Technical Manual

Manual on Mulberry Gene Bank operations and Procedures

Alok Sahay, K. Jhansilakshmi,
P. Saraswathi & S. Sekar

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Foreword

Jewel in the Crown of CSGRC, Hosur the nodal center of Mulberry Germplasm Resources is what is to be the phrase that occurred to my mind to describe this manual on Mulberry Gene Bank Operations and Procedures. The contents of the manual are found to be most painstakingly assembled systematically by the team of sericulture scientists, Dr Alok Sahay, Dr K Jhansi Lakshmi, Dr S Sekar and Dr P Saraswati. This manual with no room for uncertainty would go a long way in serving the scientific community dealing with mulberry germplasm across the country and globally.

This invaluable information bulletin deals comprehensively on variety of practical issues concerning germplasm collection, characterization, conservation, evaluation and utilization. There is no other source of practical information to access from anywhere on practical issues covering many dimensions of mulberry genetic resources management. The most effective way to conserve mulberry genetic resources is to put them to continuous use meeting competing demands of the society. Realizing this and multiple uses of mulberry germplasm resources for crop improvement, CSGRC Hosur initiated number of steps for its conservation and utilization way back in early 1990s and this manual would form another milestone in that direction. Registration, exchange, quarantine and mulberry germplasm database are various dimensions of sericulture science towards the utilization, helping the farmers, industry and the country as a whole earning revenue and foreign exchange.

I have read the draft of this book with complete concentration and have found the contents to be informative, educational and interesting. I once again congratulate Dr Alok Sahay and his co-authors on this publication. Now, turn on the pages and let them introduce to you the science of mulberry plant genetic resources weaved attractively in simple and lucid form of English language.

Dr. B Sarath Babu

Principal Scientist and Head
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Hyderabad

Preface

I am pleased to present this Manual of Operations and Procedures of the Mulberry Gene bank. This Manual describes how and why we carry out the various operations and procedures of collection, conservation, characterization, evaluation of mulberry genetic resources and other activities involved in germplasm maintenance at CSGRC, Hosur. This manual is intended to guide new researchers who involve in handling different activities of mulberry germplasm conservation. We felt that development of a genebank operation manual will facilitate and streamline the work at the genebank itself and enhance genebank activities and approaches. It will also allow scientists, especially new scientists, to assume their tasks and responsibility more easily.

Gene banks are biodiversity reservoirs and sources of alleles for sustainable genetic enhancement of any crop plant. In Mulberry, efforts have been made to collect landraces, cultivars that were becoming obsolete, genetic stocks, and wild species important for crop improvement. *Ex situ* field gene bank with diversified collections from different geographical areas were assembled, followed by characterization, preliminary evaluation and obtained national accession numbers from NBPGR, New Delhi. Since breeding gains rely on access to useful genetic variation in the crop genepools, well-documented information about mulberry genetic resources allow genebank curators to offer specific accessions with the desired characteristics to plant breeders and other researchers who can then select material as per their objectives.

This manual of Gene bank Operations and Procedures provides information in 6 chapters regarding germplasm assembly, conservation, plant quarantine, characterization and preliminary evaluation, registration and taxonomic classification. I hope, this manual would help researchers in other institutes involved in germplasm maintenance in their work for preserving mulberry biodiversity in *ex situ* field gene banks. We welcome any suggestions to improve the manual in further editions.

Alok Sahay
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Introduction

Genetic resources are the essential raw materials for improving crops for yield and quality, to safeguard against losses from abiotic and biotic stresses and developing new value added products. Mulberry genetic resources are important resources at the heart of sustainable mulberry production. Their efficient conservation and use is critical to safeguard sericulture development, now and in the future.

In contrast with the large number of local cultivars being used by earlier generations, a few high yielding varieties are used in the present day sericulture and these often have a narrow genetic base. If we do not consciously increase genetic improvement with broad genetic base, it may have serious consequences, especially when facing a changed climate due to global warming. Organized preservation of mulberry genetic resources is a prerequisite for future generations to be able to develop new mulberry varieties and face new challenges. Future mulberry varieties need to be part of more environmentally friendly cultivation system, suitable for mechanization, be of better quality and have improved resistance to different abiotic and biotic stresses.

The genetic resources without accompanying information on its characteristics are of limited value. The information generated through characterization and evaluation on each of the mulberry genetic resources and identifying important specific traits in different genetic resources will be of immense value particularly in mulberry that is having wider adaptability and utilized for multiple uses. Mulberry is a unique multipurpose tree where in leaves can be feed to cattle, goats, sheep and rabbits; fruits can be eaten fresh, preserved, venified or dried for winter use; Provides excellent timber mainly used for sports goods and local needs of furniture; branches used as raw material for paper production and media for mushroom production; thin twigs can be used for basket making; leaves, root bark, fruit and latex have medicinal value.

Hence, germplasm characterization and evaluation are important operations for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to be in the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection. Well-managed genebanks safeguard genetic diversity and make it available to breeders.

Genebanks around the world hold collections of a broad range of plant genetic resources, with the overall aim of long-term conservation and accessibility of plant germplasm to plant breeders, researchers and other users. Sustainable conservation of these plant genetic resources depends on effective and efficient management of genebanks through the application of standards and procedures that ensure the continued survival and availability of plant genetic resources. This manual narrates different activities to be carried out, technical aspects, selected references and protocols as appropriate, supports for following standard operational procedures.

Germplasm Collection

The first step in conservation of genetic resources is collection of germplasm. This activity is done by:

A. Survey, Exploration and collection from farmers' fields and wild habitats, particularly in areas known as centers of diversity, and

B. Procuring materials of interest through correspondence from other institutes or organizations

The standards provided by FAO (2014) for acquisition of germplasm are

- All germplasm accessions added to the genebank should be legally acquired, with relevant technical documentation.
- All material should be accompanied by at least a minimum of associated data as detailed in crop passport descriptors.
- Propagating material should be collected from healthy growing plants whenever possible, and at an adequate maturity stage to be suitable for propagation.
- The period between collecting, shipping and processing and then transferring to the field genebank should be as short as possible to prevent loss and deterioration of the material.
- Samples acquired from other countries or regions within the country should pass through the relevant quarantine process and meet the associated requirements before being incorporated into the field collection

A. Germplasm collection by survey, exploration and Collection

The fundamental objective of collecting plant genetic resources is to capture the maximum amount of useful genetic variation (Marshall and Brown, 1975) available in nature. The basic parameter for measuring variation in a given population is allelic richness: the number of distinct alleles at a single locus (Brown and Marshall, 1995). Germplasm collection requires theoretical knowledge on sampling and also practical knowhow of plant diversity and environment including the socio-economic and cultural aspects of the farming societies. Tactics, logistics, preparations and procedures have been elaborately dealt with by Bennett (1970), Harlan (1975), Hawkes (1976, 1980), Arora (1981) Chang (1985) and Rao and Bramel (2000).

Owing to the origin of the genus *Morus* in sub-Himalayan belt of Indo-China, mulberry is available in natural form in this region. In India, four species of mulberry viz., *Morus indica, M. alba, M. laevigata and M. serrata* were reported to be available in nature. M.serrata is confined to higher altitude of North Western Himalayas and sparsely distributed in North East India. It is known as Himalayan mulberry and found up to an altitude of 2200 mts above

MSL particularly in Salna, Urgam valley, Joshimath (approximately 1220 years old mulberry tree protected as sacred grove), Chakrata (Ravindran et al., 1997). Dandin *et al*., (1993) reported their occurrence of mulberry in Jammu division, Kangra, Chamba, Nahan and Kullu districts of Himachal pradesh and Garwal Himalayas of Uttaranchal.

M. alba and M. indica are available throughout India mostly in cultivated forms. However, occurrence of natural distribution of *M. alba* was also observed in Surari Garhal of Himalayan region up to an elevation of 1365 mts above MSL and in Ladakh Himalayan region up to an altitude of 3300 m above MSL. The cultivated forms of *M. indica* and *M. alba* are available in Gujarat, Rajasthan, Uttar pradesh, West Bengal, Sikkim, Assam, Meghalaya, Arunachal Pradesh (Chandrasekar, 2001, Saraswat, 2001, 2002, Borpujari, 2009)

M. *laevigata* is distributed in many parts of the country naturally distributed in sub-himalayan regions of India and Andaman Islands. Besides, it has been located as developed gene pool reserves in Central India in Joara, Dhar, Shivapuri, Gwalior, Bilaspur, Pachmarhi reserve forests (Dandin et al., 1995; Jain and Kumar, 1989 and Ravindran et al., 1997) and in isolated pockets at Shevoroy hills of Salem district of Tamilnadu (Yadav and Pavan Kumar, 1996).

Apart from naturally available mulberry, mulberry genetic resources are observed to be maintained as avenue trees on road side, as shade tree, sacred grooves, under on-farm conservation mainly in coffee and tea states, farm bunds and back yards.

Why to collect germplasm ?

- To avoid loss of genetic diversity -The replacement of land races and local cultivars with a few high yielding mulberry varieties has resulted in reduced genetic diversity. The variability available in the natural habitats particularly in centre of diversity regions may be lost permanently due to increased deforestation, urbanization and natural hazards.
- when diversity is missing or insufficiently represented in an existing collection
- Need based - to collect material with attributes such as adaptation to high temperature, drought stress, salinity, alkalinity, cold tolerance, low soil fertility or wild relatives
- Opportunistic reasons – germplasm containing striking features or is found under unusual circumstances

Planning for collection of germplasm

Germplasm collecting work is time consuming, requires expert personnel, finance, permission from biodiversity authorities and cooperation from local people. It is also time specific. In a limited geographic area one can find several crop environments associated not only with altitude, soil type, hill slope, but also with disease and insect pressure, and with rainfall. It is expected that different material will arise from different environments. Plant material from diverse locations may look alike, morphologically but might differ in reactions to biotic and abiotic stress factors. These factors should be taken into consideration while organizing the collection mission (Pundir and Mangesha, 1991).

Collection missions should be planned at least one year in advance. Consulting state and regional reports, flora and latest published works to get familiarized with climate, ecology and vegetation is essential. Studying herbarium material, particularly of wild species is required to get visual impression of taxa targeted for collection. The explorer must synthesize all available information such as:

a) Germplasm already available in the genebank for the area,

b) Antiquity of the crop - It is believed that the longer a species has been present in an area, the more diverse will it be,

c) Extent of germplasm erosion in the area as a result of the introduction of more remunerative crops and high yielding cultivars, which pose a threat to the native plant material. The germplasm should be collected and preserved before the new crops and newly bred cultivars can wipe out the native landraces,

d) Urbanization, which can also lead to the wiping-out of native plant species, and

e) Drought, flood, fire, and civil war - These unfortunate tragedies, natural or man-made, can cause total loss of the native plant species. All possible efforts should be made to collect if some of these disasters are predicted in that area.

Subsequent to the coming into force of CBD, the access to genetic resources is subject to prior informed consent by biodiversity authorities. Specific permission is needed in regions where there is political sensitivity, for instance at a border area between countries. Letters of introduction, particularly to government authorities, should be carried out. These may be particularly useful when the mission runs into some difficulty. Identifying collaborating agencies and scientists is also important, which requires contacting them well in advance. Gemplasm collection will be successful best when it is taken up jointly with well established institutions like NBPGR or BSI, which are closely associated with different activities of germplasm and are having networks

through out the country. An itinerary and provisional route should be established using information gathered from above.

Organizing an expedition

Collecting germplasm requires meticulous planning. The explorer needs to be in the right area at right time. Prospective collectors should identify local or national collaborators, and have prior discussion or correspondence with them on practical arrangements which includes priorities of collection, information to be gathered during collection, processing and conservation arrangements, and financial aspects of the mission.

To make a successful collecting mission, attention must be paid to the following points

a) Collecting teams should always be small: never more than three persons. The collection team usually consists an expert on mulberry from the center and a local expert. If there is a team leader, he/she should preferably be a botanist or a plant breeder. Whenever possible it is advisable to include an experienced plant breeder in the collecting team, especially in the case of targeted collecting missions, to benefit from specific knowledge of the crop species and thereby ensuring breeders' needs.

b) Route: Route planning is essential and detailed printed road maps and list of rest houses or hotels should be collected. Regional, climatic and soil maps should also be consulted. Local experts must be consulted during planning about feasibility of the selected routes. Travel on less important roads can hamper the mission during rains or while bridges or culverts are repaired. Therefore, one should discuss travel feasibility with local bus or truck drivers every day during a trip.

c) Time of collection: Team should be in collection place at the right time. As mulberry is deciduous tree and also enters into dormancy during winter particularly in low temperature regions, it is essential to know the right season to collect the material. Without leaves, it is difficult to identify mulberry during winter. Therefore, timing of the collection mission is important. At the same time, if it is early spring after sprouting of the buds, shoot may not be mature enough for collecting the stem cuttings for multiplication of the sample. Hence, information on sprouting, maturity of shoot and dormancy need to be collected from regional stations near by. If possible, it is better to survey during autumn season and collect the material before severe winter starts. There is always a need to allow more time for an expedition than would normally have been thought necessary. A minimum 10 days are required for fruitful explorations. A few days are required to get acquainted with the surroundings, vegetations, local cultivation and cultural aspects.

d) Transport: Mode of transport will vary depending upon the location and resources available. However, in normal circumstances, the best is with four-wheel drive, long wheel base, heavy duty springs, covered and lockable. The vehicle should have spares such as a complete tool box, spare wheels and tyres, fuel cans, engine driven winch and a chain or nylon rope.

e) Equipment

i) Camping equipment: The team should plan their travel in such a way that' they reach some city, town or institution by the end of each day to obtain accommodation and food. Team should carry large and small water containers, electric torch and lamp with spare batteries, matches candles, sun glasses, food, utensils, strong high boots for snake infested areas, lightweight jackets, long sleeve shirts with several of pockets and brimmed hat. High altitudes can be very cold during nights and in the mornings even if it is warm during the day, hence suitable cloths needs to be carried.

ii) Scientific equipment: Geographical Positioning System (GPS), altimeter, field compass, two cameras, pocket lenses, pH meter and binoculars are required,

iii) Sampling equipment: secateurs, small crow bar for collecting the samples, gunny thread, twine thread for making bundles, absorbent paper for pressing specimen drying, collector's notebooks, rubber bands for closing bags, aluminum labels for labeling specimens, plant press with corrugated aluminum sheets, pencil, pens and permanent markers of different colors, stapler and staples, pocket knife, gloves, binoculars, Permit for collecting germplasm or other required permits, printed slips with institute's address collection data sheets, herbarium sheets.

iv) Medical supplies: Team members must have all necessary vaccinations in advance. They should be careful with unfamiliar food and drinks. Also, they should carry antiseptic cream or liquid insecticide sprays or repellent creams pain-killer pills antipyretics (paracetamol or aspirin), antacid tablets and anti-diarrhea pills and bandages.

Agro-ecological information

It is useful to breeders/ explorers who would be using the material if they know characteristics of the environment to which the material is specifically adapted. Therefore, it is desirable to collect information on climatic, ecological and farming conditions of the place of each collection.

The following observations are required to be documented

a) Name of the place and its longitude and latitude, or the exact location based on a permanent landmark

b) Topography, altitude, precipitation, rainfall-distribution, and drainage conditions

c) Soil type, depth and estimate of salt status, pH and electrical conductivity, whenever possible,

d) Irrigation facilities, other crops and associated vegetation and ground water table,

e) Specific local conditions,

f) Peculiarities, e.g., crop utilization,

g) Indications of hybridization and introgression with wild and weedy forms,

h) Taxonomic notes

Collection technique

A comprehensive technical guide on collecting plant genetic resources providing many practical and managerial suggestions was published by Guarino *et al*., (1995).

The fundamental objective of collecting plant genetic resources is to capture the maximum amount of useful genetic variation in the smallest number of samples (Marshall and Brown, 1975).

Since mulberry is propagated by stem cuttings, mature stem cuttings are to be collected. Sufficient material need to be collected for multiplication by stem cuttings as well as through grafting as the rooting ability of the collected material is not known. Also, there is possibility of loss/decrease in rooting ability of the collected material during transport. A minimum of 10 mature shoots of 2 feet length need to be collected. If several samples are collected in a given area, the extent of genetic divergence and the total genetic variation among the samples is important (Schoen and Brown, 1991).

Engelmann (1997) specified several cases in which *in vitro* collecting can be advantageous. These include long missions to remote areas or mature stem cuttings are not available. This can be advantageous particularly while collecting the wild species whose rooting ability is very less. Even, the grafts may not be successful after long transportation due to moisture loss from the material. Also, shoot tips or nodal explants or dormant buds can be collected if mature stem is not available. Though this is not practiced in mulberry, it can be made it a practice in future explorations. In general, use of budwood is practiced in cocoa (Yidana, 1988); use of stem nodal cuttings for cotton and related species

(Altman et al., 1990); and use of herbaceous plantlets as explants described for some forage grasses (Ruredzo, 1989).

Sampling strategy

- Collections should not be made from sites that are less than 10 km apart, unless: landraces grown are morphologically different, there is marked change in altitude or cropping systems, a formidable barrier such as mountain or a river exists, or local people are ethnically different from previous collection site.
- Samplings must be made over as many different environments and regions as possible.
- Within the unit of collection/genepool, to have full range of variation represented, common types are collected through random collection and rare types through biased collection.
- Collect away from major routes since introduction of advanced cultivars begins in regions close to major roads.

Documentation of the collection site and environment through photography

Documentation of collection site, ecosystem where the collection is made, nature of growth of collection and its surroundings will give idea about its possible uses, probable source for stress resistance/tolerance. It will be of immense benefit to the breeder and other researchers for understanding the adaptability and uniqueness of collection.

Useful tips when collecting

- It is advisable to start work in the morning after an early breakfast. Carry packed lunch. Get back to the camping site before sunset.
- Do not plan to cover more than 100 or 200 km on "bad" roads and 300 km on "good" roads.
- If the team is coming back to the same camping site, it is advisable to follow a circuitous route so that more villages can be covered.
- Spare time for market survey, backyard surveys, visit to farmers' homes to see for other observations, if available to collect samples. This may reveal potential use of mulberry for non-sericultural purposes in that area or specific mulberry genetic resources they have conserved.
- Note down meter reading before the vehicle starts for work each day. Keep record of distances covered daily, petrol filling and other expenses incurred
- Hold discussions with local officers, village extension workers, old farmers, school teachers in area in the area of survey and assemble relevant

information on crops and locations for collecting diversity. Do this each evening a day ahead of the collecting itinerary and prepare a tentative program to be followed — villages/route/distances to be covered by jeep and on foot.

- On reaching the camp each evening, take out the collection, check and label them properly, press herbarium specimens, and complete notes in the field data book.
- Make it a principle to complete your daily work the same evening and before retiring, re-equip your bag with items needed for the next day's collecting.
- Data gathering is an important part of collection. Absolute minimum information to be recorded is:

    a) collectors' and collection number,

    b) date and site of collection,

    c) Documentation

    d) status of sample (wild, weedy, cultivated)

    e) source of collection (field, road side, backyards, farm bund, forest)

    f) and labeling of the collection bags both within and outside.

Geo-referenced data are very useful as they give a precise account of the location of the original collecting sites and help to identify accessions with specific adaptive traits in accordance to the agro-climatic conditions of the original collecting sites. Passport data are crucial in identifying and classifying each accession and will function as an entry point in selecting and using the accession. The forms developed by CSGRC, Hosur for collecting the passport data and also passport data collection form of NBPGR are enclosed as Annexure 1.1 and Annexure 1.2.

- For convenience in the field, carry standardized collecting record books and printed labels. The label inside accompanies the sample. The outside label helps in sorting of the samples.
- Document information on traditional knowledge from the farmers growing the varieties, including: farmers' name and description of environment, landrace or cultivars characteristics as described by the farmer, end use of the landrace or cultivars and its specific properties, normal cultural practices used with the landrace or cultivars, and history of the landrace or cultivars with the farmer.
- Be inquisitive to acquire information on anything interesting.
- Money may have to be paid occasionally to collect the desired germplasm from farmers.
- Never forget to convey your gratitude to the farmer before leaving the place.

Identification of unique samples for acquisition

Maintaining a sample in the gene bank is expensive; therefore, the genebank curator should carefully check if the sample already exists in the collection before deciding on acquiring it. Carrying the information on the availability of genetic diversity available in the gene bank in digital form may help to compare the existing variability for the trait of interest.

Handling of the samples

It becomes necessary to travel for collecting new material while holding the already collected germplasm. The exploration team should ensure safety of the collected material until the time the collection ends and it is transported back to the genebank.

Maintain the material at optimum temperature and see that moisture content of the collected material is lost even when the distance for transportation is short, see that no damage is done to the cuttings during transport. Exposing cuttings to unfavorable environmental conditions during transportation can be very damaging. Therefore care must be taken. Engage courier boys to accompany the team when collecting germplasm in in remote places on long expeditions and send material to the base camp for onward transmission to headquarters.

If the local extension agents or NGOs are requested to make the final collection, special instructions need to be given for sampling and handling of the material.

B. Acquiring unique germplasm from other genebanks

- Prior to the collection, obtain the complete passport information of the collection, especially alternate names or identification numbers, pedigree, original source, etc from the donating institute. Most often, errors are made during data entry, especially with spaces, hyphenation, case and spelling, which require careful checking when comparing databases to identify duplicate accessions. For this purpose, ICRISAT has special software package "MATCH" which helps to identify the duplicates easily. At CSGRC, similar package need to be developed.
- Prepare the final list of unique accessions to be acquired.
- If the material is to be received in India from abroad, obtain an Import Permit from the National Plant Quarantine Service, Government of India by applying to the Director, NBPGR, New Delhi on a prescribed form (Annexure 1.3).
- The original Import Permit and a Phytosanitary Certificate (PSC) issued by the National Plant Quarantine Services of the exporting country must accompany the material.

- Ask the consignor to complete and send the "Form for Advance Intimation of Import of Samples to India" as advance intimation of export of the proposed propagation material (Annexure 1.4).

C. Germplasm assembly from internal programs

Acquisition of germplasm which includes rare recombinants in the segregating material, induced polyploids, other breeding material, mutants, genetic stocks and prebreeding material from different institutes/organization which are involved in mulberry genetic improvement will also serve as important raw material for crop improvement. These include sources of resistance to biotic and abiotic constraints, and other genetic stocks which can be helpful in developing the molecular tools. Elite germplasm generated in the breeding programs for specific traits or with proven high yield may also be acquired by the genebank. While acquiring, ensure that the material has complete pedigree information and key morphological data.

D. Current policy on germplasm acquisition

The genebank should have a clear policy on acquisition so that the volume of material acquired is within limits of the management capacity of the genebank. When space or the resources to maintain the collections are limiting, germplasm should be acquired based on priority. Emphasis by germplasm collecting missions is thus laid on an understanding of prevalent genetic diversity in different areas and the field tactics involved. The prevalent diversity at specific, infra-specific and genepool level, considering the variation available in diverse populations/different kinds of genetic resources, would fall in the following germplasm categories (Frankel and Soulé, 1981; Hawkes, 1983).

Prioritization of germplasm usually consists of the whole range of genetic variation found in the crop, contained in:

a) primitive cultivars,
b) landraces,
c) wild and weedy forms,
d) genetic stocks,
e) elite breeding material, and
f) improved varieties (both obsolete and modern)

The relative importance of all these kinds of genetic resources, also including breeding lines and genetic stocks, are important though first categories would demand prioritization in national context in germplasm collection. Acquisition of germplasm should be based on value or perceived threat of extinction. Value can be assessed by the usefulness of traits, and adaptation to unique environments. Landraces, wild and weedy species should receive high priority for acquisition due to the imminent threat of replacement, followed by genetic stocks.

Plant Quarantine

Plant quarantine helps in safe introduction of new germplasm samples from other countries. The work is done with the help of NBPGR, the plant quarantine authority of the Government of India. Plant quarantine measures aim at providing protection to the agriculture of a country or region against the likely ravages of alien pests/pathogens get introduced and established. Quarantine not only helps to ward off the threats of exotic pests, but also aim to eliminate and prevent further spread of pests/pathogens (both indigenous and introduced) with restricted distribution within the country (domestic quarantine).

As the National Bureau of Plant Genetic Resources (NBPGR), New Delhi has been designated as the national nodal agency for exchange of germplasm material of agri-horticultural and agri-silvicultural crops for research purposes in the country, it has also been entrusted with the quarantine responsibilities in respect to germplasm of these crops. The Director of NBPGR has been empowered to issue 'Permits' for import of seeds/planting materials for research purpose. NBPGR has a separate Division of Plant Quarantine to meet the quarantine requirements in respect of the germplasm materials being exchanged through it. The Division has trained scientific and technical staff representing the disciplines of entomology, nematology and plant pathology, well equipped laboratories, green houses and post-entry isolation growing field facilities to discharge its quarantine responsibilities efficiently. Since, 2007, Director, NBPGR is empowered to issue Import Permit for germplasm after undertaking PRA to facilitate import of planting material.

The main features of the existing plant quarantine regulations in India are as follows:

1. No consignment of seeds/planting materials shall be imported into India without a valid 'Import Permit', which is to be issued by a competent authority, to be notified by the Central Government from time to time in the Official Gazette.
2. No consignment of seeds/planting materials shall be imported into India unless accompained by a 'Phytosanitary Certificate', issued by the official Plant Quarantine Service of the source country.
3. All consignments of plants and seeds for sowing/propagation/planting purposes shall be imported into India through land customs station, seaport, airport at Amritsar, Bombay, Calcutta, Delhi and Madras, and such other entry points as may be specifically notified by the Central Government from time to time, where these shall be inspected and, if necessary, fumigated, disinfested/disinfected by authorised plant quarantine officials, before quarantine clearance.
4. Seeds/planting materials requiring isolation growing under detention, shall be grown in post-entry quarantine facility approved and certified by

the Designated Inspection Authority (DIA) to conform to the conditions laid down by the Plant Protection Adviser to the Govt. of India.

5. Hay, straw or any other materials of plant origin shall not be used as packing material.
6. Import of soil, earth, sand, compost, and plant debris accompanying seeds/planting materials shall not be permitted. However, soil can be imported for research purposes under a special permit issued by the Plant Protection Adviser to the Govt. of India.

The planting material package should not be addressed to CSGRC, carried on one's person or brought as undeclared accompanied baggage at the port of entry. Exceptionally, when bringing the package to CSGRC, a certificate of fumigation/examination from plant quarantine officials must be obtained at the port of entry. Additional declarations are required for entry of seeds as listed below:

Plant materials received at NBPGR are subjected to visual and microscopic examination. Once a pest, pathogen or weed is detected, appropriate eradication treatments such as fumigation, heat treatment or chemical dressing are given before release of the material.

The Genebank should not acquire imported seed, unless it is cleared by National Plant Quarantine Services. Multiplication will be done under the close supervision of the Plant Quarantine Officer. Optimum number of plants (minimum sample size) are grown to Optimal planting and crop management practices are used

The detailed guidelines are plant quarantine is available at
http://plantquarantineindia.nic.in/pqispub/html/PQO_amendments.htm

Domestic quarantine is as important as the international quarantine and, therefore, planting material should be moved from one state to another or from one place within a state to another under strict phytosanitary conditions. For intra-country or internal quarantine of plants/ planting material, domestic quarantine regulations have been promulgated for regulating inter-state movement of agricultural commodities under the Destructive Insects and Pests (DIP) Act of 1914.

Any planting material brought from collection will be subjected to visual and microscopic examinations, planted in isolated nurseries and healthy saplings used for further multiplication and inclusion in the field gene bank. The soil samples collected from the collection area are also subjected to microscopic examination for presence of pathogens. If soil borne pathogens are observed, the collections will be subjected to more inspection and care before inclusion in the field gene bank.

Characterization and preliminary evaluation

Characterization of mulberry genetic resources

Characterization is the description of plant germplasm. It determines the expression of highly heritable characters ranging from morphological, reproductive, anatomical to molecular markers. Characterization can be carried out at any stage of the conservation process. It is essential that the germplasm being conserved is known and described to the maximum extent possible to assure their maximum use by plant breeders (FAO, 2014). Therefore, characterization should be carried out as soon as possible to add value to the collection.

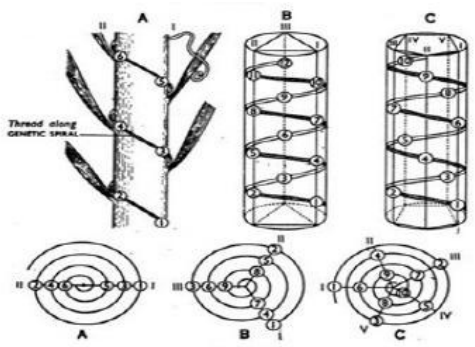
Reliability of data might vary among data collectors if they are not well trained and experienced. Therefore, trained technical staff in the field of plant genetic resources should be available during the entire growth cycle to record and document characterization data as per the gene bank standards for characterization defined by FAO,2014. Access to expertise in taxonomy, plant pathology (in-house or from collaborating institutes) during the process of characterization is desirable. Characterization is very labour-intensive and requires sufficient funding to allow for good quality data.

Standards developed by FAO (2014) for characterization of genetic resources

- All accessions should be characterized
- For each accession, a representative number of plants should be used for characterization.
- Accessions should be characterized morphologically using internationally used descriptor lists where available.
- Molecular tools are also important to confirm accession identity and trueness to type.
- Characterization is based on recording formats as provided in internationally used descriptors.

In mulberry, CSGRC, Hosur developed descriptors for morphological, reproductive, anatomical characterization in consultation with expert groups. For characterization, mulberry plantation is maintained as tree type, with the spacing 2.4m between plants and rows and 3.0m between accessions and 4 plants per accession are maintained. The recommended cultural practices for tree type mulberry are followed. The plants are trained at 1.5 m crown height following two prunings per year which will be followed by characterization. The data/ observations are recorded from 3 plants using 1 plant as one replication per accession.

a) Morphological characterization

S. No.	Descriptor	Growth stage / time of recording	Classification	Method
1	Growth nature	90 days after pruning	1 Erect 2 Spreading 3 Drooping	Visual observation of the angle between the foliage branches to the main trunk.
2	Branch nature/curve of the shoot	90 days after pruning	1 Straight 2 Slightly curved 3 Curved	Visual observation on longest shoot.
3	Colour of young shoot	30 days after pruning	1 Green 2 Greenish purple 3 Purple	Visual observation on the upper 1/3rd portion of shoot with the help of standard colour charts
4	Colour of mature shoot	90 days after pruning	1 Brown 2 Grey 3 Greyish Green 4 Greyish Brown 5 Purplish Brown 6 Greenish Brown	Visual observation on the lower portion of the longest shoot with the help of standard colour charts
5	Phyllotaxy	75 days after pruning	1 Distichous or ½ Phyllotaxy 2 Tristichous or 1/3 Phyllotaxy 3 Pentastichous or 2/5 Phyllotaxy	Observations on the middle portion of the shoot.
A. Distichous: Leaf on the second node is just opposite the leaf on the first node, the third leaf is above the first leaf, the fourth above the second **B. Tristichous**: leaves are three-ranked (three orthostichies); the fourth leaf is above the first leaf, fifth above second, sixth above third and so on. Three leaves lie in one circle of the genetic spiral **C. Pentastichous :** 6th leaf is found above the 1st leaf, 7th above the 2nd and so on. The genetic spiral completes two circles in passing these five leaves or five orthostichies  				
6	Lenticel density/	90 days after	1 Sparse (<5)	Observation on

S. No.	Descriptor	Growth stage / time of recording	Classification	Method
	(sq.cm)	pruning	2 Medium (5-10) 3 Dense (>10)	middle portion of stem surface Place one sq.cm cut film roll around the stem surface and count the number of lenticels in that area. Three observations /branch.
7	Lenticel shape	90 days after pruning	1 Round 2 Elliptical 3 Oval	Observations on mature lenticels from the middle portion of stem.
8	Bud shape	90 days after pruning	1 Round 2 Acute triangle 3 Long triangle 4 Spindle shaped	Round – buds are circular Acute triangle – tip of the bud is acute Long triangle – tip of the bud is slightly elongated Spindle shaped – basal portion of the bud is tapered to look like a spindle.
9	Bud length (cm)	90 days after pruning	1 Short 2 Medium 3 Long	Length of the mature bud from the basal portion to the tip of the bud
10	Bud attachment	90 days after pruning	1 Adhering to branch 2 Slanting outward 3 Tilting to one side 4 With one accessory bud 5 With dorsal accessory bud	1- Buds are attached to the stem 2- Buds are projected away from the stem 3- The tip of the bud is tilted to one side 4- The main bud is supported by one or two accessory buds 5- The accessory bud is on the dorsal side
11	Leaf apex	60 days after pruning	1 Acute 2 Acuminate 3 Caudate 4 Obtuse	Collect leaves from the middle portion of the longest shoot and observe on the upper 25 percent of the leaf.

S. No.	Descriptor	Growth stage / time of recording	Classification	Method
				Acute: Margins straight to convex forming a terminal angle 45 -90 . **Acuminate:** Margins straight to convex forming a terminal angle of less than 45 **Caudate:** Acuminate with concave margins. Leaf tip extended greately forming a tail like structure. **Obtuse:** Margins straight to convex, forming a terminal angle more than 90.
12	Leaf margin	60 days after pruning	1 Crenate 2 Serrate 3 Dentate 4 Repand	Observation on the leaves from middle portion of the longest shoot
	Crenate: the edge of the leaf has blunt, rounded teeth	A Serrate margin is when a leaf has sharp, "saw-like" teeth. Serrations are pointed with their axis approximately perpendicular to the trend of the margin	A Dentate margin is when a leaf has triangular, "tooth-like" edges. Dentations are pointed with axis approximately perpendicular to the trend of the margin	Margins form a smooth line or arc without noticeable projections
13	Leaf base	60 days after pruning	1 Truncate 2 Cordate 3 Lobate	Collect leaves from the middle portion of the longest shoot and observe on the lower 25 percent of the leaf.
	Truncate: Leaf base is perpendicular to the petiole and relatively straight across	Cordate: Heart shaped leaf base that attaches at the notch of the base.	Lobate: Base with lobes. leaf base small to large rounded projections whose inner margins towards the petiole and are in partly concave shape	
14	Leaf surface	60 days after pruning	1 Smooth (Glabrous) 2 Slightly rough 3 Rough	Observations based on feeling by touch. Rub the upper surface of the leaf

S. No.	Descriptor	Growth stage / time of recording	Classification	Method
			4 Hairy (Pubescent)	blade gently with the fingers and feel. Leaf surfaces can be smooth and hairless (glabrous) or rough or hairy
15	Leaf texture	60 days after pruning	1 Membranous 2 Charatacious 3 Herbacious 4 Coriaceous	**Membranous:** Thin and semi-translu-cent; membrane-like. **Charatacious:** Papery, opaque and thin. **Herbacious:** Soft and succulent. **Coriaceous** leaves are thick, stiff, or leathery.
16	Stipule nature	60 days after pruning	1 Bud scale 2 Free-lateral 3 Foliaceous	**Bud scale:** stipules are very short, slender and pointed **Fee lateral:** Stipules are free handing from both sides of the nodal portion **Foliaceous:** Stipules are broad and look like miniature leaves
17	Stipule duration	60 days after pruning	1 Caducous 2 Persistent	**Caducou**s: Stipules fall off with the maturity of the leaves **Persistent**: stipules stay for longer period
18	Shape of the scar	90 days after pruning	1 Circular 2 Elliptical 3 Triangular	Observe the leaf scars after leaves are harvested from the matured lower portion of the stem.
19	Leaf nature	60 days after pruning	1 Homophyllus a) Lobed b) Unlobed 2 Heterophyllous	**Homophyllus:** All the leaves in a plant are similar either lobed or unlobed **Heterophyllous:** Some leaves are lobed and some are unlobed either in the same branch or different branch

S. No.	Descriptor	Growth stage / time of recording	Classification	Method
20	Leaf lobation	60 days after pruning	0 – lobed Lobation with 1-5 lobes	Count the number of lobes of individual leaves
21	Leaf lobation type	60 days after pruning	Shallow Lobed Medium Lobed Deeply Lobed	**Shallow lobed:** Lobation is restricted to margin **Medium lobed:** Lobation is extended upto the middle of the leaf blade **Deeply lobed:** Lobation is very deep and extended utpo the midvein
22	Leaf colour	60 days after pruning	1 Light green 2 Green 3 Dark green	The natural colour of the mulberry leaves to be recorded visually using standard colour chart
23	Leaf glossiness	60 days after pruning	1 Nonglossy 2 Slightly glossy 3 Strongly glossy	Observations on the leaves on the middle of the shoot. Observe leaf shining on the upper portion of the leaf surface
24	Leaf wrinkleness	60 days after pruning	Smooth Slightly wrinkled Wrinkled	Smooth- no sign of waviness on leaf surface Slightly wrinkled- leaf surface is wavy Wrinkled- leaves are fully wrinkled
25	Leaf angle	60 days after pruning	1 Acute (<35°) 2 Semierect (>35°-75°) 3 Horizontal (>75°-90°) 4 Drooping (>90°)	Angle of the leaf blade to the mainstem
26	Lamina length (cm)	75 days after pruning	1 Short (<10) 2 Medium (10-20) 3 Long (>20)	Observations on leaves from the longest shoot from 7-9th position. The length from the leaf base at the juncture of the periole attachment to the leaf tip leaving the extended portion of the tip

S. No.	Descriptor	Growth stage / time of recording	Classification	Method
27	Lamina width (cm)	75 days after pruning	1 Narrow 2 Medium 3. Broad	The width of the leaf to be recorded from the widest point
28	Leaf shape	75 days after pruning	1 Lanceolate 2 Narrow ovate 3 Ovate 4 Wide ovate 5 Cordate	Lanceolate leaf is at least 3x longer than wide Narrow ovate: L/W ratio = 2:1 Ovate - described as being broadest below the middle (L/W ratio = 1.5:1 Wide ovate: L/W=1.2:1 Leaves that are Cordate-shaped leaves are broad to the point and then turn upwards at the base, forming a notch. Cordate leaves are "heart-shaped.
29	Petiole length (cm)	75 days after pruning	1 Small (<2) 2 Medium (2-4) 3 Long(>4)	Observations from the leaf petioles from below 1/3rd portion on the shoot
30	Petiole width (mm)	75 days after pruning	1 Small 2 Medium 3 Large	Observations from the leaf petioles from below 1/3rd portion on the shoot
31	Petilole groove	75 days after pruning	1 Present 2 Absent	Observe the lower portion of the petiole for presence or absence of the groove
32	Trichome density (No./sq.mm)	75 days after pruning	1 Sparse (<50) 2 Medium (50-100) 3 Dense (>100)	The density of the trichomes from both adaxial and abaxial leaf surface (on veins an veinlets) to be recorded from cuticular replicas obtained by thin coating thermocol-xylol solution on the leaf surface and after peeling the dried opaque film and observe in the microscope

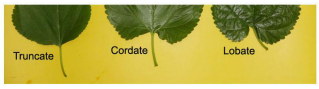
Erect

Spreading

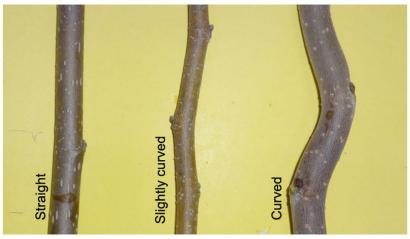
Drooping

Growth Nature

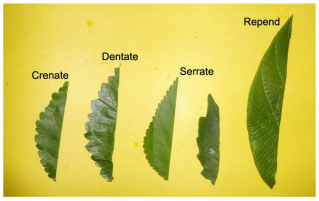
Leaf apex



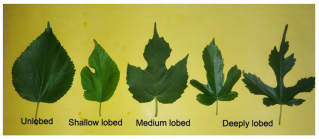
Leaf base



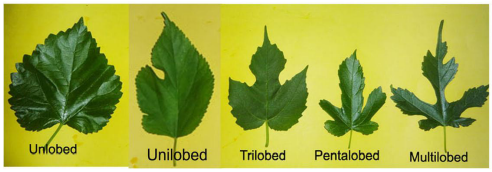
Curve or straightness of the stem



Leaf margin



Lobation type



No. of lobation

Leaf and Stem morphological features in Mulberry

b) Anatomical characterization

In sericulture, the quality and quantity of mulberry leaf production plays direct role. Also the leaf is the vital organ in plant where the major physiological activities take place and hence its anatomical structural features are important. Stomatal characters influence stomatal resistance, transpiration rate and photosynthetic rate of leaf and also influence plant adaptation. Further, number of chloroplasts in the guard cells of the stomata is directly correlated to the ploidy status. The leaf anatomical characterization of mulberry is highly essential not only for the phylogentic studies but also for the identification of stress tolerant accessions.

The following points are to be considered while making anatomical characterization

- Fully expanded leaves from 7^{th} to 9^{th} position in descending order from three months old shoots after pruning are to be collected in the morning.
- Small rectangular pieces should be taken from the central portion of the leaf blade avoiding veins and vein lets and need to be preserved in the Formalin-Acetic Acid -Alcohol (FAA) solution (Formaldehyde (38%) 5 ml, Glacial Acetic Acid 5 ml and Absolute alcohol 95 ml).
- For studying the stomatal size and idioblast frequency, the preserved leaf material are taken out from the vials and washed in running water and dried on a blotting paper. A thin layer of Wimble quick fix should be applied on abaxial side (down) for stomatal studies and on adaxial side (Upper) for the idioblast studies. After 15 minutes, when the quick fix is dried the impression has to be taken out carefully and place it on clean slide and cover it with the cover slip and observe it under microscope.
- For counting the number of chloroplasts in the stomata, collect 1 to 5 leaves of the apical part of the plant, the samples are placed in a petri dish containing a filter paper moistened with distilled water covering the bottom of the lid. Then, epidermal peels are taken from the abaxial side near the vein structure using a pair of fine tweezers, stain in 2% potassium iodide and Iodine solution (KI+I) solution for 5 minutes and put on the slide for observation.
- Dissolve 6g potassium iodide in about 200 ml distilled water and then add 3g iodine crystals and make the solution up to 1 litre with distilled water. It is essential to prepare it 24 hours before it is required, as iodine is slow to dissolve.
- For measuring the idioblast project length, cystolith length, breadth and thickness of different layers such as cuticular, epidermal, palisade, spongy and total leaf thickness, thin hand section of the preserved leaf material has to be taken and stained with 1% safranin and mounted in 50% glyecerine.

- For all the characters, three leaves per plant and three plants per accession are to be considered.

Microscopic measurements

- Place the ocular micromieter in the eyepiece with 10x magnification of the microscope
- Place the stage micrometer of 0.01 mm unit size under the objective.
- Adjust the focus and observe how many ocular divisions are coinciding with the stage micrometer divisions
- Calibrate each ocular division equals to how many µm

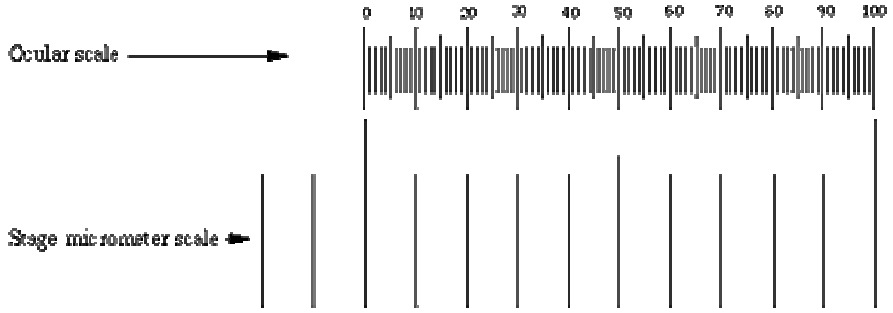
Ocular and Stage Micrometers

Calibrating an ocular micrometer is only reliable for one objective on one scope. Changing the objective lens or using a different scope than your assigned scope may yield varying results. Always calibrate your micrometers when using new objectives or different microscopes.

Each division of the stage micrometer = .01mm.

Formula:

[(# divisions counted on stage micrometer)(one division of the stage micrometer in mm)/(# divisions counted on ocular micrometer)][1000]



Example 1

.01mm stage micrometer
stage micrometer = 12 divisions to 22 divisions on the ocular micrometer
Step 1: [(12)(.01)/(22)][1000]
Step 2: (.12/22)(1000)
Step 3: (.0055)(1000)
Equals: 5.5µm per ocular division

Example 2
.01mm stage micrometer
stage micrometer = 1 divisions to 7 divisions on the ocular micrometer

Step 1: [(1)(.01)/(7)][1000]
Step 2: (.01/7)(1000)
Step 3: (.0014)(1000)
Equals: 1.4µm per ocular division

After calibration of ocular micrometer, count divisions of the ocular micrometer as the length of an individual specimen. This should be converted to length by using the following formula

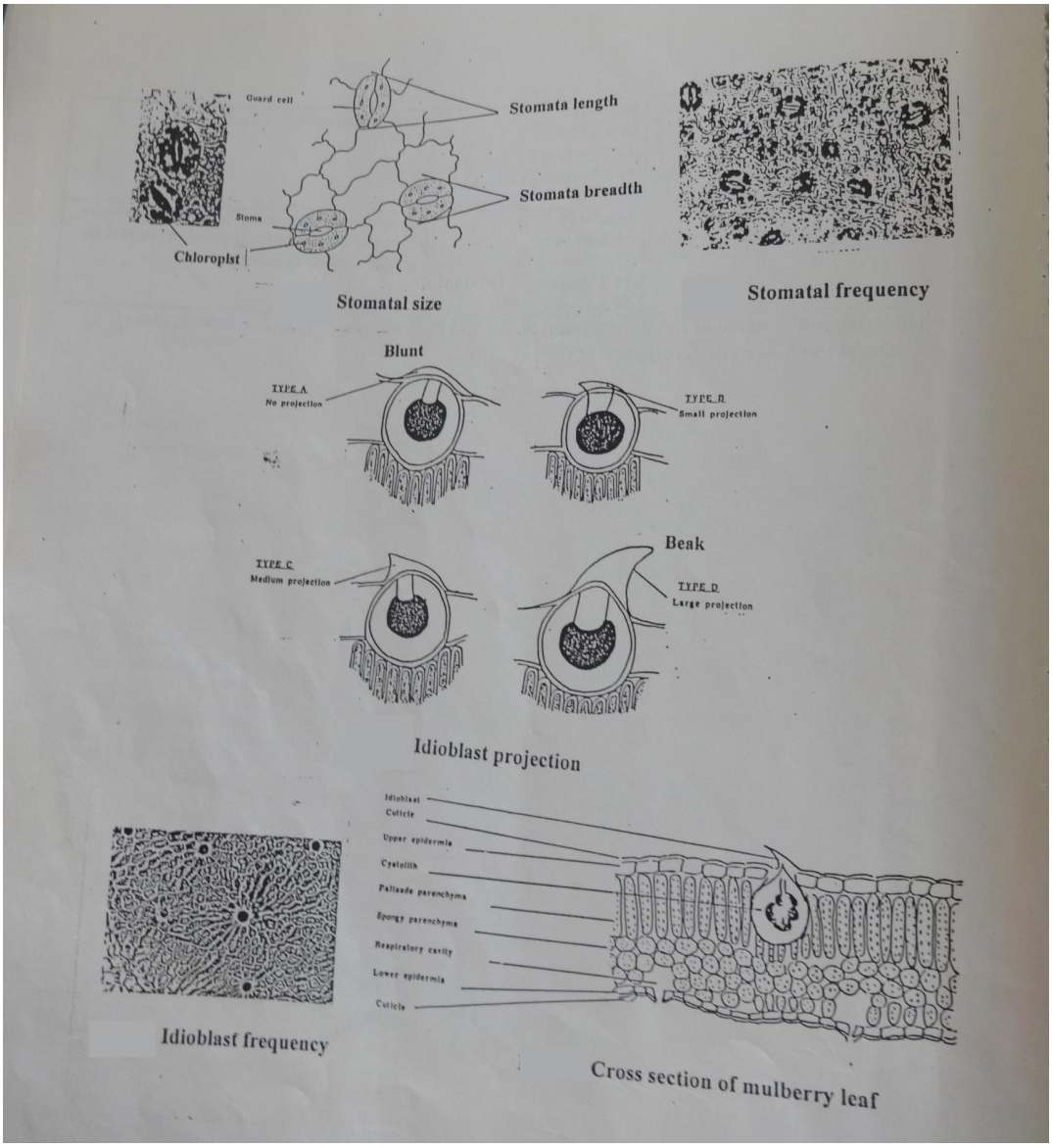
Formula: Total specimen length (x) = (length of one division of the ocular micrometer)(number divisions counted as length of an individual specimen).
Example:
Step 1: x = (1.4µm)(5 divisions)
Step 2: x = 7µm

Descriptors for anatomical characterization

S.No	Descriptor	Classification unit	Method
1	Stomata size (sq. m)	1 Small (≤200) 2 Medium (200-400) 3 Large (>400)	The length and breadth of stomata to be recorded using ocular micrometer and then convert to sq.µm.
2	Stomatal frequency (no./sq.mm)	1 Low (≤400) 2 Medium (400-800) 3 High (>800)	To be recorded preferably under low magnification (10x45) so as to accommodate more number of stomata per microscopic field. Stomatal frequency to be recorded in an unit area under microscope in nine observation fields. The number counted in an unit area has to be Giant stomata are to be avoided while counting. Minimum three microscopic fields per leaf and three leaves per accession to be considered.
3	No. of chloroplaststs / stomata	1 Less (≤12) 2 Medium (12-16) 3 More (>16)	Total number of chloroplasts including both the the guard cells of the stomata are to be counted. Minimum nine stomata are to be considered for counting.
4	Idioblast length (µm)		To be recorded under 10x45 magnification using the eye piece ocular micrometer. Free hand section are made from fixed leaf material for microscopic observation. The total length of the idioblast projection which protrude from the epidermis is to be measured. A total of nine observations from three

S.No	Descriptor	Classification unit	Method
			leaves are to be considered.
5	Idioblast type	1 Blunt 2 Beak	The type of idioblast to be recorded by observing the nature of idioblast projection from the upper epidermal cells of the leaf surface.
6	Idioblast frequency (no./sq.mm)	1 Less (≤15) 2 Medium (15-30) 3 High (>30)	To be recorded preferably under low magnification (10x10) so as to accommodate more number of idioblasts in the microscopic focus
7	Cystolith length (µm)	1 Small (<50) 2 Medium (50-75) 3 Large (>75)	The same idioblast which are considered for measuring the projection length should be taken for measurement of the length of cystolith under 10 x 45 magnification. The cystoliths, which contains calcium carbonate crystals in them are embedded in the upper epidermis. The length of the cystolith without the idioblast projection to be measured using the ocular micrometer and then convert to µm.
8	Cystolith width (µm)	1 Small (<50) 2 Medium (50-75) 3 Large (>75)	Width of cystolith to be measured similar to length
9	Upper cuticular thickness (µm)	1 Thin (≤4) 2 Medium (4-8) 3 Thick (>8)	To be recorded under 10x45 magification using ocular micrometer and then convert into µm. Minimum three leaves per accession from 7^{th} to 9^{th} position to be considered.
10	Upper epidermal thickness (µm)	1 Thin (≤20) 2 Medium (20-40) 3 Thick (>40)	As described above
11	Palisade thickness (µm)	1 Thin (≤50) 2 Medium (50-75) 3 Thick (>75)	As described above. In some of the accessions the palisade layers are more than one (multilayered). The thickness of all the layers are to be recorded to get the total palisade thickness.
12	Spongy thickness (µm)	1 Thin (≤50) 2 Medium (50-75) 3 Thick (>75)	As described above
13	Lower epidermis thickness (µm)	1 Thin (≤5) 2 Medium (5-10)	As described above

S.No	Descriptor	Classification unit	Method
		3 Thick (>10)	
14	Lower cuticle thickness (µm)	1 Thin (≤2) 2 Medium (2-4) 3 Thick (>4)	As described above
15	Total Leaf thickness (µm)	1 Thin (≤150) 2 Medium (150-175) 3 Thick (>175)	The individual thickness of each layer from upper cuticular to lower cuticular thickness is added to get the total leaf thickness.



d) Characterization for reproductive traits

The knowledge of floral structure and reproductive behaviour of mulberry accession are of utmost importance for maximum utilization of genetic resources in any breeding programme for crop improvement. Also, this information is important for classification of mulberry accessions into different species

The following aspects must be kept in mind while recording the reproductive characters

- Fully bloomed catkins should be randomly collected from different plants of the same accession in the morning around 10.00 AM in plastic bags.
- The time period from male flower initiation in the auxiliary bud to anthesis and maximum receptivity of the female flowers is generally for about 10-16 days.
- Fully ripened sorosis should be collected and different fruit characters like fruit length, breadth, pedicel length, weight, colour and taste. Fruit ripening generally takes place within 25-30 days after fertilization

Descriptors of reproductive characterization

S.No	Descriptors	Growth stage	Classification	Method
1	Flowering time	On three years old plants	1 Spring (Feb-March) 2 Autumn (Oct-Nov) 3 Both spring and autumn	Observe main flowering season before the plants are pruned
2	Sex expression	Before pruning during flowering season	1 Dioecious-Male 2 Dioecious Female 3 Monoecious 3.1 Male and bisexual 3.2 Female and bisexual 3.3 Male and Female 3.4 Male, female and bisexual	Observe inflorescences in the all the branches in a plan and record the observation based on the presence of inflorescences (Fig.)
3	No. of inflorescence/ meter	During main flowering season	1 Low 2 Medium 3 Many (>40)	Observations recorded by counting the no. of inflorescences in meter length on longest shoot
4	Inflorescence length (cm)	During main flowering season	1 Small (<2) 2 Medium (2-4) 3 Long (>4)	Observations to be recorded on fully bloomed inflorescence of different male (before dehiscence), female (at receptive

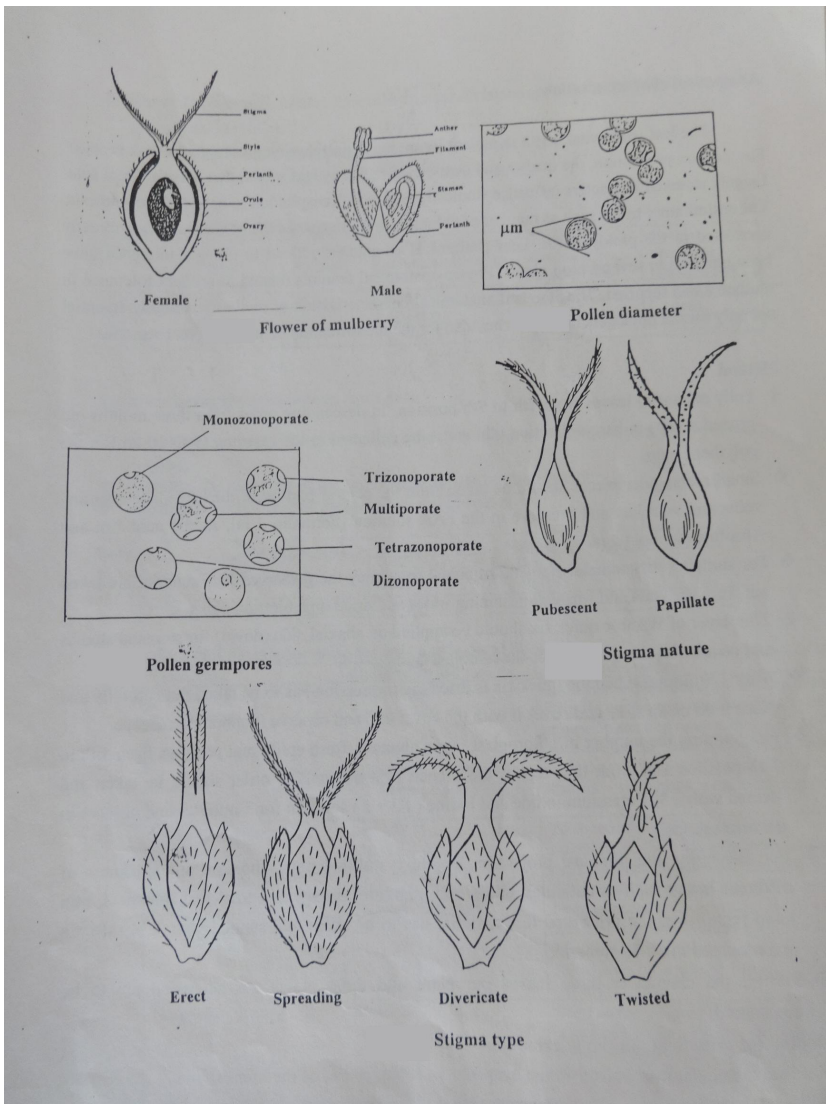
S.No	Descriptors	Growth stage	Classification	Method
				stage) and bisexual catkins depending upon the sex expression. Minimum three inflorescence/plant and three plants per accession need to be considered for recording observations.
5	No. of flowers/inflorescence	During main flowering season	1 Low 2 Medium 3 Many	Observations should be done on the same inflorescence considered for measuring the length are to be used. All the individual floweres around the rachis of the inflorescence should be dissected and counted. In case of bisexual inflorescence, the different frequencies of male, female and bisexual flowers are to be counted separately.
6	Stamen length (mm)	During main flowering season	1 Short (<4) 2 Medium (4-6) 3 Long (>6)	The individual male flowers from fully bloomed male inflorescence collected before dehiscence of anthers are dissected under stereo microscope and the individual stamens (filament and anther) are stretched and the length of the stamen is recorded using ocular micrometer and then converted to mm. Minimum of nine readings to be taken considering three plants
7	Anther length (mm)	During main flowering season	1 Short (<0.5) 2 Medium (0.5-1) 3 Long (>1)	The same stamens are used for recording the anther length to be

S.No	Descriptors	Growth stage	Classification	Method
				considered for recording anther length
8	Pollen viability	During main flowering season	1 Low (<50) 2 Medium (50-75) 3 High (>75)	The mature pollen grains should be dusted on a clean slide from the male inflorescence and stained with 2% aceto carmine solution for 30 minutes and Pollen viability= (No. of pollen grains stained / Total number of pollen grains observed) x 100
9	Pollen diameter (µm)	During main flowering season	1 Small (<15) 2 Medium (15-30) 3 Large (>30)	The fully stained pollen grains are to be considered for measuring the diameter using the ocular micrometer under 10x 45 magnification
10	No. of germ pores/pollen grain	During main flowering season	1 Monozonoporate 2 Dizonoporate 3 Trizonoporate 4 Tetrazonoporate 5 Mixed	The frequency of mono (single germ pore) to pentazonoporate (five germ pores) pollen grains to be counted separately in each micropscopic fields and record the observation
11	Style length (mm)	During main flowering season	Short (<0.5) Medium (0.5-0.75) Long (>0.75)	To be recorded when the stigma of the flowers is in receptive stage and before the ovary is fertilized. It is the length from the tip of the ovary to the base of the stigma. The style length has to be recorded under stereo microscope using ocular micrometer
12	Stigma length (mm)	During main flowering	1 Small (<2) 2 Medium (2-4)	To be recorded when the stigma are in

S.No	Descriptors	Growth stage	Classification	Method
		season	3 Long (>4)	receptive stage. The length of both of the bifid stigma should be taken separately and then finally added to get the total stigmatic length. The stigma length has to be recorded in stereo microscope using ocular micrometer.
13	Stigma nature	During main flowering season	1 Pubescent 2 Papillate	Pubescent- stigma with long hairs Papillate – Stigma surface is plain without any hairs or covered with minute hairs
14	Stigma type	During main flowering season	1 Erect 2 Spreading 3 Divericate 4 Twisted	Erect- stigmas are straight and erect Spreading- When both the stigmas are spreading forming wide angle from the style Divericate-Both the stigmas are bent to form a curve like structure Twisted- When the both the stigmas are twisted around each other
15	Fruit length (cm)	During main flowering season	1 Small 2 Medium 3 Long	Data should be recorded when the fruits are in full ripening stage. The length of the fruit including penducle. Minimum of nine fruits from three plants to be considered for observations
16	Fruit width (cm)	During main flowering season	1 Small 2 Medium 3 More	The same fruits which are used for recording length to be used
17	Fruit weight (gm)	During main flowering season	1 Low (<2) 2 Medium (2-4) 3 High (>4)	The same fruits which are used for length and width are to be used for taking

S.No	Descriptors	Growth stage	Classification	Method
				the weight of the individual fruits
18	Fruit colour	During main flowering season	1 Black 2 White 3 Greenish White 4 Purple	The colour of the fruits to be recorded when the fruits are in full ripen stage and graded on visual observation
19	Fruit taste	During main flowering season	1 Sour 2 Less sweet 3 Sweet	The fruit taste is to be recorded when the fruits are in full ripen stage
20	Seed shape	During main flowering season	1 Round 2 Elliptical	The seed shape has to be recorded after the seeds are harvested from the ripened fruits by observing under the stere-omicroscope
21	Seed colour	During main flowering season	1 Light yellow 2 Light brown 3 Yellowish brown 4 Dark brown 5 Blackish brown	The seed colour has to be recorded after the seeds are harvested from the ripened fruits by observing under the stereo-microscope
22	Hundred seed weight (mg)	During main flowering season	1 Low (<100) 2 Medium (100-200) 3 High (>200)	The data has to be recorded after the seeds are harvested from the ripened fruits (after initial drying on the blotting sheets)
23	Percentage of seed set (%)	During main flowering season	1 Low (<50) 2 Medium (100-200) 3 High (>200)	It is the ratio of number of healthy seeds to the total number of achenes per fruit expressed in percentage
24	Apomictic nature	During main flowering season	1 Weak apomictic 2 Medium apomictic 3 Strongly apomictic	The data has to be recorded from the pure diecious female accessions, which are bagged before initiation of flowering to avoid cross pollination. Based on the seed set % and seed germination the apomictic nature to be decided. However, the apomictic nature is to be confirmed

S.No	Descriptors	Growth stage	Classification	Method
				through embryological means.



d) Cytological characterization

The basic chromosome number in mulberry is n= 14. Mulberry is also characterized by different ploidy levels ranged from 2n=2x=28 to 2n=2x= 308. Among the different ploidy levels, triploids are considered to be superior in most of the economical characters and wider adaptability to different agro climatic conditions. Most of the genotypes which belong to *M. alba L. M. indica L.* are diploids (2n=2x=28). The wild species of mulberry belong *to M. laevigata Wall. M. serrata Roxb.* are available in different ploidy level. Tetraploid mulberry varieties in *M. wittiorium Handel-Mazett M. bonninnesis Koidz* was also reported Hexaploid (2n=6x=84) mulberry varieties belong to *M. serrata Roxb, M. tiliafolia and M. cathayana* and octoploidy (2n=8x=112) in *M. cathayana* and highest ploidy (2n=22x= 308.) in *M. nigra* and polysomaty in M*. multicaulis and M. alba* was also reported. Haploid mulberry (n=14) can be obtained through *in vitro* anther culture and gynogenic haploid mulberry through ovary culture. The karyotype morphology will help in understanding the phylogenetic relationship among the different species and its distribution has a great value in modern taxonomy.

Method for cytological observations in shoot tip (Dandin *et al.* 1986)

1. For studying the somatic chromosome the shoot tips are to be collected in the mornning 10 am.
2. Pre treat with 0.002M 8-hydroxy quinoline for 3 hours
3. Wash the pre-treated material with running water for three times
4. After thorough washing they are to be hydrolysed in HCl for seven minutes at 40°C
5. After thorough wash stain with 1 % propionic oricin for 30 minutes
6. Squash preparations are to be made in 45 % acetic acid and observe in the microscope

The detailed karyotype studies can be made by selecting the well spread mitotic plates and all the chromosome measurements like short arm length, long arm length, total chromatin length, satellite bearing chromosomes are to be made from the camera lucida drawings or from photomicrographs in mm and then converted to microns. All the measurements are to be tabulated separately and homologous pairs are to be arranged in the descending order of total length (Dandin et al 1986). For determining the type of chromosomes the terminology described by Leven *et al* 1964 may be followed.

Sl.No.	Descriptors	Classification unit	Methods
1	Chromosome number	1. Haploid (n=14) 2. Diploid (2n=28) 3. Triploid (3n=42) 4. Tetraploid (4n=56) 5. Pentaploid (5n=70) 6. Octaploid (8n=112) 7. Decosoploid (22n=308) 8. Aneuploid (Any addition or deletion in the diploid chromosome number 28)	To determine the ploidy level of each accession minimum 20 well spread mitotic plates to be counted for the total number of chromosomes per each cell
2	Karyotype	M= centromere in median point (r=1) m= centromere in median region (r=1.1 to 1.7) sm= centromere in sub median region (r=1.8 to 3.0) st= centromere in sub terminal region (r=3.1 to 7.0) t= centromere in terminal region (r=7.1 to infinity) T= centromere in terminal point (r= infinity) **centromere index (r)= 1/s** Where, l= length of long arm s= length of short arm	The well spread mitotic plates are to be considered for studying the karyotype of the mulberry accessions

Molecular characterization

With the advances in biotechnology, molecular marker technologies and genomics are increasingly used for characterization (De Vicente et al., 2004), in combination with phenotypic observations because they have advantages in the estimation of uniqueness of a source of variation within or among accessions. Genotypic data obtained from characterizing germplasm using molecular techniques have the advantage over phenotypic data in that variations detected through the former are largely devoid of environmental influences (Bretting and Widrlechner, 1995). There are many markers and techniques available (e.g. SSR, expressed sequence tags - simple sequence repeats [EST-SSR], amplified fragment length polymorphisms [AFLP]) but, for characterization purposes, only well-established, repeatable markers such as SSR should be used. For many crops, a wide range of marker primers suitable for their use in characterization has been developed; However, in mulberry minimum sets of key markers for characterization purpose are yet to be finalized. In order to ensure that the results of different analysis batches are comparable, some genebank accessions should be included as reference on each batch. The inclusion of reference accessions in molecular characterizations also plays an essential role for comparison among differerent genebanks.

Evaluation

Evaluation is the recording of those characteristics whose expression is often influenced by environmental factors. It involves the methodical collection of data on agronomic and quality traits through appropriately designed experimental trials. Evaluation data frequently includes insect pest resistance, plant pathology and quality evaluations (e.g. protein content) and environmental traits (drought/cold tolerance and others). Adding this type of information allows more focused identification of germplasm to meet prospective client needs. Such data should then be included in the genebank's documentation system. These data sets are all highly desired by users to incorporate traits into breeding programs and improve utilization of collections. The traits for which the germplasm accessions are assayed are defined in advance by crop experts in collaboration with gene bank curators. Reliable evaluation data that are easily retrievable by plant breeders and researchers facilitate greatly the access to, and use of, plant germplasm accessions. Germplasm may be systematically evaluated using a network approach.

Obtaining evaluation data by genebanks is time consuming and frequently more expensive than obtaining characterization data. Curators should make all possible efforts to obtain records of evaluation data. One possible source is evaluation records produced by users to whom germplasm have been distributed. The genebank should solicit the user to share the evaluation data at least after a given time period that the user has published the evaluation results. Practical arrangements in this regard should be worked out between the gene bank and the recipients/users of the material (FAO, 2014).

Data collection should be conducted by trained staff using as much as possible calibrated and standardized measuring formats with identified check accessions (controls) and published crop descriptor lists. The results of greenhouse, laboratory or field evaluations, following standardized protocols and experimental procedures are usually presented as either discrete values (e.g. scores for severity of disease symptoms; counting) or continuous values (based on measuring). The data need to be validated by curators and documentation officers before being uploaded into the genebank database and made publicly available. The participation of multi-disciplinary teams with expertise plant pathology, pest resistance, environmental tolerances, both in-house and from collaborating institutes, during the process of evaluation is desirable.

CSGRC takes up preliminary evaluation for growth and yield, biochemical parameters and natural incidence of foliar fungal diseases in the *ex situ* field gene bank which were planted for characterization purpose. Propagation traits are evaluated in the nursery. The descriptors for evaluation of growth and yield are as follows.

Evaluation of mulberry genetic resources for growth and yield

All the measurements on growth and yield attributing characters are recorded on 70th day after pruning. Measurements are made on 3-5 plants/accession. In tropical conditions, leaf fall starts 70 days after pruning. Hence, delay in observations may lead to experimental errors.

Descriptors of growth and yield attributing characters

Sl. no	Descriptor	Classification	Method
1	Number of branches	1 Low(<20) 2 Medium(20-40) 3 High(>40)	Total number of branches /plant are counted. Donot count small branches that are less than 1/3 rd length of the longest shoot of that plant.
2	Length of longest shoot (cm)	1 Low(<75) 2 Medium(75-150) 3 High(>150)	Length of the longest shoot from the base of the branch to the tip of the branch is measured.
3	Total shoot length (cm)	1 Low(<1500) 2 Medium (1500-3000) 3 High(>40)	Total length of all the branches are measured
4	Inter nodal distance (cm)	1 Low(<3) 2 Medium(3-6) 3 High(>6)	Measure the length of 10 internodes on the longest shoot leaving 1/3 rd top portion and calculate internodal distance.
5	Weight of the hundred leaves (gm)	1 Low(<300) 2 Medium(300-600) 3 High(>600)	Collect equal number of top (leaving first five leaves) middle and lower leaves on the longest shoots and record weight of 100 leaves.
6	Leaf area(sq.cm)	1 Low(<200) 2 Medium(200-400) 3 High(>400)	Measure the leaf area of the sample collected for recording leaf weight and calculated leaf area. $\text{Leaf area} = \frac{\text{Total leaf area}}{\text{number of leaves}}$
7	Lamina petiole ratio by length	1 Low(<2.5) 2 Medium(2.5-5) 3 High(>5)	It is the ratio between lamina length (excluding the petiole) to petiole length
8	Lamina petiole ratio by weight	1 Low(<5) 2 Medium(5-10) 3 High(>10)	It is the ratio between lamina length (excluding the petiole) to petiole length
9	Leaf yield/plant (kg)	1 Low(<2) 2 Medium(2-4) 3 High(>4)	Harvest all the leaves in the plant and record the weight
10	Leaf-shoot ratio	1 Low(<1) 2 Medium(1-1.5) 3 High(>1.5)	Prune the stems after harvesting the leaves and record stem weight. Add leaf weight and stem weight and record as shoot weight/plant. Divide leaf yield by Shoot weight. This gives leaf shoot ratio. This is harvest index in mulberry.
11	Moisture content	1 Low(<60)	Collect the leaf sample (or use leaves

Sl. no	Descriptor	Classification	Method
		2 Medium(60-70) 3 High(>70)	which were collected for recording the 100 leaf weight) from the longest shoot, record the fresh weight, oven dried for 48 h at 60°C and record dry weight . Calculate Moisture content as per the formula given below **Moisture content (MC)** **=** (Fresh weight-oven dry weight) x 100 Fresh weight
12	Moisture retention capacity (%)	1 Low(<60) 2 Medium(60-70) 3 High(>70)	Collect leaf sample from the longest shoot, record fresh weight, weight after 6 h, oven dry for 48 h at 60°C and record dry weight of the sample. Calculate Moisture retention capacity **Moisture retention capacity (MRC) =** (6 hours weight-oven dry weight) x 100 Fresh weight –dry weight

Evaluation for biochemical parameters

The mulberry genetic resources are evaluated for leaf nutritional status particularly for leaf chlorophyll content from the fresh leaves, water soluble protein and carbohydrates from dry leaf powders. The procedures followed are described below.

Estimation of chlorophyll content

1. Collect leaf samples from 7-9th position of leaf from longest branch from three plants
2. Take 5ml of DMSO to the test tube
3. Make 1cm diameter leaf discs with cork borer (or weigh 50g fresh leaf). Discard leaf discs with veins, take fresh weight of 5 leaf discs and add to the DMSO in the test tubes
4. Keep it for overnight in dark chamber (leaf discs become colourless)
5. Read absorbance of the solution at 645nm and 663nm
6. Calculate amount of chlorophyll present in the extract and express mg chlorophyll per g fresh leaf tissue using following equations

Chlorophyll a (mg/g F.W)= (12.7 A_{663} -2.69 A_{645})x V/1000x w

Chlorophyll b (mg/g F.W) = (22.9 A_{645} – 4.68 A_{663}) x V/1000x w

Total chlorophyll (mg/g F.W) = (20.2 A_{645} + 8.02 A_{663}) x V/1000x w

where: A_{645} = absorption value at 645 nm, , A_{663}= absorption value at 663 nm,

V = total volume of chlorophyll extract (i.e DMSO)

w= weight of leaf tissue

Note: Making leaf disc and recording fresh weight helps in avoiding drying of the leaf sample, same cut surface and also allows to calculate chlorophyll per unit area.

Reference:

Arnon, D. 1949. Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta Vulgaris*. *Plant Physiology*, V.24 (1), pp. 1-15.

Hiscox JD, Israelstam GF, 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot*.57:1332-1334.

Estimation of water soluble protein in the leaf by Lowry method

Leaf sample collection and processing: Leaf samples (equal number of tender, medium an coarse leaves) from the longest shoots are collected at 65-70 days after pruning, shade dried and keep in the oven at 60°C for 48 hours. After complete drying, make the dry powders and store in butter paper covers for biochemical estimation.

Extraction:

Weigh 100 mg of dry leaf powder, add to 5ml of distilled water in the centrifuge tube and centrifuge it at 10000 rpm for 20 minutes.

Reagents

1. Reagent A - 2% Na_2CO_3 in 0.1 N NaOH
2. Reagent B - 1% solution of $CuSO_4\ 5H_2O$ in distilled water
3. Reagent C - 2% sodium potassium tartarate
4. Reagent D - Prepare fresh before use by mixing reagents B and C in 1:1 ratio
5. Reagent E - Prepare by adding 1 ml of reagent D to 50 ml of reagent A
6. Reagent F - 1 N Folin-Ciocalteau reagent (Mix Folin-Ciocalteau reagent 2N and distilled water in 1:1 ratio)

Procedure

1. Precipitate soluble proteins in the enzyme extract by 20% TCA by keeping 5ml extract and equal amount of 20% TCA in centrifuge tubes for 1h.
2. Then, centrifuge at 10,000 rpm for 20 minutes.
3. Remove supernatant and redissolve residue in 5 ml of 0.1N NaOH.
4. Take 0.2 ml of the sample and add 5 ml of reagent E, mix well and keep at room temperature for 10 min.
5. Add 0.5 ml of phenol reagent (reagent F) and mix the content immediately on a vortex mixer.
6. Keep it in the dark and read the color intensity at 660 nm after 30 min. in spectophotometer
7. Calculate the equivalent amount of protein from a standard curve prepared by using bovine serum albumin (20-200 µg ml^{-1}) and express in %.

Estimation of water soluble carbohydrates by Anthrone method

Extraction:

Weigh 100 mg of dry leaf powder, add to 5ml of distilled water in the centrifuge tube and centrifuge it at 10000 rpm for 20 minutes.

Reagents:

0.2% Anthrone reagent:

Prepare 72% H_2SO_4 solution one day before the experiment or at least 4 hours before preparation of Anthrone reagent. Dissolve 200mg of anthrone reagent in 72% H_2SO_4 with the help of magnetic stirrer and make up the volume to 100 ml and keep it refrigeration to make it ice cold. Prepare fresh every day.

Procedure

- Take 0.2 ml of extract and add water to bring the volume to 1ml
- Add 4ml of anthrone reagent and mix the contents as well and cover the test tubes
- Keep it in boiling hot water bath (100°) for 10 min
- Cool the test tube to the room temperature and measure the absorbance at 620nm
- Calculate amount of water soluble carbohydrates present in the sample using standard curve prepared with Glucose.

References

Hansen J, Moller IB (1975) Anal. Biochem. 68: 87-94.

Marshall (1986) Plant and Soil, 52-54.

Evaluation of mulberry genetic resources for natural incidence of foliar diseases

There are so many diseases caused by fungi in mulberry varying from place to place depending upon climatic conditions of the area and susceptibility of the genotype. However, the most common foliar diseases are leaf spot, powdery mildew and leaf rust. The characteristics of the diseases are as follows:

Leaf Spot: The disease is caused by *Cercospora moricola* Cooke that is very common in rainy season (June - December) and prevails up to (January to February).

Symptoms: In the initial stage small brownish irregular spots appear on the surface of the leaves. As the disease become severe, the spots enlarge, coalesce and shot holes are formed. Severely affected leaves become yellowish and fall-off prematurely.

Powdery Mildew: This disease is caused by *Phyllactinea corylea* (Pers.) Karst, is the most common and widespread. The disease appears mostly in winter season.

Symptoms: This can be easily identified by appearance of white powdery patches on the lower surface of leaves. As the disease advances, the patch spreads to entire leaf surface and turns blackish in color.

Leaf Rust: This is caused by *Ceretelium fici*, which appears during winter (November-February) season. Matured leaves are more susceptible resulting in premature defoliation.

Symptoms: The pathogen produces numerous pinhead sized circular to oval, brownish to black eruptive lesions/spots on the surface of leaves. The affected leaves become yellowish in color. As the disease become severe, the leaves wither off prematurely.

Bacterial Leaf blight: This disease is caused by *pseudomonas mori.*. It is a serious disease which could cause 5-10% leaf yield loss during rainy season (June- October)

Symptoms: Numerous irregular water soaked patches appear on the lower surface of leaves. In severe condition, the leaves become curled, rotten and turn brownish black in color.

Scoring for disease incidence in mulberry germplasm

Grading based on visual symptoms is given by selecting (from top to bottom) 5 random leaves (L1, L2, L3, L4 and L5) per branch. Four such branches (B1, B2, B3 and B4) from different directions are selected in plant. Observations for 100 leaves per accession. The grades for disease severity are as below:

Infection status	Grade
No infection	0
0-5% leaf lamina covered by spots/patches	1
6-25% leaf lamina covered by spots/patches	2
26-50% leaf lamina covered by spots/patches	3
51-75% leaf lamina covered by spots/patches	4
76-100% leaf lamina covered by spots/patches	5

The percentage of disease index (PDI) will be calculated as follows

$$PDI = \frac{\text{Total number of infected leaves X Grade value given}}{\text{Total number of leaves X Maximum grade value}} \times 100$$

The tolerance or susceptibility based on the total PDI is interpreted below

Total PDI	Interpretation
0	Immune
0.1 - 5.0	Resistant
5.1 - 20.0	Moderately resistant
20.1 - 50.0	Susceptible
50.1 - 100	highly susceptible

Evaluation of mulberry genetic resources for propagation traits

Mulberry is generally propagated vegetatively through stem cuttings. Evaluation of propagation traits is carried out mainly to understand genetic potential of mulberry accessions for their rooting ability and root characters which helps for establishment. To evaluate mulberry accessions for propagation traits, 6 months old mulberry shoots are used to prepare the cuttings with 3-4 buds and planted in the nursery in RBD, in 3 replications along with the checks. A minimum of 20 cuttings per replication is planted with the spacing of 10 cm between the cuttings of the same row and 20 cm between the rows. Usually, Kanva-2 and V-1 are used as checks for evaluating indigenous mulberry accessions and Kosen is used as check for exotic mulberry accessions. The evaluation are to undering taken during rainy season and after winter. Observations are recorded at 90 days after planting the cuttings from 3 randomly selected plants/replication. Saplings are uprooted carefully without damaging the roots for recording the observations on roots.

Descriptors for evaluation of propagation parameters are

S No.	Descriptors	Method
1	Sprouting nature	Fast / Medium / Slow
2	% sprouted on10th day	No. of cuttings sprouted on 10 day to the total no. of cuttings sprouted
	% sprouted on10th day	No. of cuttings sprouted on 20 day to the total no. of cuttings sprouted
1	Rooting ability (%)	No. of samplings survived at 90 days after planting to the number of cuttings planted expressed in %
2	Sapling height (cm)	Length from the base of the sapling
3	Root proliferation on 90^{th} day	

	a) Dry weight of roots / sapling (g)	Roots are washed and excess water is removed with blotting paper, oven dried at 60°C for 72 hours to record dry weight
	b) Longest root length/ sapling (cm)	The longest root length of sapling is measured
	c) No. of roots / sapling	No. of roots per saplings is counted.
	d) Root volume	All the roots from a sapling are taken and dipped in water in a measuring cylinder to record the volume of water displaced in millilitres, to get the root volume of saplings
4	Total dry biomass of the sapling (g)	The dry weights of shoot and root weight are added and recorded as total dry biomass of the sapling. It indicates the vigour of the adult plant
5	Root shoot ratio based on length	It is the ratio of root length to sapling height
6	Root shoot ratio by based on dry weight	It is the ratio of root weight to shoot weight. It is calculated based on fresh and dry weights.

Evaluation for specific traits that associated with different abiotic and biotic stress conditions will be done in collaborative projects/ network projects with the main institutes of CSB, State agricultural universities having sericulture as one of the subject areas as per the requirement with multidisciplinary teams.

Taxonomic classification

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Urticales

Family: Moraceae

Genus Morus L.

Morus L. (Moraceae) is a temperate and sub-tropical genus distributed in Asia, Africa, Europe, North, Central and South America. Despite its broad distribution and economic importance (with a long history of cultivation for sericulture and edible fruits), species delimitation within Morus is poorly understood. the taxonomy of *Morus* has been disputed (Vijayan and Sarathchandra, 2011) because of its wide geographical distribution, morphological plasticity (Grey and Grey, 1987), hybridization among species (Burgess et al., 2005), long history of domestication, and introduction and naturalization of species [8]. Different studies have recognized a variable number of *Morus* species, leading to an uncertain taxonomy.

Linnaeus (1753) established the genus with seven species: *M. alba, M. indica, M. nigra, M. papyrifera, M. rubra, M. tartarica and M. tinctoria. Two of these, M. papyrifera and M. tinctoria,* were later moved to *Broussonetia* and *Maclura*, respectively. Linnaeus discussed the characters of fruit color, leaf shape and leaf hairs and gave a short diagnosis of each species. The first comprehensive treatment of the genus Morus was presented by Bureau (1873) and was primarily based on features of the leaves and pistillate catkins. He recognized five species with 19 varieties and 13 sub-varieties.

Koidzumi (1917) presented the most recent genus-wide treatment and recognized 24 species in two sections: Dolichostylae Koidz. and Macromorus Koidz. These sections were recognized based on the character of the style length: the former with the long style and the latter section with the short style. In his classification, Koidzumi promoted some of Bureau's varieties to the level of species.

In addition to traditionally employed characters in Morus, several attempts have been made to identify additional taxonomically useful characters that would enable workers to better distinguish the species. For example, Hotta (1954) studied variation in shape and position of leaf cystolith cells in *M. alba, M.*

australis and M. mongolica. Katsumata (1971) studied size and shape variation of leaf ideoblast and used this character to classify several races of *M. alba and M. australis.* However, their results showed that these characters were variable and often transcended the species boundaries. Venkataramana (1982), in his review article on wood phenolics of the family Moraceae, discussed three types of bark flavonoids present in *M. rubra* but absent in Asian species examined (*M. alba, M. serrata, M. laevigata and M. indica*). He further showed that some other flavonoids in the bark such as mulberrin, mulberrochromene, cyclomulberrin, cyclomulberro-chromene and three other unnamed pigments present in Asian species were absent in *M. rubra*. There were five other wood phenolics found to be present in different concentrations in Asian species and absent in *M. rubra*.

According to Tojyo (1985), M. nigra (2N=308), introduced to Japan from western Asia, had been hybridized with the native species to produce varieties exhibiting several ploidy levels. Many introduced Morus species including M. alba are easily and vegetatively propagated and can be opportunistically apomictic (Griggs and Iwakiri, 1973), which may increase their adaptability in the novel habitats.

More recently, Zhou and Gilbert (2003) described 16 species in *Morus*, 12 of which were found in China. Because of this variable taxonomy, 260 validated published names in *Morus* have been deposited in the International Plant Names Index (http://www.ipni.org/), with many of them being synonymous. Therefore, although as many as 68 (Sanjappa, 1989; Datta, 2000) or 150 species have been reported in *Morus*, only 10–16 are generally cited and accepted (Berg, 2005).

Characteristics of different species of *Morus* (Tikader, 2013) that are naturally distributed in India are presented below

Characteristics of *Morus indica* Lin.

- A medium sized deciduous tree
- Leaves length 3-4 inches long
- Ovate, acuminate often caudate, serrate or crenate
- Base truncate rounded or sub-cordate and 3 nerved
- Petiole 0.4 – 0.6 inches long, shortly cylindrical, pubescent on short peduncles
- Female spikes ovoid 0.2 – 0.3 inches long
- Styles long, hairy, connate or ¼ of their length
- Fruit 0.2 – 0.5 inch long, ovoid or cylindrical, black when ripe (Ref: A.E. Osmaton (1927), A forest flora of Kumaon, pp502-503)

Characteristics of *Morus alba* Lin.

- A deciduous monoecious tree, 30-40 ft height, cultivated as shrub
- Branchlets, petioles and leaves beneath pubescent

- Leaves 2.5-3.5 inches, rather membranous, petiole 0.6-1 inch, 5 nerved leaves
- Sub-sessile spikes, densely hariy perianth and styles short or absent
- Fruits white, pink or red
- Available in temperate and sub-tropical Himalayan from Kashmir to Sikkim ascending to 7000 ft
- Wild and cultivated form in Bengal, Assam, Burma and also distributed in China and Japan
- (Ref: J.D. Hooker (1885) Flora of British India, pp.491-493.

Characteristics of *Morus serrata* Roxb.

- A medium sized deciduous tree
- Girth upto 9 ft, height up to 70 ft
- Bark dark grey, reddish brown
- Ex-foliating irregular wood strips and scales, exuding mulky juice
- Leaves variable, broadly ovate, acuminate or caudate
- Often 3-5 lobed on young trees, vigorous shoot
- Sharply serrate, teeth usually unequal and cuspidate, base round truncate or cordate
- 3-5 nerved, pubescent or tometose when young, petiole usually 1-2 inch long
- Flowers dioecious, greenish, male spike 1.5-3.5 inches long, female spike 0.4 – 0.5 inches long shortly cylindrical, Flowers:
- April-May, Fruit:May-June
- Fruit 0.4 inch long 0.35 inch diameter, sweet, mucilage, white pink, purplish
- Available throught the hills between 8500-9000 ft
  Ref: (Ref: A.E. Osmaton (1927), A forest flora of Kumaon, pp502-503)

Characteristics of *Morus laevigata* Wall.

- A medium sized deciduous tree up to 10 ft girth and 80 ft height
- Bark dark reddish brown, rough and reddish brown
- Ex-foliating irregular wood strips and scales, exuding milky juice
- Branches and young stem smooth and thickly covered with circular lenticels
- Leaves variable, usually 5-10 by 3-7 inches
- Ovate, elliptic ovate, acuminate usually caudate

- In general entire but sometimes deeply 3-5 lobed, finely crenate-serrate
- Petiole usually 1-2 inches long
- Dioecious, flowers greenish, in drooping cylindrical spikes
- Male spikes 1.5-2.5 inches long, dense, villose
- Female spike 1.5-2.5 inche long
- Fruits 2-3.5 inch long, white, dark purple, sweet
- Available throught the sub-Himalyan tract and hill range from 1000-2000 feet.
  Ref: (Ref: A.E. Osmaton (1927), A forest flora of Kumaon, pp502-503)

Characteristics of other *Morus* spp.

***Morus bombycis* Koidz.**

- Female flower with distinct long flower
- Stigma papilose or pilose
- Leaves serrate, crenate
- Bud scale glaborous

***Morus rotendiloba* Koidz.**

- Style long
- Stigma lanceolate minutely papillose
- Leaf margin serrate
- Leaves trilobite, apex are rotundate

***Morus australis* Poir.**

- Style long
- Stigma paillose, pilose
- Shoot erect, under surface light green and slightly hairy
- Leaves ovate, apex caudate,
- Leaf margin serrate with short prickles

***Morus sinensis* Hort.**

- Style long, stigma short with blunt hair
- Leaf margin ovate, smooth
- Leaf margin serrate, apex acuminate, base truncate

***Morus lhou (Ser)* Koidz.**

- Style indistinct
- Stigma papilose
- Leaf entire, smooth glossy, shriveled
- Leaf margin serrate, base lobate

***Morus multicaulis* Perr.**

- Style absent / indistinct
- Stigma papilose (protuberances inside)
- Leaf cordate generally unlobed, surface wrinkled

- Leaf margin serrate, apex sharp obtuse, base cordate

***Morus nigra* Lin.**

- Style absent
- Stigma pubescent
- Leaf generally ovate or cordate, apex caudate
- Leaf margin serrate have erose serrate
- Leaf base truncate or shallow cordate

***Morus tiliefolia* Makino**

- Style absent or indistinct
- Stigma densely pubescent
- Leaves tomentose on the undersuface
- Leaf surface covered with thick hair

***Morus cathayana* Hemsl.**

- Style absent, stigma pubescent
- Leaf cordate, ventral side covered with thick hair
- Leaf margin sharply serrate
- Leaf base cordate or truncate

The most recent studies on systematics in Mulberry by Madhav (2008). recognized 13 species of the genus *Morus*. A key to the species recognized is presented below

Key to the species of *Morus*

1. Leaf ovate to orbicular, secondary venation (from the mid-rib) less prominent and scalariform except two to three pairs towards the leaf apex. Peduncle longer than inflorescence. .....**7. *M. mesozygia***

1. Leaf ovate to lanceolate, secondary venation prominent and not scalariform. Peduncle shorter or equal to inflorescence (2)

2(1). Pistillate flowers with distinctly long style (>1 mm) (3)

2. Pistillate flowers with no or short style (<1 mm) (5)

3(2). Leaf margin with acute dentation characterized with a short to long seta…..**9. *M. mongolica***

3. Leaf margin without seta as mentioned above (4)

4(3). Infructescence elongated, < 2 cm (excluding peduncle), leaf shape variable ……**2. *M. australis***

4. Infructescence cyndric, 2-4 cm, leaf broadly ovate with cordate base…….. **11. *M. notabilis***

5(2). Infructescences longer than 2 cm (excluding peduncle) (6)

5. Infructescences less than 2 cm. (8)

6(5). Infructescences 2-5 cm..... **3. *M. cathayana***

6. Infructescence 5 -16 cm or longer. (7)

7(6). Axillary bud minute, petiole 1- 2.5 cm, leaf blade usually lanceolate to elliptic, margin minutely serrate to subentire, peduncle <0.5 cm……**5. *M. insignis***

7. Axillary bud larger, petiole 2.5-6 cm, leaf blade ovate to broadly ovate, margin sub-entire to minutely serrate, peduncle >0.5 cm……**6. *M. macroura***

8(5). Leaf blade usually bright green, adaxially usually glabrous, abaxially sparse pubescent along the veins, leaf margin irregularly dentate, leaf apex usually obtuse.....**1. *M. alba***

8. Leaf blade usually dull green, adaxially slightly scabrous, abaxially pubescence all over, leaf margin with acute serrations, leaf apex acute to subcaudate.(9)

9(8). Leaf margin with regularly spaced triangular teeth, bud scales and stipules semi persistent .....**13. *M. serrata***

9. Leaf margin not as above, bud scales and stipules immediately cauducous (10)

10(9). Leaves broadly cordate at base, glabrous adaxially or slightly scabrous, sparsely pubescent along the veins abaxially, the leaf margin with wider teeth. Infructescence oblong, 1.5-2.5 cm wide, up to 2.5 cm; stigma long pubescent……..…..**10. *M. nigra***

10. Leaves deeply cordate at base, densely pubescent along the veins adaxially, sparsely pubescent in the interveinal areas. Infructescence cylindric, 0.5-2 cm wide, up to 2 cm; stigma short pubescent (11)

11(10). Leaf blade abaxially pubescent, adaxially usually scabrous. Stem branches are horizontally spread in a characteristic pattern. Fruits compactly arranged in a fleshy cylindrical infructescence .....**12. *M. rubra***

11. Leaf blade adaxially slight to harsely scabrous, fruits loosely arranged, globose or capitate, not as fleshy as in *M. rubra* (12)

12(11). Shrub to small tree, mature leaf blade less than 6 cm, ovate to ovato-lanceolate, abaxially scabrous or pubescent, infructescence small (ca. 0.5 cm; excluding the peduncle) adaxially harshly scabrous.....**8. *M. microphylla***

12. Small to big tree, mature leaf up to 4-20 cm, abaxially harshly pubescent to scabrous, oblong to lanceolate, base usually unequal to cordate, adaxially glabrous to slightly scabrous. Infructescence 1-2 cm sometime longer ....**4. *M. celtidifolia***

Germplasm conservation

In the long run, the major threats for loss of diversity are replacement of local cultivars and land races of traditional agro-ecosystems by a few genetically uniform modern varieties, deforestation, urbanization, pollution, habitat destruction, fragmentation and degradation, spread of invasive alien species, climate change, over-grazing and changes in land-use pattern (Kaviani, 2011). Therefore, essential measures must be taken for the conservation of the genetic variability. Different strategies for conservation are

A. In situ conservation

B. *Ex situ* conservation

A. *In situ* conservation

The conservation of species in their natural habitats is considered the most appropriate way of conserving biodiversity. *In-situ* or on site conservation refers to situations where the material is maintained in its natural habitat, within the community, of which it forms a part. The aim of *in-situ* conservation, thus, is to allow the population to maintain/perpetuate itself within the community environment, to which it is adapted so that it has the potential for continued evolution (Anon., 1989. It is on site conservation in which the genetic resources are is preserved by protecting the ecosystem in which it occurs naturally. The Indian government has established 18 Biosphere Reserves in India and among them Nandadevi, Manas, Nokrek, and Great Nicobar are the potential for *in situ* conservation sites for mulberry. Namdapha of Arunachal Pradesh and North Andaman also are important areas among the potential sites for Biosphere Reserves selected by the Ministry of Forests and Environment.

Since ages, mulberry is being worshiped by tribals of Uttaranchal in the Himalayan region and conserved as "Sacred grooves". The sacred mulberry tree (M. serrata) at Joshimath (2000 above MSL) worshipped by the pilgrims of Badrinath, is said to be the oldest (1200 years) and biggest mulberry tree (21.6 mts circumference) in the world (Rau, 1967 and Tikader et al., 2000). The great Indian sage Adiguru Shri Shankarcharya is said to have meditated under this tree. Due to this fact, *M. serrata* trees are protected at several places namely, Shirmoli near Almora, Ulkadevi temple at Pithoragarh, Gwaldam, Garhwal and Kumaon regions of Himalayas.

However, CSGRC plays only gives information about *in situ* conservation to biodiversity authority and only place a advisory role since these *in situ* conservation places are under the control of state Forest Departments.

B. Germplasm conservation in *ex situ* field gene bank

Ex situ conservation is the preservation of components of biological diversity outside their natural habitats. This involves conservation of genetic resources collected from natural habitats/wild, climate stressed regions and cultivated lands at a designated site. Off site conservation may include part of

the organism from which the organism concerned can be reproduced is preserved or whole organism where a stock of individuals of the organism concerned is kept outside its natural habitat in a plantation, botanical garden, gene bank etc. CSGRC being the Nodal Agency and National Active Germplasm Site (NAGS) for mulberry genetic resources, conducted more than 80 survey and exploration trips in India which includes cold deserts of Ladakh Himalayan region, arid and semiarid regions of Rajasthan, saline regions of Andaman Islands and managed habitats of south India. At present, CSGRC is maintaining 1269 (999 indigenous and 270 exotic) mulberry accessions which were collected from different geographical areas of the world as dwarf trees in its *ex situ* field gene bank. It represents 13 *Morus* species collected from 27 countries.

Establishment and Management of *Ex situ* field gene bank

The key principles at the core of genebank operation are the preservation of germplasm identity, maintenance genetic integrity, and the promotion of access. This includes associated information to facilitate use of conserved plant material in accordance with relevant national and international regulators.

The major underlying principles (FAO, 2014) explain why and for what purpose plant genetic resources are being conserved are described in the section below.

Identity of accessions

Care should be taken to ensure that the identity accessions conserved in gene banks is maintained throughout the various processes, beginning with acquisition through to conservation and distribution. Proper identification samples conserved in gene banks requires careful documentation of data and information about the material. This begins with recording passport data and collecting donor information if applicable. Where possible, such information should also be recorded for older collections in gene banks for which passport data was not previously recorded or is incomplete. Herbarium voucher specimen often plays an important role in the correct identification samples. Field labeling needs to be complemented with field layout plans, which should be properly documented in order to ensure proper identification of accessions in field gene banks. Field labels are prone to loss due to various external factors, e.g. bad weather conditions. Modern techniques, such as accession labels with printed barcodes, Radio- Frequency Identification (RFID) tags and molecular markers, can greatly facilitate the management of germplasm by reducing the possibility of error, further ensuring the identity of accessions.

Maintenance of genetic integrity and health of germplasm

Gene banks, to the extent possible, should ensure that collected germplasm is genetically representative of the original population as well as take into account the number of live propagules, such that sample quality is not compromised. A monitoring system should be in place to check the viability status of stored

samples at appropriate intervals. Field gene banks are vulnerable to the impacts of environmental factors such as weather conditions, incidence of pests, etc. Various molecular techniques, including surveys of possible epigenetic changes that may or may not be reversible, are needed to assess whether genomic stability has been maintained, particularly when samples are retrieved from cryostorage. Maintenance of genetic integrity is equally as important for germplasm conserved in vitro, especially in view of the risk of somaclonal variation.

Physical security of collections

An underlying principle of germplasm conservation is that the physical structures of the gene bank facilities in which germplasm are conserved are of adequate standard to secure the materials from any external factors, including natural disasters and human-caused damage. Adequate security systems are required to ensure that gene bank cooling equipment, as well as backup generators and equipment to control power outages, are in good running condition. Furthermore, it is vital that levels of LN are maintained in cryovessels.

Availability and use of germplasm

Conserved material must be available for current and future use. It is, therefore, important that all processes in gene bank operations and management contribute to this goal. Although there are a few individuals of accessions in field gene banks, and thus a limited capacity for distributing to users, the genebank should have a strategy in place to multiply quickly any germplasm for distribution.

Availability of information

In order to ensure communication of information and accountability, essential, detailed, accurate, and up-to-date information – including historical as well as current information, especially in relation to the management of individual accessions subsequent to their acquisition – should be recorded in electronic databases. Access, availability and sharing of this information should be treated with high priority, as it leads to better and more rational conservation. Search-query interactive databases containing phenotypic evaluation data can assist germplasm clients in the targeting of germplasm requests and, in turn, feedback of further evaluation data adds to the value and utility of the collection. If information on the conserved germplasm is made easily available and accessible, it will enhance germplasm use

Proactive management of gene banks

Sustainable and effective conservation of genetic resources depends on active management of conserved germplasm. Proactive management is critical for ensuring that germplasm is efficiently conserved and made timely available and in adequate quantity for further use by plant breeders, farmers, researchers and other users. It emphasizes the importance of securing and sharing material

as well as the related information, and sets in place a functional strategy for management of human and financial resources for a rational system. It includes a risk management strategy and encourages collaborations with third parties in providing services to gene banks in the efforts to conserve biodiversity.

Standards defined by FAO (2014), while acquisition of material to field gene bank

- All germplasm accessions added to the genebank should be legally acquired, with relevant technical documentation.
- All material should be accompanied by at least a minimum of associated data as detailed crop passport descriptors.
- Propagating material should be collected from healthy growing plants whenever possible, and at an adequate maturity stage to be suitable for propagation.
- The period between collecting, shipping and processing and then transferring to the field gene bank should be as short as possible to prevent loss and deterioration of the material.
- Samples acquired from other countries or regions within the country should pass through the relevant quarantine process and meet the associated requirements before being incorporated into the field collection

Passport data (based on International Plant Genetic Resources Institute [IPGRI]/FAO Multi-crop passport descriptors)

Documenting the information received with the sample is an important activity. Much of the information will be passport data, which is recorded when the sample was originally collected or the data accompanying the sample when it is received from other sources. Include information relating to the identification of each accession in the genebank.

Accession number: This number serves as a unique identifier for accessions and is assigned when an accession is entered into the collection. Once assigned this number should never be reassigned to another accession in the collection. Even if an accession is lost, its assigned number should never be reused.

Collecting number: Original number assigned by collector(s) of the sample, normally composed of initials of the collector(s) followed by a number. It should be unique and always accompany subsamples wherever they are sent.

Genus: Genus name for the taxon. Initial uppercase letter required.

Species: Species name in lowercase letters plus authority.

Subtaxa: To store additional taxonomic information. Following abbreviations are allowed: "subsp. (for subspecies); "van" (for variety); "race" (for race).

Accession name: Either a registered name or other formal designation given to the accession. First letter uppercase. Multiple names separated with semicolon.

Country of origin: Name of the country in which the sample was originally collected or derived. Use the ISO 3166 extended codes.
Location of the collecting site: Location information below the country level that describes where the accession was collected starting with the most detailed information. Such information may include distance in kilometers and direction from the nearest town, or village.
Latitude of collecting site: Degrees and minutes followed by N (North) or S (South) (e.g., 1030S). Missing data (minutes) should be indicated with hyphen (e.g., 10-S).
Longitude of collecting site: Degrees and minutes followed by E (East) or W (West) (e.g., 07625W). Missing data (minutes) should be indicated with hyphen (e.g., 076-W). **Elevation of collecting site (masl):** Elevation of collecting site expressed in meters above sea level.
Collecting date of original sample (YYYYMMDD): Collecting date of the original sample where YYYY is the year, MM is the month, and DD is the day.
Biological Status of sample: 1 Wild 2 Weedy 3 Traditional cultivar/Landrace 4 Breeder's line 5 Advanced cultivar 0 Unknown 99 Others
Collecting source: 1 Wild habitat 2 Farm 3 Market 4 Institute/research organization 0 Unknown 99 Others
Donor institute code: Code for the donor institute. The code consists of a 3-letter ISO 3166 country code of the country where the institute is located.
Donor number: Number assigned to an accession by the donor. Letter should be used before the number to identify the genebank or national system.
Other number(s) associated with the accession: Any other identification number known to exist in other collections for this accession. Letters should be used before the number to identify the genebank or national system.
Remarks: The remarks field is used to add notes or to elaborate on descriptors with value "99" (= Others). Prefix remarks with the field name they refer to and a colon (e.g., Collecting source = roadside). Separate remarks referring to different fields are separated by semicolon

Standards for establishment of collection

- A sufficient number of plants should be maintained to capture the genetic diversity within the accession and to ensure the safety of the accession.
- A field genebank should have a clear map showing the exact location of each accession in the plot.
- The appropriate cultivation practices should be followed taking into account micro-environment, planting time, watering regime, pest, disease and weed control.

Standards for field management

- Plants and soil should be regularly monitored for pests and diseases.
- Appropriate cultivation practices such as fertilization, irrigation, pruning and weeding should be performed to ensure satisfactory plant growth.
- The genetic identity of each accession should be monitored

Mulberry *ex situ* field gene bank is maintained as tree type, with the spacing 2.4 m between plants and rows and 3.0 m between accessions and 4 plants per accession are maintained. The plants in the conservation block are trained at 1.5 m crown height following one pruning after the onset of monsoon (June-July). Immediately after pruning, manures are applied. This is followed by intercultural operations for weed management and basins preparation. Chemical fertilizers @ 100:50:50 kg NPK/ha/yr are applied after sprouting (20-25 days after pruning). Irrigation is provided through drip irrigation system during non-rainy days as per requirement based on the season. Special attention is required for accessions that are planted by grafting. Basal branches from the root stock are regularly removed. Plant protection measures are taken as per the requirement.

Standards for regeneration and propagation

- Each accession in the field collection should be regenerated when the vigour and/or plant numbers have declined to critical levels in order to bring them to original levels and ensure the diversity and genetic integrity is maintained.
- True-to-type healthy plant material should be used for propagation.

Standards for safety duplication

- A risk management strategy should be implemented and updated as required that addresses physical and biological risks identified
- A gene bank should employ the requisite staff to fulfil all routine responsibilities to ensure that the gene bank can acquire, conserve and distribute germplasm according to the standards.
- Every field gene bank accession should be safety duplicated at least in one more site and/or backed up by an alternative conservation method/ strategy such as *in vitro* or cryopreservation where ever possible.

Common problems in mulberry field gene bank maintenance and suitable measures to be taken are presented below.

Nature of problem	Precaution/Action to be taken
Poor establishment of mulberry genetic resources in the field gene bank and training the plant as dwarf tree	Young saplings should not be used for plantation in the field gene bank. Healthy and saplings of 10-12 months old saplings are to be used for planting. In case of weak stems, support should be given until tree attains sufficient girth and strong.
Soil erosion and maintenance of soil fertility and health	Because of wider spacing in the field gene bank, there is every possibility of loss of top soil during heavy rains. Hence, attention need to be paid on the leveling of land, proper bunds and minimum tillage etc. In addition, attention to be paid on organic content of the soil by addition of sufficient manures, raising of green manure crops, addition of green leaf manure etc. Special attention to be paid to the old plantations.
Termites control	Continuous drought years and low organic content in the soil will aggravate termite attack. By the time the symptoms appear on the above ground, there may be severe damage in the root zone and basal portion of the trunk becomes hallow. Hence, much attention to be paid on maintenance of organic content of the soil and if any termite attack is observed, immediate control measures has to be followed. Extracts of *Agave Americana* found to be effective to control termites as well as stem borer. 25 kg of Agave can crushed and soaked in 100 litres of water and sprayed on the stem with the help of rock sprayer. As this will not have any harmful effect on the plant or person engaged for this work, it is advised to follow. In severe cases, imidochloprid @0.75 ml/litre can be applied.
Damage by ant hills	If neglected, the ant hills in the basins of the plant can damage the tree. Ant hills after rains forms big holes near trunk of the tree which expose roots, tree losses the support and in extreme cases may die.

Removal of parasitic plants	Parasitic plants need to be removed immediately if observed otherwise reduces the vigour of the mulberry trees.
Formation of dead wood and poor sprouting	Pruning of the trees in the field gene bank should be carried out by skilled workers. Some mulberry accessions can not with stand repeated pruning or harsh pruning. It should be ensured to have clean cut surfaces which is possible by sharp pruning saw or chain saw. Bark should not be peeled off or stem should not be split. Sickle should not be used as the cut will be slant and exposes more surface area and may reduce the sprouting due to harsh pruning. The plants showing poor growth should be pruned every year. To regenerate such kind of old trees, dead wood should be removed through power chain saw and ensure full and frequent irrigation to allow latent buds to sprout
Mixed branches from root stock and scion	Monitoring is required to avoid branches from the root stock and death of the original accession which was raised through graft. Removal of basal branches at frequent intervals leads helps for proper maintenance.

ii) Accessioning of germplasm

As soon as any new genetic resource is added in the field gene bank, it should be given number to identify that accession. It should be unique and should be given it for any other accession. CSGRC, Hosur gives continuation number and starts with "MI" for indigenous collections and "ME" for exotic collection and plants.

iii) Obtaining National accession number

To avoid maintenance and multiplication of same mulberry accession with different names by different institutes and to avoid duplicity of the material, NBPGR provides national accession number upon the request by the national active germplasm sites along with the supportive information. After obtaining the national accession number, it is to be used while distribution of germplasm for utilization. If the accession is received from other institutes, the number

should be informed to them for using the same in their publications and documentation of data etc.

C. *In vitro* conservation

The required germplasm can be conserved *in vitro* by addition of growth inhibitors in the culture medium to reduce the no. of cycles of regeneration per year

Standards defined by FAO (2014) for in vitro culture and slow growth storage

a. Identification of optimal storage conditions for *in vitro* cultures must be determined according to the species.
b. Material for *in vitro* conservation should be maintained as whole plantlets or shoots, or storage organs for species where these are naturally formed.
c. A regular monitoring system for checking the quality of the *in vitro* culture in slow-growth storage, and possible contamination, should be in place.

D. Long term conservation through Cryo-preservation

As a alternative conservation strategy, mulberry genetic resources are conseverved in the National cryogene bank at NBPGR, New Delhi. Mostly, winter dormant buds are cryopreserved following dehydration and slow freezing protocol. The accessions which can not withstand dehydration are cryopreserved through encapsulation and vitrification techniques.

Cryopreservation of mulberry

Protocol 1: Cryopreservation of winter-dormant buds by dehydration and slow freezing.

Axillary winter-dormant buds are collected from 8-10 old shoots after pruning during January–February and cryopreserved in liquid nitrogen (−196°C) following dehydration in silica gel and slow freezing (Niino et al. 1995). For this purpose, the following protocol (Anandarao *et al*., 2007) is to be followed.

- Dormant buds along with bark tissue are dissected from stem cuttings,
- Remove the the bark tissue and the outer five to seven bud scales without damaging the bud.
- The moisture content of the dormant buds of fresh samples are determined and desiccated in silica gel (500 g/150 mm internal diameter) for 4–6 h to attain the moisture content of 12-15%.
- The slow freezing is achieved by storing the buds at sequentially lower temperatures of −5°C/day up to temperature of −30°C (Niino *et al*. 1995) and placed in 2.5 ml polypropylene cryo-vials and stored in the cryocan containing liquid nitrogen (−196°C).

Regeneration from cryopreserved winter buds

- For recovery, the cryopreserved axillary buds are taken out from cryo tanks and thawed rapidly at 38°C in a water bath for 5 min.
- A rehydration treatment lasting 24 h is given by keeping the buds in sterile filter papers in the desiccators filled with sterile soil-rite mixture.
- The rehydrated buds are surface-sterilized with liquid detergent Tween-20, disinfected with 70% ethanol for 25 s followed with 0.1% mercuric chloride for 6 to 8 min depending on the size of the bud, rinsed with repeated three sterile water washes, and placed in culture tubes (25×150 mm, Borosil make, fitted with polypropylene caps) with Murashige and Skoog (MS; Murashige and Skoog 1962) medium with 3% (w/v) sucrose and gelled with 0.7% (w/v) agar. The medium is supplemented with 1.0 mg l−1 6-benzyl amino purine (BA) initially.
- After bud break and emergence of three to four young leaves, a dark treatment of 1 wk will be given to initiate stem elongation.
- The sprouted buds which did not elongate were subcultured in the MS medium with 1.0 mg l−1 BA, 0.2 mg l−1 gibberlic acid
- Initially, the cultures were kept under dark for 4–7 d and then transferred to normal growing conditions at 25±1°C with 55–60% relative humidity and light intensity of 65±10 µmol m−2 s−1 with 16 h photoperiod in vitro culture room.
- In vitro sprouted buds were subcultured after 30 d of inoculation to the fresh MS medium with cytokinins and auxins
- The accessions, which have high phenolic exudation, were repeatedly subcultured in the medium with activated charcoal 400–500 mg l−1.
- The well-developed micro shoots are transplanted to 1/2 MS medium with indole butyric acid (IBA) 0.5 mg l−1 and activated charcoal (AC) 100 mg l−1 and incubated at 25±1°C with 55–60% relative humidity and light intensity of 65± 10 µmol m−2 s−1 for inducing rooting.
- The in vitro rooted plants were hardened in plastic pots using autoclaved soilrite mixture covered with polyethylene bags and kept in the poly house with 75% relative humidity.
- The plants were watered once in 2 d with 1/2 MS liquid medium.

Protocol- 2: Cryopreservation of shoot-tips by encapsulation and dehydration

For this purpose, the *in vitro* grown shoot tips can be used. Shoot cultures are hardened at 5°C at 8 h/day photoperiod for 3 weeks. The ge matrix should be prepared by adding 4% (w/v) sodium alginate, and 3% sucrose, 100 mg l^{-1}, myoinositol in MS medium without $CaCl_2$. A solution of $CaCl_2 2H_2O$ (100mM) in

sterilized distilled water should be prepared separately. The *in vitro* shoot tips/ sterilized dormant buds should be dropped in the sodium alginate gel matrix solution and mixed thoroughly and keep for 5 minutes. The explants kept in sodium alginate gel matrix be picked up with tweezers and dropped into sterile solution of calcium chloride ensuring one bead to contain one bud and allow for 30 minutes for complete polymerization. The alginate beads with the plants tissue should be treated with sucrose (0.5M to 1M) for 24 hours and air diried in the laminar flow for 4 hours and cryopreserved in liquid nitrogen. The procedure for *in vitro* regeneration of cryopreserved encapsulated buds after thawing in water bath at 37°C is similar to the above.

Protocol- 3: Cryopreservation of apical meristems by vitrification

The apical meristems of in vitro grown shoot tips can be used in this process. The cultures are harden at 5°C at 8 hours / day photoperiod for 3 weeks. The meristematic tissue with two leaf primorida has to be dissected carefully using microscope under aseptic condition. The explants should be pretreated in loading solution with 0.7M sucrose for one day and to be given plant vitrification, PVS2 (comprising 1m l of standard growth liquid medium containing 0.4M sucrose, 30% (w/v) glycerol, 15% (w/v) ethylene glycol, 15% (w/v) dimethyl sulfoxide in 2.5 ml polypropylene cryo vials) treatment for 90 mintures. The explants along with fresh PVS2 solution in the cryo vials should be preserved in LN. For recovery, the explants in the cryo vials should be removed from the cryo tank, thawed immediately at 37°C, treated with unloading solution (1.25M sucrose) after decanting PVS2 solution and subjected to *in vitro* tissue culture techniques.

Registration of germplasm

To accord due recognition to the persons/institutions who are associated with the development and identification of improved or unique potentially valuable germplasm, genetic stocks, improved varieties, mutants, polyploids, prebreeding material or any host plant material of sericigenous insects identified for its uniqueness and significance in crop improvement , CSB is implementing registration of germplasm through CSGRC. The form to be submitted for registration and guidelines for filling the form are provided below.

Check-list for screening of applications

The Member-Convenor, Germplasm Registration Committee at CSGRC shall screen all applications and make recommendations to the Germplasm Registration

Committee for *inter alia* the following points:

1. Whether this is a new application or a revised one? (Yes/No)

2. Whether same or similar material has been registered earlier? (Yes/No)

3. Whether unique or distinguishing characteristics of potential value merit consideration for registration? (Yes/No)

4. Whether documentary evidence or data is provided in support of the claim on potential value of germplasm? (Yes/No)

5. State, if possible any other economic potential value of germplasm. (Yes/No)

CSGRC viewpoint about the candidate germplasm

1. Whether applicant, institution, university, or centre has given a commitment for maintenance and supply of germplasm for use? (Yes/No)

2. Whether appropriate size of sample of germplasm for long term storage at National Genebank or for conservation and maintenance

at the concerned National Gene Bank has been sent? (Yes/No)

3. Whether maintainer line has been sent by the applicant to the National

Genebank, in case of parental lines of hybrids? (Yes/No)

4. Whether acknowledgement receipt of germplasm from concerned National Gene Bank for conservation and maintenance of germplasm is attached, wherever required? (Yes/No)

5. Whether complete address of the corresponding person is given?

(Yes/No)

Germplasm utilization

Utilization refers to use of germplasm in crop improvement programmes. CSGRC, Hosur promotes utilization of germplasm by publishing the characterization and evaluation data in the form of catalogues, brochures and research publications. The researchers who indent the germplasm will be supplied the required germplasm subjected to the clearance by the Germplasm Supply Committee.

Germplasm Supply Committee comprises

1. The Director, CSGRC, Hosur – Chairman of the committee
2. The Director (Tech), CSB - Member
3. Divisional Head of Mulberry Division - Member
4. Divisional Head of Silkworm Division - Member

At the time of supply, the indenter is required to submit Material Transfer Agreement (MTA). A Material transfer agreement (MTA) is a contract that governs the transfer of tangible research materials between two organizations, when the recipient intends to use it for his or her own research purposes. The MTA defines the rights of the provider and the recipient with respect to the materials and any derivatives.

The mulberry germplasm supply is made mainly by supply of 6-8 months old stem cuttings of the required material. To supply for distant places, the material will be supplied through courier services. The mature stem cuttings of 2 feet long are labeled with thin aluminum labels, dipped the cut ends in paraffin wax to prevent moisture loss from the cut ends, wrapped with cora cloth, sealed with sealing wax will be send by courier.

The researcher/ indenter is followed up for the utilization of the supplied material and information on utilization and outcome of the research will be collected through feed back form. The MTA and feed back forms are presented in Annexure 7.1 and 7.2 respectively.

CENTRAL SERICULTURAL GERMPLASM RESOURCES CENTRE, HOSUR

PASSPORT DATA FORM

Team leader ……..…

Team members …….……..……

Region/Zone ……..……..……..…

Assigned Code No……..……..…...

Year ……..……..

1 ACCESSION DATA

1.1	a) ACCESSION NUMBER	:	
	b) Cultivated/Wild/Improved variety	:	
	c) Country and place of origin		
1.2	DONOR NAME *(Name and address of the institute responsible for donating the Germplasm)*	:	
1.3	DONOR IDENTIFICATION NUMBER *(The number or name assigned by person or institute as above)*	:	
1.4	OTHER NUMBER ASSOCIATED WITH ACCESSION	:	
1.5	SCIENTIFIC NAME (Genus, Species, Sub-species, Botanical variety)	:	
1.6	DATE OF ACQUISITION (dd/mm/yyyy)	:	
1.7	ACCESSION SIZE(Number of Plants in collection/ accession at donor site)	:	
1.8	TYPE OF MAITENANCE	:	Low bush / high bush / dwarf tree / tree
1.9	GENETIC ORIGIN(whether by self pollination, intraspecific hybrid, clonal selection, bud spontaneous mutation, bud induced mutation, open pollination, others)	:	
1.10	CURATOR	:	
1.11	VERNACULAR NAME	:	
1.12	DISTRIBUTION	:	
1.13	YEAR OF PLANTATION	:	
1.14	SYNONYMS (if any)	:	

2. COLLECTION DATA

2.1	SAMPLE NUMBER (Assigned by the Collector)	:	
2.2	COLLECTING INSTITUTE :	:	
2.3	DATE OF COLLECTION OF ORIGINAL SAMPLE (dd/mm/yyyy)	:	
2.4	PLACE OF ORIGIN(With full address)	:	
2.5	LOCATION OF COLLECTION SITE (in case of wild collection)		
	(a) Collected in the wild (No. of KM and direction from nearest town, village or map grid reference)	:	
	(b) Postal address (For material collected at a clearly identifiable postal address)	:	
2.6	LATITUDE	:	
2.7	LONGITUDE	:	
2.8	ALTITUDE (Elevation above MSL in meters)	:	
2.9	COLLECTION SOURCE	:	Wild / Farm Land / Backyard / institute
2.10	STATUS OF SAMPLE	:	Wild / breeder's line / primitive / cultivar / advanced cultivar etc.
2.11	LOCAL / VERNACULAR NAME	:	
2.12	NUMBER OF PLANTS SMAPLED (No. of plants from which cuttings are made)	:	
2.13	PHOTOGRAPH	:	Yes/No
2.14	HERBARIUM SPECIMEN	:	Yes / No
2.15	TYPE OF SAMPLE		Vegetative / reproductive (seeds) / both
2.16	NATURE OF VEGETATATIVE SAMPLE cuttings / grafts / rooted plants / tissue cultures / others	:	
2.17	DISEASE STATUS (Visible sign of diseases)	:	
2.18	END USE, GENERAL	:	
2.19	FRUIT USE	:	
2.20	PLANT USE (Whether used for clonal rootstock, pollinator, multipurpose use , other uses)	:	

2.21 **PLANT DATA**

a) HABITAT	:	
b) GROWTH HABIT	:	Erect / semi erect / drooping / weeping
c) COLOUR OF SHOOT	:	
d) PHYLLOTAXY	:	
e) LEAF CHARACTER	:	
(i) Lobation (Unlobed, if lobed whether : Lobed / Unlobed	:	
(a) Palmatifid		
(b) Palmatiparrite		
(ii) Shape : Ovate / cordate / others	:	
iii) Margin : Serrate / dentate / crenate	:	
(iv) Apex : Acute / acuminate / caudate	:	
(v) Base : truncate / cordate / lobate	:	
(vi) Colour : Light green / green / dark green	:	
vii) Surface : Smooth / hairy	:	
(viii) Texture Chartaceous / coriaceous / membranaceous	:	
(f) INFLORESENCE AND FLOWER	:	
(g) FRUIT	:	

2.23 OTHER NOTES FROM COLLECTOR :

Annexure-1.2

ICAR-National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi-110 012
PASSPORT DATA FORM

Collector's Name and Address:
Collaborating Institute: Name of Scientist(s) and Address:
Area Explored:
Duration of Exploration: From To:

Sr. No.	Collector No.	IC No.	Crop's common name	Botanical name	Vernacular name	Landrace name	Biological status	Type of material	Date of collection	Collecting site /acquisition source	Frequency
1.											
2.											

Sr. No.	Collector No.	Sample type	Sampling method	Habitat	Site of collection				Latitude (N)	Longitude (E)	Altitude (m)	Ethnobotanical information/ Ethnic group	Remarks (Trait-specific characters)
					Village	Mandal/Taluk/ Tehsil	District	State					
1.													
2.													

The completed sheets for allotting IC number should be sent along with samples (2000/4000 seeds of self/cross pollinated crops) to:
The Head, Division of Plant Exploration and Germplasm Collection, ICAR- National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110 012
E mail address: exploration@nbpgr.ernet.in (please also send soft copy of passport data in MS Excel through e mail), Phone: 011-2584 8405 (O)
For issuance of IC number to vegetatively propagated crops/species, also furnish the certificate of conservation/maintenance in field gene bank/NAGs

Useful tips

Collector number: denotes a unique/primary identity assigned by the collector at the time of collection, given in abbreviated form of collectors name followed by accession number (for example: KCB/KP/231 (expanded form is: collector number assigned in an exploration by KC Bhatt as team leader and K Pradheep as collaborator/associate; germplasm sample sequence/ serial number is 231)
Biological status: (***Wild***-All wild species that are related and part of the genepool from which genetic introgression into cultivated species is possible using conventional methods; ***Weedy***-Weedy form of cultivated species occurring in companionship (fields) of some other cultivated species; ***Landrace/Traditional/ Primitive cultivars/ Farmers variety***- All cultigens under cultivation in farmer's field with/ without specific names frequently associated with unique traits identified by farmers; ***Breeding line***-Semi-finished products or segregating material generated out of hybridization programme to meet specific breeding objectives; ***Elite line/Advanced/ Improved cultivar***- Selection from population, from coordinated trial (AVTII line), improved cultigens of common knowledge in commercial cultivation (extent, released by institution/organization/State) but not notified from the Central Sub-Committee on crop standards, Notification and Release of Varieties of Agricultural and Horticultural Crops and Parental lines of hybrids; ***Released cultivar/ Hybrid***-Varieties/hybrids notified and released by the Central Sub-Committee on crop standards, Notification and Release of Varieties of Agricultural and Horticultural Crops; ***Genetic stock/ Registered germplasm***- Trait and gene specific germplasm experimentally developed/identified through scientific interventions (e.g. sources of resistance, mutant, cytogenetic stock etc.) which is registered for unique trait(s) at ICAR-NBPGR; ***Others***-Doubtful or material with unknown biological status)
Type of material: Seed/fruit/inflorescence/root/underground parts/cuttings/live plants
Collecting site/acquisition source: Farmer's field/ threshing yard/ fallow/ farm store/ wild/ orchard/home garden/ market/ aquatic/Institute name (if others, give source name)
Frequency: Abundant/frequent/occasional/rare;
Sample type: Population/pure line/Individual; **Sampling method:** Bulk/random/non-random
Habitat: Cultivated/disturbed/partially disturbed/rangeland/forest/aquatic habitat

Annexure 1.3

NATIONAL BUREAU OF PLANT GENETIC RESOURCES

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
NEW DELHI, INDIA

APPLICATION FOR PERMIT TO IMPORT SEEDS/PLANTING MATERIALS
(FOR RESEARCH PURPOSE)
(Please type/write in block letters)

The Director
National Bureau of Plant Genetic Resources
Pusa Campus
New Delhi 110 012

I hereby apply for a permit authorising the import of seeds/planting materials for research purpose as per details given below:

1. Name and address of the Applicant :

2. Exact description of seed/planting material to be imported :

   a) common name and botanical name :

   b) germplasm / variety / hybrid / composite / ; synthetic

   c) form of material required : (seed / rooted plants / scions / tubers / cuttings /bulbs etc)

   d) parentage, if known :

   e) place of collection / origin of the material to be imported (country / state)

   f) name and address of the organisation/ Institution producing the material :

3. Quantity to be imported (separately for each accession/variety/hybrid) :

4. Suggested source of availability of material including published reference, :

5. Whether the aforesaid germplasm/variety/ hybrid was imported by you earlier? If so, details thereof (year, quantity, source etc.) Was the material shared with other scientists National Gene Bank at the NBPGR? :

6. Expected date and mode of arrival in India (Airmail/air freight/accompanied baggage) :

7. Place where imported seeds/planting material will be grown and scientists under whose supervision the seeds/planting material will be grown :

DECLARATION

I hereby declare that the germplasm under import has no commercial value /exclusive ownership and may be shared freely for research purposes.

Place: Signature of the Applicant

Date: Address:

Annexure 1.4

Form for Advance Intimation of Export of seed/Plant material to India

To:
The Director
National Bureau of Plant Genetic Resources
IARI Campus
NEW DELHI 110 012
INDIA

The following consignment has been dispatched separately to you for plant quarantine clearance and forwarding to CSGRC, Hosur.

1. Name and address of consignor
2. i) Crop (with botanical name)
   ii) No. of boxes/bags/cartons
   iii) Distinguishing marks
3. Weight
4. Mode of dispatch
5. Particulars of Phytosanitory certificate
6. General health, pest incidence/intensity on crop at the time of seed collection
7. Date(s) of collection
8. Remarks, if any

Cc: To The Director, CSGRC, Hosur, Tamil Nadu, India

Note: Duplicate copy of Phytosanitory Certificate should be attached with this letter to facilitate release of the seed material.

Form A

Application for Registration of Silkworm and Host Plant Germplasm

(To be submitted to The Director, CSGRC, Hosur – 635 109)

1. Application status (Code) N R	For use of CSGRC
2. Silkworm/ Host Plant	**i)** Application number
3. Binomial name	**ii)** Date of application
4. Species code	**iii)** Whether new or revised?
5. Biological status of the material to be registered	**iv)** If revised, Date of 1st Application
6. Criteria for registration [Unique feature(s)]	**v)** If validation test suggested, whether report attached **vi)** **Action** taken **a)** Forwarded for registration
	b) Sent for validation
	c) Incomplete, sent for revision
7. Nature of genetic material(Code)	**vii)** Whether registered or rejected
8. Quantity deposited (Actual)	**viii)** Date of registration or rejection
9. Value referred to (Code)	**ix)** Registration Number

10. Basis of eligibility (Code)	**x)** Notified : on:
11. Validation test suggested	**xi)** Remarks

12. Particulars of the scientist(s)/person(s) who developed germplasm/genetic stock*

Name (Dr./Ms/Mr.) :

Designation :

Address :

Tel. : Fax : E-mail:

(*Please attach separate sheet for additional name(s) and address(es) of co-authors (persons responsible).*

13. Name and address of the corresponding person

Name (Dr./Ms/Mr.) :

Designation :

Address :

Tel. : Fax : E-mail: :

(A) Passport information of germplasm/genetic stock

IC/EC No.	Other Identity	Source	Place of origin Tehsil/Distt/Province	Remarks

(B) Pedigree of the genetic stock/germplasm

Pedigree	Breeding method (Code)

14. Salient Characteristics/Chief botanical and morpho-agronomic /morpho economicdescription (attach details)**

S.No.	Trait Description
1.	
2.	
3.	

15. Year of seed/cutting/egg | **Location of seed production** | **Quantity of seed available**

15. Additional Information/Remarks (if any)

UNDERTAKING

I/We undertake to ensure long term conservation of the aforesaid germplasm/genetic stock at the National Genebank, CSGRC/,Concerned CSB Institutes and also its sustainable use by maintaining appropriate quantity of Active/Working Collection and providing access as appropriate on prior informed consent and on mutually agreed terms. I/We also agree to provide any further information or data pertaining to the description and unique characteristics to the CSGRC/Concerned CSB Institutes in a transparent manner.

COUNTERSIGNED

Signatures
Full Name
Designation & Address

✓
SIGNATURES OF THE DEPOSITOR
Name
Designation & Address

SEAL

Guidelines for filling Form A and description of Codes

1. Use capital letters or write legibly. All items are self-explanatory. Give minimal explanation for a particular item in "Remarks*" (Item 16),* wherever needed.

2. Be to the point for *Item 6*, give only the most salient features, traits or alleles, considered suitable for consideration of registration.

3. On the other hand, give detailed description of traits & characteristics of the material in *Item 14*. Follow the format of variety release application or AIMSGEP data sheets for respective crops. Use separate sheet if needed.

   Give main botanical and morpho-agronomic characteristics in description in case of host plants and morpho economic traits in case of silkworm Include isozyme or DNA profile or other chemical/biochemical characteristics, if available.

4. Use Codes for filling in *Item 1,4,5,7* [actual], *9,10*, 11 and 13AB [Breeding Method]. In case of the code "Other" fill in specific details in *Item 16*.

5. For filling silkworm / host plant name (*Item 2*) give English or Hindi name, if known. In case a local name is given then also specify in parenthesis the language or dialect in which this name is used.

6. Give name(s) of all persons associated with development of the material in *Item 12*. Use separate sheet and fill in additional names along with designation, address and phone/fax/email, etc. beginning with Sr.No. 2 on new sheet, in the same format

7. *Item 13* has two alternate parts, (A) and (B) to fill in:
   (a) In case Nature of the material to be registered as given in *Item 13* is "Germplasm" then you must give its basic passport information, that should include National Identity (IC/EC) given by CSGRC or other Identity Number allotted and maintained locally. In latter case, CSGRC will provide a unique National Identifier Number (IC), based on passport data provided.
   (b) In case the nature of the material to be registered is Genetic Stock, then clearly give its Pedigree, including parentage, year of crossing or selection. Also give breeding method used in codes described below.

8. Give particulars of developers in *Item 12* over and that of corresponding person in *Item 13* as the applicant and developer may not be always the same as the first person responsible for development of the material.

9. Undertaking to the effect ensuring long term conservation and maintenance of active material for facilitating access and sustainable use has been given, which may be read and implied before putting signatures.

Codes for filling information in Col. 1,4,5,7,8 [actual], 9,10, and 14 (B) [Breeding Method]

Item 1 : Application Status	
N	New
R	Revised
Item 2 : A. HOST PLANTS	
ML	Mulberry
TT	Tropical Tasar
OT	Oak Tasar
MH	Muga
ER	Eri
OF	Other food plants
B. SILKWORM	
MS	Mulberry
TS	Tasar
OS	Oak
MU	Muga
ES	Eri
OS	Others
Item 5 : Biological status of material to be registered	
GP	Germplasm collection
GS	Genetic Stock
RE	Recombinant
MT	Mutant
OG	others
Item 7 : A. Host Plant	
SD	Seed
VP	Vegetative propagule
WP	Whole Plant
OP	Others
B. Silkworm	
EG	Egg
CO	Cocoon
OS	Others

Item 8 : Quantity deposited with application	
	Actual
Item 9 : Value referred to by applicant	
SC	Scientific
CM	Commercial
AC	Academic
Item 10 : Basis of eligibility for registration	
PR	Published with Peer review
AD	AIMSGEP Data
AR	Institute annual report
OR	any other report
Item 11 : Validation Test suggested	
Y	Yes
N	No
Item 14 B : Breeding method used	
IN	Introduction & selection
MS	Mass selection
PS	Pedigree selection
PL	Pure line selection
RS	Recurrent selection
BC	Backcross method
OM	Other (Specify in Item19)

To

The Director
Central Sericultural Germplasm Resources Centre (CSGRC)
Central Silk Board
P.B. No. 44, Thally Road
Hosur – 635 109
Krishnagiri District
Tamil Nadu
Ph. 04344 – 222013, 220698; Fax : 04344 – 220520
e.mail : csgrchos.csb@nic.in, csgrchosur@gmail.com

Requisition for supply of seed/planting material for research from/through CSGRC, Hosur

1. Details of seed/planting material required for research

Sl. No.	Variety Name	Scientific Name	Accession Number (IC/EC)	Required Quantity	Purpose (Screening/breeding/ evaluation /augmentation/ multiplication /others [mention details])

2. Thesis / Project title for which request is made(name the funding agency) …….

……

……

3. Objective/Activity for utilization of indented material : (Please attach sheet if required): ……

……

……

4. Material Transfer Agreement(enclosed): Yes/No

5. Feedback report submitted on germplasm received earlier (if applicable) : Yes/No

6. Have you or your Institute developed any variety based on germplasm supplied by CSGRC? Yes/No
(If yes, please let us know the details)

7. If required by CSGRC, will you be able to supply multiplied material of the above mulberry accessions in sufficient quantity for conservation: Yes/No

8. Signature of the indentor:

Name : ……..….. Designation ……

Address of the Institute …….

……

Phone (with STD code) ……..….M ……., Fax ……, e-mail …….

9. Signature of the Competent Authority with seal : …….
(PI/Head of the Department/Director of the Institute)

Material Transfer Agreement

Central Sericultural Germplasm Resources Centre
Central Silk Board, Thally Road, Hosur – 635 109

Agreed between
Central Sericultural Germplasm Resources Centre, Central Silk Board, Hosur – 635109 under Ministry of Textiles, Govt. of India being the first party (Provider of the Material)
And[1]
…….. ……. …….
Being the Second Party (Recipient of the Material)

For the Supply/Exchange/Transfer of Genetic Resources for Sericulture / Germplasm / Genetic Material / Genetic Components for Research[2]

- Within India, not covering persons as described in Section 3(2) of the Biological Diversity Act, 2002 (18 of 2003) (BDA).
- Within India, wholly or partly covering persons as described in Sec. 3(2) of BDA.
- Outside India, with Members of the International Treaty for Food and Agriculture 9ITPGRFA), and wholly or partly covering persons as described in Sec. 3(2) of BDA.
- Outside India, with Non-Members of ITPGRFA, and wholly or partly covering persons as described in Sec. 3(2) of BDA.

AS follows:

Recipient Name	
Recipient Institution/Organisation/ Agency/Centre	
Recipient Full address with PIN code	
Phone number	
Fax	
E-mail	
Nature of activities	
Germplasm material (specify)[3]	
Supply made through	CSGRC
For official use of supplier	1. Germplasm Identity (Species name, common name, etc.) 2. Accession Number 3. Short description of the material 4. Passport data

[1] Mention Name and address of the Second Party
[2] Tick mark the appropriate box
[3] Specify the type of material involved for supply/transfer e.g. cuttings, saplings, leaf samples, tissue culture , DNA etc.

I/We agree to abide by the following terms of the MTA and certify that:

i) The germplasm MATERIAL (S) transferred herein as above shall be used only for the purpose of research under my/our direct/close supervision and will not be used for commercial purposes or profit making whatever, without prior written approval of the NBA[4]/MoEF[5]/DARE[6]/ICAR[7], Government of India as the case may be. The importer/recipient (Second party) agrees to provide a concept note of research project in which the MATERIAL(S) will be used, including the manner in which to be used. The importer/recipient(Second party) agrees to cease any use of the material in case of suspension of research project at the instance of either party or due to factors beyond the control of either party. Upon such suspension of further research, both parties will mutually agree for adopting a suitable provision for their preservation. In case of failure of the parties to arrive at an agreement, the materials including derivatives will be destroyed upon 90 days notice from CSGRC.

ii) All information and material supplied by CSGRC shall be deemed to have been disclosed or provided to the recipient in confidence. The recipient agrees to preserve the confidential status of the material and information.

iii) The germplasm MATERIAL(S) or its (their) part(s), components or derivatives (including live or dead tissue/DNA) that can be used to retrieve whole DNA/fragment or sequence or any other genetic information shall not be distributed or transferred to any third country/party, except those directly engaged in research under direct supervision of the recipient(second party), without prior written approval of the CSGRC/CSB/NBA/MoEF/ICAR/DARE, Government of India as the case may be.

iv) Any development of commercial product based on research on gene manipulation/selective breeding programme for genetic improvement shall not be undertaken without written consent of CSBRC/CSB/NBA/MoEF/ICAR/DARE, Government of India as the case may be. Modalities of undertaking any such work will be worked out before its conduct.

v) If any third country/party is to be associated with any commercial development arising out of the germplasm accessed, permission from NBA shall be sought.

vi) The recipient agrees to acknowledge explicitly the name, original identity and source of the material, if used directly or indirectly, in all research publication(s) or other publications, such as monographs, bulletins, books, etc. and shall send a copy of each of the publications to CSGRC, CSB, Hosur.

……..

[4]National Biodiversity Authority
[5]Ministry of Environment and Forests
[6]Department of Agriculture Research and Education
[7]Indian Council of Agricultural Research

vii. The recipient agrees to supply the feed back information on the performance /utilization/research outcome of the material(s) to the CSGRC, Hosur.

viii. The recipient agrees not to claim any intellectual property right over the MATERIAL (S) received including its related information and knowledge without prior written approval of the CSBRC/CSB/NBA/MoEF/ICAR/DARE, Government of India as the case may be.

ix. The intellectual property protection or benefit sharing in respect of derivatives of the material(s) received/accessed, where applicable, shall be as per the Indian IPR/Biodiversity laws.

x. The recipient agrees to hold the entire responsibility for the quarantine/SPS clearance of the material accessed as specified herein above. The recipient shall abide by the bio-safety guidelines of …… …… …….. (Name of the importing country/organization) and shall not hold CSGRC,CSB/ICAR/DARE, Government of India responsible for any identity/quality/viability/purity/quarantine/bio-safety related or any other related matter/hazard that may be attributable to the release of genetic material/resource accessed as specified in this Agreement. The recipient agrees to hold entire responsibility for the importer/indenting country's bio-safety and any other related hazards due to release of genetic material. The receipt agrees to waive all claims against CSGRC,CSB/ICAR/DARE, Government of India and to defend and indemnify them from all claims and damages /recoveries arising from the use, storage or handling of the material.

xi. The recipient also agrees that the material is for experimental use and is being supplied without any warranties, whatsoever.

xii. The MTA is non-assignable. The recipient agrees to abide by any other conditions that may be set in and conveyed to them from CSGRC, CSB in respect of this germplasm access/exchange or any Law, Rules, Regulations, etc. enacted by Government of India from time to time.

xiii. In case of any dispute between the parties to this MTA, the dispute shall be referred to the Sole Attributor to be appointed by the Secretary, DARE, Government of India. The decision of the Sole Attributor shall be final and binding on the Parties. The Arbitration proceedings shall be governed by the Arbitration and Conciliation Act, 1996. The Arbitration proceedings shall be in New Delhi.

AGREED RECIPIENT	PROVIDER
Authorised Officer's Name: Designation: Organisation/Institute/University Address: Signature : Date	Authorised Officer's Name: Designation: Organisation/Institute/University Address: Signature : Date
Recipient Scientist/Person's Name: Designation: Organisation/Institute/University Address: Signature : Date	Provider Scientist/Person's Name: Designation: Organisation/Institute/University Address: Signature : Date

DEFNITIONS

Extract from Section 3(2) of BDA-2002-

a) a person who is not a citizen of India;
b) a citizen of India, who is a non-resident as defined in clause (30) of Section 2 of the Income-Tax Act, 1961 (43 of 1961);
c) a body corporate, association or organization-
    i) not incorporated or registered in India; or
    ii) incorporated or registered in India under any law for the time being in force which has any non-Indian participation in its share capital or management.

Annexure 7.2

PROFORM FOR COLLECTION OF FEED BACK INFORMATION ON THE UTILISATION OF SERIGENETIC RESOURCES RECIEVED FROM CSGRC, HOSUR

1. BASIC INFORMATION:

Name	
Institution	
Address **e-mail-Id** **Phone No.**	

2. Names of the germplasm accessions received

Date of receipt	**Details of germplasm (Mulberry/Silkworm)**

3. Major objectives for which germplasm indented/received

Sl No	**Objective**	**Mark the objective**
1	Conservation programme	
2	Breeding	
3	Research	
4	Horticulture purpose	
5	Education/demonstration	
6	Others (please specify)	

4. Do you conserve the germplasm received Yes/No

(If answer is yes, please specify purpose of conservation viz., propagation/multiplication/breeding material/genetic stock and others)

5.General information:

a) Whether germplasm received is further characterized and or evaluated at your institute (Please specify the characterization or evaluation with results)

Traits Characterized	Specific characters If any observed	Results
Traits evaluated	**Specific characters If any observed**	**Results**

b) Breeding (Mulberry/Silkworm)

Please specify the germplasm received used for breeding purpose : **Yes/ No**

Name of accessions	Details of breeding (Including development of elite breeds showing the parental material of germplasm)

c) Research (Mulberry/Silkworm)

Type of study/Title	Names of germplasm accessions used	Results in brief and related publications

(Please furnish Photostat copies of the publication /documentation, if any for having utilized germplasm)

d) Horticulture purposes (Mulberry Germplasm)

Name of germplasm accessions used	Purpose of utilization (Please indicate details)

e) Others (please specify) (Mulberry/Silkworm)

6) Major approaches you use to acquire information about seri-enetic resources

a) Annual report
b) Catalogues published by CSGRC, Hosur
c) Scientific journals
d) Web site etc.,

7) Rating of the germplasm received by your institute

Mulberry

Supply as per your Indent	Yes/No
Quality of germplasm received Spouting/Rooting Any other Information)Please Specify)	Very good/Good/Poor Very good/Good/Poor

Silkworm

Supply as per your indent	Yes/No
Quality of germplasm received	Very good/Good/Poor
Hatching	Very good/Good/Poor
Performance	Very good/Good/Poor
Any other information (Please specify)	

8) **Whether the germplasm material re-distributed Yes/No,**
If yes furnish details.

Particulars	Details
Aim	
Institution	
Purpose	
Frequency	

Date:

Signature and Seal

Note : - Please fill this form and send to the Director through e-mail
csgrchosur@gmail.com