-simple sequence repeat primers, the genetic distance of eleven mulberry cultivars from six different Indian states encompassing a large geographic range was examined (Vijayan, and Chatterjee, 2003) [24]. Random Amplified Polymorphic DNA (RAPD) and Directed Amplification of Minisatellite DNA (DAMD) technologies based on Polymerase Chain Reaction are used for the molecular characterization of varieties. These have been used to determine variation among nine varieties of *Morus* spp. (Bhattacharya and Ranade, 2001) [7]. Jiao *et al*. (2020) [12] reported a high-quality, chromosome-level domesticated mulberry (*Morus alba*) genome. *M. alba* is a diploid with 28 chromosomes, as determined by karyotype and genomic investigations (2n = 2x = 28). Domesticated mulberry was divided into three regional groupings based on a population genomic study using resequencing of 134 mulberry accessions: Taiho Basin of Southeast China (Hu mulberry), Northern and South-western China, and Japan. Several abiotic stress conditions, such as drought, salt, heat, and cold stress, negatively impact the productivity and quality of mulberry leaves. A trustworthy and popular technique for locating genes and molecular mechanisms in many plant species that are susceptible to abiotic stress is quantitative real-time PCR (qPCR). For qRT-PCR studies that normalize gene expression, choosing appropriate reference genes is crucial. Eight prospective reference genes in mulberry have been chosen to study the analysis of the stability of their expression under various abiotic stress conditions, including drought, salt, heat, and cold stresses (Shukla *et al*., 2019) [21].

Genetic engineering

Recent genetic engineering developments have accelerated the mulberry breeding program. Using *Agrobacterium tumefaciens* and particle bombardment, effective protocols have been created for direct plant regeneration from explants and the insertion of desired genes into the plant genome (Bhatnagar *et al*., 2002, 2003) [5]. There have generated transgenic mulberry plants with several desirable genes (Lal *et al*., 2008) [16]. The transgenic plant overexpressing HVA1, a group-3 LEA protein identified and described from barley, demonstrated enhanced cell membrane stability, higher relative water consumption efficiency, and growth under salt stress (200 mM NaCl) in mulberry. (Lal *et al*., 2008) [16]. This transgenic mulberry plant performed significantly better than the non-transgenic plant when exposed to salinity (200 mM NaCl) and drought (2% PEG, MW 6000) induced stressors, according to physiological, biochemical, and molecular analyses. Under salinity and water stress, transgenic plants demonstrated improved cell membrane stability, photosynthetic productivity, reduced photo-oxidative damage, and high relative water content.

Improved mulberry varieties

The details of improved varieties are given in table 1 and depicted in figure 1.

Table 1: Detailed description of mulberry varieties

Varieties	Characteristics
Victory1 (V1) Selection from a hybrid of S-30 and Ber	1) Erect branching habit, Thick, Succulent, smooth, glossy, ovate, and truncate base. 2) Good rooting ability, superior in yield.
S36	1) Short internodes, semi-erect habit, unlobed, glossy, pale green with a smooth surface. 2) High moisture and high nutrient quality of leaves. 3) suitable for chawki rearing
S 13 Selection from hybrid Kanva-2	1) Short internodes, high branching habit, leaves are thick, unlobed, pale green with a smooth surface. 2) Recommended for rainfed areas and can withstand water scares conditions.
S 34 A hybrid between S-30 and Ber	1) Leaves are medium to large in size, unlobed, and suitable under rainfed conditions. 2) Fast-growing, Deep root system, resistant to water stress conditions and good water retention capacity.
Sahana A hybrid between K-2 and Kosan	1) Short internodes, medium branching, fast-growing, large, thick, glossy, and dark green leaves. 2) Suitable for intercropping with coconut, it performs under limited shade.
Resource Constraint (RC-1) A hybrid between Punjab local and Kosan	1) Short internodes, medium branching habit, slightly spreading habit, leaves are thick, glossy and dark green. 2) Grow well under reduced irrigation and fertilizer condition.
Resource Constraint (RC-2) A hybrid between Punjab local and Kosan	1) Short internodes, fast-growing, slightly spreading, leaves are thick, unlobed, dark green, and glossy. 2) Grow well under reduced irrigation and fertilizer conditions.
AR12	1) Short internodes, medium branching, leaves are unlobed, thick, slightly rough surface.

A hybrid between S-41 (4x) and Ber	2) High rooting ability even under alkaline conditions.
G2 Selection from hybrids of *M. multicaulisin* and S-34	1) Leaves are smooth, glossy and dark green in color. 2) Best suitable for chowki gardens.
G4 Selection from hybrids of *M. multicaulisin* and S-34	1) Short internodes, fast-growing, straight branches, and leaves are thick and wavy margins. 2) Suitable for late-age silkworms.
Vishwa	1) Developed by selection 2) Recommended for irrigated conditions
Vishala	1) Developed by selection 2) Six harvests can be taken per year, with good moisture retention capacity
Suvarna 2 & Suvarna 3	1) Developed by selection 2) Good moisture retention capacity, Recommended for irrigated conditions

Source: (Central Sericultural Research & Training Institute, Mysore, Karnataka; Karnataka state sericulture research and development institute)

Fig 1: Improved mulberry varieties.

Conclusion

The mulberry (*Morus* L.), which is important economically, is grown for its leaves, which are used to raise the silkworm *Bombyx mori* L. Mulberry leaves, especially those of the white mulberry, are crucial because they are the only source of food for *Bombyx* spp. Increased mulberry leaf production is required to boost sericulture productivity, and this can be accomplished by creating new varieties that produce more leaves while being more adaptable. It is crucial to have appropriate knowledge of the genetics and genomics of the plant in order to change the mulberry's genetic makeup, which enhances yield. In order to improve the sustainability and profitability of the silk industry in India, concentrated efforts will be made to merge conventional breeding with recent technological advancements.

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