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Conservation and utilization of plant genetic resources with special emphasis on mulberry (*Morus* spp.)

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Abstract
Mulberry is originated in the foothills of Himalayas and later spread to Asia, Africa, America and Europe etc. It belongs to genus *Morus* and family Moraceae. Mulberry (*Morus*), a fast-growing deciduous woody tree of the family Moraceae, is grown widely in Asian countries for its leaves to feed the silkworm *Bombyx mori*, which feeds exclusively on mulberry leaves. Hence, mulberry is one of the most important components that decide the sustainability of this multibillion dollar industry. There are more than 70 countries which produce silk, among which China, India, Vietnam, Uzbekistan, Brazil, Thailand, and Bangladesh are the leaders. Although, the maximum utilization of mulberry is in Asia, this does not mean that mulberry is restricted only to Asian countries. Though, more than 68 species have been widely recognized, the taxonomy of mulberry is still a matter of great dispute and intense research due to the high rate of natural hybridization among the species. Hence, a large number of the so-called species are hybrids and their true taxonomic identity is difficult to deduce properly.

Keywords: Plant genetic resources, mulberry, Mulberry *(Morus)*

Introduction
All crops, whether traditional varieties selected and harvested by farmers or modern varieties bred by professional plant breeders, descend from wild and improved genetic resources (also called germplasm) collected around the world. Biodiversity encompasses all organisms, species, populations, the genetic variation and assemblages of communities and ecosystems among these organisms. The variations in biodiversity occurring in nature, is what has sustained the harmonious existence of life on earth. Although, biodiversity in general has enormous importance, plant genetic resources (PGR) constitute the foundation upon which agriculture and world food security is based. As the threat from urbanization, climatic changes, out breaks of new diseases and pests, and the frequent occurrence of natural calamities increases as a result of global warming, conservation of plant genetic resources is seen widely as a necessary step to preserve the world's germplasm for posterity. Conservation strategies for vegetatively propagated plants are different from those adopted for conserving annual crops including mulberry. This article summarizes the strategies that are best suited for better and safer conservation of precious genetic germplasm of mulberry, an economically important tree of Asia.

Plant genetic resources: Definition and importance
Genetic diversity simply means all the variety of genes that exist in a particular variety or species. 'Biological diversity' or 'biodiversity' refers to all forms of life - plants, animals and microorganisms – and the ecosystems in which they exist and interact. Biological diversity exists at three levels– ecosystem, species and variety level [1]. As far as PGR are concerned, genetic diversity within species is often more important than the diversity between species. Germplasm refers to the hereditary materials transmitted to the offspring through the germ cells. It is the total content of genes that serves as the raw material for the breeder to develop different crops. In other words, germplasm can be defined as living tissue from which new plants can be grown. It can be a seed or another plants part; a leaf, a piece of stem, pollen or even just a few cells that can be turned into a whole plant. Germplasm is the living genetic resources such as seeds or tissue that is maintained for the purpose of animal and plant breeding, preservation and other research uses.

These resources may take the form of seed collections such as seed banks, trees growing in nurseries, animal breeding lines maintained in animal breeding programmes or gene banks etc. It contains the information for a species genetic makeup, a valuable natural resource of plant diversity. Germplasm collections can range from collections of wild species to elite, domesticated breeding lines that have undergone extensive human selection. The main objective of germplasm collection is preservation of genetic diversity of a particular plant or genetic stock for its use in the future.

Types of germplasm
Germplasm can be organized in six different categories based on their station or advancement in the agro-ecosystem. These include.

Advanced (Or elite) germplasm
1. "Cultivarsor cultivated varieties" which are suitable for planting by farmers, either recently developed cultivars or„ Obsolete "cultivars that are no longer grown.
2. Advanced breeding materials that breeders combine to produce new cultivars (sometimes referred to as "breeding materials").

Improved germplasm
This is any plant material containing one or more traits of interest that have been incorporated by scientific selection or planned crossing.

Landraces
These are varieties of crops improved by farmers over many generations without the use of modern breeding techniques. Within a modern breeding programme, landraces are sometimes used for resistance traits and generally required before their genes can be used in a final variety.

Wild or weedy relatives
Are plants that share a common ancestry with a crop species but have not been domesticated. These plants can serve as another source of resistance traits, but these traits can be very difficult to incorporate on final varieties.

Genetic stock
These are mutants or other germplasm with genetic abnormalities that may be used by plant breeders for specific purposes. Genetic stocks are often used for highly sophisticated breeding and basic research.

Benefits of germplasm collection
Collection of germplasm has been found to yield many advantages and these benefits are outlined below:
- Cell and tissue culture of many plants species can be cryopreserved and maintained in a viable state for several years and used when required.
- Plant material from endangered species can be conserved using this method.
- It is an ideal method for long term conservation of cell cultures producing secondary metabolites such as antibiotics
- Recalcitrant seeds (seeds which lose their viability on storage) can be maintained for a long period of time.
- Disease free plants material can be frozen and propagated whenever required.
- Conservation of soma clonal variations in cultures.
- Rare germplasms developed by using somatic hybridization and other genetic manipulation techniques can be stored.
- Pollen conservation for enhancing longevity.
- Germplasm banks facilitate the exchange of information at international level.

Germplasm preservation and propagation
Through the knowledge of germplasm, various breeding techniques of plants have been developed. Hence the storage or preservation of germplasm is important. Conventionally seeds were used to store the germplasm, but in case where seeds cannot be used for regeneration of plants or in case where shoot and root tissue is not stable, it is then important to preserve them. Germplasm preservation can be done by two broad methods namely, *in-situ* preservation and *ex-situ* preservation". In situ preservation includes the organization and/or servicing of natural supplies where species are permitted to stay in maximum environment with the lowest of management. On the other hand, *ex-situ* preservation includes the use of botanic landscapes, field farms, seeds shops and gene financial banks. However, within each type of preservation, there are numerous techniques/mechanisms and associated problems. These are discussed as follows.

Cryopreservation
The freeze preservation of cells or tissues in liquid nitrogen at -196 ^{0}C is known as cryopreservation. This technique involves four steps.

a) Freezing
The procedure of freezing may be conducted slowly, rapidly or initial freezing by dropping temperature slowly and followed by a rapid decrease in temperature. In order that the plants are affected by the sudden decrease in temperature, treatment of cells with plant verification solution helps cells and tissue to overcome the harsh temperature. The medium was added with cry protectant like DMSO, glycerol and proline to the culture medium to protect cells from injury. The addition of cry protectant protects the cell by prevention of large crystals inside cell, protect from water loss from cell. The frozen cells are stored in a refrigerator containing liquid nitrogen. The temperature of such refrigerator is maintained at or below -130 ^{o}C. Organized tissues like shoot tips, somatic and zygotic embryos are usually chosen for storage. Attentively cells can be immobilized in sodium alginate and then cryopreserved.

b) Thawing
Thawing of culture is done in a rapid process. The freeze preserved culture is dipped in a water bath containing water at about 37-40 ^{o}C for 90 seconds. This process is done rapidly so that no ice crystals are formed. The thawed culture is washed several times to remove cryoprotectant. In the recent times, the cryoprotectant is removed by diluting. This is done by fixing the culture along with a cryoprotectant onto a disk and is kept on a suitable medium. The disk is frequently transferred into a fresh medium. This frequent transfer dilutes out the cryoprotectant.

c) Re-culture
The culture which is freeze preserved nee/d to be thawed and cultured to bring it back to normal life. The optimum conditions of freeze preserved plants have to be determined

for developing a successful re-culture. After cryopreservation, some plants tend to show special requirement for growth which was not necessary under normal propagation of the corresponding plants. For example, tomato shoots tips when cryopreserved, thawed and re-cultured, the culture required some levels of abscisic acid in their medium in order to initiate and develop shoot tip from callus formed.
It has been found that mostly meristematic cells survive cryopreservation than other cells. In parts where the germplasm cannot be stored in seeds or other parts the cryopreservation provides a good option of storage and future usage.

Slow growth cultures: This is another method that can be used in germplasm preservation and propagation. It involves limiting the conditions of growth so that the culture does not grow and propagate in ordinary pace. This can be achieved by limiting the factors affecting the growth. This provides an attractive alternative to cryo-preservative as the procedure is cost effective and simply comparatively. There is also reduction of contamination and gene modification. The various factors affecting the growth of cultures are temperature, nutrient restriction, growth regulation and osmotic concentration. Other factors that can affect growth of cultures include oxygen concentration, type of culture vessel used as well as restriction of illumination received by cultures.

DNA clones: The germplasm can also be preserved in DNA segments cloned into appropriate vectors but the process demands high expertise and is costly.

Artificial seeds: Another mechanism of germplasm preservation is by desiccating embryos and storing it as artificial seeds. This has proved to be an effective technique, but was possible only with somatic embryo and in certain cases by shoot tips. This process of germplasm preservation offers several advantages like cost effective, availability of germplasm of specific plants to propagate, small storage space, and longer terms of storage. In addition, it reduces risks such as cell damage by cryopreservation, and does not involve high technology associated with other methods.

d) *In-vitro* storage

This is another method of germplasm preservation which is now routinely used for germplasm of some crops like cassava. The in-vitro cassava gene bank at CIAT, Colombia, comprises nearly 5000 clones, in an area of 50 square meters, with transfer (subculture) intervals of 12 – 14 months [2]. While in-vitro storage thus offers some advantage over field genebanks, such as requiring less space and limited labour cost (Towill, 1988) [2], however, the management of large collections remains problematical, due to the requirement for periodic subculture. The possible introduction of genetic variants during culture may be a risk with some types of culture.

Cold storage: In this technique, bulbs, tubers and rhizome of certain crops can be stored at 0 -15 ^oC under high humidity for several months or up to one year. However, it is also a type of short- term storage.

Super-cold storage: This technique allows the embryos, tissues and pollen grains to be stored for long periods in liquid nitrogen at -196 ^0C. However, its practical use is yet to be developed as crops require different cooling and thawing treatments and have different viability level.

Field conservation: Germplasm materials such as fruit trees, potatoes and grasses are grown in nurseries field for preservation. Field nurseries can be maintained at different elevations above sea level.

Mulberry genetic resources: Distribution, conservation and utilization

Origin, importance and distribution of mulberry

Mulberry is originated in the foothills of Himalayas and later spread to Asia, Africa, America and Europe etc., [3]. It belongs to genus *Morus* and family Moraceae. Mulberry (*Morus*), a fast-growing deciduous woody tree of the family Moraceae, is grown widely in Asian countries for its leaves to feed the silkworm *Bombyx mori*, which feeds exclusively on mulberry leaves [4]. Hence, mulberry is one of the most important components that decide the sustainability of this multibillion dollar industry [5]. There are more than 70 countries which produce silk, among which China, India, Vietnam, Uzbekistan, Brazil, Thailand, and Bangladesh are the leaders [6]. Although, the maximum utilization of mulberry is in Asia, this does not mean that mulberry is restricted only to Asian countries. Though, more than 68 species have been widely recognized [7], the taxonomy of mulberry is still a matter of great dispute and intense research due to the high rate of natural hybridization among the species [8, 9]. Hence, a large number of the so-called species are hybrids and their true taxonomic identity is difficult to deduce properly [10]. To complicate things further, different ploidy levels ranging from diploids with 28 chromosomes to docosaploid with 308 chromosomes are very common among most species [11]. Out of these 68 species, only a few, mostly belonging to white mulberry (*Morus alba*), are used for sericulture while a few other species such as red mulberry (*Morus rubra*) and black mulberry (*Morus nigra*) are used for fruits. Considering the great economic value attached with mulberry, several countries have already made extensive efforts to collect and conserve mulberry [12]. In mulberry, a total of 150 *Morus* species were recognized but only 68 species were given more importance, based on their use in silkworm rearing, medicinal value and sweetness of fruit. Each species has its own unique importance such as *M. alba, M. indica, M. latifolia, M. nigra* and *M. multicaulis* are cultivated for silkworm rearing, while *M. rubra* and *M. nigra* for fruits. The *Morus* species such as *M. multicaulis, M. alba* and *M. atrpurpuria* are widely distributed in different provinces of south, north and west China [13]. *M. bombycis* are distributed and largely cultivated in cold region and *M. latifolia* in warm places of Japan [14]. *M. macroura* Miq. is in the north western part of India, certain parts of south India [15] and West Bengal [16]. The genotypes of *M. alba* L. were distributed in Punjab, North western part of Himalayas and Western Tibet [16]. The natural and cultivated forms of *M. indica* L. is widely distributed extending from temperate to subtropical Himalayas, Arunachal Pradesh, Kashmir to Sikkim ascending to 2100m ASL. It is also distributed in Uttar Pradesh, Assam, West Bengal, Meghalaya, Karnataka, Tamil Nadu and Kerala [16]. The two wild species, *viz*., *M. serrate* Roxb. is confined mostly to the high altitude region of North western part of India [16] and *M. laevigata* is distributed throughout India both under natural (Andaman and Nicobar islands) and managed habitats [17].

During the last few years many accessions (14) belonging to *M. indica* and *M. alba* were brought from extreme cold regions (Ladakh, Meghalaya, Himachal Pradesh etc.) and maximum number of accessions (75) belongs to *M. indica*, *M. alba* and *M. laevigata* were brought from extreme dry hot regions (Rajasthan, U.P, M.P. and Bihar) and maintained in CSGRC, Hosur [18, 19]. Availability of different *Morus* species in four major countries (species wise) have been reported and most of the species occur in Asia, especially in China followed by Japan, India and Korea. Number of germplasm accessions available in each species in four sericulture progressing countries were recorded by different authors are given in Table 1 and the characteristic features of *Morus* species available in India are given in Table 2.

Table 1: List of *Morus* species and mulberry accessions in each species in four main countries

Species	Japan	China	India	Korea
M. bombycis Koidz.	583	22	15	97
M. latifolia Poir.	349	750	19	128
M. alba L.	259	762	93	105
M. acidosa Griff.	44	–	–	1
M. wittorium Hand-Mazz.	–	8	–	–
M. indica L.	30	–	350	5
M. mizuho Hotta	–	17		–
M. rotundiloba Koidz.	24	4	2	–
M. kagayamae Koidz.	23	–	–	1
M. australis Poir.	–	37	2	–
M. notabilis C.K.Schn.	14	–	–	–
M. mongolica Schneider		55	–	–
M. boninensis Koidz.	11	–	–	–
M. nigriformis Koidz.	3	–	–	–
M. atropurpurea Roxb.	3	120	–	–
M. serrata Roxb.	3	–	18	–
M. laevigata Wall.	3	19	32	1
M. nigra L.	2	1	2	3
M. formosensis Hotta.	2	–	–	–
M. rubra L.	1	–	1	–
M. mesozygia Stapf.	1	–	–	–
M. celtifolia Kunth.	1	–	–	–
M. cathayana Hemsl.	1	65	1	–
M. tiliaefolia Makino	1	–	1	14
M. microphylla Bickl.	1	–	–	–
M. macroura Miq.	1	–	–	–
M. multicaulis Perr.	–	–	15	–
Morus spp. (unknown)	15	–	106	259

Source: Pan, 2000; Kazutoshi *et al*., 2004; Annual Report, CSGRC 2012-13 & www.silkgermplasm.com

Table 2: Characteristic features of *Morus* species available in India

***Morus* species**	**Characteristic features**
Morus indica	Teeth of leaves unequal, the lateral nerves running straight into the teeth or forked within the margin, leaves usually medium to long, male spike less than 2.5 cm long, style is long, hairy, fruit ovoid or cylindrical, unripe fruit colour red, becomes black when ripe. Bud colour dark brown, elongated, triangular in shape.
Morus alba	Teeth of leaves uniform, usually blunt, segments of the perianth of female flowers in four numbers, the two outer keeled, style very short, fruit colour white, pink and black. Bud colour brown, oval round in shape.
Morus laevigata	Teeth of leaves fine, the lateral nerves abruptly curved upwards within the margin, style is very short. Fruit is long greenish, white, dark purple. Bud colour brown, elongated, lengthy and elliptical in shape.
Morus serrata	Teeth of leaves usually coarse, somewhat unequal and sharp, leaves velvety, coarse, full of minute hair on both the side, segments of the perianth of female flowers 2-4, usually 3, all similar, style is medium, fruit colour white, pink and mucilage fruit. Bud colour dark brown, round and bigger in size.

Conservation of mulberry genetic resources in India

Present status of germplasm maintenance

Survey, exploration, collection and introduction of mulberry germplasm resources is the prerequisite for conservation and exploitation of mulberry genetic resources for their further use. Realizing the importance of impending global climatic changes and threatened sustainability of biodiversity wealth in India at faster rate, systematic survey and exploration for collection of mulberry biodiversity have gained greater momentum in the recent past. Consequently, CSGRC at Hosur has been aptly established in the year 1990 by Central Silk Board (CSB), Ministry of Textiles, and Government of India under prestigious National Sericulture Project. It is the nodal agency for mulberry germplasm management in India and recognised as National Active Germplasm Site (NAGS) for mulberry by National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India under Indian National Plant Genetic resources System (IN PGRS). CSGRC, Hosur so far conducted 46 survey and exploration trips covering more than 50 districts from Himalayan belt to Andaman and Nicobar Islands covering forest areas, biosphere reserves, national parks, back yards, kitchen gardens, agricultural lands and farmhouses etc. and collected 516 diverse mulberry germplasm resources which mainly belong to 4 Indian species

(Table- 2). Out of 4 species of genus *Morus* reported in India, *M. laevigata* is distributed throughout India both under natural and managed habitats and *M. serrata* is confined to northwest India in wild condition. *M. indica* and *M. alba* are mostly available in cultivated forms [16]. The sericulture research institutes like Central Sericultural Research and Training Institute (CSRTI) at Berhampur (West Bengal), Mysore (Karnataka) and Pampore (Jammu and Kashmir) have started collection of *Morus species* in Central Himalayas, North-Eastern India, North-Western Himalayas, Kumaon and North Eastern India and Western Ghats and Kerala. All these germplasm resources are collected and introduced in the field gene bank.

The purpose of conservation of mulberry accessions in field gene bank is to maintain integrity of the material conserved for prolonged period of time [20]. Several activities are involved in conservation of mulberry such as collection, characterization and evaluation for agronomic traits and to utilize the genetic resources for breeding and other research activities. In India, the Central Sericultural Germplasm Resources Centre (CSGRC) was established during 1990 at Hosur and currently conserves 1254 mulberry accessions (984 indigenous and 270 exotics), which includes wild species, exotics (unadopted) landraces, modern elite cultivars, polyploid mutants, open pollinated hybrids and cross pollinated hybrids. The performance of each accession conserved is recorded morphologically, agronomically, biochemically, anatomically, physiologically etc., The details of each accession are documented in Mulberry Germplasm Information System (MGIS) data base [21], which can assist the breeder for selection of parents (donor and receptor) with desired traits and it is most crucial in pre-breeding programme.

Conservation strategies of mulberry genetic resources

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

***In-situ* conservation**

Plants that are conserved in their original habitat and it allows natural selection, mutation, population structuring etc., and thereby promoting free evolution of the species. It should be protected from all human interference and disturbing activities. *In-situ* conservation promotes the conservation of eco-system and natural habitats and the maintenance and recovery of viable population of species, which can survive, and best perpetuate in their natural microclimate. It also simultaneously permits continued evolutionary development under natural selection pressures, thereby promoting the fitness of the species. In situ conservation demands the establishment of nature or biosphere reserve and national parks to protect the endangered species. The National Committee on Environmental Planning and Coordination (NCEPL) and Man and Biosphere (UNESCO) already identified 14 Biosphere reserves in India and among them Uttarkhand, Nandadevi, Namdapha, Kaziranga, Manas, Nokrek, North Andaman and Great Nicobar are the potential reserves for *in situ* conservation of mulberry [20]. Keeping this in view, efforts have been made to collect information on the location of availability of mulberry germplasm with details on "declared protected area network of India" including biosphere reserves, national parks, wild life sanctuaries etc. Mulberry is not fully protected under Indian Forest Act (1972) in many parts of India except in some states of North-East India. Survey map, exact location, landowner with postal address, survey number and its jurisdiction are not available with CSB units or State department. Under the circumstances, a suggestive and advisory role can be contemplated with greater thrust on repeated survey and exploration.

***Ex-situ* conservation**

Since, mulberry is out breeding and highly heterozygous in nature and easily propagated through stem cuttings, the common conservation method are field germplasm banks or preserving vegetative buds in the *in-vitro* conditions. Collection of mulberry germplasm are maintained for evaluation of accession for economic traits and for supply of genetic resources to research institutes or to breeders for breeding programs. It was reported that many population of mulberry are migrated from its places of origin to far away places [22]. The *ex-situ* mulberry germplasm, which contains 1254 mulberry accessions that are maintained at CSGRC, Hosur. *In-vitro* conservation is classified in two categories. The plant material (nodal explants and dormant buds) can be stored for many years in the nutrient media (growth limited) under tissue culture conditions, however sub-culturing has to be done periodically and it all depend on type of the species and its *in-vitro* regeneration capacity. The interspecific hybrid embryos are sometimes very weak, which can be rescued by keeping them in a suitable nutritional media and allowing them to grow. Therefore, *in-vitro* technique can be used to rescue or save the F1 hybrid's developed through pre-breeding. Also *in-vitro* technique can be used for rapid screening of genotypes for resistance to salinity, alkalinity and drought.

Field gene bank

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

On-farm participatory conservation

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

Botanical gardens and national herbarium

In the context of PGR, the herbarium provides the basic material for detailed monographic and phyto-geographical information of the species. In India there are many herbarium canter's and some of the main herbarium canter's like: Botanical Survey of India with its regional centre's,Presidency College, Chennai; Baltter Herbarium, St. Zavier College, Mumbai; St. Josephs College, Tiruchirapalli and National Herbarium, NBPGR, New Delhi maintain *Morus* species herbarium. Botanical Survey of India, Pune preserves the oldest herbarium of genus *Morus* dates back to 1886. Seri-biodiversity museum of CSGRC, Hosur maintains large number of herbaria and it serves as National Herbarium Centre for *Morus* species in India. In India there are about 33 botanical gardens and the some of them like: Indian Botanical garden, Howrah; Lloyd Botanical garden, Darjeeling; National Botanical Garden, Lucknow maintain mulberry arboreta.

Conservation using biotechnology *in-vitro* and cryopreservation

The plant material preferably winter dormant buds can be stored in liquid nitrogen (-196 °C) for long period. It requires less space, labor, cost effective and disease free. cryopreservation is a step wise process, wherein the buds will be dehydrated in silica gel and subjected to slow freezing -5 to -30° C and finally it is transferred to Cryo-Can containing liquid nitrogen (-196C) [23]. Cryopreservation procedures have been standardised for about 100 different plant species cultured in various ways including cell suspension, callus, apices and zygotic and somatic embryos. The advanced techniques of cryopreservation comprise many stages ranging from tissue culture, pre-growth, cryoprotection by plant vitrification solution, slow and fast freezing, thawing recovery and invitro regeneration [23]. The National gene bank at National Bureau of Plant Genetic Resources (NBPGR) in New Delhi, the nodal agency for plant germplasm conservation, has a state of the art facility presently and conserves a total of 1,783 accessions (*in vitro* conservation) and 8,000 accession (cryo-banking with temperatures between -160 C and -196C) of diverse plant germplasm including 338 mulberry accessions collected from diverse geographical region [23].

Conservation of mulberry germplasm in the field gene bank is simple and technically less demanding. However, maintaining a large collection is costly, requiring huge resources. Besides, it is risky since it is exposed to different biotic and abiotic stresses. Cryopreservation technology forms an alternative to ex situ conservation strategy for crop like mulberry, which is vegetatively propagated utilizing liquid nitrogen (LN) at ultra-low temperature of -150°C(vapour phase). Cryopreservation method ensures the genetic stability of the germplasm and provides an alternate cost effective non-dependence on electricity long-term strategy for conservation of mulberry germplasm. Keeping this in view, establishment of *in vitro* and cryopreservation laboratory facilities at CSGRC, Hosur is planed in collaboration with NBPGR, New Delhi. Efforts have been made to standardize the efficient techniques on cryopreservation of winter buds, embryonic axes, pollen of mulberry accessions comprising different species, landraces, local cultivars, wild and polyploid accessions in liquid nitrogen (-196 °C) [24]. *In-vitro* conservation techniques involve conservation of active collections under active growth stage and base collection germplasm under suspended growth stage using growth retardants. Even though lot of work on mulberry regeneration with or without callus formation in in vitro has been reported. Very little work has been carried out in in vitro conservation of mulberry genetic resources. Single shoots of *M. nigra* L. stored on multiplication medium at 4°Cfor 16-hour photoperiod survived only for six months. Survival was enhanced to 42% at nine months by storing them at 25°Cwith activated charcoal as supplement. High viability (80%) for six months was observed in 15 genotypes of *M. alba* stored at 4°Cdark in shoot proliferation medium [24]. Rooting was observed in all the shoots and shoots retained their multiplication potential.

Cryopreservation, possibility for the first time was demonstrated using mulberry twig [26]. Since then, considerable work on cryopreservation of mulberry has been undertaken especially in Japan. Shoot tips of pre-frozen winter buds of *M. bombycis* Koidz was able to withstand storage in LN. However, grafts and cuttings did not survive in LN. With modification of this method it was able to regenerate plants of *M. multicaulis* P. through tip culture of frozen winter buds. Shoot segments were pre-frozen at -3°Cfor 10 days, - 5°Cfor three days, - 10°Cfor 1 day and -20°Cfor one day before immersion in LN. Buds were cultured on MS medium after thawing in air at 0 to 20 °C. Survival rate was 55 to 90%. Excised shoot tips from winter buds of *M. bombycis* Koidz. pre-frozen at 10°Cper day. Prior to pre-freezing at -20°Cpartial dehydration to 38.5% improved the recovery rates. The survival rates of the winter buds stored in LN from the month to 3-5 years did not change. Direct dehydration with silicon gel at 25°Cof excised shoot tips (2 mm long) from winter bud could be done before immersion in LN. With decreasing water content shoot formation increased and at about 19% of water content, a maximum of 80% survival rate was observed. Encapsulation by alginate coating of winter hardened shoot tips of many *Morus*species had 81% of shoot formation with 22-25% water content. In vitro grown shoot tips of thirteen cultivars of mulberry were tested for cryopreservation. Slow freezing (0.5°C/min. to - 42°C), vitrification (PVS2, 90 min) and air- drying (24% water content) or encapsulation dehydration (33% water content) was tested for survival, which ranged from 40-81.3%. It was also reported the long-term storage of mulberry winter buds by cryopreservation. Winter buds from *M. bombycis* with about 10 mm vascular tissue were kept at 0°C for 1 day before freezing. Buds were cooled to 10°C steps at daily intervals from 0 to 30°Cprior to immersion in LN or before transferring to -135 °C. After storage, buds were rapidly thawed at 37°Cin a water bath and then cultured on MS medium supplemented with 2% fructose and 1 mg/l with 6-BAP. Rate of shoot formation did not vary much in buds stored in LN or deep freezed at -135°Cafter a storage period of 3-5 years.

In India, the application of *in-vitro* technique for mulberry conservation has been recently attempted. *In vitro* technique has been attempted in mulberry mainly for propagation of popular mulberry varieties and poor rooting materials, evolution of some clonal variants and elite materials and screening of genotypes for tolerance to salt and somatic stresses [27]. A developed method for propagation of *Morus indica* L. has been done by culturing encapsulated shoot buds [28]. Conservation of mulberry germplasm accessions through cryopreservation technique has been recently attempted in India.

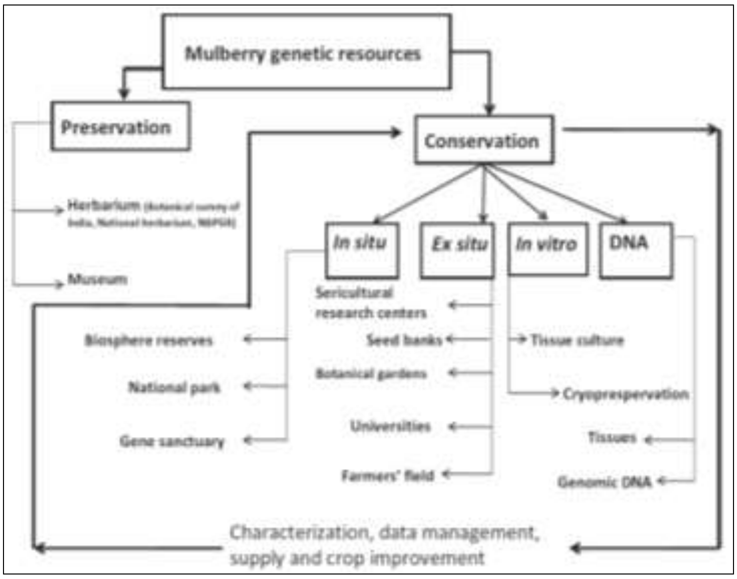

Fig 1: Schematic presentation for conservation of mulberry genetic resources

Utilization of mulberry genetic resources

The germplasm are viewed as source of genetic diversity to support crop improvement. The level of genetic diversity in different *Morus* spp. and accessions has been analysed using morphological characters [30]. The information on the genetic diversity of *M. species* could help breeders to understand and predict which combination would produce the best hybrids. To evolve region specific or improved mulberry varieties for desirable characters large number of germplasm is required [30], which can be used in breeding programme to enhance genetic variability in the germplasm and also to harvest new desirable alleles required for crop improvement.

Utilization of germplasm efficiently for any breeding programme depends on the core collection (10% of entire collection) of germplasm in several crops [31]. The main goal of the core is to present the genetic diversity of a crop species and its relatives with a minimum repetitiveness [32]. Core collection have been used to identify genetically diverse trait from specific germplasm with resistance to abiotic and abiotic stresses and for agronomic traits [31]. In recent years, several mulberry accessions were collected from extreme cold regions (Ladakh, Meghalaya, Himachal Pradesh and Arunachal Pradesh etc.,) and extreme dry hot arid regions (Rajasthan, Madhya Pradesh, Uttar Pradesh and Bihar etc.,), which are maintained at CSGRC, Hosur [18, 19]. These accessions may possess certain hardy genes or genes for salt, cold and drought tolerance, which can be used in pre-breeding for the development of pre-breeding lines, which later may be used in the breeding programme for development of new variety with resistance to abiotic stress.

The utilization of different *Morus* species was attempted and inter-specific hybridization was conducted to incorporate the desirable characters for crop improvement. But sometimes, it is observed that interspecific crosses do not perform well due to various reasons. The main reason may be incompatibility, geographical isolation among the species. The performance of untried wild species like *M. serrata*, *M. laevigata* and *M. tiliaefolia* were tried at inter and intra-specific level. At national and international level *M. alba*, *M. indica; M. multicaulis* and *M. bombycis* are extensively used for commercial purposes. Both *M. laevigata* and *M. serrata* are grown as tree in forest area and people are using these trees for multipurpose use other than sericulture. But till date these species have not been used for mulberry crop improvement due to its non-availability or suitability for silkworm industry. The cultivated species exhibits considerable genetic diversity, but the diploid mulberry showed narrow genetic base and threat to genetic erosion. In order to broaden the genetic base, new gene pools have to be incorporated into the gene pool of cultivated forms. *M. serrata* and *M. laevigata* possess several agronomic important traits including resistance to abiotic stresses like drought and frost. Some authors tried to study the crossing ability among different *Morus* species and its inheritance pattern. All the reports are of preliminary in nature and have attempted to get successful hybrids of wild species of *M. laevigata* and *M. serrata* with cultivated species like Kanva–2 and Kajli [9]. In sericulture, the mulberry variety plays an important role. The leaf of *M. laevigata* is not used for silkworm rearing due to its thick and rough nature. But in the F1 hybrid, leaf is soft, palatable to silkworm and rearing performance is like commercial varieties. The vigour and growth performance are better in male parents than female parents and suitable for selection. The rooting performance on lesser side, which has to be improved by back crossing or treatment with root hormones like IBA, IAA and commercial hormones.

Mulberry biodiversity in Jammu and Kashmir

Jammu and Kashmir state is located in the silk belt at the same altitude in which leading sericulture countries lie. Sericulture is practiced in all areas of the state except cold arid zones like Ladakh and Gurez. The growth pattern in J&K is prioritized for good harvest of quality bivoltine cocoon crop. It is a well-established fact that, in commercial sericulture, more than 60% of the total cost of cocoon production goes towards mulberry production alone [33]. Therefore, prime importance should be given to have improved mulberry varieties with quality mulberry leaf in sericulture industry. Though mulberry grows luxuriant under temperate climatic conditions, however the major drawback of mulberry propagation under temperate condition is poor rooting ability of popular mulberry genotypes besides the long dormancy period of six months (October-March) hinders the growth of saplings. Conventionally it takes 2-3 years for a sapling to be ready for transplantation. J&K is well known for the existence of several mulberry varieties belonging to different species of *Morus*; such as *M. alba*, *M. indica*, *M. laevigate*, *M. serrata*, *M. nigra*, *M. rubra*, etc. Sericulture in temperate areas of J & K is being practiced utilizing mostly Ghoshoerami mulberry variety belonging to *M. multicaulis*; Chinese white, KNG and Ichinose mulberry varieties belonging to *M. alba*. Along with these some indigenous local varieties such as Brentul Kashmir, Chattatul, Janglitul, Krenantul, Nadigam, Kablitul, Lajward, Hamtul etc. are also in practice to some extent.

Natural mulberry is abundant in J&K and is suitable for sericulture. Introduced and cultivated mulberry is also abundantly available. The mulberry resources should be used judiciously to better the sericulture industry in J&K. Wild mulberry available in various regions of J&K like Kargil-Ladakh, Gurez, Kupwara, Rajouri etc., needs prime importance for characterization since, lot of diversity is available in the mulberry wealth found in these regions. As mulberry is found growing in these regions through seed dispersal as such collections from these regions must be highly heterozygous, hardy with noble genes for cold/frost/drought tolerance which needs immediate attention of breeders for evaluation and their further utilization in breeding programs for mulberry crop improvement. With the development of sericulture industry and the recent increase in the technical know-how, it has become very essential to evolve better performing mulberry varieties to fulfil the demand of Sericulturist's.

Table 3: Mulberry germplasm maintained at CoTS, SKUAST-Kashmir, mirgund

<table>
<tr><th>Indigenous</th><th>Exotic</th><th>Selections</th></tr>
<tr><td>Bruntul</td><td>Ghosoerami</td><td>SKM-04</td></tr>
<tr><td>Botatul</td><td>Ichinose</td><td>SKM-07</td></tr>
<tr><td>Chinarpati</td><td>Kokuso-20</td><td>SKM-10</td></tr>
<tr><td>Chatatul (Zangir)</td><td>Kokuso-21</td><td>SKM-19</td></tr>
<tr><td>Chatatul (Mirgund)</td><td>Kokuso-27</td><td>SKM-20</td></tr>
<tr><td>Cherry</td><td>K.N.G</td><td>SKM-25</td></tr>
<tr><td>Local mulberry</td><td>Kanva-2 (Indian)</td><td>SKM-26</td></tr>
<tr><td>Robeshsarnal</td><td>Kasuga</td><td>SKM-27</td></tr>
<tr><td>Zagtul</td><td>Kairyoroso</td><td>SKM-29</td></tr>
<tr><td>Shahtul (Iran)</td><td>Lemoncina</td><td>SKM-30</td></tr>
<tr><td colspan="2">Mukey</td><td>SKM-31</td></tr>
<tr><td colspan="2">Rokokyoso</td><td>SKM-33</td></tr>
<tr><td colspan="2">Senmetsu</td><td>SKM-35</td></tr>
<tr><td colspan="2">Serpentina</td><td>SKM-36</td></tr>
<tr><td colspan="2">China white</td><td>SKM-44</td></tr>
<tr><td colspan="2">Enshutakasuka</td><td>SKM-45</td></tr>
<tr><td colspan="2">France</td><td>SKM-48</td></tr>
<tr><td colspan="3">Japani-Mirgund</td></tr>
<tr><td colspan="3">Japani-Thuj</td></tr>
<tr><td colspan="3">Lajward</td></tr>
<tr><td colspan="3">Suka-sukawa</td></tr>
</table>

Takawase
Tomeiso
Ichihei
Zust
Tr-10
S-1531
Mandalya
C-1708
Obawase
Royal china
Shimonouch
Sujanpur
C-1608
V1

Need for conservation of elite temperate mulberry germplasm

Mulberry as stated above, is grown in varied climatic conditions ranging from temperate to tropical. In past, sericulture was prevalent in the temperate regions only. However, now tropical regions dominate the industry. In India most states have taken up sericulture as an important agroindustry, with good results. As mentioned above, there is rich diversity of mulberry available, and attempts have been made to conserve the plant species through *ex-situ* approaches in the form of germplasm banks. In India, mulberry biodiversity is maintained through various germplasm banks spread across the country. An exclusive centre for the established in Tamil Nadu (Central Sericultural Germplasm Resource Centre, Central Silk Board) maintains more than thousand mulberry accessions, including those of temperate origin. The germplasm banks maintained under temperate conditions in Kashmir valley do not, however, represent all the available temperate genotypesin the country. With the global environmental change having a marked effect on the cultivation of important agricultural crops, the conservation of mulberry needs more focus. In addition, various abiotic and biotic factors can have sudden drastic effects on elite genotypes. Owing to this; there is a chance of losing some of the important temperate species of mulberry. Hence, alternative approaches for germplasm conservation are needed. Initiatives taken by the Norwegian Government and Global Crop Biodiversity Trust (Salbard Global Seed Bank), and the Defense Research and Development Organisation (DRDO), India for establishing cryo-banks are praiseworthy. Establishing various *ex-situ* germplasm banks helps in the conservation of various indigenous and exotic varieties. Already, some of the selected temperate germplasm samples have been submitted to the cryo-bank of National Bureau of Plant Genetic Resources, New Delhi. However, complete sub-zero storage facility is required to preserve the valuable mulberry germplasm. Similarly, non-conventional approaches for regeneration after long-term storage should be given priority. The success and utility of this technology will depend on its acceptance by the scientific community and policy makers. We believe it will be a muse for those working towards the conservation of some important crop plants in developing countries.

Future thrust

Conservation of plant genetic resources is rapidly becoming increasingly important, especially for sustainable agriculture to feed the needs of ever-increasing populations. Land would be a limiting factor in future and through utilization of PGR, a continued increase in agricultural productivity would be possible. Keeping this in view, and also the act that environmental changes in future may require genes for adaptability, the conservation of genetic reservoir of plants need to be given high priority in policy planning**.** Exploration of selected minor and underutilized species for germplasm collections and assessment of genetic diversity of landraces, should have priority. There is need to develop better methods of characterization and evaluation of germplasm collections, to improve strategies for conservation, and to increase the utilization of PGR. Although seeds of sexually propagated plants can be conserved easily, conservation of vegetatively propagated plants requires species techniques and attention. With reverence to mulberry, traditional conservation strategies like *in-situ* and *ex-situ* conservation should be complemented with modern techniques like cryopreservation and DNA banking as *ex-situ* conservation of mulberry entails huge investment in the form of labour and space. Well-developed protocols are now available and a number of mulberry accessions, but the genotypic effect on the survival percentage of the warrants further effort to for fine tuning the protocol to accommodate most of the species. DNA banks for biodiversity and plant genetic resource evaluation and conservation are important, although no such bank exists for mulberry. Efforts are now under way to establish DNA banks to integrate them into a network for easy exchange of DNA materials and better co-ordination for utilization of the information being generated within them. The data being collected from DNA banks should be made compatible with those from other morphological and biochemical studies so that effective and efficient plant genetic conservation strategies can be formulated and implemented. Thus, concerted efforts are to be made urgently to integrate all these techniques to conserve precious genetic resources of this very important tree crop of Asia. The mulberry resources in J&K should be used judiciously to better the sericulture industry in J&K. Natural mulberry available in various regions of J&K like Kargil, Ladakh, Gurez, Kupwara etc., needs prime importance for characterization since, lot of diversity is available in the mulberry wealth found in these regions. As mulberry is found growing in these regions through seed dispersal as such collections from these regions must be highly heterozygous, hardy with noble genes for cold/frost/drought tolerance which needs immediate attention of breeders for evaluation and their further utilization in breeding programs of mulberry crop improvement for sustainable sericulture industry in the valley.

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