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Assessment of Biodiversity and Strategies for Conservation of Genetic Resources in Mulberry (*Morus* spp.)

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ABSTRACT

Mulberry (*Morus* spp.), an important tree cultivated in most Asian countries, is a vital component of the sericulture industry as the silk-producing insect *Bombyx mori* L. feeds only on its leaves. Mulberry is believed to have originated in the northern hemisphere and spread to the tropics of southern hemisphere. More than 150 species of mulberry have been reported, though their identities are still a matter of great debate. Since most of the cultivating varieties were developed from *M. alba*, other species were mostly neglected; as a result the gene pool of the cultivated mulberry has drastically reduced. As it is essential to protect all whole genetic resources comprising both cultivated and wild relatives, efforts have recently been initiated to assess the biodiversity of this crop in different countries, especially in south Asian countries. In this review, the genetic resources of mulberry available in different countries and the measures being taken for its conservation are discussed. Biodiversity analyses with both morphological and modern biotechnological methods along with the conservation strategies such as *in-situ*, *ex-situ*, field gene bank, on-farm participatory and cryopreservation are reviewed and discussed.

Keywords: biodiversity, biotechnology, *Bombyx mori*, cryopreservation

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INTRODUCTION

Genetic resources comprising useful living organisms fulfill our basic needs of food, shelter and clothing, provide valuable medicine, spices and materials for industrial products and help in maintaining and ameliorating the environment. The wide distribution of mulberry indicates its adaptability and genetic plasticity to various environments.

About 15,000 plant species occur in India of which around 160 species are of economic importance (Srivastava *et al*. 2003; Tikader and Dandin 2006). Over 300 wild relatives of crop plants are also reported from Indian subcontinent (Srivastava *et al*. 2003). Looking into the status of endangered, rare and threatened species particularly with reference to economic plants carries importance. The species should be collected and conserved before they finally disappear. India is recognized as one of the 12 mega-biodiversity centers of the world and covers 11.90% of the world's flora (Srivastava *et al*. 2003). In India, mulberry is not only cultivated for sericulture but for fruit, timber, fuel and fodder. Mulberry, a perennial deciduous plant is reported to have originated in China, the primary center of the plant origin (Vavilov 1926) apart from the Indian species namely *M. indica*, *M. alba*, *M.* serrata and *M. laevigata*, which are considered as indigenous (Hooker 1885; Brandis 1906). Many mulberry species were introduced in India from other countries i.e., *M. multicaulis*, *M. bombycis*, *M. nigra*, *M. alba*, *M. rotundiloba*, *M. cathayana* and *M. tiliaefolia* are the prominent ones (Tikader and Kamble 2007). Thus, the conservation of mulberry genetic resources has become very much essential to meet the desired objectives of long-term management and utilization.

Mulberry, which is mainly used as exclusive silkworm feed for silk production has been introduced in different countries of the world (Tazima 1978). The mulberry spread from the temperate areas of North West and Central Asia, Europe and North America through the tropics of Asia, Africa and Latin America to the southern hemisphere (South Africa and South America) (Sánchez 2000). Mulberry varieties grow in various environments from sea level to altitudes of 4000 m and domestication of mulberry must have started several thousand of years ago been for silkworm requirement (FAO 1990). Mulberry also grows from humid tropics to semi-arid lands like in the near East with 250 mm of annual rainfall and South West of the USA (Tipton 1994).

Mulberry is supposed to be native of Indo-Chinese border area and distributed in the lower slopes of Sub-Himalayan region up to the elevation of 3300 m. Brandis (1906) and Hooker (1885) have reported 4 species viz., *Morus indica*, *M. alba*, *M. laevigata* and *M. serrata*, which

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Invited Review

Table 1 Mulberry utilization in different Asian countries.

Country	Utilization				Research		
	Sericulture	**Fruit**	**Forage**	**Other***	**Agronomy**	**Breeding selection**	**Animal nutrition**
Afghanistan	--	--	#	--	--	--	--
China	#	--	--	# 2	#	#	--
India	#	--	#	# 3	#	#	#
Indonesia	#	--	--	--	--	--	--
Japan	#	#	#	# 2	#	#	#
Korea	#	--	#	--	#	#	--
Kyrgystan	#	#	--	--	#	--	--
Malaysia	#	--	--	--	#	--	--
Pakistan	#	--	#	--	#	--	--
Philippines	#	--	--	--	#	--	--
Syria	--	#	--	--	--	--	--
Tajikistan	#	--	--	--	#	--	--
Turkey	--	#	--	--	--	--	--
Turkmenistan	#	#	--	--	#	--	--
Vietnam	#	--	#	--	#	#	--
Uzbekistan	#	--	--	--	#	--	--

= Involved in the respective field, Other* = # 2 (Medicinal and infusion), # 3 (Handicrafts and cabinet works)
Source: Sánchez 2000

Table 2 Mulberry utilization in different African countries.

Country	Utilization				Research		
	Sericulture	**Fruit**	**Forage**	**Other***	**Agronomy**	**Breeding Selection**	**Animal nutrition**
Egypt	#	#	--	--	#	--	--
Ethiopia	#	--	--	--	#	--	--
Kenya	#	#	--	--	#	--	--
Madagascar	#	--	--	--	#	--	#
Tanzania	--	#	--	--	--	--	#
Tunisia	#	#	--	--	#	--	--

= Involved in the respective field
Source: Sánchez 2000

Table 3 Mulberry utilization in different American countries.

Country	Utilization				Research		
	Sericulture	**Fruit**	**Forage**	**Other***	**Agronomy**	**Breeding selection**	**Animal nutrition**
Argentina	--	--	--	# 1	--	--	--
Bolivia	--	--	--	# 1	--	--	--
Brazil	#	--	#	--	#	#	#
Colombia	#	--	#	--	#	--	--
Costa Rica	--	--	#	--	#	--	#
Cuba	--	--	#	--	--	--	--
El Salvador	--	--	#	--	--	--	#
Dominican R	--	--	#	--	--	--	--
Guatemala	--	--	#	--	--	--	#
Honduras	--	--	#	--	--	--	--
Mexico	#	#	#	# 1	#	--	#
Panama	--	--	#	--	--	--	--
Peru	--	--	--	# 1	--	--	--
Saint Vincent	--	--	#	--	--	--	--
USA	--	--	--	# 1	--	--	#

= Involved in the respective field, Other* = # 1 (Landscape and gardening)
Source: Sánchez 2000

occur in India. Most of the Indian varieties belong to *M. indica* and *M. laevigata*, which are available in wild and cultivated forms whereas *M. serrata* is confined to North Western Himalayan region as wild isolated trees. *M. alba* is available in wild and cultivated forms throughout India. In India, at present 1120 mulberry accessions are maintained in an *ex-situ* field gene bank at Central Sericultural Germplasm Resources Centre (CSGRC), Hosur and evaluation, characterization, information on useful traits is documented (Thangavelu *et al*. 2000; Tikader *et al*. 2006). Due to changes in global silk production and marketing, the utilization of mulberry is being focused on diversified fields (Sánchez 2000). The different countries of the world are already involved for multipurpose use on mulberry (Sánchez 2000) (**Tables 1-4**). In India, mulberry is cultivated for sericulture and phase wise multipurpose uses are also tried for maximum exploitation of mulberry genetic resources (Tikader and Dandin 2006).

Plant genetic resources comprise diversity of genetic material contained in traditional varieties, land races, modern cultivars, weedy relatives and wild species. These diverse genetic materials provide farmers and plant breeders with the options to develop, through selection (Tikader and Kamble 2007) and breeding, new and more productive varieties that are resistant to virulent pests and diseases and have better adaptability to the changing environments. It is important to note, in this context, that the development of modern high yielding varieties would not have been possible if the earlier landraces were not available to plant breeders. With this realization, it is possible to understand the importance of conserving these same species and strains for use in future years of plant breeding. Since the white mulberry (*M. alba*) dominates the sericulture, farmers and researchers have mostly been concentrating on the development of new mulberry varieties using genotypes from *M. alba* and its close relative diploid, neglecting other wild species (Tikader and Kamble 2008). This monogenic research and farming trend, in turn, has resulted in conside-

Table 4 Mulberry utilization in different European countries.

Country	Utilization				Research		
	Sericulture	Fruit	Forage	Other*	Agronomy	Breeding selection	Animal nutrition
Bulgaria	--	--	--	--	#	--	--
France	--	--	--	# 1	#	--	#
Greece	--	--	--	# 1		--	--
Italy	--	--	--	# 1	#	--	#
Poland	--	--	--		#	--	--
Spain	--	--	--	# 2		--	--

= Involved in the respective field, Other* = # 1 (land scape and gardening), #2 (medicinal and infusion)
Source: Sánchez 2000

rable loss of genetic diversity among the diploid mulberry species as indicated by Tikader and Dandin (2007). Further, due to the increased urbanization and industrialization, large areas of the natural habitat of mulberry have been destroyed and many populations have been reduced below the size needed for their continued survival without management (Tikader and Dandin 2006). Hence, the wild mulberry species like *M. serrata*, *M. laevigata* and many other species with higher ploidy are to be conserved urgently to prevent further genetic erosion. Likewise, the red mulberry is also considered as the most endangered tree species in Canada. Consequently, much emphasis has now been placed for its proper conservation in USA and Canada. The importance of biodiversity conservation provides multiple benefits as follows:

- Increase in productivity and food security
- Reduce pressure of agriculture on fragile areas, forests and endangered species
- Build stability, robustness and sustainability of farming system
- Reduce/spread risks to individuals; communities and nation
- Help maximum effective use of the resources and environment
- Reduce dependency on external inputs
- Increase nutritional values and provide sources of medicine and vitamin.

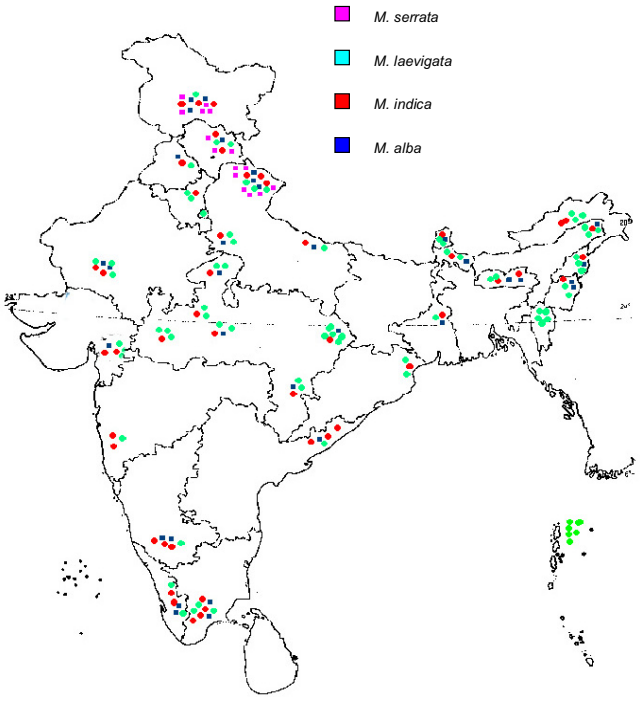

Fig. 1 Distribution of *Morus* species in India.

Mapping mulberry biodiversity

Biological diversity is being viewed as the potential resource capital of a state, region or country that possesses it (Ganeshaiah and Uma Shaankar 2003). Achieving this requires a clear understanding of what resource we have and where they exist. Such information based on the biological resources and their geographic distribution, besides helping the states in deciding a need based allocation of conservation efforts, facilities ascribing and claiming, appropriate rights over these resources. In this regard a comprehensive database to be set up on the biological resources and their geographic distribution for the entire country. The work on mulberry mapping is under progress with the help of remote sensing technology and global information system.

Understanding the spatial distribution of biological diversity is the foremost pre-requisite for meaningful conservation of any natural ecosystem. The construction of biodiversity maps reflecting the spatial distribution would serve several purposes such as locating hotspots of diversity, assigning conservation values of different areas, information on the structure and dynamics of the vegetation and eventually formulating strategies for sustainable utilization of the resources. However, such map of the area rarely available and hence, there is a critical need to map the biological resources at local, regional and national level throughout the country and abroad (Ganeshaiah and Uma Shaankar 1998).

Development of habitat conservation map is important to know the macro and microhabitats, sensitiveness of the habitats, ecological significance and the biological value of the habitats. The map needs to be developed hierarchically for micro-macro habitats, forest zones and ecosystems in that order. The target species to be covered may be categorized. The broad areas covered for each species are taxonomy, systematic, distribution, vernacular names, uses, produces and processes, economically important bio-chemical components, ethno botany, phenology, reproductive biology, economic status in India also to be featured in database. In addition distribution map for all plant species using Global Information System tools can be developed which will depicts the probable recovery areas and density distribution of the species. The information should be accessible on the internet with suitable security features for protection of sensitive information from the unauthorized users. Any plant genetic resources are required to document and assess biological diversity available. The mapping of biological resources is needed to make future programme to conserve or restoration. The genetic diversity of *Morus* species is well documented in Indian context along with distribution of different species (**Fig. 1**). The mulberry genetic diversity and its distribution documented in countries like Japan and China who are the major stakeholders. More information is provided in the section Global distribution of mulberry. The plant materials are also categorized based on their availability, performance, habitat and distribution. There are various techniques and tools for managing biological diversity i.e., analysis using GIS technique, mapping of forests based on biological diversity and tools for measuring biological diversity (Ganeshaiah and Uma Shaankar 2003). The study was carried out for general biodiversity including mulberry wherever it is available and the same has referred.

Threats to mulberry biodiversity

Plant diversity is the fundamental for agricultural production, maintains ecological niche, produces humus on soil and used by other microorganisms. However, in the present day scenario, plant genetic resources associated with livelihood are being rapidly eroded and disappearing throughout the world due to various developmental changes. The present changes jeopardize productivity, threaten food security and result in high losses. Moreover, the loss of biodiversity in natural habitats occurs with expansion of agricultural production into new frontiers. In India, because of habitat fragmentation, destruction, over exploitation, land use for agriculture and other purposes, a large number of species have already been lost and mulberry is one of them (Tikader and Thangavelu 2002a; Tikader and Dandin 2006). Seventeen species have been lost as reported by The International Union for Conservation of Nature and Natural Resources (IUCN). The other reason of biodiversity loss is due to genetic erosion (monoculture, over exploitation and addition of improved variety), genetic vulnerability (diseases and pests) and genetic wipe out (Vijayan *et al.* 2006). Natural calamities such as landslides in floods, cyclones have wiped out rare species of plants and animals in natural and protected habitats (Tikader and Kamble 2008), which suggests for complementary comprehensive conservation strategy (CCCS) for both *in-situ* and *ex-situ* conservation of mulberry genetic resources.

Global information on mulberry

The number of genotypes classified in accordance with the places of origin is recorded for different countries (Koyama *et al*. 2002). These are Japan (1375), India (1120), China (2600), South Korea (208), France (70) Argentina (2), Colombia (4), Mexico (5), Peru (2), USA (23), Iran (2) and Italy (50) and of unknown origin (27). The available mulberry in different countries is shown in **Table 5**.

The important countries, which are engaged in sericultural activities, are described below and the species status of important countries is presented in **Table 6** (Koyama *et al*. 2002; Tzenov 2002; Pan 2003; Tikader and Dandin 2006).

India: In India, large-scale *ex-situ* mulberry germplasm are maintained in CSGRC, Hosur, which holds more than 1120 (Indigenous-856 and Exotic-264) mulberry accessions. The species available in India are presented in **Table 7** (Chandrasekhar *et al*. 2009; Saraswat *et al*. 2009). Besides this, small-scale germplasm are available in Central Sericultural Research and Training Institutes in Mysore (Karnataka), Berhampore (West Bengal) and Pampore (Jammu and Kashmir). The CSGRC, Hosur has been acting as the nodal agency for mulberry germplasm in India and is maintaining the entire mulberry germplasm available in the country (Tikader and Dandin 2006).

China: China, being the major silk producing country in the world, has more than 2600 germplasm accessions comprising of 15 species. The accessions are maintained in various provinces like Zhejiang, Jiangsu, Guangdong, Guangxi, Shandong, Sichuan, Anhui, Hubei, Hunan, Hebei, Shanxi, Shuanxi and Xinjiang etc. The national mulberry gene bank of the Sericultural Research Institute, Chinese Academy of Agricultural Sciences (CAAS), Zhenjiang, Jiangsu province, China ranks No.1 in the world on mulberry germplasm conservation (Pan 2000). There are four ways for collecting the mulberry germplasm resources in China. They are (1) collection of local indigenous varieties, i.e., most of them are selected or bred through natural or artificial selections giving emphasis to their suitability to local condition such

Table 5 Mulberry germplasm accessions available in different countries.

Country	Mulberry accession
Argentina	2
Brazil	---
Bulgaria	140
China	2600
Colombia	4
France	70
India	1120
Indonesia	5
Italy	50
Japan	1375
Mexico	5
Peru	2
South Korea	208
Taiwan	5
USA	23

Reproduced from Tikader and Dandin 2006.

Table 6 Species wise distribution of mulberry germplasm in Japan, China and Korea.

Japan		China		Korea	
Species	**Accessions**	**Species**	**Accessions**	**Species**	**Accessions**
M. bombycis Koidz.	583	*M. bombycis* Koidz.	22	*M. bombycis* Koidz.	97
M. latifolia Poir.	349	*M. multicaulis* Perr.	750	*M. latifolia* Poir.	128
M. alba L.	259	*M. alba* L.	762	*M. alba* L.	105
M. acidosa Griff.	44	*M. wittorium* Hand-Mazz.	8	*M. acidosa* Griff.	1
M. indica L.	30	*M. mizuho* Hotta	17	*M. indica* L.	5
M. rotundiloba Koidz.	24	*M. rotundiloba* Koidz.	4	*M. rotundiloba* Koidz.	--
M. kagayamae Koidz.	23	*M. australis* Poir.	37	*M. kagayamae* Koidz.	1
M. notabilis C.K.Schn.	14	*M. mongolica* Schneider	55	*M. mongolica* Schneider	--
M. bionensis Koidz.	11	*M. bionensis* Koidz.	--	*M. bionensis* Koidz.	--
M. nigriformis Koidz.	3	*M. nigriformis* Koidz.	--	*M. nigriformis* Koidz.	--
M. atropurpurea Roxb.	3	*M. atropurpurea* Roxb.	120	*M. atropurpurea* Roxb.	--
M. serrata Roxb.	3	*M. serrata* Roxb.	--	*M. serrata* Roxb.	--
M. laevigata Wall.	3	*M. laevigata* Wall.	19	*M. laevigata* Wall.	1
M. nigra L.	2	*M. nigra* L.	1	*M. nigra* l.	3
M. formosensis Hotta.	2	*M. formosensis* Hotta.	--	*M. formosensis* Hotta.	--
M. rubra L.	1	*M. rubra* L.	--	*M. rubra* L.	--
M. mesozygia Stapf.	1	*M. mesozygia* Stapf.	--	*M. mesozygia* Stapf.	--
M. celtifolia Kunth.	1	*M. celtifolia* Kunth.	--	*M. celtifolia* Kunth.	--
M. cathayana Hemsl.	1	*M. cathayana* Hemsl.	65	*M. cathayana* Hemsl.	--
M. tiliaefolia Makino	1	*M. tiliaefolia* Makino	--	*M. tiliaefolia* Makino	14
M. microphylla Bickl.	1	*M. microphylla* Bickl.	--	*M. microphylla* Bickl.	--
M. macroura Miq.	1	*M. macroura* Miq.	--	*M. macroura* Miq.	--
Morus spp. (*Unknown)*	15			*Morus* spp. (Unknown)	259
Total	1375		1860		614

Sources: Koyama *et al*. 2002; Tzenov 2002; Pan 2003; Tikader and Dandin 2006

Table 7 Species-wise collection of genus *Morus* in India.

Species	Indigenous accessions	Exotic accessions	Total
M. indica	350	---	350
M. alba	58	35	93
M. laevigata	32	---	32
M. serrata	18	---	18
M. sinensis	2	---	2
M. latifolia	4	15	19
M. australis	---	2	2
M. rubra	---	1	1
M. nigra	---	2	2
M. cathayana	---	1	1
M. bombycis	---	15	15
M. multicaulis	---	15	15
M. pendulata	---	1	1
M. rotundiloba	---	2	2
M. moretti seringe	---	3	3
M. lhou seringe	---	2	2
M. tiliaefolia	---	1	1
Unidentified	---	100	100
Total	464	195	654

Sources: Tikader and Rao 2002a; Tikader and Dandin 2006

as weather, soil and high resistance to pest and disease (Pan 2003). The percentage of the local varieties accounts for about 65% of total resources in China. (2) Introduced varieties, viz., mainly from Japan, the former USSR, Korea, India, Thailand, Vietnam etc. (3) Evolved varieties, which constitutes those developed through line selection, traditional breeding, mutation breeding and biotechnology. These varieties have a wider acceptability, thus they occupy more than 30% of the mulberry genetic resources in China currently. (4) The wild types have less economic value but have many special characteristics such as resistance to pest, diseases and abiotic stresses.

Japan: Japan has a long tradition of sericulture. A number of mulberry germplasm accessions have been collected and maintained in various research institutes. The National Institute of Sericultural and Entomological Science (NISES) maintain 1300 germplasm accessions (Machii *et al*. 1999; Koyama *et al*. 2002). The National Institute of Agrobiological Sciences (NIAS), which is now responsible for maintaining and conserving the mulberry genetic resources, presently has 1502 genotypes in the Gene bank (Okino *et al*. 2004). These genotypes are preserved on the campus field and greenhouse and are being used to dissect the morphological and agricultural traits to improve mulberry cultivars for beneficial uses. These genetic resources are classified into five groups based on their origin such as wild type, domestic type, bred type, popular cultivar and the source unknown type. These genetic resources are also classified into working collection, base collection and active collection. Working collection is utilized for field examinations and investigations while the base collections are preserved for long-term investigations. Genetic resources that are judged to distribute out of base collection are grouped as active collection, which are mostly domestic varieties. At present, 430 genotypes belong to working collection and 945 genotypes including 780 genotypes of active collection are base collection.

Bulgaria: In Bulgaria, naturally growing mulberry has been reported since the ancient times and now *M. alba*, *M. bombycis*, *M. multicaulis*, *M. kagayamae*, *M. rubra* and *M. nigra* are growing in different parts of the country. The Sericulture Experiment Station (SES) in Vratza collects, characterizes and evaluates both indigenous and exotic mulberry varieties. Currently more than 140 mulberry accessions are maintained in the germplasm at SES-Vratza (Tzenov 2002).

Korea: In Korea, the department of sericulture and entomology has collected more than 615 accessions of mulberry comprising of 205 indigenous, 151 exotic and 259 unclassified strains. These mulberry accessions are being evaluated for various morphological and agronomical characters. The exotic accessions are from nearly all countries practicing sericulture from the temperate and sub-tropical belt like Japan, Iran, China, Uzbekistan, Pakistan, India, Canada, Taiwan, America, Philippines and Lebanon (Sanchez 2000).

Turkey: Although mulberry has been cultivated in Turkey for more than 400 years (Ercisli and Orhan 2007) and all the three major types of mulberry viz., *M. alba*, *M. nigra* and *M. rubra* have been cultivating (Gökmen 1973; Yaltirik 1982), till date no mulberry cultivar was registered in Turkey. Each region of Turkey has its own local mulberry genotypes with different names. Therefore, efforts are now under way to conserve these precious genetic resources. As a result, germplasm collections have been set up in Adana, Tokat and Malatya regions (Gunes and Cekic 2004; Koyuncu 2004). The Malatya Fruit Research Institute has a vast number of mulberry accessions mainly from *M. alba* and *M. nigra*, which were collected from different parts of Turkey.

Biodiversity in Indian mulberry

1. Wild Morus species

M. serrata, the natural Himalayan mulberry is confined to North West India. Depending on the agro-climatic distribution, the morphological parameters showed wide variation. The bark colour varied from red, brown to dark brown and blackish brown. Leaf varied from unlobed to multilobed, thin to thick, rough, tomentose and velvety (Tikader and Kamble 2008). Phyllotaxy is mixed type i.e., 1/2, 1/3, 2/5. Internodal distance varied from 3.5 to 7.5 cm. In *M. laevigata*, the bark colour varied from grayish to reddish and leaf is mostly unlobed. Phyllotaxy is unique i.e., 1/2. Internodal distance is more i.e., from 6.0 to 8.0 cm (Tikader and Thangavelu 2000a). In *M. alba*, the bark colour varied from light brown to dark brown, leaf is smooth and in general unlobed. Phyllotaxy is 1/2, 1/3, 2/5 and mixed. Internodal distance is short (3.5-5.0 cm). In *M. indica*, the bark colour varies from dark brown to blackish, leaf is slightly rough and is mixed i.e., lobed and unlobed. Phyllotaxy is mixed type. Internodal distance varies from 4.0-6.0 cm (Tikader and Dandin 2009b).

2. Ploidy status

The chromosome count of the collected samples indicates that *M. serrata* is available in polyploid form in nature (Basavaiah *et al*. 1989). Mulberry, in general, is diploid (2n=28). But in nature, diploid (2n=28), triploid (2n=42), tetraploid (2n=56) and hexaploid (2n=84) species are found (Tikader and Kamble 2008). Ploidy level has been used to trace the genesis of plant endemism. The paleo-endemics studies showed high chromosome number and are assumed to be ancient polyploids and grow in higher altitude (Dhar 2002). In *M.serrata* similar observation was also noted. The ploidy level of *M. laevigata* Wall. varies from diploid to tetraploid and grows in higher altitude ranges from 700 to 2200 m (Tikader and Kamble 2008). In general the cultivated varieties like *M. alba* and *M. indica* are diploid.

3. Reproductive variability

The inflorescence length is higher in *M. serrata* than other cultivated species in India. The maximum collections of *M. serrata* possess male flower. If the chromosome number increases, the pollen size also increases. The pollen study indicates that the diameter of the pollen spores varies from 16.00 to 25.70, 21.00 to 28.00, 23.00 to 30.50, and 25.00 to 33.00 µm in diploid, triploid, tetraploid and hexaploids, respectively. The number and diameter of the pollen pores showed 2 to 5 porate pollen grain (Tikader *et al*. 2000a). Male and female flowers are borne on separate plants i.e.,

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 2002a).

M. laevigata shows dioecious flowers i.e., male and female flower borne on separate plants. In wild species, dioecious conditions are available. Male inflorescence length varies from 3.72 to 11.30 cm. The anther length varies from 0.90 to 1.16 mm and stamen length from 2.02 to 5.19 mm. The pollen diameter and viability varies from 15.32 to 21.54 µm and 55.21 to 94.25% (Tikader and Kamble 2008). *M. laevigata* has a lengthy inflorescence and is used as key character to identify the species. The female inflorescence length varied from 5.00 to 12.00 cm. The flower/catkin varies from 132.00 to 244.00, style length 0.02 to 2.35 mm, fruit length 6.10 to 10.27 cm and fruit weight 2.02 to 6.37 g. Fruit colour varied from black, white, pink and greenish. The fruit is usually lengthy and seedless, very sweet to taste (Tikader *et al*. 2000a). Rare abnormality i.e., the male inflorescence showed branching, which varies from 2-3 branches. The collection was made from Andaman and Nicobar Islands and was recorded (Tikader and Thangavelu 2003).

In *M. alba*, the male inflorescence length varies from 2.98 to 3.43 cm; anther length from 0.87 to 1.02 mm; pollen diameter from 17.24 to 20.12 µm and pollen viability from 92.62 to 97.42%. The female inflorescence length varied from 1.35 to 3.48 cm. The flower/catkin varies from 16.33 to 61.00, style length 0.17 to 1.18 mm, fruit length 1.45 to 4.13 cm and fruit weight 0.67 to 1.80 g. Fruit colour varied from black, white and pink. The fruit are sweet to taste (Tikader and Dandin 2009a).

In *M. indica*, the male inflorescence length varies from 1.92 to 3.62 cm, anther length from 2.80 to 3.97 mm; pollen diameter from 16.67 to 19.42 µm and pollen viability from 88.00 to 95.172%. The female inflorescence length varied from 0.85 to 2.88 cm. The flower/catkin varies from 10.33 to 67.00, style length 0.25 to 1.30 mm, fruit length 1.30 to 5.62 cm and fruit weight 0.35 to 3.27 g. Fruit colour varied from black and pink. The fruits are sweet and sour in taste (Tikader and Dandin 2009b).

4. Leaf histological variability

The stomata frequency ranges from 255.00 to 416.00/mm² whereas idioblast frequency ranges from 14.00 to 36.00/ mm². 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 12.00 to 32.00. The chloroplast number/stomata is the indirect way to group mulberry accessions in different ploidy levels (Yang and Yang 1995; Tikader *et al*. 1999a; Tikader and Rao 2001a). In *M. serrata*, the chloroplast number per stomata varies from 10 to 20 in triploid, 10 to 26 in tetraploid and 12 to 30 in hexaploid. The variation of leaf anatomical parameters in *M. laevigata* ranges from 18.95 to 30.38 µm stomata length, 15.55 to 20.65 µm and stomata width, 266.90 to 943.70 mm² in stomatal frequency, idioblast length (4.25 to 39.35 µm), idioblast width (8.67 to 36.68 µm) and idioblast frequency (10.73 to 23.66 mm²). The palisade layer thickness (49.03 to 96.53 µm), spongy layer thickness (55.57 to 128.66 µm) and leaf thickness (140.90 to 267.75 µm) showed variation among the accessions. The chloroplast number/stomata in *M. laevigata* varies from 9.50 to 16.00 and indicates some of the accessions are diploid, triploid and/or tetraploid (Tikader and Rao 2001a).

In *M. alba*, the different anatomical parameters showed variation i.e., stomata length (17.23-25.65 µm), stomata frequency (394.02-971.70 mm²), idioblast length (6.06-12.35 µm) and idioblast frequency (12.01-24.25 mm²). The other parameters i.e., palisade layer thickness (36.67-74.13 µm), spongy layer thickness (47.22-87.57 µm), leaf thickness (132.37-192.70 µm) and chloroplast number/stomata (9.00-12.00). In *M. indica* different anatomical parameters showed variation i.e., stomata length (16.97-25.32 µm), stomatal frequency (541.22-1009.40 mm²), idioblast length (3.35-18.77 µm) and idioblast frequency (15.27-21.35 mm²). The palisade layer thickness (34.57-70.95 µm), spongy layer thickness (40.05-77.67 µm), leaf thickness (121.85-206.38 µm) and chloroplast number/stomata (9.58-11.77).

5. Growth performance

The growth behaviour is important for effective utilization of mulberry germplasm for sericultural purposes. Wide range of variation was observed in *M. serrata* among the different growth traits (Tikader and Kamble 2008). 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 to 8.17%, respectively. Different growth traits of *M. laevigata* showed variation among the accessions. Number of shoot varies from 10.17 to 23.33 followed by length of the longest shoot (80.83-205.33 cm), internodal distance (4.56-9.15 cm), 100-leaf weight (381.73-1093.02 g), total shoot length (572.67-3277.83 cm), moisture content (68.97-73.30%), moisture retention (61.11-83.62%) and leaf yield per plant (0.39-3.00 kg) (Tikader and Dandin 2008b). Information on the field performance of *M. serrata* was assessed and found significant variation among the accessions. The accession provides the information on variability, association and grouping pattern. As mulberry grows in natural condition with the component of stress related factors, it might be utilized in mulberry crop improvement programme through breeding (Tikader and Dandin 2005a, 2005b).

Variation in *M. alba* was observed for different parameters like number of shoots (9.17-40.83), followed by length of the longest shoot (90.17-194.17 cm). Likewise, other parameters also varied with ranges in internodal distance (3.40-5.61 cm), 100-leaf weight (63.42-535.28 g), total shoot length (544.17-4346.17 cm), moisture content (65.29-72.18%), moisture retention capacity (30.22-74.80%) and leaf yield/plant (0.41-3.34 kg). The different growth parameters of *M. indica* showed variation i.e., number of shoot varies from 8.83 to 49.10, length of the longest shoot from 132.67 to 197.50 cm. Likewise, internodal distance varies from 3.48 to 5.09 cm, 100-leaf weight (143.83-783.33 g), total shoot length (1097.00-5941.00 cm), moisture content (66.91-74.01%), moisture retention capacity (45.04-76.76%) and leaf yield/plant (0.51- 3.95 kg).

6. Genetic variability

Divergence analysis at species level in mulberry was conducted on morphological, anatomical and reproductive traits. Based on these traits the species are grouped into different clusters (Tikader *et al.* 1999b). *M. alba* alone formed individual cluster for all the traits where *M. laevigata* formed separate cluster for morphological and reproductive traits. *M. macroura* and *M. rotundiloba* are grouped in one cluster for anatomy and reproductive traits except morphological traits that shows their close association (Tikader *et al.* 1999b). When the genetic distance was calculated between species, it was seen that the inter-specific distance was highest between *M. serrata* Roxb. and *M. indica* L. (0.465) that is followed by *M. serrata* and *M. alba* L. (0.457), *M. serrata* and *M. macroura* Miq. (0.401). The genetic distance between *M. macroura* Miq. and *M. indica* L. was 0.307, which was higher than that of *M. macroura* Miq, and *M. alba* L. (0.339). The interspecific distance between *M. indica* L. and *M. alba* L. was 0.168. The intraspecific variability as calculated by averaging the genetic distance among the genotypes of a single species was highest in *M. serrata* Roxb. (0.180) and lowest in *M. alba* L. (0.147). The genotypes of *M. serrata* Roxb. and *M. macroura* Miq. can be used for breeding with cultivated varieties, which are capable of high leaf yielding but are sensitive to biotic and abiotic stress to confer stress resistance to these varieties

(Vijayan *et al.* 2006). The promising genetic resources of *M. serrata* Roxb. and *M. macroura* Miq. should be preserved properly for the genetic advancement of the presently cultivated varieties of mulberry.

Sixteen populations collected from natural sources of the wild mulberry, *M. serrata* Roxb. were analyzed for their genetic diversity with the aim to use them in introgressive breeding programme with cultivated relatives. Significant amount of genetic diversity (0.165) was observed among these populations for morpho-anatomical as well as DNA markers. The result indicates significant amount of genetic diversity among the collections of *M. serrata.* The 17 ISSR primers generated a total of 95 DNA markers, 51 of which were polymorphic revealing 67% polymorphism among the populations (Vijayan *et al.* 2004).

7. Breeding performance

The breeding objective is to produce or create a broadly adapted population with sufficient genetic buffering to face a wide variety of environmental conditions producing good yield. In order to broaden the genetic base, new gene pools have to be incorporated into the mulberry cultivars (Tikader and Dandin 2001b). *M. serrata* possesses 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 are resistance to drought and frost (Tikader and Thangavelu 2002b). Initial breeding performance of *M. serrata* at inter-specific level with *M. indica* was found to be suitable for crop improvement. More number of crosses at inter-specific and intra-specific level with *M. laevigata* and *M. serrata* through introgression breeding is required. F1 hybrid thus developed of *M. indica* and *M. laevigata*, *M. indica* and *M. serrata* for further utilization (Tikader and Dandin 2008a). *M. serrata* and *M. laevigata* provides scope for target oriented selective breeding to incorporate the characters of drought, frost, and disease resistance into cultivated cultivars. Moreover, the breeding performance of *M. laevigata* showed positive results (Tikader and Thangavelu 2005). The wild species retain their characteristics after crossing and expressed in hybrids (Tikader and Dandin 2007).

8. Propagation

In general, mulberry is propagated through vegetative cuttings, but the wild species (*M. laevigata* and *M. serrata*) are poor in rooting and show limitations in studying the material. The alternative method of propagation is grafting (Tikader and Thangavelu 2006). The wild collection of *M. serrata* is generally higher in ploidy i.e., triploid, tetraploid, hexaploid, etc. When a graft is made, the adjustment of chromosome number poses incompatibility and results in unsuccessful grafting. The graft union formed at the initial stage with apparent success gradually develops distress symptoms with time due to failure in producing the callus parenchyma, which is the main reason for low percentage of successful grafts and difficulty in multiplication. *M. laevigata* also showed grafting incompatibility like *M. serrata*. Therefore, all the grafting was not successful due to graft incompatibility.

9. Bioassay

M. serrata was used for bioassay study and results indicate that it is suitable for late stage and from hatching to cocoon harvest; the crop yielded 32 kg/100 disease free layings. The farmers can get additional income from the silkworm rearing as they are using the plant as fodder. The plants are growing in the bund areas and landless farmers can take up rearing on such species to supplement their income as additional crop or as alternate food during the last stage of rearing (Singh *et al*. 2006).

The rearing performance of *M. laevigata* was assessed throughout rearing and was found that larval period (26 days), weight of 10 mature larvae (32.02 g), yield/10,000 larvae (5077 no.), 6400 g by weight, single cocoon weight (1.28 g), shell weight (0.223 g) and silk ratio (17.33%), which is comparable with commercial cultivars (Tikader 1993). The quality of leaf is good but mortality of the worm was little high. Effect of feeding leaves of *M. alba* and *M. laevigata* were assessed on larval growth and silk yield of *Bombyx mori* L. The result indicated that *M. laevigata* leaf was better in rearing performance than *M. alba* (Tikader 1993).

10. Special characters

M. serrata 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 (Tikader and Thangavelu 2002a). *M. laevigata* harbors a number of agronomically important traits such as bigger leaf size, higher leaf thickness, moisture retention, resistance to biotic and abiotic stress like drought, saline and frost (Ravindran *et al*. 1999). Due to above positive characters, *M. laevigata* showed scope for introgression breeding between wild and cultivated species (Tikader and Dandin 2005a, 2005b; Vijayan *et al.* 2006).

11. Variability in cultivated species

M. indica: The different growth parameters showed variation i.e., number of shoot is highest in *Morus* Indigenous (MI), MI-0346 (49.10) and lowest in MI-0431 (8.83), longest shoots in MI-0346 (197.50 cm) and shortest in MI-0316 (132.67 cm). Likewise, internodal distance range from 3.48 to 5.09 cm, 100-leaf weight (143.83-783.33 g), total shoot length (1097.00-5941.00 cm). Moisture content (66.91-74.01%), moisture retention capacity (45.04-76.76%) and leaf yield/plant (0.51-3.95 kg) (Tikader and Dandin 2009b).

M. alba: The variation was observed for different parameters like number of shoots (9.17 to 40.83), followed by length of the longest shoot (90.17 to 194.17 cm). Likewise other parameters also varied which ranged from internodal distance (3.40-5.61 cm), hundred leaf weight (63.42-535.28 g), total shoot length (544.17-4346.17 cm), moisture content (65.29-72.18%), moisture retention capacity (30.22-74.80%) and leaf yield/plant (0.41-3.34 kg) (Tikader and Dandin 2009a).

Utilization and conservation strategies for mulberry genetic resources

Most of the wild and weed relatives of domesticates have not been collected, studied and conserved. Wild progenitors and other close relatives of crop species – the secondary gene pools have assumed an important role as genetic resources used by plant breeders (Tikader and Dandin 2007). Gene transfer by genetic engineering is opting up tertiary gene pools. The cultivated species exhibits considerable genetic diversity, but the diploid mulberry showed narrow genetic base and threat to genetic erosion (Tikader and Kamble 2008). In order to broaden the genetic base, new gene pools have to be incorporated into the gene pool of cultivated forms. In depth analysis of these species is required towards utilization in mulberry improvement and conservation programme.

The maintenance of biodiversity depends on the nature of the material to be conserved, its ecological status, life cycle, mode of reproduction and population size (Virchow 1999). The conservation strategies can be either *in-situ* conservation of genetic resources within their ecosystem or natural habitat or *ex-situ* conservation of components of genetic materials outside their natural habitat. The conservation of mulberry is a holistic concept that encompasses a wide spectrum of activities ranging from establishment of protected areas to building of DNA libraries (Vijayan *et al.*

2009). The basis of all conservation actions is sufficient knowledge of the diversity of the plant concerned and the ecosystem in which they occur. There are many conservation methods, but some of the most important strategies followed in mulberry are its original habitat, managed condition, farmers field, alternative field area, *in vitro* culture and cold treatment.

In-situ conservation

The conservation of biodiversity in nature by setting aside the natural reserve, where species are allowed to grow in their natural habitat by maintenance of ecological continuum (Ravindran *et al.* 1999; Tikader and Thangavelu 2002b). However, there is always a threat of a species becoming extinct or declining due to genetic drift and inbreeding, demographic and environmental variations, habitat loss, competition from invasive alien species, disease or over exploitation, human disturbances etc. In such circumstances the only way to conserve directly among and within species for posterity is to maintain them as *ex-situ* collection (Tikader *et al*. 2000b). *In-situ* conservation of plant genetic resources covers biosphere reserve, national parks and gene sanctuary.

Merits of *in-situ* is good for i) species about to extinct, ii) good for wild relatives of crop plants, tree crops, forest crops etc. where *ex-situ* conservation is not effective and iii) It is specially fit for those species that cannot grow in *ex-situ* outside their habitat (Arora 1991). In case of mulberry *in-situ* conservation is already initiated with different agencies like state government, non-governmental organization etc. for proper implementation in the country.

1. Habitat conservation

Many species survive and best perpetuate only in their own niche or microclimate available in the wild habitat itself. The advantages of such approach are that it does not require detailed knowledge of the flora available in that habitat (Koshoo 1995). The Namdapha Biosphere Reserve of India in Arunachal Pradesh for *Coptis teeta*, Demabeyang Valley in North Sikkim for *Panax* pseudo ginseng and Nanda Devi Biosphere for rhododendron, pine etc. including *Morus serrata*, which is available in that area (Tikader *et al*. 2000b).

2. Biosphere reserves

The biosphere reserve establishes a balanced relationship between human and biosphere. The biosphere is the site of excellence for foster economic and human development, which is a socio-culturally and ecologically sustainable with an logistic support for demonstration projects, environmental education and training cum research (Rana 1993). The National Committee on Environmental Planning and Coordination (NCEPC) and Man and Biosphere (UNESCO) are involved in designated areas as Biosphere Reserves (Nayar 1997). Out of 14 biosphere reserves in India, the ones at Nandadevi (Uttarkhand), Namdapha (Arunachal Pradesh), Kaziranga (Assam), Manas (Assam), Nokrek (Meghalaya), North Andaman and Great Nicobar (A&N islands) have been utilized for *in situ* conservation of mulberry (Naik and Mukherjee 1997). Accordingly, efforts have now been made to collect information on the location of availability of mulberry genetic resources with details on the "declared protected area network of India" including biosphere reserves, national parks, wild life sanctuaries, etc. (Rao 2002). Similarly, in Canada, efforts to conserve red mulberry have received strong support from land managers and naturalists. A recovery plan has recently been developed for the species *M.rubra* found in Hamilton's Royal Botanical Gardens, Ball's Falls Conservation Area, Niagara Glen, Rondeau Provincial Park, Point Pelee National Park, Fish Point Provincial Nature Reserve, Pelee Island, Middle Island and East Sister Island. The biosphere provides i) continued evolution, ii) wide genetic base of species conserving large range of alleles, iii) allows population to maintain or perpetuate itself within community environment, other species part of system also conserved, iv) can serve several disciplines at any time for example breeding, forage, forestry, wild-life, etc. v) it facilitates research on species natural habitat, mechanism of genetic variation and evaluation of species and vi) it also facilitates better evaluation of species and utilization (Pareek 2003). But disturbance of habitat causes degradation of vegetation that may disturb the stability of plant components.

3. Gene sanctuaries/National parks

The significant approaches for *in-situ* conservation are to declare the highly rich communities and habitats under protected areas. The protected area can be easily maintained with the help of communities residing in that area. India has one of the largest networks of protected areas including National park (81), wildlife sanctuaries (441) and wetland and mangroves (31) providing *in-situ* conservation of many plant species including *Morus* species. Five of the protected areas have been designated as world's Heritage Sites under UNESCO's World Heritage Programme namely Kaziranga National Park, Keoladeo Ghana national park, Manas wild life Sanctuary, Nanda Devi National Park and Sunderban National Park.

4. Sacred grooves

From time immemorial plants are worshiped like Gods or as their blessings in India. Many tribal communities live in complete harmony with nature; their feeling to cut a plant might cause evil effects on their family (Pareek 2003). These communities have developed their own system of conserving forests or habitats by naming them as sacred grooves. These are untouched virgin forests with a taboo that even taking a dead wood or fallen fruits may cause harm to the person. There exist more than 500 sacred groves in the tribal inhabited area of Northeastern region; Maharastra, Western ghats, Nilgiri, Orissa and Uttarakhand, Khashi and Jaintia hills and Baster area in central India are rich in such diversity. Mulberry species is also available in that area. *M. serrata* is being worshiped in Uttarakhand; Himachal Pradesh as sacred grooves and do not use the mulberry wood, leaves and fruits (Tikader and Kamble 2008). The oldest mulberry tree of about 1200 years old is being worshiped in Joshimath of Uttarakhand state (Rau 1967).

Ex- situ conservation

The *ex-situ* conservation refers to the man made gene bank conservation that includes *ex-situ* seed conservation in seed gene bank; *ex-situ* plant conservation in field gene bank; *ex-situ in vitro* conservation of explants or organs in *in vitro* bank and cryo bank, DNA library and DNA bank (Vijayan *et al*. 2009). Resources are conserved outside the area where it naturally occurs, it is called *ex-situ* conservation. The techniques of *ex-situ* conservation often form an important and indeed critical element in comprehensive conservation programs. Conservation of plants in botanical gardens, on-farm conservation by farmers with traditional agricultural systems or Arboreta or field gene banks, all comes under the umbrella of *ex-situ* conservation. Traditionally, plant genetic resource management involved conserving germplasm as seeds at low temperature, or as field plantings for vegetatively propagated plant species. Since, mulberry is a perennial out-breeding tree that exhibits high degree of heterozygosity, conservation of the germplasm accessions through preservation of seeds is not a viable method (Tikader and Dandin 2007). Hence, mulberry genetic resources are conserved mainly through maintenance of the whole plant in either the field or preserving vegetative parts in a viable form or using both ways (Tikader and Dandin 2005a). *Ex-situ* field gene banks are developed through planting of stem cuttings/saplings or by grafting the buds on appropri-

ate rootstocks (Tikader and Thangavelu 2006). The ideal genetic resource conservation program consists of active collections that are available for distribution or characterization and base collections held for the sole purpose of long-term preservation. to maintain the purity, viability and also for security reasons duplicates of base collections are usually maintain in geographically different locations (Tikader and Kamble 2009c).

1. Ex-situ field gene bank management in India

The management of collections is important, as it has to be preserved for posterity. The utility of germplasm is only realized if the germplasm stored in, has been clearly defined, evaluated, characterized scientifically and documented (Saraswat *et al*. 2009). The development of an effective data management, therefore also forms gene bank manager's responsibility (Sekar *et al*. 2009). Central Sericultural Germplasm Resources Centre (CSGRC), Hosur (India) is situated around 12.45° N and 77.51° E, altitude of 942 m with dry tropical climate. The average rainfall ranges from 700–1000 mm per annum. CSGRC, Hosur maintain 1120 accessions (Ind-856; Exo-264) in the field gene bank as *ex-situ* conservation (Tikader and Kamble 2009c). All the accessions are maintained as dwarf tree with spacing of 2.4 × 2.4 m. Pruning followed once in a year for conservation and twice for recording data.

2. Classification of existing germplasm

The contribution of gene banks to crop improvement is of paramount importance. The gene banks provide a continuous service to plant breeder by regularly supplying materials with new traits (Saxena 1993). While searching for a trait, breeders usually turn first to their working collections because the plants are agronomically superior and are thus closer to what farmer wants (Tikader and Dandin 2006). Only when the desired traits are not found in the elite breeding lines, scientists search them in gene banks. Sources of disease and pest resistance may also be available in the tropical germplasm bank.

The genetic resources centers undertake the responsibility of conserving the resources is being followed as mentioned below:

i) ***Active collections*****:** These collections are also called working collections for multiplication, regeneration, evaluation, documentation, distribution and use by plant breeders. These collections have a higher rate of usage and thus more frequent used for multiplication (Tikader and Dandin 2006; Tikader and Kamble 2007).

ii) ***Base collections*****:** The primary objective of a base collection is to preserve indefinitely specific germplasm accessions in order to maintain as broad germplasm base as possible for the future. These collections are stored for long-term periods and conditions of storage are more exacting so as to reduce the risk of genetic changes introduced through frequent regeneration and prevent genetic erosion of the stored germplasm accession resulting from excessive deterioration (Tikader and Kamble 2009d).

iii) ***Duplicate base collections*****:** These collections are duplicates of the base collections that are housed in geographically different locations for security purposes. This is also called a safety back up of the base collections. The objectives and methods of storage are essentially the same as for the base collections (Naik *et al*. 2002; Tikader and Dandin 2007).

3. On-farm participatory conservation

The other form of conservation is the on-farm conservation linked with farmers' participatory breeding (FPB), which gives special emphasis on sustaining and utilizing on-farm biodiversity by the farmers (Eyzaguirre and Iwanaga 1996). 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 and hence, conservation of potentially interesting alleles and development of diversity is promoted. In mulberry, the wild species like *M. laevigata* and *M. serrata* and others do not get attention in the formal sector for cultivation for sericulture purposes (Tikader and Dandin 2005a). However, these wild species have been used for other non-sericultural purposes such as horticulture and agroforestry. Farmers/aboriginals largely use fruits and timbers of these species as a livelihood. Thus, the biodiversity of these species are conserved through the on-farm participation of aboriginals and farmers (Tikader and Kamble 2007).

4. Botanical garden/herbaria

Botanical gardens are being maintained from thousand of years when herbal doctors, healers, sages maintained gardens of their own for medicinal plants. Even today, one can notice many ashrams in and around Haridwar, Rishikesh (Uttarakhand) and Himalayan region where medicinal plants have been maintained including mulberry (Pareek 2003). The major botanical gardens in India are Tropical Botanical Garden and Research Institutes, Thiruvanathapuram (Kerala); Medicinal and Aromatic Plant Garden and Herbarium, Pune; Lalbagh Botanical Garden, Bangalore; Royal Botanical Garden, Kolkata; Lloyd Botanical Gardens, Darjeeling (West Bengal). The Government of India and Provincial Government together run and maintain 33 Botanical gardens, which maintains the diversity in the form of plant or plant populations. Biodiversity has also been preserved in the form of Herbarium specimens (Saxena 1993). Botanical Survey of India (BSI) has the largest holding of 1,500,000 specimens (Pareek 2003). There are many more herbaria in India maintained in different Research Institutes where *Morus* species is also stored. The National Germplasm on mulberry also maintained more than 1000 samples of different *Morus* species.

5. Arboreta

Traditionally arboreta have been regarded as assemblages of tree for scientific purposes generally with some sort of economic imperative in the not too distant background. The arboreta and botanical gardens had more utilitarian purpose – to find species that would benefit the new colonies and to establish which trees would provide wood, fruit, foliage for future needs (Pareek 2003). Community can get use of such arboretum in different ways. The arboreta help in maintaining genetic conservation of valuable rare and endangered trees. Arboreta can reflect changing values in society where they are becoming something for all to enjoy rather than the domain of select few and serve the purpose of the society. The mulberry plants are conserved as arboreta in different botanical gardens (Tikader and Thangavelu 2002a).

6. Ex-situ conservation in the forms of plant parts

The plant materials can be stored in the form of gene, seed gene or pollen banks and other forms. The vegetatively propagated crops like tubers, rhizomes, corms and cuttings. The mulberry is conserved as cuttings, pollen and seeds.

Use of tissue culture technology in conservation**:** The rich biodiversity in plant species would lay a strong foundation for development of new cultivars presently and more so in future allowing alteration and reconstruction of existing ones to suit the changing human needs and environment. It is satisfying that germplasm value in the third world is also very impressive and Indian gene center is uniquely rich in this respect (Chandel and Bhat 1992). Thus, a worldwide interest in germplasm collection, conservation and utilization using conventional breeding approaches as well as new emerging techniques of tissue culture and biotechnology

has assumed importance. Efforts are being made currently at global level to preserve biodiversity. Today efforts are being directed world wide to diversify methods and new strategies are being integrated to achieve gene conservation. Tissue culture (*in vitro*) technology and cryopreservation (freeze preservation) employing cryogenic gases such as liquid nitrogen at ultra low temperature (-146-196°C) are being used for mulberry cryopreservation (Vijayan *et al*. 2009).

***In vitro approaches*:** Plant tissue culture techniques have extensively been used in mulberry for its propagation and genetic improvements mentioned below:

In vitro conservation indicates maintenance under aseptic condition using culture of plants. *In vitro* technology offers unique strategy for clonal multiplication and conservation particularly for vegetatively propagated crop plant species. Preservation of germplasm diversity using *in vitro* technology can be accomplished in the vegetatively propagated crop plants using meristem/shoot tip cultures, axillary buds and nodal segments (Mandal 1999). Although most of the tropical mulberry species are propagated through stem cuttings, many hybrids developed through crossing between tropical and temperate species are hard to propagate due to their poor rooting ability. Such high yielding varieties were propagated through micropropagation techniques. In mulberry micropropagation is mostly achieved by growing axillary buds under in vitro conditions (Jain *et al*. 1990; Sharma and Thorpe 1990; Yadav *et al.* 1990; Pattanaik *et al.* 1996; Pattanaik and Chand 1997).

Regeneration of plantlets through organogenesis and embryogenesis from callus tissue was also reported in a number of species. Ghugale *et al*. (1971), Oka and Ohyama (1973, 1975, 1981) and Kim *et al*. (1985) are the pioneers who contributed much to the development of highly efficient protocols for plant regeneration in mulberry. Attempts were also made to develop synthetic seeds by encapsulating the apical/axillary buds or somatic embryos with 3-5% sodium alginate and 100 mM calcium chloride solution as complexing agent (Bapat *et al*. 1987). Sodium alginate solution is mixed with tissue culture medium containing all necessary ingredients essential for proper growth. Chand *et al.* (1994) successfully developed a technology for artificial seeds of mulberry. The potential of somaclonal variation is enormous in asexually propagated plants like mulberry due to the easiness by which the plants can be propagated through vegetative means. A somaclonal variant (SV1) isolated from plants regenerated from internodal callus of *M. alba* var. S1 was isolated and found superior to the control variety S1 in the leaf yield and number of branches (Narayan *et al.* 1993). The somaclonal variant found phenotypically stable after multiplication and experimantation in different conditions. The result of the experiment concluded with recommendation that SV1 can be utilized for irrigated condition, as its performance was good under such conditions (Chakroborti *et al.* 1999).

***DNA library*:** Conserving the extracted DNA is a very recent approach. The genome of the accession is the main source of the genes required in various crop improvements. The present day DNA banking offers tremendous opportunities of practical and academic value (Mandal 1999). Infact, DNA as a gene bank resource has emerged out of revolution in genomic information. The uses of DNA like genomic construction of DNA libraries, collection of segments of DNA containing several copies of the part of genome, which includes cDNA, cosmid, PAC (Plasmid derived artificial chromosome) etc. DNA can offer important resources for several applications i.e., characterization of source materials and understanding genetic and evolutionary relationship between taxa, functional analysis of genes comparative genomic and plant breeding in mulberry (Vijayan *et al.* 2009). DNA bank is a particular type of genetic resources bank that preserves and distributes the DNA samples and provides associated information.

***DNA storage*:** DNA is a highly stable molecule degradation kinetic models suggests that fully hydrated DNA kept at room temperature takes about 10,000 years to depolymerise into small fragments. An alternative approach is to store cells and tissues rather than purified DNA (Mandal 1999). Further stored cells and tissues have added advantages of providing a continuous supply of DNA and enabling biochemical and molecular studies of living cells. Depending upon the available facilities, the reference sample may be in the form of live plant in field repository, a propagule conserved in a gene bank, which can be recovered into a full plant or a herbarium specimen. DNA banks around the world are maintained at Australia, Brazil, USA, Great Britain and South Africa. The DNA bank at Royal Botanical Gardens, Kew (UK) maintained nearly 23,000 samples of plant genomic DNA stored at -800°C. The bank has a large collection of orchids. DNA samples and samples of rare and endangered species are maintained (Vijayan *et al.* 2009).

***Opportunities of DNA banks*:** There are number of areas in which DNA banks could make an impact which are as follows:

Germplasm characterization and management which combines phenotypic and molecular markers improves management and maintenance of germplasm mainly on identification of gaps in collection, duplicates and redundancy, provides information about molecular diversity, genetic and evolutionary relationship, identification of unique genotypes of special importance to gene banks and breeders.

Marker assisted selection (MAS): Application of molecular techniques to identify genes controlling specific traits in collection of cultivated species and wild relatives (Vijayan *et al.* 2009).

***DNA bar coding*:** Global efforts to produce DNA barcoding of all the plant species on earth DNA banks can support such type of study by avoiding time-consuming collection trips.

***Exchange of genetic resources*:** It is easier to exchange genetic resources as DNA samples, rather seed or vegetative propagules. Exchange of DNA samples can overcome time consumption, other formalities avoiding phytosanitary certification etc. A novel method of DNA distribution has been developed recently where DNA clones or PCR products are pasted directly on the pages of books for distribution to users. The National Institute of Agrobiological Sciences, Japan has constructed a DNA book for rice containing 32,000 clones. The DNA can be extracted from the paper and analysed for various purposes

Cryopreservation of mulberry germplasm

Cryopreservation of plant materials has proven to be a potentially ideal method for long-term preservation, because it requires a minimum space, labor, medium and maintenance.

The techniques for cryopreservation that are currently in use varied greatly and include the older classical technique based on freeze-induced dehydration of cells as well as newer techniques based on vitrification (Englemann 2000). Classical cryopreservation techniques involve slow cooling down at a controlled rate (usually 0.1-4°C/min) down to about -40°C, followed by rapid immersion of samples in liquid nitrogen. They are generally operationally complex, as they require the use of sophisticated and expensive programmable freezers. In the new hyperhydricity-based procedures, cell dehydration is performed prior to freezing by physical or osmotic dehydration of explants. This is followed by ultra-rapid freezing which results in hyperhydricity of intracellular solutes, i.e. formation of an amorphous glassy structure without occurrence of ice crystals, which are detrimental to cellular structural integrity. These techniques are less complex and do not require a programmable freezer, hence are suited for use in any laboratory with basic faci-

lities for tissue culture. Cryopreservation involves storage of plant material in ultra-low temperatures in liquid nitrogen (-196°C). At this temperature, cell division and metabolic activities remain suspend and the material can be stored without changes for long periods. Thus, cryopreservation method ensures genetic stability of the mulberry germplasm and requires limited space, protects material from contamination, involves very little maintenance and is considered a cost-effective method for conservation of mulberry germplasm. In fact, cryopreservation is the only available method for long-term conservation of vegetative propagated plant like mulberry. In mulberry the most appropriate material for cryopreservation was found to be winter buds, though embryonic axes, pollen, synthetic seeds have also been used (Niino and Sakai 1992a; Niino *et al.* 1992a, 1992b, 1993; Niino 1995) Keeping this in view, many laboratories across the world has established cryopreservation laboratories. For instances, the cryopreservation facilities at CSGRC, Hosur is actively involved in preservation of its 908 mulberry germplasm accessions (Rao *et al.* 2007). Success has been achieved in the cryopreservation of several accessions belonging to *M. indica*, *M. alba*, *M. latfo1ia*, *M. cathayana*, *M. laevigata*, *M. nigra*, *M. australis*, *M. bombycis*, *M. sinensis*, *M multicaulis* and *M. rotundiloba*. Likewise, with the epoch making research on cryopreservation by Sakai (1960), Japan has undertaken cryopreservation of mulberry in a large scale. About 450 germplasm accessions within several species have been cryopreserved in liquid nitrogen tanks in mulberry gene bank now (Okino *et al*. 2004). Shoot tips of pre-frozen winter buds of *M. bombycis* Koidz. was able to withstand long storage in liquid nitrogen. The general procedure for cryopreservation of shoot tips is that the shoot segments were first pre-frozen at -3°C for 10 days, -5°C for three days, -10°C for 1 day and -20°C for one day before immersion in liquid nitrogen. Buds were cultured on MS medium after thawing in air at 0 to 20°C. Survival rate was 55 to 90%. Prior to pre-freezing at -20°C partial dehydration of the bud up to 38.5% has found improving the recovery rates. The survival rates of the winter buds stored in liquid nitrogen up to 3-5 years did not change significantly. Encapsulation of winter-hardened shoot tips of many mulberry species with calcium alginate coating was also tested successfully. In addition, Yakua and Oka (1988) conducted experiments on cryopreservation of intact vegetative buds of mulberry *(M. bombycis*) attached to shoot segments by prefreezing and storing in liquid nitrogen. The buds were later thawed, and the meristems were excised for culture on MS (Murashige and Skoog) medium supplemented with 1 mg l^{-1} BA to regenerate plants. Either prefreezing at -10 or -20°C along with rapid thawing at 37°C or prefreezing at -20 or -30°C along with slow thawing at 0°C was a suitable condition for high percentages of survival and shoot regeneration.

CONCLUSION AND FUTURE ACTION

India is fortunate to have a high and varied diversity of flora. Mulberry the host plant of silkworm is available in different parts of the country (**Fig. 1**) and produces large quantity of silk. The assessment of biodiversity in *Morus* species is to go a long way. The latest technology/tools may be used for mapping the *Morus* diversity using GIS tools, contour mapping, remote sensing information, species density, uniqueness of macro and micro habitats, ecological significance and formulating strategy for sustainable utilization of resources. *In-vitro* technology is suitable for vegetatively propagated species especially *Morus*. The tissue culture technology is yet to be standardized, as the technique is unable to provide commercial utilization. The haploid mulberry is needed for mulberry genetics to use in crop improvement but is not readily available. Cryopreservation is the cheapest way to conserve the material where limited space can be used for large number of accessions and periodically can be checked for viability after cryopreservation but the technology is yet to used in mulberry at commercial level. DNA banking could constitute a complementary conservation strategy for safeguarding the genetic diversity of a crops gene pool especially if combined with *in-vitro* or cryopreservation. DNA bank can also serve as back up or safety duplicate of the physical seed, field or *in vitro* collection, in case of catastrophic loss. Use of single conservation technology may not be able to meet the present day requirement. Therefore, complementary conservation strategies including *in-situ* and *ex-situ* conservation method should be used. In *ex-situ* conservation, a balance of seed, field, *in vitro* and cryo-gene bank as well as preservation of seed, organs, pollen cells and DNA would be needed. Therefore, various approaches should be treated as complementary to each other and a combination of more than one technology should be utilized.

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