**Applications of Biotechnology in Conservation of Mulberry Genetic Resources**

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**ABSTRACT**

Mulberry is regarded as a unique plant on this earth due to its broader geological distribution across the continents; ability to be cultivated in different forms; multiple uses of leaf foliage and its positive impact in environmental safety approaches such as eco-restoration of degraded lands, bioremediation of polluted sites, conservation of water, prevention of soil erosion and improvement of air quality by carbon sequestering. The modern interest on the cultivation and use of mulberry forage crop for animal feed and medicinal uses has been started due to search for alternative uses of mulberry once the sericulture decayed due to competition from foreign countries. In addition to the major utilization of leaves as silkworm feed, it has many excellent and beneficial bioactive compounds which can be explored for using it as rich feed supplement to animals. This has opened a new vista to think about other uses of mulberry apart from silkworm feed. In India, mulberry biodiversity is maintained through various germplasm banks spread across the country. The germplasm banks maintained under temperate conditions in Kashmir valley do not, however, represent all the available temperate genotypes in the country. With the global environmental change having a marked effect on the cultivation of important agricultural crops, the conservation of mulberry needs more focus. In addition, various abiotic and biotic factors can have sudden drastic effects on elite genotypes. Owing to this, there is a chance of losing some of the important temperate species of mulberry. Hence, alternative approaches for germplasm conservation are needed. The aim of this chapter is to highlight the conservation approaches of mulberry as a potential forage crop for its sustainable use as livestock feed supplement.

**Key Words:** Biotechnological tools,Conservation,Genetic Resources, Mulberry (*Morus* S*pp*.).

**Introduction**

Conservation of mulberry germplasm resources is the prerequisite for exploitation of mulberry genetic resources for their further use. Realizing the importance of impending global climatic changes and threatened sustainability of biodiversity wealth in India at faster rate, systematic survey and exploration for collection of mulberry biodiversity have gained greater momentum in the recent past. Consequently, CSGRC at Hosur has been aptly established in the year 1990 by Central Silk Board (CSB), Ministry of Textiles, and Government of India under prestigious National Sericulture Project. It is the nodal agency for mulberry germplasm management in India and recognised as National Active Germplasm Site (NAGS) for mulberry by National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India under Indian National Plant Genetic Resources System (IN PGRS). CSGRC, Hosur so far conducted 46 survey and exploration trips covering more than 50 districts from Himalayan belt to Andaman and Nicobar Islands covering forest areas, biosphere reserves, national parks, back yards, kitchen gardens, agricultural lands and farmhouses etc. and collected 516 diverse mulberry germplasm resources which mainly belong to 4 Indian species. Out of 4 species of genus *Morus* reported in India, *M. laevigata* is distributed throughout India both under natural and managed habitats and *M. serrata* is confined to northwest India in wild condition. *M. indica* and *M. alba* are mostly available in cultivated forms (Ravindran *et al*. 1977). The sericulture research institutes like Central Sericultural Research and Training Institute (CSRTI) at Berhampur (West Bengal), Mysore (Karnataka) and Pampore (Jammu and Kashmir) have started collection of *Morus species* in Central Himalayas, North-Eastern India, North-Western Himalayas, Kumaon and North Eastern India and Western Ghats and Kerala. All these germplasm resources are collected and introduced in the field gene bank. The purpose of conservation of mulberry accessions in field gene bank is to maintain integrity of the material conserved for prolonged period of time (Ananda Rao, 2002). Several activities are involved in conservation of mulberry such as collection, characterization and evaluation for agronomic traits and to utilize the genetic resources for breeding and other research activities. In India, the Central Sericultural Germplasm Resources Centre (CSGRC) was established during 1990 at Hosur and currently conserves 1254 mulberry accessions (984 indigenous and 270 exotics), which includes wild species, exotics (unadopted) landraces, modern elite cultivars, polyploid mutants, open pollinated hybrids and cross-pollinated hybrids. The performance of each accession conserved is recorded morphologically, agronomically, biochemically, anatomically, physiologically etc.

**Mulberry as Potential Forage Crop**

The foliage of mulberry (Morus spp.), traditionally used for silkworm rearing, is known for its high protein content with good amino acid profile, high digestibility, high mineral content, low fibre content and very good palatability. The high biomass yield of the plant together with its low tannin content make it an attractive fodder resource for ruminants particularly, as a supplement to low quality basal diets. There is evidence that mulberry foliage compares favourably to commercial concentrates, whilst maintaining optimum animal performance through improvements in the rumen functions. Research conducted on mulberry in different countries have focused mostly on the utilisation of the leaves, which like most forages, contain greater nutrient concentrations than the whole plant. The whole plant, when harvested during the early growth stages can be a potentially valuable supplemental feed resource to poor quality diets. Hence, conservation of mulberry for sustainable forage production is very important in context to changing global climatic conditions. The leaves of the mulberry are known for its high protein content (15-28%), good amino acid profile (> 46%), high digestibility (>80%), high mineral content with ash values up to 25%, low fibre content (7.1-8.1%) and excellent palatability. The high biomass yield of the plant, together with its low tannin content, makes it an attractive resource for livestock. Moreover, mulberry is an excellent species to overcome pasture shortage during summer, which is a common issue reported in many Mediterranean countries.

**Mulberry Genetic Resources and their Distribution**

Mulberry, a fast-growing deciduous woody tree of the genus Morus family Moraceae, is grown widely in Asian countries for its leaves to feed the silkworm *Bombyx mori*, which feeds exclusively on mulberry leaves. There are more than 70 countries which produce silk, among which China, India, Vietnam, Uzbekistan, Brazil, Thailand, and Bangladesh are the leaders. Although, the maximum utilization of mulberry is in Asia, this does not mean that mulberry is restricted only to Asian countries. Though, more than 68 species have been widely recognized (Datta, 2000), the taxonomy of mulberry is still a matter of great dispute and intense research due to the high rate of natural hybridization among the species. Hence, a large number of the so-called species are hybrids and their true taxonomic identity is difficult to deduce properly. To complicate things further, different ploidy levels ranging from diploids with 28 chromosomes to docosaploid with 308 chromosomes are very common among most species. Out of these 68 species, only a few, mostly belonging to white mulberry (*Morus alba*), are used for sericulture while a few other species such as red mulberry (*Morus rubra*) and black mulberry (*Morus nigra*) are used for fruits. Considering the great economic value attached with mulberry, several countries have already made extensive efforts to collect and conserve mulberry (Tikader and Vijayan, 2010). In mulberry, a total of 150 morus species were recognized but only 68 species were given more importance, based on their use in silkworm rearing, medicinal value and sweetness of fruit. Each species has its own unique importance such as *M. alba, M. indica, M. latifolia, M. nigra and M. multicaulis* are cultivated for silkworm rearing, while *M. rubra* and *M. nigra* for fruits. The Morus species such as *M. multicaulis, M. alba* and *M. atrpurpuria* are widely distributed in different provinces of south, north and west China (Yong Kang Huo, 2000). *M. bombycis* are distributed and largely cultivated in cold region and *M. latifolia* in warm places of Japan (Machii *et al*., 2000). *M. macroura* Miq. is in the north western part of India, certain parts of south India (Yadav and Pawan Kumar, 1996) and West Bengal. The genotypes of *M. alba* L. are distributed in Punjab, North western part of Himalayas and Western Tibet. The natural and cultivated forms of *M. indica* L. is widely distributed extending from temperate to subtropical Himalayas, Arunachal Pradesh, Kashmir to Sikkim ascending to 2100m ASL. It is also distributed in Uttar Pradesh, Assam, West Bengal, Meghalaya, Karnataka, Tamilnadu and Kerala. The two wild species, viz., *M. serrata* Roxb. is confined mostly to the high-altitude region of North western part of India (Ravindran *et al*., 1997) and M. *laevigata* is distributed throughout India both under natural (Andaman and Nicobar Islands) and managed habitats (Tikader and Thangavelu, 2003). During the last few years many accessions (14) belonging to *M. indica* and *M. alba* were brought from extreme cold regions (Ladakh, Meghalaya, Himachal Pradesh etc.) and maximum number of accessions (75) belongs to *M. indica*, *M. alba* and *M. laevigata* were brought from extreme dry hot regions (Rajasthan, U.P, M.P. and Bihar) and maintained in CSGRC, Hosur (Ananda Rao *et al*., 2005 and 2011). Availability of different Morus species in four major countries (species wise) have been reported and most of the species occur in Asia, especially in China followed by Japan, India and Korea.

**Mulberry Wealth in Jammu and Kashmir**

Jammu and Kashmir is well known for the existence of several mulberry varieties belonging to different species of Morus; such as *M. alba*, *M. indica*, *M. laevigate*, M. *serrata*, *M. nigra*, *M. rubra*, etc. Sericulture in temperate areas of J&K is being practiced utilizing mostly Ghoshoerami mulberry variety belonging to *M. multicaulis*; Chinese white, KNG and Ichinose mulberry varieties belonging to *M. alba*. Along with these some indigenous local varieties such as Brentul Kashmir, Chattatul, Janglitul, Krenantul, Nadigam, Kablitul, Lajward, Hamtul etc. are also in practice to some extent. Natural mulberry is abundant in J&K and is suitable for sericulture. Introduced and cultivated mulberry is also abundantly available. The mulberry resources should be used judiciously to better the sericulture industry in J&K. Wild mulberry available in various regions of J&K like Kargil-Ladakh, Gurez, Kupwara, Rajouri etc., needs prime importance for characterization since, lot of diversity is available in the mulberry wealth found in these regions. As mulberry is found growing in these regions through seed dispersal as such collections from these regions must be highly heterozygous, hardy with noble genes for cold/frost/drought tolerance which needs immediate attention of breeders for evaluation and their further utilization in breeding programs for mulberry crop improvement.

**Conservation strategies of mulberry genetic resources**

India being signatory to both CBD and World Trade Organization (WTO) needs to develop the efficient conservation strategies of its vast seri-biodiversity best suited to India’s national interest. Exploration for collection of mulberry biodiversity and development of efficient conservation strategies has gained momentum in the recent past in India. The conservation of mulberry genetic resources includes their augmentation, safe holding for medium and long-term preservation; protection in natural habitats and it is interlinked to the sustainable utilization of germplasm to justify long-term investments on managements and maintenance of the germplasm (Anand Rao, 2002). Studying the geographical spread, distribution and genetic architecture, arborescent nature and physiological storage behaviour of mulberry, two basic conservation strategies i.e. *ex-situ* and *in-situ* methods composed of various techniques covering entire gamut of genetic diversity have been worked.

***In-situ* conservation**

Plants that are conserved in their original habitat and it allows natural selection, mutation, population structuring etc., and thereby promoting free evolution of the species. It should be protected from all human interference and disturbing activities. *In-situ* conservation promotes the conservation of eco-system and natural habitats and the maintenance and recovery of viable population of species, which can survive, and best perpetuate in their natural microclimate. It also simultaneously permits continued evolutionary development under natural selection pressures, thereby promoting the fitness of the species. In situ conservation demands the establishment of nature or biosphere reserve and national parks to protect the endangered species. The National Committee on Environmental Planning and Coordination (NCEPL) and Man and Biosphere (UNESCO) already identified 14 Biosphere reserves in India and among them Uttarkhand, Nandadevi, Namdapha, Kaziranga, Manas, Nokrek, North Andaman and Great Nicobar are the potential reserves for *in\_situ* conservation of mulberry (Ananda Rao, 2002). Keeping this in view, efforts have been made to collect information on the location of availability of mulberry germplasm with details on “declared protected area network of India” including biosphere reserves, national parks, wild life sanctuaries etc. Mulberry is not fully protected under Indian Forest Act (1972) in many parts of India except in some states of North-East India. Survey map, exact location, landowner with postal address, survey number and its jurisdiction are not available with CSB units or State department. Under the circumstances, a suggestive and advisory role can be contemplated with greater thrust on repeated survey and exploration.

***Ex-situ* conservation**

Since, mulberry is out breeding and highly heterozygous in nature and easily propagated through stem cuttings, the common conservation method are field germplasm banks or preserving vegetative buds in the *in-vitro* conditions. Collection of mulberry germplasm are maintained for evaluation of accession for economic traits and for supply of genetic resources to research institutes or to breeders for breeding programs. It was reported that many populations of mulberry are migrated from its places of origin to faraway places (Tikader *et al*., 2009). The *ex-situ* mulberry germplasm, which contains 1254 mulberry accessions that are maintained at CSGRC, Hosur. *In-vitro* conservation is classified in two categories. The plant material (nodal explants and dormant buds) can be stored for many years in the nutrient media (growth limited) under tissue culture conditions, however sub-culturing has to be done periodically and it all depend on type of the species and its *in-vitro* regeneration capacity. The interspecific hybrid embryos are sometimes very weak, which can be rescued by keeping them in a suitable nutritional media and allowing them to grow. Therefore, *in-vitro* technique can be used to rescue or save the F1 hybrids developed through pre-breeding. Also, *in-vitro* technique can be used for rapid screening of genotypes for resistance to salinity, alkalinity and drought.

**Field gene bank**

Mulberry being perennial outbreeding tree exhibits high degree of heterozygosity. Hence, for conservation of mulberry outside its natural habitat, *ex-situ* field gene bank (clonal repository) has been developed by planting vegetative clones of mulberry accessions for maintaining the genetic integrity of the conserved material. Rooted cuttings (saplings) after six-month establishment in the nursery are transplanted in the field gene bank. Saplings of some exotic accessions and wild *Morus* species with poor rooting rate, are developed through bud grafting to the local scions and then established in the base collection. The plants (four plants/accessions) are maintained as a dwarf tree with spacing of 2.4 ´ 2.4 m between plants with the crown height of 1.5 m following recommended cultural practices and plant protection measures. Pruning is being followed once in a year (June-July) to renew the germplasm with new sprouts. However, the species, which cannot sustain repeated pruning are left unpruned. The entire collections in field gene bank are fenced and protected. Presently, the *ex-situ* field gene bank of CSGRC, Hosur holds 908 mulberry accessions (Indigenous-647 and Exotic-261) from diverse genetic and geographical origin representing 13 *Morus* species collected from 26 countries. CSGRC, Hosur being the nodal agency for mulberry germplasm in India maintain the entire mulberry germplasm available in the country. However, the Sericulture Research Institutes, Universities, State Sericulture Departments are also maintaining the mulberry germplasm for research purpose, which also serves as backup conservation centres. Each accession contains permanent label with unique identification number. National Accession Numbers (Indigenous collections: IC. No: 313662- 314262 and Exotic collections: EC. No: 493758-493928) have been provided for all the mulberry accessions conserved in the ex-situ field gene bank by NBPGR, New Delhi for protection of mulberry genetic resources at global level.

**On-farm participatory conservation**

The advent of high yielding varieties of mulberry like V-1 and S-1635 and their large scale spread in the traditional sericultural zones under monocropping pattern replacing the local landraces and more particularly in the irrigated system which leads to reduced genetic base and increased the genetic vulnerability of the crop. The seri-biodiversity, otherwise greatly threatened because of unlawful habitat destruction, natural calamities, fragmentation of forests and social disruption and this large-scale genetic wipe out disturb the coexistence of sericigenous flora and fauna. Under these circumstances on-farm conservation linked with Farmers Participatory Breeding (FPB) to be given due emphasis for achieving twin goals of sustainable conservation of biodiversity and in turn utilization on-farm biodiversity. In India, rich *Morus* diversity exists under managed habitats *i.e.* in the backyards, kitchen gardens, farmhouses, horticultural gardens, agricultural lands and roadside plantations. These are the first-hand selections of the farmers and tribals for varied utilizations hence; conservation of potentially interesting alleles and development of diversity is promoted. In mulberry the wild species like *Morus laevigata* and *Morus serrata* and other wild species, which do not get attention in the formal sector for cultivation for sericulture purposes. So, these associated valuable species or otherwise utilized for non-sericultural purposes (horticulture and agroforestry) flourish well in the on-farm conservation procedures promoting farmers/tribals livelihood development while conserving *Morus* biodiversity. This particular sector of *Morus* biodiversity lies mainly in the public domain, which needs to be taken care and bring them under definite set of legal frameworks of Indian Forest Act (1972).

**Botanical gardens and national herbarium**

In the context of PGR, the herbarium provides the basic material for detailed monographic and phyto-geographical information of the species. In India there are many herbarium canter’s and some of the main herbarium canter’s like: Botanical Survey of India with its regional centres,

Presidency College, Chennai; Baltter Herbarium, St. Zavier College, Mumbai; St. Josephs College, Tiruchirapalli and National Herbarium, NBPGR, New Delhi maintain *Morus* species herbarium. Botanical Survey of India, Pune preserves the oldest herbarium of genus *Morus* dates back to 1886. Seri-biodiversity Museum of CSGRC, Hosur maintains large number of herbaria and it serves as National Herbarium Centre for *Morus* species in India. In India there are about 33 botanical gardens and the some of them like: Indian Botanical Garden, Howrah; Lloyd Botanical Garden, Darjeeling; National Botanical Garden, Lucknow maintain mulberry arboreta.

**Conservation using Biotechnological Approaches**

The plant material preferably winter dormant buds can be stored in liquid nitrogen (-196°C) for long period. It requires less space, labor, cost effective and disease free. cryopreservation is a step wise process, wherein the buds will be dehydrated in silica gel and subjected to slow freezing -5 to -30° C and finally it is transferred to Cryo-Can containing liquid nitrogen (-196C) (Anand Rao *et al*., 2009). Cryopreservation procedures have been standardised for about 100 different plant species cultured in various ways including cell suspension, callus, apices and zygotic and somatic embryos. The advanced techniques of cryopreservation comprise many stages ranging from tissue culture, pre-growth, cryoprotection by plant vitrification solution, slow and fast freezing, thawing recovery and invitro regeneration (Tikader *et al*., 2009). The National gene bank at National Bureau of Plant Genetic Resources (NBPGR) in New Delhi, the nodal agency for plant germplasm conservation, has a state-of-the-art facility presently and conserves a total of 1,783 accessions (invitro conservation) and 8,000 accession (cryo-banking with temperatures between -160 C and -196C) of diverse plant germplasm including 338 mulberry accessions collected from diverse geographical region (Ananda Rao *et al*., 2009).

Conservation of mulberry germplasm in the field gene bank is simple and technically less demanding. However, maintaining a large collection is costly, requiring huge resources. Besides, it is risky since it is exposed to different biotic and abiotic stresses. Cryopreservation technology forms an alternative to ex situ conservation strategy for crop like mulberry, which is vegetatively propagated utilizing liquid nitrogen (LN) at ultra-low temperature of -150 °C (vapour phase). Cryopreservation method ensures the genetic stability of the germplasm and provides an alternate cost-effective non-dependence on electricity long-term strategy for conservation of mulberry germplasm. Keeping this in view, establishment of in vitro and cryopreservation laboratory facilities at CSGRC, Hosur is planned in collaboration with NBPGR, New Delhi. Efforts have been made to standardize the efficient techniques on cryopreservation of winter buds, embryonic axes, pollen of mulberry accessions (Niino *et al*. 1992) comprising different species, landraces, local cultivars, wild and polyploid accessions in liquid nitrogen (-196°C). *In-vitro* conservation techniques involve conservation of active collections under active growth stage and base collection germplasm under suspended growth stage using growth retardants. Even though lot of work on mulberry regeneration with or without callus formation in in vitro has been reported. Very little work has been carried out in in vitro conservation of mulberry genetic resources. Cryopreservation, possibility for the first time was demonstrated using mulberry twig by Sakai (1960). Since then, considerable work on cryopreservation of mulberry has been undertaken especially in Japan.

In India, the application of *in-vitro* technique for mulberry conservation has been recently attempted. In vitro technique has been attempted in mulberry mainly for propagation of popular mulberry varieties and poor rooting materials, evolution of some clonal variants and elite materials and screening of genotypes for tolerance to salt and somatic stresses. (Tewary *et al*. 2000). Bapat *et al*. (1987) developed method for propagation of *Morus indica* L. by culturing encapsulated shoot buds. Conservation of mulberry germplasm accessions through cryopreservation technique has been recently attempted in India.

**Future Prospectus**

Conservation of forage genetic resources is rapidly becoming increasingly important, especially for sustainable agriculture to feed the needs of ever-increasing livestock populations. Land would be a limiting factor in future and through utilization of PGR, a continued increase in agricultural productivity would be possible. Keeping this in view, and also the act that environmental changes in future may require genes for adaptability, the conservation of genetic reservoir of plants need to be given high priority in policy planning**.** With reverence to mulberry, traditional conservation strategies like *in-situ* and *ex-situ* conservation should be complemented with modern techniques like cryopreservation and DNA banking as *ex-situ* conservation of mulberry entails huge investment in the form of labour and space. Well-developed protocols are now available and a number of mulberry accessions, but the genotypic effect on the survival percentage of the warrant’s further effort to for fine tuning the protocol to accommodate most of the species. DNA banks for biodiversity and plant genetic resource evaluation and conservation are important, although no such bank exists for mulberry. Thus, concerted efforts are to be made urgently to integrate all these techniques to conserve precious genetic resources of this very important tree crop of Asia. The mulberry resources in J&K should be used judiciously for forage production and its improvement in terms of quality and quantity. Natural mulberry available in various regions of J&K like Kargil, Ladakh, Gurez, Kupwara etc., needs prime importance for characterization since, lot of diversity is available in the mulberry wealth found in these regions. As mulberry is found growing in these regions through seed dispersal as such collections from these regions must be highly heterozygous, hardy with noble genes for cold/frost/drought tolerance which needs immediate attention of breeders for evaluation and their further utilization in mulberry breeding programs with respect to forage crop improvement for its sustainable utilization.

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