

Seri-biodiversity with Reference to Host Plants in India

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ABSTRACT

India harbours a wide range of biodiversity due to its diverse climatic and cultural practices. There are five types of natural silkworms, which feed on host plants of mulberry, arjun, asan, sal, *Quercus*, som, soalu, castor, kesseru, bar kesseru, tapioca and payam. Unlike mulberry silkworm, the wild silkworms are polyphagous and feed on several plants. Mulberry is the leading silk and 1120 host plants are being maintained with 13 species at the Central Sericultural Germplasm Resources Centre, Hosur. Tropical tasar host plant is represented by 13 species, distributed in 20 states and 124 genetic resources of host plant are being maintained in the *ex situ* gene bank at the Central Tasar Research and Training Institute, Ranchi. In temperate tasar a total of 10 species of oak are growing in the eastern part of the country and 6 species in the Western Himalayas for silkworm rearing. About 14 morphotypes of som and 10 morphotypes of soalu are maintained for muga silkworm. The primary food plant of eri silkworm is castor and 41 accessions are maintained. Although mulberry dominates silk variety in the global silk market, other non-mulberry silks also have importance in the domestic market. Besides this primary and secondary food plants have many multipurpose uses other than for silk. The availability, biodiversity, progress of work in different aspects to utilize and conserve plant genetic resources is reviewed and discussed.

Keywords: conservation, host plants, mulberry, non-mulberry, utilization

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INTRODUCTION

India is classified into 10 bio-eco geographic regions: Trans Himalayan, Himalayan, Indian desert, Semi arid, Western ghats, Decan Peninsula, Gangetic plains, North east, Islands and Coasts (GOI 1994). India harbours a wide range of bio-

diversity due to its diverse climatic and cultural practices. India is known to have more than 17,500 species of higher plants, including 168 major and minor crop species and 334 of their wild relatives. India is homeland for several serigenous insects and their host plants. There are five types of natural silks viz., mulberry, tropical tasar, temperate

(oak) tasar, muga and eri, and all these five varieties of natural silks are commercially cultivated. The word silk is synonymous with silk produced by *Bombyx mori* L. that feeds on mulberry. The other silks are known as wild or non-mulberry silk. Mulberry is cultivated not only for sericulture but also for fruit, fodder, timber, wood, etc. Mulberry, a perennial deciduous plant is reported to have originated in China, the primary centre of plant origin (Vavilov 1926). Mulberry is supposed to be native of Indo-Chinese area and distributed in the lower Sub-Himalayan region up to an elevation of 2100 m. Brandis (1906) and Hooker (1885) reported 4 species of *Morus* in India. viz., *M. indica*, *M. alba*, *M. laevigata* and *M. serrata*. *M. alba* and *M. indica* are mostly available in cultivated forms and not available in natural forest areas whereas *M. laevigata* and *M. serrata* are mostly available in wild form and *M. laevigata* is widely distributed in the forest area of Andaman and Nicobar Islands, North Eastern States, Western Ghats and Central India. *M. serrata* is confined to North Western Himalayan region and it is available only in wild form and is also threatened (Ravindran *et al.* 1997, 1999; Tikader *et al.* 2002, 2003). Unlike mulberry silkworm, the wild silkworms are polyphagous and feed on several food plants. The oak silkworm (temperate tasar) feed on several species of *Quercus*, whereas tropical tasar, muga and eri silkworms feed on several annual plants and perennial trees and some of these plants/tree species have diverse uses and are thus denoted as multipurpose trees. Some of the food plants exist only in the forest and not generally cultivated. These days, deforestation due to pressure on land has become a regular feature leading to loss of biodiversity. Therefore, the conservation of seri-genetic resources especially, the food plants of sericigenous insects has become very much essential to meet the desired objective for long term utilisation and crop improvement programme.

PRESENT STATUS OF SERI-GENETIC RESOURCES IN INDIA

The Central Sericultural Germplasm Resources Centre (CSGRC), Hosur is engaged in collection of mulberry germplasm, which are subsequently characterised after establishment in the gene bank. At present 1120 mulberry accessions (indigenous – 856, exotic – 264) are being maintained in the field gene bank collected from 26 countries, including India. There are 13 species, both cultivated and wild (Table 1).

In the case of non-mulberry (wild) silkworm host plant, *Terminalia arjuna* and *Terminalia asana* is represented by 13 species, distributed in 20 states and 124 genetic resources of host plants are being maintained at the Central Tasar Research and Training Institute (CTR&TI), Ranchi. The oak tasar silkworm feed on 16 *Quercus* species is distributed throughout the sub Himalayan region from Jammu and Kashmir in the Northwest to Manipur in the Northeast. The germplasm of *Quercus* spp. are maintained at the Regional Sericultural Research Station, Imphal, Manipur. Muga silkworm feeds on 15 host plants, which are distributed in 11 states, and this germplasm are maintained at the Regional Muga Research Station, Boko, Assam. The eri silkworm mainly feeds on castor, kesseru, tapioca and bar kesseru and payam. The germplasm of eri silkworm food plants are maintained at the Regional Eri Research Station, Mendiathar, Meghalaya.

Table 1 Collection and conservation of mulberry germplasm resources at CSGRC, Hosur.

Country name	No. of collections	State name	No. of collections
Afghanistan	2	Andaman and Nicobar	15
Australia	2	Andhra Pradesh	4
Bangladesh	5	Arunachal Pradesh	9
Myanmar	7	Assam	11
China	53	Bihar	24
Cyprus	1	Goa	4
Egypt	3	Gujarat	16
Spain	2	Haryana	2
France	32	Himachal Pradesh	26
Hungary	1	Jammu and Kashmir	36
Indonesia	6	Karnataka	101
India	856	Kerala	71
Italy	7	Manipur	12
Japan	69	Meghalaya	21
Pakistan	8	Maharashtra	32
Papua New Guinea	1	Madhya Pradesh	16
Paraguay	4	Nagaland	1
Philippines	1	New Delhi	2
Portugal	1	Orissa	1
Russia	1	Punjab	11
South Korea	6	Rajasthan	43
Thailand	11	Sikkim	3
Turkey	1	Tamil Nadu	83
Venezuela	1	Uttar Pradesh	145
Vietnam	3	Uttarakhand	8
Unidentified	25	West Bengal	148
Zimbabwe	11		
Total Collection	1120		856

COLLECTION OF MULBERRY GENETIC RESOURCES THROUGH SURVEY AND EXPLORATION

CSGRC, Hosur has collected a total of 727 mulberry germplasm from 24 states through 65 surveys covering 114 districts and 5 zones (Table 2). Maximum collection was made from North West India followed by South, North East, Western and Central India. These collections include wild, cultivated, popular, local forms, etc.

CHARACTERISATION OF MULBERRY GERmplasm

Characterisation is a part of a preliminary evaluation and this includes recording highly heritable phenotypic characters, which can be visually observed and expressed in various environment. After establishment of a plantation base in the collection and two years of growth, subsequent data are recorded on morphology (22 characters), anatomy (14 characters), reproductive characters (26 characters), isozymes, etc. To date characterisation has been completed on 928 accessions (Table 3). The data on mulberry germplasm characterisation were published in Mulberry catalogue volumes 1 and 2 (Thangavelu *et al.* 1997, 2000). The mulberry evaluation catalogue (Vol I, 2003) contains evaluation data of 316 mulberry accessions (indigenous – 236, exotic – 80) (Thangavelu *et al.* 2003).

Table 2 Progressive collections of mulberry genetic resources through survey.

Explored zone	States	Total collections	Collection (%)
North West India	Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh, Uttrakhand, New Delhi, Punjab, Haryana	241	33.15
North East India	Assam, Meghalaya, Manipur, Nagaland, Sikkim, Tripura, West Bengal, Jharkhand, Bihar	136	18.70
Central India	Madhya Pradesh, Orissa	17	2.34
West India	Maharashtra, Rajasthan, Gujarat	144	19.81
South India	Tamil Nadu, Karnataka, Andhra Pradesh, Kerala, A & N Islands	189	26.00
Total		727	100.00

Table 3 Phase-wise progress of work on characterisation of mulberry.

Characters	Mulberry accessions	1993-1997	1997-2000	2000-2003	2003-2006	2006-2009	Total
Morphology	Indigenous	212	243	107	119	110	791
	Exotic	116	57	43	31	--	247
Anatomy	Indigenous	212	243	107	119	110	791
	Exotic	116	57	43	31	--	247
Reproductive biology	Indigenous	212	243	107	119	110	791
	Exotic	114	57	43	31	--	247
Biochemical parameter	Indigenous	212	243	107	119	110	791
	Exotic	116	57	43	31	--	247
Growth traits	Indigenous	212	243	107	119	110	791
	Exotic	116	57	43	31	--	247

CLASSICAL GENETIC INVESTIGATIONS

Mulberry is highly heterozygous plant with long gestation period to establish for that reason; it is difficult to develop inbred lines for classical genetic studies. Therefore, the genetics of mulberry is still not studied well. To understand the crossing ability among the species attempts were made by different workers (Dandin *et al.* 1987; Dwivedi *et al.* 1989; Tikader 1993; Tikader and Dandin 2001). The inter-specific hybridization studies conducted with *M. alba*, *M. australis*, *M. latifolia*, *M. cathayana*, *M. nigra* and *M. sinensis* revealed different results, as in some crosses the fertility was very high while in some others it was very poor. For instance, fertility in crosses between *M. alba* and *M. indica*, *M. alba* and *M. cathayana* were high while *M. alba* and *M. laevigata* and *M. alba* and *M. serrata* were poor (Das and Krishnaswami 1965; Katagiri *et al.* 1982). Likewise, studies on combining ability of a few selected mulberry parents and heritability of major leaf yield contributing characters were made (Vijayan *et al.* 1997a, 1997b, 2008b). The studies revealed in mulberry under ideal cultural conditions, non-additive genes control the expression of most of the agronomically important traits. However, when the plants were subjected to salinity stress the effect of non-additive gene reduced but the additive gene became prominent (Vijayan *et al.* 2008b). It is observed that plant height and leaf size were under the control of non-additive gene actions in normal cultural conditions but under additive gene action in stress conditions. Thus, under stress conditions, the non-fixable components of variance give way to fixable components of variance in mulberry. This in turn, suggests the need of a change in the breeding strategy in mulberry when breeding programs are under taken for developing stress resistant varieties. For the development of salt adaptable genotypes, less cumbersome pedigree method can be of much use than the more cumbersome recurrent selection as suggested by Sheikh and Singh (1998). The details breeding achievement of last 50 years was highlighted in a review paper (Tikader and Kamble 2007a). Tikader and Dandin (2007b) based on their extensive experience on mulberry breeding suggested that pre-breeding strategies are necessary for effective hybridisation in mulberry. The authors also observed from different crosses that introgression of wild traits into cultivated species are quite possible even in the F₁ generation (Tikader and Dandin 2008).

PROFILE OF BIOCHEMICAL MARKERS IN MORUS SPP.

Isozymes are multiple forms of enzymes that share a common substrate but differ in electric mobility (Markert and Moller 1959). Isozymes are revealed when tissue extracts are subjected to electrophoresis in various types of gels and subsequently submersed in solutions containing enzyme-specific stains. Isozymes generally exhibit Mendelian inheritance, co-dominant expression, complete penetrance and are free of pleiotropic and epistatic interactions (Weeden 1989). Isozymes are fast, cheap and simple. In mulberry isozymes were first used by Hirano (1977) using peroxidase

to evaluate genetic relationships. In a significant research, Hirano (1979) demonstrated that the banding pattern of peroxidase was correlated with the leaf stalk length. Using seven isozymes, Hirano (1980) elucidated the genetic relationships among 131 mulberry varieties. Isozymes were also used for identifying mulberry with different geographic origin (Venkateswaralu *et al.* 1989a) and ploidy levels (Venkateswaralu *et al.* 1989b; Katagiri *et al.* 1994; Venkateswaralu *et al.* 1995). However, of late due to their low polymorphism it has rarely been used in mulberry. At the Germplasm Centre, isozyme banding pattern in PAGE was studied using peroxidase in 13 *Morus* species to assess the allelic variation in isozyme loci to ascertain the genetic diversity. The observed Rf values were 0.29 for major band and 0.37-0.63 for minor bands (Tikader and Thangavelu 2003).

Molecular characterisation of mulberry germplasm

The characterisation of mulberry germplasm using advanced molecular tools is important because of its precision and stability. The characterisation of mulberry species has provided very valuable species-specific marker as well as demarcated clustering of species. In molecular study, a total of 27 *M. laevigata*, 15 *Morus* species and 46 accessions were studied using RAPD and ISSR markers in collaboration with Seri-biotech Research laboratory (SBRL), Kodathi. The study indicated wide genetic distribution among wild and indigenous species. Currently, a number of molecular markers such as RAPD, ISSR, AFLP, SSR, EST and DNA sequences from both nuclear and plastid genes are being used for elucidating the genetic diversity among cultivars of the same species, inter specific variation, relationships among geographically divergent species and cultivars. RAPD was initially used to study the genetic diversity among a few cultivars in China (Xiang *et al.* 1995; Feng *et al.* 1996; Lou *et al.* 1998; Zhang *et al.* 1998). Later on a group of workers used RAPD markers for assessing inter and intra specific relation in mulberry (Bhattacharya and Ranade 2001; Chatterjee *et al.* 2004; Srivastava *et al.* 2004; Zhao and Pan 2004). Considering the inconsistencies in the results from RAPD markers, attempts were made to explore other DNA markers in mulberry. Consequently, Vijayan and Chatterjee (2003) used inter simple sequence repeat (ISSR) markers to characterize mulberry and found that (AG), (TG), (AC), (ACC), (ATG), (AGC), (GAA), (GATA), (CCCT), (GGAGA) and (GGGGT) produced excellent results. Subsequently, ISSR markers were used to estimate the genetic diversity among indigenous cultivars of *M. alba* and *M. indica* (Awasthi *et al.* 2004; Vijayan *et al.* 2004b, 2004d, 2005, 2006a) and among different ecotypes present in China (Zhao *et al.* 2006, 2008). Relationships between temperate and tropical mulberry species (Vijayan 2004), inter-specific variability (Vijayan *et al.* 2004c) and relationships between cultivated and wild mulberry species (Vijayan *et al.* 2006b) were also investigated in detail using ISSR markers. Sharma *et al.* (2000) used amplified fragmented length polymorphism (AFLP) markers for elucidating the inter-relationships of different mulberry species. In the mean time, efforts were made to develop microsatellite primers

for mulberry. Accordingly, Aggarwal and Udhaykumar (2004) and Zhao *et al.* (2005a) developed simple sequence repeat (SSR) markers for mulberry. In a related investigation, the internal transcribed spacers of nuclear ribosomal RNA (nrITS) and the cpDNA gene (trnL-F) were used for phylogenetic relationships among different species of mulberry in China (Zhao *et al.* 2005b). Recently, Tikader and Dandin (2008) explored the genetic variability among the inter- and intraspecific hybrids of mulberry and reported high level of variation among the hybrids. The fingerprints of genomic DNA indicated that deletion and addition of DNA fragments in the corresponding region of the hybrid genomes makes similar or unequal contribution of parental genetic substances in the process of fertilization, recombination, etc.

Preliminary evaluation

The preliminary evaluation of mulberry genetic resources of growth behaviour and various agronomical traits of different accessions was conducted in an *ex-situ* field gene bank. Growth attributes, disease incidence, rooting behaviour, etc. are highly influenced by agro-climatic conditions. A preliminary evaluation at Hosur agro-climatic conditions was conducted, mainly to study the relative performance of various indigenous and exotic mulberry accessions (Tikader and Rao 2002b). Based on that evaluation, selected mulberry promising accessions are subjected to detailed evaluation in different agro-climatic condition through multi-location trial. During the preliminary evaluation, mulberry accessions with special features are identified based on the leaf yield performance, relative tolerance to diseases and pests and promising accessions are further studied for relative tolerance to abiotic and biotic stresses under hotspot locations.

CSGRC, Hosur has selected 24 promising accessions each from indigenous and exotic for further test in different agro-climatic conditions in collaboration with some network units of Central Silk Board located in different agro-climatic zones and eight centres are involved in the evaluation of mulberry genetic resources. The material was tested and found suitable in different conditions and recommended for large-scale cultivation in farmers' fields.

PROPAGATION STUDIES

As part of a preliminary evaluation, a rooting study was conducted with 60 cuttings in three replications in a randomised block design and the data was recorded after 90 days of planting. A total of 527 accessions have been completed for propagation studies (17 characters) both for above and underground characters (Sau *et al.* 1995; Goel *et al.* 1998). In general, mulberry is propagated through vegetative cuttings, but the wild species are poor in rooting and shows limitation to study the material. The alternative method of propagation is grafting (Tikader and Dandin 2006; Tikader and Thangavelu 2006). *M. laevigata* and *M. serrata* are wild species and very poor in root initiation and observed rooting after grafting on local shoot stock. Wild collections of *M. serrata* generally have more chromosomes i.e., triploid (42), tetraploid (56), hexaploid (84), etc. When the graft is made with normal cultivars (28), the adjustment of chromosome number poses incompatibility and result in unsuccessful grafting. The graft union forms at the initial stage with apparent success gradually develop distress symptoms with time due to failure of producing the callus parenchyma, which is the main reason for low percentage of successful grafts. *M. laevigata* is a wild species, poor rooter and difficult to multiply through vegetative cuttings. *M. laevigata* also showed grafting incompatibility like other wild species of *M. serrata*. Therefore, all the grafting of wild species was not successful due to graft incompatibility. The modified method of grafting was effective and successfully used for multiplication of poor rooting cultivars (Tikader and Thangavelu 2006a).

SELECTION OF MULBERRY GERMPLASM FOR CROP IMPROVEMENT

In order to promote utilization of mulberry germplasm, the available accessions are grouped into different categories (Tikader and Dandin 2006b) for the convenience of breeders and other users:

1. Faster growth and related traits
2. High rooting and root proliferation
3. High leaf nutritive quality
4. High biomass production
5. High leaf yield
6. Resistance to pest and diseases.

DISTRIBUTION OF MULBERRY IN DIFFERENT AGRO-CLIMATIC REGIONS

Mulberry is distributed in different agro-climatic condition. It is necessary to know the distribution of mulberry accessions to select the germplasm for specific purpose. A total of 612 mulberry accessions are grouped based on their origin and distribution (Table 4).

Table 4 Distribution of mulberry in different agro-climatic zone.

Region	Indigenous	Exotic	Total
Tropical wet	185	52	237
Tropical dry	105	16	121
Sub-tropical (humid)	130	85	215
Semi-arid	10	----	10
Arid	4	----	4
Highland	25	----	25
Grand total	455	157	612

Preliminary evaluation in Augment Design

After characterisation, mulberry accessions are subjected for evaluation according to farmers' practice i.e. bushes type (90 × 90 cm) at CSGRC. During 1996, 316 accessions (indigenous – 234, exotic – 78) were evaluated in an Augment Design following recommended cultural practices (Thangavelu *et al.* 2003). The experiment was continued for 3 years, 4 harvests/year and for a total of 12 crops. In a perennial crop like mulberry, a minimum gestation period of 6 months is required to stabilize the leaf yield. The yield attributes were compared with the check varieties MR2 and K2 (indigenous) and Kosen and China white (exotic). The best performing accession may be further evaluated through multi location trials in hot spots. In phase II, a total of 196 accessions (indigenous – 163 and exotic – 33) of mulberry are under evaluation.

Evaluation of mulberry genetic resources under All India Co-ordinated Experiment – Authorisation of mulberry variety

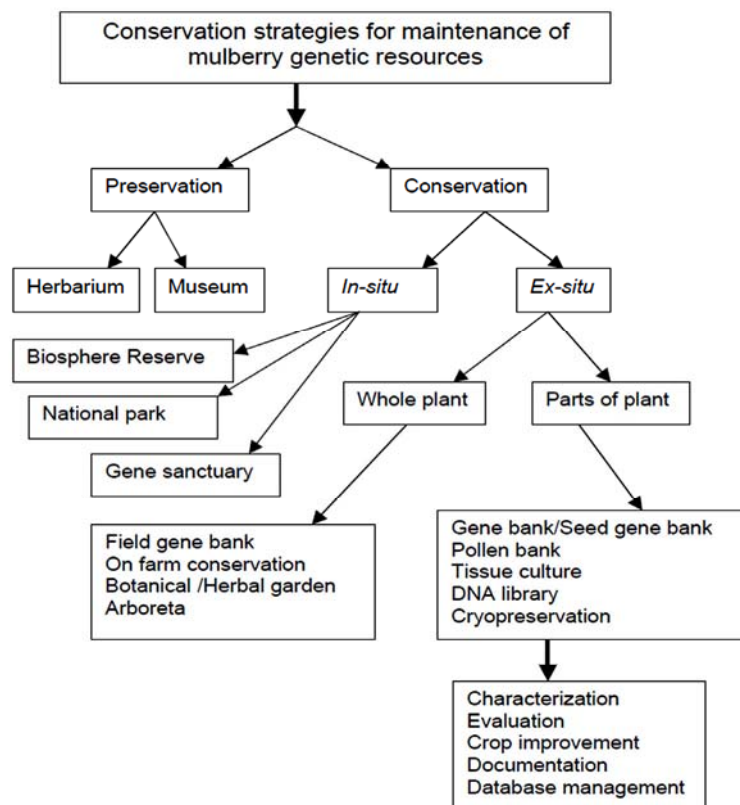
To develop a region-specific variety suitable to a particular zone, an All India Coordinated Experiment (AICE) was conducted under Central Silk Board, Bangalore. After completion of the experiment several varieties were recommended for different agro-climatic regions (Table 5). Presently, the second phase of AICE is in progress in different climatic zones with eight-mulberry genotypes viz., Victory-1, Anantha, RFS-175, C1730, C2016, C2017, Thalaghattapura (TG) and Vishala.

CONSERVATION OF MULBERRY GENETIC RESOURCES

In the broadest sense, conservation incorporates elements of enhancement. 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 conser-

Table 5 List of mulberry varieties authorized for different agro-climatic zones.

Name of the mulberry varieties authorized	Regions/Zones
S 1635 DD	North Karnataka, Andhra Pradesh, Tamil Nadu and Maharashtra (Irrigated, high temperature, black soils).
S 1635 S 36 DD	Traditional areas of South Indian states viz., Karnataka, Andhra Pradesh, Tamil Nadu and Kerala (Irrigated red soils).
S 1635 S1	Central India (Irrigated conditions)
S 799 S 1635 S1	West Bengal, Uttar Pradesh, Bihar and Assam (Rainfed conditions).
S 13 S 34	Karnataka, Andhra Pradesh, Tamil Nadu (Rainfed conditions: S 13 for red soil, S 34 for black soil).
TR10 BC259 S146	Hilly Eastern areas (Rainfed conditions).
S146 TR10 S146	Central India, Doon Valley and Himachal Pradesh (Rainfed conditions).
Chak Majra Chinese White	North Western Jammu and Himachal Pradesh (Rainfed conditions).

**Fig. 1** Conservation strategies for maintenance of mulberry.

vation of mulberry is a holistic concept that encompasses a wide spectrum of activities ranging from establishment of protected areas to building of DNA libraries. 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 are (i) *in-situ*, (ii) *ex-situ*, (iii) field gene bank, (iv) on-farm participatory, v) *in-vitro* and (v) cryopreservation (Fig. 1).

***In-situ* conservation**

In-situ conservation is defined as the conservation of ecosystem and natural habitats and the maintenance and recovery of viable population of species in their natural surroundings and in the case of domesticated or cultivated species, in the surroundings where they have developed

their distinctive properties (UNEP 1992).

In-situ conservation of wild mulberry is difficult for various technical and administrative reasons. 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 resources, national parks, sanctuaries, etc. (Tikader and Dandin 2006b).

The interesting feature of *in-situ* conservation of mulberry is that the sacred mulberry tree (*M. serrata*) at Joshimath is being worshipped by the pilgrims of Badrinath, which is said to be the oldest and biggest mulberry tree in the world and Adiguru Shri Shankarcharya is said to have meditated under this tree. Because of this reason all *M. serrata* trees found at several places namely, Shirmoli near Almora, Dhaniya enroute to Pithoragarh, Ulkadevi temple at Pithoragarh, Gwaldam etc. which are being conserved and worshipped. Similarly, trees of *M. laevigata* are con-

Table 6 Status of mulberry genetic resources maintained at other Institutes.

Sl no.	Institutes / Universities	Accessions
1	CSGRC, Hosur, Tamil Nadu	1120
2	CSR & TI, Mysore, Karnataka	342
3	CSR & TI, Berhampore, West Bengal	224
4	CSR & TI, Pampore, Jammu & Kashmir	75
5	KSSRDI, Thalaghattapura, Karnataka	376
6	RSRS, Miransahib, Jammu, Jammu & Kashmir	61
7	RSRS, Sahaspur, Dehradun, Uttaranchal	64
8	SSBC, Coonoor, Tamil Nadu	48
9	SKUAST, Srinagar, Jammu & Kashmir	55
10	Karnataka University, Dharwad, Karnataka	10
11	Mysore University, Manasgangotri, Karnataka	25
12	Pamoate Mahila Viswavidyalaya, Tirupati, Andhra Pradesh	28
13	Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu	33
14	University of Agricultural Sciences, Dharwad, Karnataka	30
15	Marathwada Agriculture University, Parbhani, Maharashtra	12

served in Government Office premises namely at Public Works Department Bungalow of Haldwani, Sujanpur (Punjab) and in the natural forest of Havelock island (Andaman and Nicobar Islands), etc. Attempts have been made to locate and identify the concentrated areas of wild/natural mulberry trees viz., Havelock Island, Chidiatapu, Interview and long island of Andaman, Laha of Arunachal Pradesh for *M. laevigata*. Pandukeshar, Chakrata, Urgan Valley, Joshimath, Mussoorie and Pithoragarh areas of Himalaya for *M. serrata*. Large number of naturally grown *M. laevigata* trees is also available in Kalimpong, Assam, Meghalaya and Manipur. Besides this, developed gene pool of *M. alba*, *M. indica* and *M. laevigata* are also available in Western Ghat, Madhya Pradesh and Mahabaleshwar, which are required to be protected.

Ex-situ conservation

Biological diversity may be conserved outside the area where it naturally occurs. *Ex-situ* conservation refers to the conservation in botanic gardens, on-farm conservation by farmers with traditional agricultural systems or arboreta or field gene banks. Mulberry germplasm maintained in different organisations are presented in **Table 6** (Tikader and Dandin 2006b). The safety back up accessions available in other institutes/organisations is presented in **Table 7**.

Table 7 Status mulberry genetic resources at CSGRC, Hosur.

Status of mulberry genetic material	Accessions
Total accessions at CSGRC, Hosur	1109
Collection through Institutes/Universities	709
Collection through exploration	400
Safety back up status	
Safer (More than one safety back up)	189 (Ind - 96, Exo - 93)
Sensitive (Only one safety back up)	217 (Ind-144, Exo - 73)
More sensitive (No safety back up)	247 (Ind-154, Exo - 93)

Field gene banks

Field gene banks are essential and reliable means of *ex-situ* conservation of genetic diversity. Gene banks usually maintain basic and long-term collection and hence require extensive facilities and infrastructure.

CSGRC, Hosur maintain 1120 accessions (indigenous - 856; exotic - 264) in the field gene bank as *ex-situ* conservation. The indigenous collection represents 24 states in India whereas exotic collections represent 25 countries. All the accessions are maintained as dwarf tree with a spacing 2.4 × 2.4 m. Pruning is done once in a year for conservation. Recommended cultural practices are followed to maintain the field gene bank (Tikader *et al.* 1999, 2000, 2001).

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. In India, rich *Morus* diversity exists under managed habitats i.e. in the backyards, kitchen gardens, farmhouses, horticultural gardens, agricultural lands and roadside plantations (Tikader and Dandin 2006b). These are the first hand selections of the farmers and local communities (tribals) for varied utilizations 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. However, these wild species have been used for other non-sericultural purposes such as horticulture and agro forestry. 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.

In vitro approaches

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). The shoot tip culture of mulberry was less problematic when they are maintained over long periods of time. The culture of tropical and temperate plant species can be maintained on normal or modified culture media at normal culture room (25 to 28°C) for a number of years with annual or biennial subculturing (Mandal 1993). The vegetatively propagated material as in the case of mulberry can be maintained in an *in vitro* repository and should become ideal back-ups for the collections of field genebank. Slow growth *in vitro* can be accomplished either by allowing cultures to survive on minimal media or by reduction in sucrose, thus restricting the availability of nutrients to cultures. Use of osmotic agent such as sorbitol or mannitol also helps in achieving the reduction of growth. *In vitro* culture techniques have been extensively developed and applied to more than 1000 species including many tropical species (Engelmann 1991, 1997). Many National and International Organizations have used *in vitro* techniques as a complementary conservation method to field gene bank for conservation of germplasm of various vegetatively propagated and recalcitrant seed species. Over 37,600 accession of germplasm of various crops have been covered worldwide (FAO 1994).

Cryopreservation

The conservation of mulberry in the field gene bank is simple and technically less demanding, but it requires vast resources like fund, manpower and land. Further, the collections in the field gene bank are exposed to natural disaster and attack of pests and pathogens. To overcome these, the alternative method is cryopreservation of vegetative buds. The possibility of storage of woody cuttings of scion material by cryopreservation, which is successfully followed in other sericultural countries, is being considered for long-term preservation under Indian condition.

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 vitrification-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 vitrification 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 facilities for tissue culture. 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 1992; Niino *et al.* 1992a, 1992b, 1993; Niino 1995). For instance, the cryopreservation facilities at the Central Sericultural Germplasm Resources Centre (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. latfolia*, *M. cathayana*, *M. laevigata*, *M. nigra*, *M. australis*, *M. bombycis*, *M. sinensis*, *M. multicaulis* and *M. rotundiloba*. Encapsulation of winter-hardened shoot tips of many mulberry species with calcium alginate coating was also tested successfully. In addition, Yakua and Oka

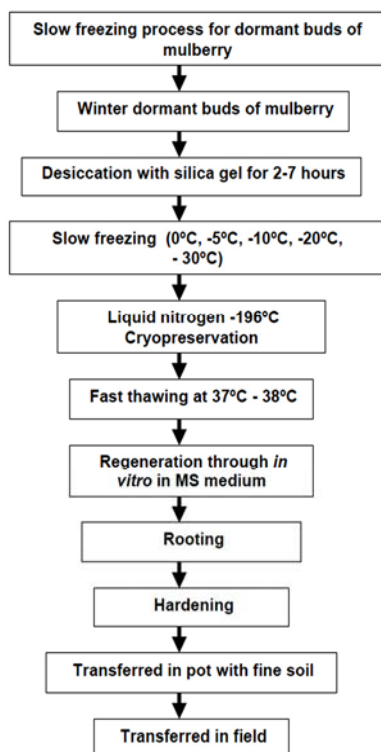


Fig 2 Cryopreservation through slow freezing.

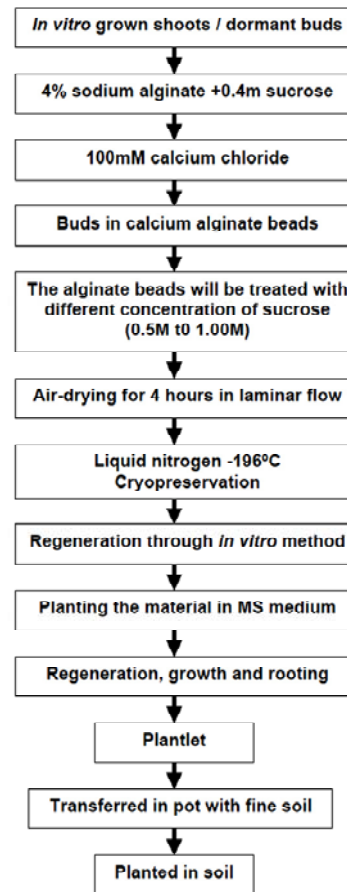


Fig 3 Encapsulation and dehydration process of cryopreservation.

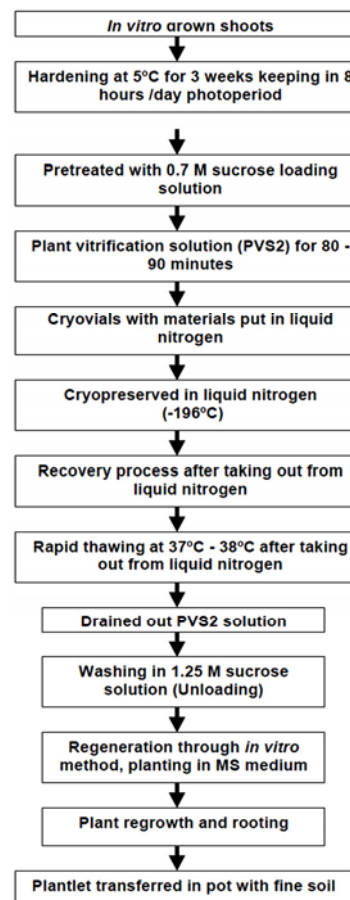


Fig 4 Vitrification process of cryopreservation.

(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 meristem was excised for culture on 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. The different methods for cryopreservation have been standardized for long-term preservation of *Morus* species (Figs. 2-4).

Supply of mulberry genetic resources

Any germplasm maintained by Institutes are meant for utilization in different form. As a part of utilisation of mulberry germplasm, CSGRC, Hosur has promoted supply of mulberry germplasm to scientists/universities/research institutes and Department of Sericulture. The supply programme initiated from 1997 and to date, a total of 1480 accessions have been supplied to 31 organisations (Tikader *et al.* 2002a). Besides, under All India Mulberry and Silkworm Germplasm Evaluation Programme (AIMSGEP) 24 accessions each of indigenous and exotic have been supplied to 8 network units of Central Silk Board. During supply of mulberry germplasm, quarantine and phytosanitary certificate is being issued to avoid the contamination due to pests and pathogens.

STATUS OF HOST PLANTS OF NON-MULBERRY (WILD) SILKWORM AND MANAGEMENT

The role of plant genetic resources in the improvement of cultivated plants has been well established. The global action plan of FAO (1994) has emphasized the characterisation, evaluation and development of core collection to facilitate the utilisation of more germplasm. High priority has been given to the development of crop specific characterisation and evaluation programme to identify suitable accessions for cultivation.

India is the only country in the world where all the five known types of commercial silks are available in wild/semi-domesticated and domesticated conditions (Thangavelu *et al.* 2000; Srivastav and Singh 2002). Tropical tasar plant is represented by 13 species, distributed in 20 states and 124 genetic resources of host plant is being maintained in the *ex situ* gene bank. One Research Institute and 4 regional stations are involved in germplasm research in tropical tasar. The oak tasar plant represents 16 species distributed throughout the sub-Himalayan region from Jammu and Kashmir in the Northwest to Manipur in the Northeast. Muga plant is distributed in 11 states with 15 types of plants. The eri plant has 16 species with 41 germplasm maintained at Mendipathar, Meghalaya.

Tropical tasar silkworm host plant

The polyphagous tasar silkworm feed on different plants. Among them the primary plants are *Terminalia arjuna*, *T. tomentosa*, *Shorea robusta* (Thangavelu *et al.* 2000a). There are several secondary and tertiary plants of tropical tasar silkworm (Table 8).

Central Tassar Research and Training Institute (CTR&TI), Ranchi is maintaining a total of 124 genotypes of *T. glabra* syn. Putative hybrids of *T. glabra*, *T. berryi*, *T. alata*, *T. coriacea*, *T. crenuleta*, *T. arjuna*, *T. tomentosa*, *T. chebula* and *T. bellerica*. Systematic characterisation and evaluation is under progress at CTR&TI, Ranchi. For descriptor recording, a *Terminalia* (section: Pentaptera) has been prepared on the basis of observations made on 130 accessions collected from Central India (Srivastav *et al.* 1997). For characterisation and evaluation, a minimal descriptor is available to record the data in respect of morphology (5 descriptors), reproductive (7 descriptors), growth (7 des-

Table 8 Food plants of tropical tasar silkworms.

Primary food plants	
1. <i>Terminalia arjuna</i>	
2. <i>Terminalia tomentosa</i>	
3. <i>Shorea robusta</i>	
Secondary food plants	
1. <i>Anogeissus latifolia</i>	4. <i>Zizyphus jujube</i>
2. <i>Hardwickia binata</i>	5. <i>Zizyphus mauritiana</i>
3. <i>Lagerstroemia parviflora</i>	
Tertiary food plants	
1. <i>Artocarpus lakoocha</i>	23. <i>Garuga pinnata</i>
2. <i>Bauhinia variegata</i>	24. <i>Lagerstroemia indica</i>
3. <i>Bombax ceiba</i>	25. <i>Lagerstroemia speciosa</i>
4. <i>Buchanania latifolia</i>	26. <i>Madhuca indica</i>
5. <i>Canthium didymum</i>	27. <i>Melastoma malabathricum</i>
6. <i>Careya arborea</i>	28. <i>Messua ferrea</i>
7. <i>Carissa carandas</i>	29. <i>Mimusops elangi</i>
8. <i>Celastrus paniculatus</i>	30. <i>Prymus domestica</i>
9. <i>Chloroxylon sweitenia</i>	31. <i>Pterocarpus marsupium</i>
10. <i>Cipadessa fruticosa</i>	32. <i>Rhizophora caseolaris</i>
11. <i>Dalbergia sissoo</i>	33. <i>Semecarpus anacardium</i>
12. <i>Diospyros lanoxylon</i>	34. <i>Shorea talura</i>
13. <i>Dodonea viscosa</i>	35. <i>Syzygium cumini</i>
14. <i>Embelica officinalis</i>	36. <i>Syzygium jambos</i>
15. <i>Ficus bengalensis</i>	37. <i>Tectona grandis</i>
16. <i>Ficus benjamina</i>	38. <i>Terminalia bellerica</i>
17. <i>Ficus hispida</i>	39. <i>Terminalia catappa</i>
18. <i>Ficus religiosa</i>	40. <i>Terminalia chebula</i>
19. <i>Ficus retusa</i>	41. <i>Terminalia paniculata</i>
20. <i>Ficus tjakela</i>	42. <i>Zizyphus rugosa</i>
21. <i>Ficus tsiela</i>	43. <i>Zizyphus xylopyrus</i>
22. <i>Gardenia lucida</i>	

criptors), biochemical (5 descriptors), biotic stress (1 descriptor) and others (2 descriptors) (Kumar *et al.* 2002). For effective utilisation of germplasm and develop superior varieties of plants, proper characterisation, documentation, maintenance, evaluation, clonal propagation and other biotechnological works are essential.

Temperate tasar silkworm host plant

Indian oak provides a unique ecosystem having both deciduous and evergreen forests and covers 29 lakh acre distributed from Jammu and Kashmir in the North west to Arunachal Pradesh in the North East. In India, more than 35 species of Oaks have been reported (Negi and Nathani 1995). Among the various species of oak, 16 species are popular and widely distributed in different parts of the country. A total of 10 species are growing in eastern part of the country and 6 species in the Western Himalayas. However, the recent report states, out of 16 species of *Quercus*, 6 species have been identified for rearing of oak tasar silkworm in different states of India (Misra *et al.* 2000). At present 5 species of oaks i.e., *Q. serrata*, *Q. griffithii*, *Q.*

Table 9 Food plants of temperate (oak) tasar silkworms.

Primary food plants	
1. <i>Lithocarpus dealbata</i> (Jr. syn. <i>Q. dealbata</i>)	
2. <i>Quercus acutissima</i> (Jr. syn. <i>Q. serrata</i>)	
3. <i>Quercus floribunda</i>	
4. <i>Quercus griffithii</i>	
5. <i>Quercus leucotrichophora</i> (Jr. syn. <i>Q. incana</i>)	
6. <i>Quercus semecarpifolia</i>	
7. <i>Quercus semiserrat</i> (Jr. syn. <i>Q. himalayana</i>)	
Secondary food plants	
1. <i>Castanopsis indica</i>	7. <i>Quercus glauca</i>
2. <i>Castanopsis lancaefolia</i>	8. <i>Quercus kamroopi</i>
3. <i>Castanopsis purpurella</i>	9. <i>Quercus lamellosa</i>
4. <i>Lithocarpus fenestrata</i>	10. <i>Quercus lanata</i>
5. <i>Lithocarpus xylocarpa</i>	11. <i>Salix viminalis</i>
6. <i>Quercus baloot</i>	

Table 10 Food plants of Muga silkworms.

Primary food plants	
<i>Litsaea polyantha</i>	
<i>Persea bombycina</i> (Jr. syn. <i>Machilus bombycina</i>)	
Secondary food plants	
<i>Cinnamomum camphora</i>	
<i>Cinnamomum tamala</i>	
<i>Litsaea citrata</i>	
Tertiary food plants	
1. <i>Actinodaphne augustifolia</i>	9. <i>Litsaea salicifolia</i>
2. <i>Actinodaphne obovata</i>	10. <i>Machilus odoritissima</i>
3. <i>Celastrus monosperma</i>	11. <i>Magnolia pterocarpa</i>
4. <i>Cinnamomum cecicodaphne</i>	12. <i>Michelia champaca</i>
5. <i>Cinnamomum glanduliferum</i>	13. <i>Michelia oblonga</i>
6. <i>Cinnamomum obtusifolium</i>	14. <i>Symplocas grandiflora</i>
7. <i>Gmelina arborea</i>	15. <i>Symplocas paniculata</i>
8. <i>Litsaea nitida</i>	16. <i>Symplocas ramosissima</i>

Table 11 Food plants of Eri silkworms.

Primary food plants	
1. <i>Ricinus communis</i>	
2. <i>Heteropanax fragrance</i> .	
Secondary food plants	
1. <i>Manihot utilitissim</i>	
2. <i>Evodia fraxinifolia</i>	
Tertiary food plants	
1. <i>Ailanthus excelsa</i>	8. <i>Jatropha multifida</i>
1. <i>Ailanthus glandulosa</i>	9. <i>Micromelum pubescence</i>
2. <i>Ailanthus grandis</i>	10. <i>Plumeria acutifolia</i>
3. <i>Ailanthus tryphysa</i>	11. <i>Sapium eugenifolium</i>
4. <i>Carica papaya</i>	12. <i>Sapium sebiferum</i>
5. <i>Coriaria nepalensis</i>	13. <i>Xanthoxylum alatum</i>
6. <i>Hodgsonia heteroclita</i>	14. <i>Xanthoxylum rhesta</i>
7. <i>Jatropha curcas</i>	

leucotrichophora, *Q. himalayana* and *Lithocarpus dealbata* are maintained at the Regional Tasar Research Station, Imphal (Srivastav and Singh 2002). There is an urgent need for systematic characterisation, evaluation and documentation of oak tasar plants. The list of primary, secondary and other plants are mentioned in **Table 9**.

Muga silkworm host plant

Muga silkworm (*Antherea assama* Westwood) is polyphagous and feeds on the leaves of several plants. *Persea boby-cina* (*Machilus bombycina*, King Jr. syn) and *Litsea poly-antha* Juss are the primary plants. The other secondary plants are *L. citrata*, *L. salicifolia*, *L. nitide*, *C. obtusifolium*, *Zanthoxylum rhesta*, *Mangolia sphenocarpa*, *Gmelina arborea*, *Celastrus momosperma* and *Zizyphus jujuba* (Thangavelu *et al.* 1988). The primary and secondary plants are presented in **Table 10**. The plants are distributed throughout

North-eastern region and also in hill tracts of Himalayan and Western ghats. Many morphotypes and their variants are available in nature. So far 14 morphotypes of som and 10 morphotypes of soalu have been identified, collected and maintained at Regional Muga Research Station, Boko, Assam. Morphology, floral biology, bioassay and chemo assay studies have been conducted in Som collections (Das *et al.* 2000; Thangavelu *et al.* 2005). Besides this, 8 distinct morphotypes have been identified and isolated on the basis of leaf morphology and floral biology from the populations collected from Boko and Borahibari, Kamrup and Sibsagar districts of Assam, India (Raja Ram *et al.* 1993). Among the variants there is significant difference in their performance for growth and bioassay. The morphotypes are useful to evolve some superior hybrids.

Eri silkworm host plant

Although mulberry dominates silk variety the global silk market, among the non- mulberry silks, eri is extensively utilized as a dazzling white natural fibre with thermal properties. The eri silkworm feeds mainly on castor (*Ricinus communis* Linn.) leaves. The eri silkworm is also polyphagous and feeds on variety of plants i.e., Castor, Kesseru, as primary plants, Tapoica, Payam as secondary plants and Borkessuru, Gulancha and Gamari as tertiary plants (Sarmah 2000). Regional Eri Research Station at Mendipathar, maintains Castor (41 accessions), Kessuru (1), Tapoica (12), Payam (1), Borkessuru (1), Gulancha (1) and Gamari (1). On the basis of morphology and growth characters 41 castor germplasm have been characterised. The plants of eri silkworm are mentioned in **Table 11**.

Registration of sericultural germplasm

The Sericultural Germplasm Registration of Central sericultural Germplasm Resources Centre (CSGRC) Hosur, Central Silk Board has developed a system for registration of sericultural germplasm. It is very important in the light of GATT agreement and patent policy to safeguard the sovereign right of the nation over the natural resources and national asset. CSGRC, Hosur has registered the following mulberry and non-mulberry host plants (**Table 12**).

CONCLUSION AND FUTURE STRATEGY

Silk production in India secured 2nd position after China and India is the only country in the world, where all the five known types of commercial silks are available in wild/semi-domesticated and domesticated conditions (Datta 2002). The progress highlights the dominance of mulberry silk and more and more research carried out on mulberry silkworm and its host plants. As the other silk is wild or semi-domesticated, more research is required to be carried out to unravel the unknown facts as well as conservation of

Table 12 List of registered silkworm host plants.

Variety/ Race	ISGR Reg.No.	Methodology
MULBERRY		
S1	02 001	Selection from clone
S1635	02 002	Selection from seedling of open pollinated seeds collected from mother plant CSRS-1
TR10	02 003	Cross between colchicines induced tetraploid of (<i>Morus indica</i> x Mandalaya) x Phillippine (2x)
BC259	02 004	Back crossing of female plant (<i>Morus indica</i> Var. Matigara x Kosen) and with recurrent parent Kosen
S799	02 005	Selection from seedlings of open pollinated seeds of unknown mother plant
C1730	02 006	Crossing with a tetraploid mother (T25) and diploid male parent (S162)
NON MULBERRY (SOM)		
MH(Som) MT-1	03 012	Selection from survey and exploration materials and designated as morphotype – 1 (MT-1)
MH(Som) MT-2	03 013	(MT- 2)
MH(Som) MT-3	03 014	(MT- 3)
MH(Som) MT-4	03 015	(MT- 4)
MH(Som) MT-5	03 016	(MT- 5)
MH(Som) MT-6	03 017	(MT- 6)
MH(Som) MT-7	03 018	(MT- 7)
MH(Som) MT-8	03 019	(MT- 8)

the food plants. The biotechnological aspects are the virgin field in case of non-mulberry silk sector and needs to be addressed properly. Maximum local people and tribals depend on the host plant as well as their livelihood on wild silkworms. Due to inadequate steps, the forest resources are decreasing day by day and the tribals are losing their lands as well as forests. Since knowledge on interspecies relationships is essential for effective strategies on conservation and utilization of genetic resources, concerted efforts are to be made to deduce the phylogenetic relationships among the species of host plants of seri-genetic resources using both molecular and other morpho-biochemical characteristics. Systematic studies on different aspects will help to chalk out proper strategy to utilize and conserve the host plant of seri-genetic resources in effective and efficient way.

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