Manual on Survey, Exploration, Collection and Conservation of Silkworm Genetic Resources

Edited & Published by
Dr. Alok Sahay
Director, CSGRC, Hosur

Compiled by
N. Balachandran, Veeranna Gowda,
M. Muthulakshmi & G. Thanavendan

CENTRAL SERICULTURAL GERMPLASM RESOURCES CENTRE
Central Silk Board, Ministry of Textiles, Govt. of India,
P.B. No. 44, Thally Road, Hosur - 635 109, Tamil Nadu

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Cover page design
S.Sekar

Central Sericultural Germplasm Resources Centre
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Introduction

The genus *Bombyx* Hubner has two species, *Bombyx mori* L. and *Bombyx mandarina* Moore. Apart from the genus *Bombyx,* there are eleven other genera in the family *Bombycidae* Hubner.

1) Genus - *Theophila* Moore,
2) Genus - *Ocinara* (Walker),
3) Genus - *Mustilia* (Walker),
4) Genus - Gunda (Walker),
5) Genus *Penicillifera* (Walker)
6) Genus - *Ernolatia* (Moore)
7) Genus - *Norasuma* (Moore)
8) Genus - *Trilocha (*Dieri),
9) Genus - *Prismosticta* (Swinhoe),
10) Genus - *Andraca* (Walker), and
11) Genus - *Ectrocta* (Hampson).

Among these genera, *Theophila* and *Ocinara* are very close to the genus *Bombyx*. The wild sericigenous species of *Bombyx*, *Theophila* and *Ocinara* are naturally distributed in the Himalayan ranges of Indo-China range and also in Andaman Islands in India, besides, Jawa, Sumatra, Borneo and Malaya Peninsular (Barlow, 1982). The wild species of these genera have not been explored for transferring the useful genes to confer resistance to diseases and tolerance to adverse agro-climatic conditions into the domesticated species, *B.mori*. The useful genes from the wild relatives of *B. mori* may be cloned and these cloned genes may be transferred into the germ cells of the silkworm to develop transgeneic silkworm. Hence, there is an urgent need to collect and conserve the wild species of *Bombyx, Theophila* and *Ocinara* and study their genetics for possible use in the breeding programme of *B.mori* and widen the genetic base as well.

The wild relatives of *Bombyx mori* L. exist in natural habitat in the Himalayas. The wild species of *Bombyx mandarina* Moore and other wild species of *Theophila* and *Ocinara* constitute the natural seri-biodiversity. These wild species also need conservation and utilization to broaden and enrich the gene bank seri-biodiversity.

Conservation of wild seri-biodiversity should be done by organizing exploration, collection and conservation followed by utilization. Otherwise, this very valuable seri-biodiversity may gradually be eroded and the very precious gene pool may be lost forever. Explorations and survey trips may be organized, particularly in the Himalayas of Indo-Chinese region, Andaman Islands, Java, Sumatra, Malaya peninsula and other geographical regions where *Morus* and *Bombycidae* genetic diversity are rich to develop repository for these wild seri-biodiversity and develop a comprehensive inventory including wild sericigenous insects.

Need for Survey

Central Sericultural Germplasm Resources Centre, Hosur was established during the year 1990 with the mandate of Collection, Characterisation, Evaluation, Conservation, Supply to promote utilization of the Serigenetic resources involving both host Plant (Mulberry) and Silkworm (*Bombyx mori*). The Institute has reached to the current status of conserving 470 SWGRs (81 MV, 369 BV and 20 mutants) completely collected from different Institutes. The scientists of this centre also undertook one exclusive survey to Andaman Islands resulting in the collection of one silk moth belonging to *Ocinara spp.* but continued conservation of the species could not be further persisted. Later, when the scientists of this centre were on survey and exploration trips for collection of mulberry accessions they have also brought different stages of silkworm resources that were reared at this centre resulting in identification of the genus of *Theophila spp.* which was maintained for a generation but later could not be continued.

In this context, it becomes important that the sericigenous insects of the North Eastern and North Western regions of the country needs to be collected, reared, identified and a document on the available sericigenous insects of the country has to be prepared. Being the conservation centre, it becomes a task of the centre to regularly embark on the survey and exploration trips to the wild areas to collect *Bombyx* genus related species and other sericigenous insects for rearing under quarantine to identify the genus and species for proper conservation. The desirable traits of these wild silkworm genuses like tolerance to abiotic and biotic stress conditions, resistance to diseases and pests, fibre qualities etc. could be utilized in the

development and evolution of new horizons in sericulture aimed towards improving productivity and quality silk.

There are 77 species of Bombycidae belonging to 25 genera that were collected from China representing 3 sub families. Therefore, there is a need to undertake survey and exploration of the availability of the species under Bombycidae in the North eastern and Sub Himalayan regions to gain indepth knowledge on the wealth of the Sericigenous Insects of the Country.

Planning area of Survey

The literature available on the survey and exploration undertaken earlier for other lepidopteron insects can be utilized as helping hand. The earlier survey reports indicate the availability of the *Bombyx spp.* in that particular area. Reports of the Royal British museum will indicate the availability of the *Bombyx spp*. in the Indian Sub continents. The survey reports of local Zoology departments and Zoological survey of India can give an idea and details of availability of the Bombycidae species and other sericigenous insects of a particular locality. Based on the reports available on hand, one can plan the area of the survey and exploration for collection of sericigenous insects.

Correspondences for survey

Careful planning and proper correspondences should be made well in advance to the State Government departments like Forest Departments and other Central Government institutes like NBAIR / Zoological Survey of India, Forest Research Institutes located in the area concerned or covering the area proposed for survey and exploration. The permission required from the Forest Department authorities is to be obtained so that, the Officer / Official concerned will lend us helping hand in conducting the survey. Their knowledge the localities, wild animal threats, tribals, types of insects and plants available etc. with facilities that could be utilized and exploited for our survey purpose. When we proceed towards the interior forest areas where there no commercial accommodation available, the Forest Department Guest houses can be of immense help to the survey team.

In addition to the other Governmental agencies, the CSB units in the state / locality can also be approached for utilizing the facilities available with them or their sub units or regional centres that will be helpful in completion of the planned survey work.

Preparation for survey

After making due correspondences and getting their consent and approval in advance, it has to be planned for the travel thoroughly with booking of tickets indicating the date and time-wise mode of travel (by train / air) for proper operation of the plan. Ensuring proper approval in case of Air travel or travel over and above the entitlement of the team persons for conducting the survey from the competent authorities should also be ensued in time with prior sanction.

Conveyance from the Airport/ railway station should be planned much before the trip and properly communicated to the persons coming for picking up to save time and negotiate in terms of hiring charges for the conveyance. Accommodation and boarding either in hotels or guest houses, as per availability, has to be booked / arranged in advance and proper communication has to be sent to the concerned authorities on the date and time of arrival and travel plan / no. of persons staying, etc. In addition to the written communication, telephonic talk to confirm the receipt of the letter of travel plan is also desired.

Vehicle booking is essential for conducting survey in remote areas. A guide in co-ordination with State Department official / local department is also very much essential while conducting the survey for better communication being a local person to guide the team. The guide should be paid suitable remuneration and treated equally like the survey team. Survey period be of not less than 10-15 days.

Survey Kit

The survey trips should be performed with standard survey kit containing all the Instruments, equipments and essential items for proper collection of samples as indicated below.

Collection methods and equipment

Collection methods

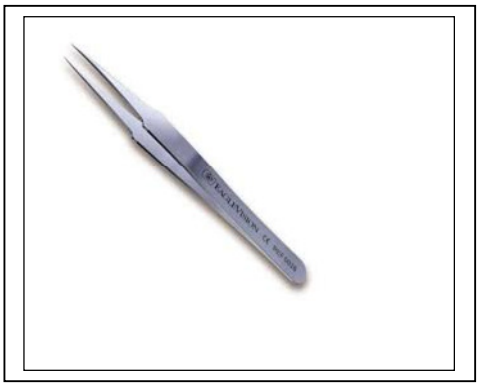
Collecting methods could be divided into two broad categories. In the first, a collector actively finds and collects the insects with the aid of nets, aspirators or whatever apparatus suits particular needs. In the second, a collector participates passively and permits traps to do the work. Both approaches should be used simultaneously. Utilization of as many different collecting methods as possible will permit a collector to obtain the greatest number of specimens in the shortest period of time. Catching specimens by hand may be the simplest method of collecting.

Some insects are not active at times and places that the collector finds convenient. Often, particular kinds of equipment and special methods are needed. Equipment and methods described here have general application. Clever collectors will make adaptations to fit their specific purposes and resources. However, additional items will permit more effective sampling of a particular fauna. Many collectors carry a bag or wear a vest in which they store equipment.

The following items usually are included in the general collector's bag.

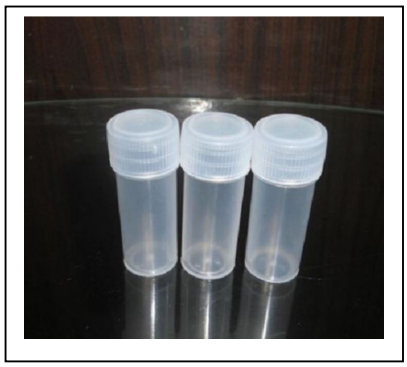
Equipments

Forceps: Forceps fine, light weight forceps are strongly recommended for any collector. Specialized forceps may be selected depending upon individual needs.



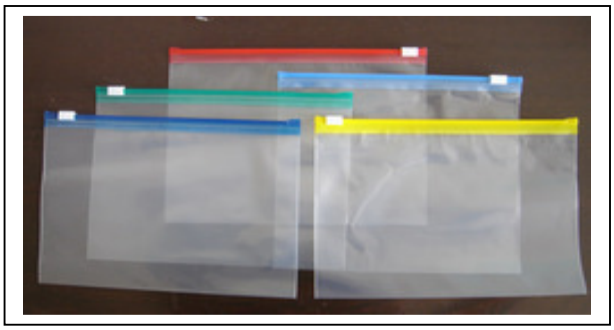
Light weight spring-steel forceps are designed to prevent crushing of fragile and small insects. Extra-fine precision may be obtained with sharp-pointed "watchmaker" forceps; however, care must be taken not to puncture specimens. When possible, grasp specimens with the part of the forceps slightly behind the points. Curved forceps often make this easier. When the forceps are not in use, their tips should be protected. This can be accomplished by thrusting the tips into a small piece of Styrofoam or cork or by using a small section of flexible robbing as a collar.

Sample vials: Sample vials of various sizes containing alcohol or other preservatives are necessary for collecting many species and life stages of insects and mites. Leak proof caps are recommended for both field and permanent storage.



Small containers: Small crush proof containers are necessary for storing and protecting specimens after collection. These containers may be made of card board, plastic or metal and should be partly filled with soft tissue paper to keep specimens from becoming damaged. Some collectors do not recommend use of cotton in storage containers because specimens become entangled in the fibres and may become virtually impossible to extricate without damage. However, some collectors of minute and fragile insects find that the specimens stored in a few wisps of cotton are better protected from damage.

Small envelopes: Small envelopes (Zip-lock covers) are useful for temporary storage of delicate specimens specially designed glassine envelopes which prevent undue dislodging of butterfly and moth scales are available from biological supply houses.

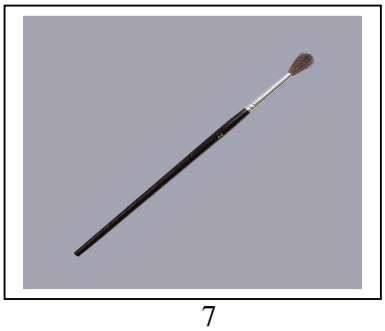


Aspirators: Aspirators are necessary for collecting many kinds of small bodied early instars of silkworm moths.

Notebook: A note book and writing equipment are essential for jotting down notes and label the data.

Tools for cutting or digging: A knife or plant clippers or both necessary for opening galls, seed pods, twigs and other kind of plant material. In addition, a small gardeners trowel for some kinds of excavation and a heavy knife or small hatchet may be helpful for searching under bark or in decaying logs.

Brush: A small fine brush (camel's hair is best) is needed to aid in collecting minute specimens.



Bags: Bags for retrieving plant material, rearing material or berlese samples are a good idea. Remember samples stored in plastic may decompose within a short duration of time. Samples must be transferred to more permanent containers immediately upon returning from the field.

Hand lens: A hand lens is helpful and will quickly become an indispensable aid to collectors. A lens worn on a lanyard is convenient and prevents its loss while in the field.

Insect box with Pins and naphthalene balls

For temporary mounting of the Insects which are caught at the shortest interval of time Insect box with Pins and naphthalene balls.

White cloth of measurements (10'X4') with straps is necessary to tie them to the support as a back drop to trap the insects near the light trap for collection.

First aid Box containing essential medicines needs to be a part of the survey kit that is necessary.

Gum boots are much essential for all the surveyors to prevent / safe-guard themselves from insect / snake bites.

A big trolley bag is very much essential to carry all the materials and survey kit.

GPS instrument is required to know the longitude, latitude and altitude of the areas surveyed.

Digital Camera with two sets of additional batteries is needed for capturing the images.

A Secateur A good quality secateur is required to cut the plants and plant parts.

Hammer and Nails: Good quality hammer and nails are needed to tie the things in trees.

Labels and stickers: Readymade labels and stickers are essential for labelling the collections.

UPS with 12 Volt batteries back up for maintenance of electricity are required, which can be hired locally.

Gum Stick trap: Using the indigenously available gum obtained from trees are used for sticking the insects at height using bamboo poles.

The above list may be modified according to the different kinds of collection planned. When collecting at night, a flash light or headlamp is essential, the latter is especially useful because it leaves both the hands free.

Much of the basic equipments may be obtained from ordinary sources, but equipment especially designed for collection often must be bought from biological supply house. The addresses of the biological supply houses may be found on the internet or in the yellow pages of the telephone directory under biological laboratory supplies or Laboratory Equipment and Supplies. Biological and entomological publications often carry advertisements of equipment suppliers. Biologists at a local university usually can recommend a supplier in the area concerned.

Observations of insect litters and feeding behaviour can be helpful in identifying their presence. During leaf shade time it becomes easy to locate the larvae or cocoons of the worms.

Safety during survey

Safety during survey is also major responsibility on the part of the surveyor. The persons doing the survey know where he is and what you will be doing. The next thing to do is to assess what the potential risks might be and to take steps to minimise them.

Information on disease-carrying mosquitoes, sand flies, black flies, tsetse flies and other harmful insects should be known and appropriate preventative and prophylactic measures should be taken care of. Even if they do not carry disease the bites of insects can be very disruptive to work programmes.

If one has to go through swamp and damp conditions, small water bodies and streams it is essential that you take precautions against blood flukes and leech.

Many species of social wasp and honey bees can pose very serious problems. Keep away from any nests you come across even if this means backtracking and going round. Honey bee colonies are very much more sensitive to nearby movement and they are much more aggressive. Despite being careful one might still accidentally damage or come too near a honey bee or social wasp colony.

The sand flea is a significant parasite of humans in dry, sandy areas if walked bare footed in sandy areas. There can be severe itching and inflammation and a number of these fleas can make walking difficult. The associated skin lesions can lead to infection and ulceration.

Remedies

- Wearing a T shirt under a shirt, even if it is very warm, is a good idea in certain habitats, such as tropical seasonal forests, during the wet season.
- Ensure one don't have an allergy and apply any insect repellent products before going into the field. Some of the insect repellents are for extreme conditions, 100% Deet (diethyl toluamide) on exposed skin and a spray of 30-50% Deet on clothing is recommended.
- The use of wellington boots and rubber gloves is obligatory while crossing water bodies to avoid liver fluke and leech attacks.
- Try standing absolutely still to avoid the sting of wasps as movement will be very clear to the wasps.
- One has to carry antihistamine injections or tablets as the medical practitioner advises to treat the honey bee and wasp multiple stings as usually around the head and neck it will produce painful swelling and the symptoms of shock. .
- As far as tree climbing is concerned, surveyor should avoid it unless he is fully qualified and trained.
- Walking bare footed is not generally good even when off duty as its quite dangerous with sharps and thorns.

- Avoid glass tubes and containers if possible as they are heavier than plastic and a common cause of accidents may happen.
- Make sure to know the properties of any chemical being using by following instructions on their use and disposal.
- It might be worthy taking a small plastic or cardboard sharps bin for the safe disposal of blunt scalpel blades, syringe needles and pins.

Survey

While on survey, the team has to be physically looking for host plants and alternate hosts while walking in the forests and the different life stages of the insects observed on the host plant may be collected. Different stages of the insects like eggs, larvae and cocoons can be collected in the day time. During night hours, light traps have to be set to collect the moths in the vicinity and the collections have to be periodically monitored and collected in separate covers and labelled so that at the immediate pause they could be preserved in the bottles and mounted in boxes.

Collection Methods

Litter observation

The surveyor while walking through the survey areas has to look for the faecal pellets of silkworm on the ground. If any observed beneath the trees whether of mulberry or alternate host plants in the case of the wild *Bombyx spp*. then observation on the trees have to be intensified for the growth stages of the silkworm. The different life stages of the insect like eggs/larvae/cocoon stages may be searched on the particular trees and also near by trees in the vicinity for collections.

Physical survey and search

All the mulberry trees, bushes in the area have to be intensively searched for occurrence of any wild species of *Bombyx spp*. forms or their life stages. The alternate host plants of the wild mulberry silkworm also have to be observed for the stages.

In these two methods of collections only we may get the growth stages of the target insects. It is ideal to collect either in egg or cocoon stages through manual (by hand) collection, whereas at the moth / adult stages it cane be collected through light traps. These traps to be set up at different sites and operated from dusk to dawn. The insect specimens should also be collected naturally by net.

Light traps

Mercury blended lamp 220-240 watts and wires of different sizes 10m, 15m, 25m and 50m with plugs and switch boxes and holders are needed to set up a lamp to serve as light at any given place.

The light trap comprised a 3 x 4 m white cloth stretched between two posts or trees, in front of which was hung a mercury vapour light bulb (Philips, B/73, ML 160W). This set-up is more suited to tropical conditions than a box or tub style traps, such as the Robinson trap or Skinner trap. A battery operated indigenous light traps with different intensity of light as per the survival strength of trap insects can be used. The survey team to camp in resorts with close proximity to forests; the light traps to be operated over night from 19:00 hrs to 05:00 hrs. The light traps will be switched off at 05:00hrs to allow the moths (and other insects) to disperse before sunrise, which may happen after 05:30hrs; this will prevent their predation by birds.

Pheromone traps

Pheromones are semiochemicals released by species that can cause certain behavioural or physiological responses of other individuals. Pheromone traps are widely used in monitoring arthropods population for pest control. Sex pheromones and aggregating pheromones are the main two types of pheromones used in these traps. Sex pheromones are semiochemicals that sexually maturity specimens produce

to attract the opposite sex for mating while aggregating pheromones are produced by species to induce gathering for feeding or attack. Pheromone traps are high selective for certain species and often gender, and they are widely used in the trapping of Lepidoptera and Coleopterans. Pheromone traps are an inexpensive and easily implemented approach in many cases, although the initial production of specific pheromones can be expensive and time-consuming. They are usually weather sensitive and often require substantial knowledge of the target species.

Collecting Nets

Collecting nets come in three basic forms, aerial, sweeping and aquatic. The aerial net is designed especially for collecting butterflies and large-bodied flying insects. Both the bag and handle are relatively lightweight. The sweeping net is similar to the aerial net, but the handle is stronger and the bag is more durable to withstand being dragged through dense vegetation. The aquatic net is used for gathering insects from water.

Several kinds of nets, including collapsible models with interchangeable bags, are available from biological supply houses. The advantage of a homemade sweep net is that its size and shape can be adapted to the needs of the user, to the kind of collecting intended, and to the material available. Net-constructing materials include the following.

The catch may be conveyed from the bag to a net in a number of ways. Single specimens are transferred most easily by lightly holding them in a fold of the net with one hand while transiting into the net with the other. While the jar is still in the net, cover the opening until the specimen is overcome; otherwise, it may escape before the jar can be removed from the net and dosed.

To prevent from damaging its wings by fluttering in the net, squeeze the thorax gently through the netting when the insect's wings are closed. This will temporarily paralyze the insect while it is being moved to the killing jar. Experience will teach how much pressure to exert.

Obviously pinching small specimens of any kind is not recommended These methods of mass collection are especially adapted to obtaining small insects not readily recognisable until the catch is sorted under the microscope.

Identification of the specimens

The collected insect specimens have to be identified based on available literature, procedure and also by sending the specimen samples to the taxonomist / subject specialist(s) available in the particular area or to zoological survey of India / National Bureau of Agricultural Insect Resources (NBAIR) / Conservation Education Centre of the Bombay Natural History Society, Mumbai.

Stages of collection of Insects

Insect specimens can be collected in all the stages *viz*., egg, larvae, pupa, cocoon and moth. But, it is ideal to collect either in egg or cocoon stages through manual (by hand) collection, whereas at the moth (adult) stages it will be collected through light traps. These traps to be set up at different sites and operated from dusk to dawn. The insect specimens should also be collected naturally by net.

Transit of specimens

The collected insect specimens can be transported / carried to the destination by using corrugated fibre boxes, insect boxes, flower baskets and well aerated plastic boxes / containers with fillers to avoid shake or damage to the specimens.

Egg stage

The egg stage is ideal for collection of new species as there will be no need of attending the collected specimens repeatedly as followed in the form of providing feeding or cleaning etc., but the problem with the egg stage is, that the stages of the embryonic growth to be found out to decide (which can be ascertained by test verifying the sample embryos using hand lens) as to whether the eggs can be preserved through the survey period or it to has to be attended immediately. If it is in

the advanced stages of development the eggs have to be either send by speed couriers or speed post to conservation centres in proper packages. If cold storage facility is available nearby in the area it can be preserved till the survey period.

Larval stage

If the collection is in larval stage, they have to be reared in perforated plastic boxes by providing leaves of mulberry /host plants as feed. Care should be taken to keep the larvae in the place of stay and maintain the required temperature and RH for the proper growth of the larvae. They need not be carried away during the survey trips. Protection from ants and other predators like lizards and squirrels are to be ensured.

Cocoon stage

Like the egg stage, cocoon stage is also ideal stage for collection of wild relatives of *Bombyx spp.* Here again the pupal development stage has to be ascertained to know the probable date of emergence of moth. The surveyor has to decide based on the development of the pupae whether to transit to the conservation centre immediately or to delay and carry along with the surveyor. Proper protection for the pupae has to be ensured during the survey period and also during transport using corrugated carton sheets and bedding materials.

Adult stage

Using light traps/nets one can collect the moth stages during night hours. But there is no information on the reproduction status of the collected moths. But the moths collected can give us an idea of the species available in the vicinity and scope for expanding the survey and collection. The moths collected could be preserved as voucher specimens for reference and fruitfulness/record of the survey which can serve as reference for future surveys.

Quarantine rearings at conservation centre

The collected specimens has to be conserved by conducting quarantine rearing under isolation by maintaining ambient temperature and humidity from egg to moth stage, if the collected specimen is egg. Whereas, if the specimen collected is at pupal stage, moth emergence should be ensured for further processing, egg production and conservation. In respect of wild insect specimen collection, as the males are hyper active they will be in flying nature and hence by using a nylon net enclosure covering the live mulberry plant so as to emulate the field conditions and the insects have to confined and conserved in a insect cage. A minimum of 30-40 number of cocoons are required to ensure further multiplication. Lesser number of cocoons may end up in males getting emerged earlier and non synchronised emergence resulting in loss of continuity of generations.

Since the population is wild in nature knowledge on the hibernating nature of the eggs is unknown the same has to be studied, suitability for acid treatment to break the hibernation using standard procedures has to be tried and documented for future usage. Once the life cycle of the collected specimen is known and procedures for continuing the life cycle is standardised and ensured disease freeness they can be accessioned and accession number can allotted at the conservation centre.

Detective and Corrective Testing Protocols

During the quarantine rearings of the newly collected silkworm breeds the different stages are to be subjected to testing to rule out the possible incidence of the pebrine disease (detective testing).

Dust examination

Collect the samples of dust (1-2 g.) before rearing and macerate in about 8-10 times volume of 0.6 % K2Co3 solution. Filter the solution, centrifuge at 3000 rpm for 3 minutes and examine the pellet under binocular microscope at 600 x magnification.

Leaf examination

Dip the collected mulberry leaf 2-3 times in a beaker containing 0.6 % K2CO3 solution. Filter the solution, centrifuge at 3000 rpm for 3 minutes and examine the pellet under binocular microscope at 600 x magnification.

Eggs/empty shells examination

Collected the unfertilized /dead/eggs showing slow development after hatching and homogenise them in 0.6 % K2CO3 solution (1g: 4 ml) using mortar and pestle. Filter the solution, centrifuge at 3000 rpm for 3 minutes and examine the pellet under binocular microscope at 600 x magnification.

Larval examination

The weak and lethargic larvae suspected to be diseased to be starved for 24 hours homogenise them in 0.6 % K2CO3 solution (1g: 4 ml) using mortar and pestle. Filter the solution, centrifuge at 3000 rpm for 3 minutes and examine the pellet under binocular microscope at 600 x magnification.

Whereas in the case of new collections from wild the number of worms may less and conducting a successful crop is a must therefore instead of larvae the exuviae after moult may be collected and they can be examined after the above procedures.

Faecal matter examination

Soak small quantity (1-2 g) of faecal matter in 8-10 times volume of 0.6 % K2 CO3 solution. Add a few drops of 1N HCL and homogenise using mortar and pestle. Filter the solution, centrifuge at 3000 rpm for 3 minutes and examine the pellet under binocular microscope at 600 x magnification.

Pupal examination

As we cannot kill the live pupae in the case of wild collections for want of numbers the dead and diseased pupae may be cut from the cocoons and homogenise them in 0.6 % K2CO3 solution (1g: 4 ml) using mortar and pestle. Filter the solution, centrifuge at 3000 rpm for 3 minutes and examine the pellet under binocular microscope at 600 x magnification.

All the above procedures could be employed for ruling out (Predictive) the incidence of the diseased wild silkworm genetic materials during the course of the generations so that at any stage if the samples revealed infection the y could be avoided.

Even if, there are no incidences revealed during the stages of lifecycle then also the mother moth examination have to be done 100 % to rule out the possible incidence of disease from mother moth transovarially to the next generations through eggs.

Individual mother moth examination

After egg laying homogenise the single moth in 4 ml of 0.6 % K2CO3 solution using mortar and pestle. Filter the solution, centrifuge at 3000 rpm for 3 minutes and examine the pellet under binocular microscope at 600 x magnification.

Dissolution of sediment

After centrifugation the supernatant have to be discarded without disturbing the sediment formed at the bottom of centrifuge tube and 2-3 drops of 0.6% K2CO3 solution to the sediment. Dissolve the sediment well over a cyclo mixer using a glass rod.

Smear preparation

Use the glass rod to place a tin smear on a clean glass micro slide and cover it with cover slip. Smear should not be too thick or too thin

Microscopic examination

Examine under binocular microscope at 600 X magnification. Examine five fields in each smear by two examiners. Grade the infection based on (No. of spores / field). 1-3 : ± ; 4-10 : 1+ ; 11-30 : 2 + ; 31-100 : 3+ ; 101-300 : 4+ ; > 300 : ∞

Preservation of insects

The light trap collected insect specimens / moths may of varied nature comprising unidentified species, moths of other than *Bombyx* genus should be properly put into the killing bottle and with out damaging them they have to be mounted on the mounting boards. The wings have to be spread in stretching boards, pinned with entomological pins from the dorsal of mesothorax and dried in oven at 55º C. The dried specimens were then kept in wooden insect boxes. Few naphtha balls can be placed at the bottom of the box for preventing fungal attack.

Voucher specimen

Moths attracted to the light traps to be photographed and documented, a maximum of five voucher specimens per species should be collected for identification and future reference. These specimens are to be retained in the study laboratory by the first author at the Conservation centre. Besides this, data collected through light traps, photographic evidences have also to be considered when listing of the species. Identification of moths and compilation of distribution ranges has to be carried out with the assistance of literature sources.

Report preparation and documentation

A detailed survey and exploration report comprising all the aspects of survey viz., area surveyed, route map, itinerary of the survey, mode of conveyance, survey team, methodologies adopted, details of insects collected, stages of insects collected, difficulties encountered and suggestions for future improvement all should be included in the document. The description of the samples including all morphological

features like colour and pattern of wing, size of the moth, taxonomic details along with pictorial representation should be part of the survey and exploration report.

COLLECTION OF DATA

1. Collector's number (Original number assigned to the sample)
2. Collecting institute:
3. Date of collection of original sample:
4. Country/province/state of collection:
5. Location of collection site:

Wild: distance & direction from the nearest town/village:

6. Latitude of collection site:
7. Longitude of collection site:
8. Altitude of collection site:
9. Collection source (wild/farm/institution):
10. Local/vernacular name:
11. Number of specimens sampled:
12. Photograph:
13. Museum specimen:
14. Disease status:
15. End use:

Acknowledgement

Proper acknowledgements to be accorded to the respective station officer, forest officers, local state department officials, local guide, farmers / tribal people and scientists from concerned survey area(s) and the donor organisation for funding.

Characterisation

Characterisation of silkworm germplasm helps in the identification and determination of a genotype and in classifying the genotypes in various ways in different groups for utilization. It can also be utilized for establishing the relationship of different morphological characters with that of commercial traits for utilizing in breeding studies. Also, characterization will be useful in identifying the variability in the germplasm for various parameters and separate / weed out the duplicates.

1. Morphological Characterisation Descriptors

Morphological characterisation is concerned with the description of characters, which are stable in their phenotypic expression irrespective of environmental influence so as to typify the germplasm amongst the genetic resources. Depending upon the economic importance of the character, morphological characterisation forms an indirect basis of selection of germplasm for understanding and evaluating the germplasm to use the same for breeding purpose. Morphological characterisation has direct or indirect relation with various quantitative and qualitative traits. For instance, variations in egg colour, larval markings, cocoon colour, cocoon shape, pupal shape, moth shape and colour have close association with geographical distribution of genetic resources. Nature of integument of larva and wing fasciation in moth governs the tolerance to diseases and survival rate, respectively. The shape and size of cocoon play a major role in important reeling parameters like filament length, breakage during reeling process, evenness, etc. Further, morphological traits along with correlation parameters help to identify and group similar performing germplasm for effective conservation in the gene bank.

1. Egg Characters

(i) **Egg Shape**: Eggs shapes such as spherical, kidney shaped, spindle, clumpy (shapeless), ellipsoidal or short elliptic, slightly attenuated shapes to be observed Dimples and hollowness are either present or absent depending upon the hibernation character.

Method of observation: Egg shape to be observed under stereo dissection microscope under diffused sunlight conditions.

(ii) Colour of eggshell: The eggshell colour after eclosion to be observed.

Method of observation: Eggshell colour is observed under stereomicroscope after eclosion under diffused sunlight.

(iii) Yolk Colour: The yolk of *Bombyx mori* eggs is normally colourless. Yolk with light yellow and dark yellow is also common.

Method of observation: Yolk colour is observed by piercing open the eggshell and placing the yolk content over a glass slide with white background under diffused sunlight. The colour can be represented as colourless, yellow (bright yellow or light yellow) and faint yellow with greenish tint [FYGT] and greenish yellow.

(iv) Serosa Colour: The presence of serosa colour is a unique feature of bivoltine silkworm genotypes. The serosa is a single layer membrane that covers both yolk and embryo, lying underneath the chorion. In hibernating eggs pigments appear in the serosal cells on the second day after oviposition and the colour is fully developed in 3 to 4 days. In non-hibernating eggs the serosal layer is colourless. The colour of the serosa shows the inheritance pattern in some race and affected by the genes for translucency of the larval skin. Variations in the serosal colour are brown, olive green, grey and grey with intermediate tint of green or brown.

Method of observation: The sample (5 DFLs / accession) is observed visually under diffused sunlight on 4^{th} day of oviposition. Serosa colour is classified into brown, grey, greyish brown, olive green, mixed and not applicable (since serosal layer do not develop in non-hibernating eggs as in multivoltine silkworm).

(v) Presence of Glue: Normally egg is attached to the substratum with the help of gelatinous collateral glandular fluid coating while oviposition. In most of the cases (except under any atrophication, which interfere the secretion and oviposit egg without glue), the egg possesses glue.

Method of observation: It is observed by ascertaining the firm attachment of eggs over egg sheet and represented as glue present, fairly present (detach easily) or absent.

2. Larval Characters

(i) Colour of Neonate: The colour of larva soon after hatching (neonate) is usually brown to dark brown. Colour variability such as grey, black and brown with varying hues is also observed.

Method of observation: It is observed immediately after hatching under stereomicroscope in diffused sunlight and scored as brown and dark brown.

(ii) Types of Bristles in Neonate: Bristles or setae are found on the dorsal and pleural region of the body with varying length and colour such as silvery, pale yellow and dark brown. These bristles may be short or long with varying density of bristles.

Method of observation: It is observed immediately after hatching under stereomicroscope in diffused sunlight and scored as normal, short and long

(iii) Larval Markings: Epicuticular markings are grouped into eyespot, crescent, star and quail markings. Larvae possessing all or either of these markings are designated as "Marked" and those without any markings are called "Plain

Method of observation: Larval markings are observed during V instar under diffused sunlight and represented as dark, faint and absent under each category viz., eye spot, crescent and star. In some accessions where the populations show both marked and plain larvae, the descriptor state "mixed" is used.

(iv) Body shape: Represents shape of body segments with special features. Various body shapes in *B.mori* are given as below.

i)	Elongate	- I and II abdominal segment elongated
ii)	Stick	- Slender and firm to touch
iii)	Geometrid	- Very long and slender
iv)	Stony	- Hard to touch and bamboo-like in shape
v)	Narrow brest	- Pot belly shaped
vi)	Constricted	- Each segment constricted in the middle
vii)	Compressed	- Short and fat abdominal segments
viii)	Apodal	- Thoracic legs degenerated
ix)	Knobbed	- Presence of knot-like dermal protuberances
x)	Swollen	- Bulged and bag-like

Method of observation: Observe on 3rd day of V instar larva.

(v) Body Colour: The colouration of the cuticle is generally white with bluish, or yellowish or pinkish tint.

Method of observation: Observe on 3rd day of V instar larva and described as white, bluish white (BW), ash, dirty and mixed.

(vi) Haemolymph Colour: The haemolymph of the yellow cocoons varieties is yellow, while it is colourless in most of the white cocoons varieties, but the silkworm with colourless haemolymph spinning greenish yellow cocoons and silkworm having yellow Haemolymph spinning white cocoons is also reported.

Method of observation: Observation to be done in 3rd day old V instar larva. The entire population can be characterised by observing the colour of prolegs and grouped as colourless, yellow including intermediate hues and mixed (both colourless and yellow as found in sex-limited genotypes)

(vii) Nature of Integument: The integument of the silkworm larva is usually opaque, containing whitish urate crystals. Most of the bivoltine germplasm possess opaque integument and multivoltines have semi-translucent integument. In addition, there are known several mottled translucent strains in which translucent and opaque skin are intermingled. Translucent larvae are thought to have less ability than the normal type to retain uric acid in various organs and tissues of ectodermal origin.

Method of observation: Observation to be done in 3rd day old V instar larva and designated as translucent, semi-translucent (ST), opaque, oily and mixed.

3. Cocoon Characters

(i) Cocoon Colour: White cocoons types are very common in China and Japan; flesh yellow cocoon types in Europe and greenish yellow types in India. It is not much known about the cocoon colour of the first domesticated silkworm. However, it is presumed that the original colour was light yellow, because the ancestral silkworm *Bombyx mandarina* spins light yellow cocoons. Conventionally, white is considered as normal. Other than white, colours such as chrome yellow (=golden yellow), light

yellow, inner layer yellow, flesh, pink, yellowish brown, rusty, green with yellowish tint are also available.

Method of observation: The entire population is studied through visual scoring immediately after harvest. The cocoon colour is categorised into white including intermediate hues, golden yellow, yellow including intermediate hues, greenish yellow, flesh and mixed white ♂ and yellow ♀.

(ii) Cocoon Shape: Several types of cocoon shapes and size have been known. Though oval shape is very common, there are many Chinese races, which spin spherical cocoons, and Japanese races with peanut shaped cocoons. Most Indian strains spin peaked cocoons attenuated at one or both the ends. There is also wide range of variations in the size. Within constriction also the depth of constriction varies from light to deep. Peaked cocoons also exhibit broad base as well as narrow base.

Method of observation: The entire population can be studied through visual scoring immediately after deflossing. The cocoon shape is classified into oval, elongated with constriction (EC), elongated without constriction (ENC), spindle, spatulate and dumb-bell (DB). For better understanding intermediate variations within oval and elongated shapes were narrowly grouped

(iii) Cocoon Grains: Variations are also seen in cocoon grains *i.e.,* the number of ridges and pits per unit area on the shell surface. Some have smooth velvety surface (where grains are not clearly visible), in other cases it is observed as coarse, medium and fine. Majority of peaked cocoons (e.g. Pure Mysore, Nistari etc) are always associated with more floss on the shell and designated as flossy.

Method of observation: A sample of 20 cocoons per replication will be studied by observing the shell surface with hand lens. The grains are classified into fine, medium, coarse and flossy.

4. Pupal Characters

(i) Pupal Colour and Shape: The colour and shape of pupa vary according to the species. Generally the pupa is obtect type; the imaginal buds of wings and legs protrude from the thorax extending posteriorly down to the second abdominal segment on the ventral side.

***Method of observation**:* For characterising pupal colour entire population is observed. A sample of pupae in each sex is observed with magnifying glass for development of wing pad and legs. They are grouped as brown and black including intermediate hues.

5. Adult Characters

(i) Body Colour: Moths are covered with scales on body and wing. Hence, the colouration of the moth is determined by the colour of scales, such as creamy white. Black moth and wild melanisim (moth are dark grey). In the domesticated silkworm the body of the female moth is creamy white or white with faint yellow tint, male tinged slightly dark brown as secondary sexual character. The intensity of pigmentation (or banding) differs. There are several strains differing in the intensity of pigmentation.

Method of observation: Entire population is observed during emergence under diffused sunlight and characterised for body colour as creamish white (CW), dull white and dirty

(ii) Body Shape: This denotes the shape of the body (head, thorax and abdomen), excluding fore and hind wings.

***Method of observation**:* The entire population has to be studied for its shape.

(iii) Wing Fasciations and Venation: Wing colours are classified in many types such as white, white with dark fasciations, dark wing pattern, black and some intermediates. Dark types are inherited polygenically. Majority of the bivoltine accessions are characterised with plain wing, while multivoltine with faint to medium dark having dark ante median and post median fasciation. The wing venation typically follows the bombycid pattern. However, degeneration of vein sometimes may occur in the radius, cubitus and anal regions.

***Method of observation**:* Moth in each sex is observed under stereomicroscope after clearing the scales. The fasciation is described based on the presence or absence of ante median and post medial wavy markings on wings. The fasciation is observed visually and represented as dark ante-median and post-median fasciations [AM+PM (D)], faint ante-median and post-median fasciations [AM+PM (F)] and no ante-median and post-median fasciations [AM+PM (A)]

(iv) Shape of Antenna: The shape of antenna in *Bombyx mori* is bipectinate. However, atrophication in flagellar region is found in some species, thus giving short-sized antennae.

Method of observation: The shape of antennae is observed with the help of magnifying glass for a sample of moths.

(v) Eye Colour: Normally the eye colour is black in *Bombyx mori*. There are several mutants for eye colour having pink, dark red, white, green, chocolate etc. The character is usually controlled by the same gene, which controls the serosa colour of egg.

Method of observation: A sample moths is studied under stereomicroscope in diffused sunlight.

Biochemical characterization

Allozyme / isozyme markers are the most widely used biochemical markers as allelic variations through isozyme studies aids in more accurate estimation of genetic diversity than morphological traits. Electrophoresis identifies alleles at the co-dominant loci that code for the enzymes and heterozygotes can be scored directly. The studies on storage proteins, alpha and beta esterases and alkaline as well as acid phosphates provide insight into the genetical identities among the accessions. The enzymes G6PD, alpha and beta esterases were found to be highly polymorphic and suitable for genetic variability studies. The heat shock profile analysis of various accessions reveals which accessions can be more thermo tolerant. Subsequently studies can also be carried out to identify enzyme resistance of esterase and mid gut alkaline protease against the chemical inhibitor Phenyl methane sulfonyl fluoride (PMSF) among promising silkworm genetic resources and their association for grouping them into higher resistance groups for their selection by breeders in silkworm crop improvement breeding programmes. The breeds thus identified with genetic hardiness could be utilized by breeders for crop improvement. Analysis of digestive amylase enzyme activity will give an indication of the hardy genotypes as generally higher amylase enzyme activity is attributed to hardy breeds and vice versa.

Molecular characterization

Molecular markers are known to have many advantages over morphological and biochemical markers, because these markers are stable and independent of

environmental influences Mulberry silkworm (*Bombyx mori* L.), the most important silk producing insect and exhibits wide diversity in morphological and biometric characters. Characterization of vast genetic resources based on the morphological and quantitative traits is not solely dependable as the phenotypic traits are influenced by environment. Molecular tools can be effectively utilized to analyze phylogenetic relationship and heterozygosity in silkworm. Therefore it is imperative to make molecular characterization of the breeds to analyze genetic diversity. Molecular characterization is very much related to conservation of germplasm as the extended cold preservation of genetic stocks is important for reducing the cost of conservation and genetic erosion and their crop cycles. An innovative technique in this direction will be much helpful for proper conservation of them for longer period with stability of the gene organizations.

Evaluation

Evaluation of silkworm genotypes can be done in pre-cocoon, cocoon and post-cocoon stages by following the descriptors.

Evaluation Descriptors

Evaluation of germplasm resources is the most important aspect in a gene bank, which predisposes the suitability of the germplasm to be utilized for various breeding programmes. Evaluation of germplasm genotypes under gene bank conditions forms a preliminary approach to assess the potential range of quantitative traits of germplasm, whereas evaluation at different location gives us an opportunity to observe the full expressivity of the desired trait(s). Based on the evaluation data, each germplasm can be identified and labeled for the special characters on rearing, grainage and reeling parameters such as effective rate of rearing (ERR), disease tolerance, superior silk quality etc. Breeders look for well-evaluated genetic materials to meet the specific needs of their breeding programmes. Data on preliminary evaluation provides opportunity to short list the right germplasm for further detailed evaluation. The preliminary evaluation is normally directed towards measurement or rating on mainly physiological traits which are easy to evaluate. Based on the preliminary evaluation data, germplasm accessions are selected for further evaluation considering additional descriptors thought to be useful in breeding programme. For evaluation of silkworm

germplasm accessions at CSGRC follows set descriptors incorporating more than 70 traits. These descriptors include core and secondary characters formulated by the committee framed for the maintenance of gene and genotypes of silkworm. Based on the data generated on various descriptors, the silkworm germplasm accessions are classified, grouped and data are documented for better understanding of the germplasm and effective utilization of the same.

Egg Characters

(i) Fecundity

The total number of eggs laid by a female moth is recorded as egg fecundity.

(ii) Hatching (%)

The mean number of larvae hatched out of the total eggs laid by a moth recorded and represented in percentage.

$$\text{Hatching \%} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs laid by single moth}} \times 100$$

(iii) Unfertilised Eggs (%)

The mean number of unfertilized eggs present (which remains as yellow due to no embryogenesis) to the total eggs laid by a moth recorded and represented in percentage.

$$\text{Unfertilised Eggs \%} = \frac{\text{No. of unfertilised eggs}}{\text{Total No. of eggs laid by single moth}} \times 100$$

(iv) Dead Eggs (%)

The mean number of dead eggs (which don't hatch and become black inside the shell) present to that of total eggs laid by a moth recorded and represented in percentage.

$$\text{Dead Eggs \%} = \frac{\text{No. of dead eggs}}{\text{Total No .of eggs laid by single moth}} \times 100$$

Larval Characters

(i) Weight of 10 Grown Larvae

Mean larval weight recorded in grams for 10 randomly selected 5days old V instar larvae (both males and females together).

Vth Instar Larval Duration

The duration of larva from IV moult-out to pre-spinning (cessation of feeding) recorded in hours.

Total Larval Duration

The mean larval span recorded in hours from hatching to pre-spinning stage including moulting duration.

Cocoon Characters

Single Cocoon Weight (g)

The mean single cocoon weight in grams recorded from 25 cocoons each of males and females randomly selected and represented separately for each sex.

$$\text{Single cocoon weight (g)} = \frac{\text{Weight (g) of 25 cocoons (in each sex)}}{25}$$

Single Shell Weight (g)

The mean single shell weight in grams recorded from 25 shells taken from male and female cocoons randomly selected in an accession and represented separately for each sex.

$$\text{Single shell weight (g)} = \frac{\text{Weight (g) of 25 cocoon shells}}{25}$$

Shell Ratio (%)

The mean ratio of shell weight to that of cocoon weight calculated from mean cocoon and shell weight taken together for both male and female sexes and represented in percentage

$$\text{Shell Ratio \%} = \frac{\text{Mean weight (g) of shell}}{\text{Mean weight (g) of cocoon}} \times 100$$

Cocoon Length

The mean length (longitudinal) of cocoon recorded in centimetres arrived from cocoons selected

Cocoon Width

The mean width (transverse length) of cocoon recorded in centimetres arrived from cocoons selected

Melt Cocoons (%)

The mean number of dead pupa containing cocoons, obtained to that of number of larvae retained after III moult in a replication and represented in percentage

$$\text{Melt cocoons \%} = \frac{\text{No. of melt cocoons}}{\text{No. of larvae retained after III moult}} \times 100$$

Double Cocoons (%)

The mean number of double pupae containing cocoons obtained to that of larvae retained after III moult in a replication and represented in percentage.

$$\text{Double cocoons \%} = \frac{\text{No. of double cocoons}}{\text{No. of Larvae retained after III moult}} \times 100$$

Open-end Cocoons (%)

The mean number of open-end cocoons obtained to that of larvae retained after III moult and represented in percentage.

$$\text{Open-end cocoons \%} = \frac{\text{No .of open-end cocoons}}{\text{No. of Larvae retained after III moult}} \times 100$$

Stained Cocoons (%)

The mean number of stained cocoons obtained to that of larvae retained after III moult in and represented in percentage.

$$\text{Stained cocoons \%} = \frac{\text{No. of stained cocoons}}{\text{No. of larvae retained after III moult}} \times 100$$

Floss (%)

The quantity (g) of floss obtained from the cocoons shells samples taken for both sexes (25 in each sex) and represented in percentage

$$\text{Floss \%} = \frac{\text{Floss weight (g) [25 males + 25 females cocoon shell]}}{\text{Weight (g) of 25 males + 25 female cocoons}} \times 100$$

Yield Parameters

(i) Cocoon Yield/10,000 Larvae by Number

The mean number of cocoons harvested to that of larvae retained after III moult in a replication and converted for 10,000 larvae.

$$\text{CocoonYield/10,000larvaeby Number} = \frac{\text{Actual yield by number}}{\text{No. of larvae retained after III moult}} \times 10{,}000$$

(ii) Cocoon Yield/10,000 Larvae by Weight (kg)

The mean weight of cocoons harvested in kilogrammes to that of larvae retained after III moult and converted for 10,000 larvae.

$$\text{CocoonYield/10,000 larvae by weight (kg)} = \frac{\text{Total Cocoon yield by weight}}{\text{No. of larvae retained after III moult}} \times 10{,}000$$

(iii) Pupation Rate (%)

The mean number of live pupae obtained to that of larvae retained after III moult and represented in percentage.

$$\text{Pupation Rate (\%)} = \frac{\text{No. of live pupae}}{\text{No. of larvae retained after III moult}} \times 100$$

(iv) Pupal Weight (g)

The mean weight (g) of single pupa computed by subtracting the shell weight from cocoon weight for 25 males and females selected randomly per accession

$$\text{Pupal weight (g)} = \frac{\text{Weight of 25 Pupae males + 25 female pupae}}{50}$$

Post-Cocoon Parameters

(i) Filament Length (m)

Silk filament length indicates the reelable length of silk filament from a cocoon. It is the average length of the silk reeled from the total number of cocoons.

$$\text{Avg. filament length} = \frac{\text{Length of raw silk reeled (m) x No. of cocoons maintained /end}}{\text{No. of reeling cocoons}}$$

(ii) Non-broken Filament Length (NBFL)

It is the length at which cocoon replacement or break occurs in that interval.

$$\text{NBFL} = \frac{\text{Length of the silk reeled x No. of cocoons maintained / end}}{\text{No. of feeding end}}$$

(ii) Denier (d.)

The size of the cocoon filament expressed as weight in grams of 9000 meters length of silk filament.

$$\text{Denier (d.)} = \frac{\text{Wt.(g) of raw silk reeled x 9000 (m) single cocoon filament}}{\text{Length (m) of silk reeled x No. of cocoons maintained/end}}$$

(iii) Reelability (%)

It is the ease with which a cocoon yields the cocoon filament. It is ratio of non-broken filament x filament length expressed in %.

$$\text{Reelability \%} = \frac{\text{No. of reeling cocoons}}{\text{No. of feeding ends}} \times 100$$

(v) Renditta

It is the quantity of cocoons (Kg) required for producing one kilogram of raw silk.This character is inversely proportional to silk percentage. This was estimated by using the formula.

$$\text{Renditta} = \frac{\text{Quantity of fresh cocoons used in kg.}}{\text{Quantity of raw silk obtained in kg.}}$$

(v) Raw Silk Recovery (%)

It is the ratio of the raw silk present to the shell ratio % or it is quantity of raw silk recovered from the cocoon shell.

$$\text{Raw silk recovery \%} = \frac{\text{Raw silk \%}}{\text{Shell ratio \%}} \times 100$$

(vii) Raw Silk (%)

Quantity of raw silk reeled in relation to the quantity of fresh cocoons utilized, expressed in percentage.

$$\text{Raw silk (\%)} = \frac{\text{Conditioned wt. of raw silk reeled (Kg.)}}{\text{Wt. of fresh cocoons taken for reeling (Kg.)}} \times 100$$

(viii) Neatness (%)

Imperfection in raw silk yarn, which are smaller than those described, as minor cleanness defects are known as neatness defects. Nibs are small-thickened places or spots in the yarn less than 2 mm in length. Loops are small open places in the yarn caused by the excessive length of one or more cocoon filaments, less than 10 mm in length when measured along the filament. Hairiness and fuzziness are the conditions of yarn, which show small loose ends of less than 10 mm and fine particles of cocoon filaments protruding from the yarn. Small knots are knots, which have loose ends, less than 3 mm in length. Fine corkscrews are places in which one or more cocoon filaments are longer than the remainder and give the appearance of a spiral.

Apparatus and equipment: The Standard Photographs for neatness defects, Seriplane and lighting equipment.

(ix) Cleanness (%)

The object of this test is to determine kind and no. of cleanness defects of raw silk. Defects are divided into 3 groups, Super-major defects, Major defects and minor defects such as long knots, long loops, corkscrews, bad castings, waste, slugs, etc.

Apparatus and equipment: Standard Photograph for cleanness, Seri plane and lighting equipment.

(x) Degumming loss (%)

The purpose of the degumming loss on raw silk is to determine the percentage of sericin and water-soluble substance which the silk contains.The loss of silk weight due to removal of sericin after degumming process, which is expressed in percentage.

$$\text{Degumming loss \%} = \frac{\text{Initial* wt. of sample} - \text{Final** wt. of sample}}{\text{Final** wt. of sample}} \times 100$$

* Weight before degumming, ** Weight after degumming

xi) Evenness variation – I (Stripes)

Evenness variation is defined as the defective portion of raw silk threads on an inspection board, which appear as stripes caused by variation in the size of raw silk, is to determine the degree and frequency of size variation occurred in the raw silk thread and is noticeable by visual inspection. There are 3 types of evenness variations viz., I, II and III. The degree of variation (Intensity of variation) is compared with the standard board consisting of standard variation photographs, viz., Vo, V1 and V2.

Apparatus and equipment: Seri plane (127 x 457 mm), Standard Photographs and Illumination room.

(xii) Tenacity (g/d.)

Tenacity (load-bearing capacity) is strength of raw silk expressed as grams per denier to test the strength of the raw silk, the breaking point (g per denier) and the

degree of elongation (percentage) is carried out on the Serigraph. This test is conducted in a room maintained at a standard temperature of 25±2 ºC and humidity of 65%.

Apparatus: Serigraph, sizing reel and scale.

(xiii) Elongation (%)

Elongation represents strechability of raw silk when pulled to the breaking point, which is expressed as percentage to test the degree of elongation (percentage) is carried out on the Serigraph. This test is conducted in a room, which is maintained at a standard temperature of 25±2 ºC and a humidity of 65 %.

Apparatus: Serigraph, sizing reel and scale.

(xiv) Cohesion (Strokes)

By means of the Duplan cohesion tester, the number of frictions required to split silk thread for the purpose of examining the state of cocoon filaments sticking together, can be counted. This test is conducted in a room kept at standard temperature and humidity.

Apparatus: Duplan cohesion tester.

The objective of this test is to determine the degree of agglutination of cocoon filament forming the thread. The instrument used to test the cohesion is Duplon cohesion tester. The number of strokes required to open the individual filament are recorded, lesser the strokes poorer the cohesive property and vice-versa. Cohesive property is important it directly impairs the fabric quality if the yarn is not having sufficient cohesiveness.

Conservation

Conservation of wild silkworm stocks through *in-situ* means is a difficult task since various agencies like Forest Department, Sericulture Department and other related departments of the concerned states are stakeholders. However, during the survey and explorations as a National level advisory unit, CSGRC will make the local people and concerned State Department authorities thoroughly aware of the importance of the wild seri-genetic silkworm species as natural resources of our country, the necessity of *in-situ* conservation to ensure loss of precious seri-biodiversity resources as well as the

modalities of *in-situ* conservation for their benefit. In view of this difficulty, *ex-situ* / *in-vitro* conservation has to be adopted for which the protocols established for conservation of domesticated silkworm stocks including consigning the eggs under short-term or long-term preservation schedules have to be modified and standardized. Such standardized protocols will also be useful for maintenance of the collected wild silkworm stocks through conservation rearing to avoid loss of genetic resources.

Conservation of domesticated sericultural germplasm resources requires a separate rearing package to maintain silkworm accessions true to their passport data. Rearing package comprises of composting of about 1000 eggs / accession for rearing, retaining 500-600 larvae after III moult and rearing in two replications till spinning. During rearing, morphological characterization of egg, larvae and cocoon should be conducted as per standard descriptors. After spinning is completed, cocoons should be processed for egg production by ensuring disease freeness at moth stage by conducting quarantine rearing. After the preparation of the silkworm eggs, depending on voltinism, disease free eggs should be preserved in cold storage under different hibernation schedules.

Multivoltine silkworm genetic resources

The multivoltine accessions at CSGRC are reared five times in a year and the eggs are preserved in the cold storage for 35 days at 5°C. Another set of all the MV accessions are preserved at 5°C for extended period of 46 days also. This will serve as the safety back up. If there is any loss of accessions due to diseases or in any unforeseen circumstances accessions preserved under 45 days will be released and once again the backup rearing will be conducted and disease free layings will be conserved. One rearing cycle of multivoltine accessions takes 75 - 80 days including egg preservation.

Bivoltine silkworm genetic resources

The bivoltine accessions are divided into three batches with nearly 120 accessions in each batch and grouped as I, II & III batch respectively and the rearing of these three batches are conducted in July-Aug, Sept-Oct, Jan-Feb. respectively, and the eggs are preserved under 10 months schedule in the cold storage. One rearing cycle of bivoltine accessions takes 360 - 370 days

Preservation schedules followed for conservation of bivoltine silkworm genetic resources (being a diapausing stock) is 10 months for regular rearing with 12 months as back up. The dfls prepared are being preserved in two cold storages one set at CSGRC, cold storage Hosur and another at NSSO-Cold storage, Mysore to come over the unforeseen situations arising due to disease incidence while rearing and cold storage break down resulting in loss of accessions. The studies are on to prolong the cold preservation beyond 12 months as it would result in avoiding loss of genetic resources, genetic erosion arising out of repeated rearings and huge savings in the cost of conservation in terms of men and materials.

Replenishment of silkworm accessions

The germplasm accessions are brushed every time after preparing two composite samples at of about 1000-1500 eggs consisting a piece of about 50 eggs from all the 20 Dfls preserved pin head stage mainly to prevent inbreeding depression due to continuous rearing for many generations. These composite Dfls are reared as cellular beds and after third moult 250 worms per accession are maintained in two replications till spinning. Though this procedure is being adopted in regular conservation program periodical (once in 5-10 years) replenishment of silkworm accessions from breeders stock/donors are also in practice to avoid inbreeding depression. Apart from this CSRTI, Pampore, Berhampore and Mysore are maintaining some of working silkworm germplasm which can serve as safety backup.

Cryopreservation

The sericigenous insects are becoming extinct very rapidly due to deforestation, urbanization and Industrialization and there is an urgent need to preserve these genetic pool for future use. Insects rely on a variety of ecological and physiological adaptations to survive low temperatures, making cryopreservation technique significantly complex. Mulberry silkworm (*Bombyx mori* L.) eggs are cleidoic with (approximately 20-25µm thick) chorion. Preservation of non-diapause eggs to a limited period is practiced usually for delayed hatching. The advantage of early embryonic periods having resistance to low temperature is utilized for chilling of eggs and preservation for longer periods. However, technique for cryopreservation of silkworm eggs is being developed and identification of precise embryonic stage and chill sensitivity is necessary for

effective silkworm cryopreservation. Studies on the chill-sensitivity and tolerance of non-diapause silkworm embryos of mulberry silkworm at various embryonic stages are reported. Silkworm embryo of 48h-age are relatively chill-sensitive as compared to other embryonic ages. This is vital information for the development and standardization of long term cryopreservation protocol for silkworm eggs.

Multilocational trials

Evaluation of silkworm germplasm in different agro climatic regions is imperative to find out the true genetic potential of the elite bivoltine silkworm germplasm identified and adaptability in the different locations to classify parental lines region and season specific breeds.

In the multilocational trail studies, based on the performance of bivoltine germplasm resources were evaluated and identified as better performers having wider adaptability in different agro-climatic zones and different seasons for utilizing them as zone-wise and season-wise breeding materials.

All India Mulberry and Silkworm Germplasm Evaluation Programme (AIMSGEP)

Overall analysis of AIMSGEP data based on rearing and post-cocoon parameters showed that BBE-222 & BBE-183 performed better in temperate regions, while BBE-197 for sub-tropical regions and BBE-183 & BBE-187 for tropical regions. Accession BBE-183 showed wider adaptability for both temperate and tropical regions. Hence, BBE-183, BBE-187, BBE-197 and BBE-222 is recommended as the best performing bivoltine silkworm germplasm having wider adaptability to different climatic conditions of India with higher potential for rearing and post-cocoon parameters. Accessions BBE-0266 and BBE-0178 have performed better than both the local ruling breeds SH-6 (Sahaspur) Jam-25 (Jammu) and national control CSR-2 for rearing and reeling parameters at high temperature and high humidity prevailing during the autumn can be exploited by breeders to evolve hardy races for autumn rearing. The outcome of the collaborative project revealed high temperature / high humidity and high temperature and low humidity conditions identified accessions are BMI-045, BMI-025, BMI-027, BMI-060 for Ananthapur, Chamarajanagar and Salem zones and BMI-040,

BMI-025, BMI-027 and BMI-016 for abiotic and BMI-027 for biotic stress for Jorhat zone.

AISGEP-II study reveal accessions BBI-0348, BBE-0329 BBE-0266, BBE-216 and BBI-0348 are better performers identified having wider adaptability in different ago climatic conditions and different seasons. These accessions can be included in future breeding programs as genetic resource materials for further studies in these regions.

Silkworm Genetic Resources maintained / conserved at CSGRC along with National Accession Numbers

#	Acc. No.	National Accession No.	Race Name
1	BBE-0001	NBAII-CSG-BOM-0000001	ALPS JAUNNE
2	BBE-0002	NBAII-CSG-BOM-0000002	ALPS YELLOW
3	BBE-0003	NBAII-CSG-BOM-0000003	CEVENESE YELLOW
4	BBE-0004	NBAII-CSG-BOM-0000004	ASCOLI YELLOW
5	BBE-0005	NBAII-CSG-BOM-0000005	MEIGITSU
6	BBE-0006	NBAII-CSG-BOM-0000006	B-36
7	BBE-0007	NBAII-CSG-BOM-0000007	B-37
8	BBE-0008	NBAII-CSG-BOM-0000008	B-39
9	BBE-0009	NBAII-CSG-BOM-0000009	B-40
10	BBE-0010	NBAII-CSG-BOM-0000010	J-112
11	BBE-0011	NBAII-CSG-BOM-0000011	J-122
12	BBE-0012	NBAII-CSG-BOM-0000012	YAKWEI
13	BBE-0013	NBAII-CSG-BOM-0000013	CHAUNG NAUNG
14	BBE-0014	NBAII-CSG-BOM-0000014	C-122
15	BBE-0015	NBAII-CSG-BOM-0000015	C-108
16	BBE-0016	NBAII-CSG-BOM-0000016	C-110
17	BBE-0017	NBAII-CSG-BOM-0000017	CHUKWEI
18	BBE-0018	NBAII-CSG-BOM-0000018	CHINESE FARMER
19	BBE-0019	NBAII-CSG-BOM-0000019	CHINESE GOLDEN-70
20	BBE-0020	NBAII-CSG-BOM-0000020	CHINESE GOLDEN-80
21	BBE-0021	NBAII-CSG-BOM-0000021	CHINESE GOLDEN-90
22	BBE-0022	NBAII-CSG-BOM-0000022	HAULAK
23	BBE-0023	NBAII-CSG-BOM-0000023	KING HAUNG

#	Acc. No.	National Accession No.	Race Name
24	BBE-0024	NBAII-CSG-BOM-0000024	HAUCHI
25	BBE-0025	NBAII-CSG-BOM-0000025	NAN NAUNG 6A
26	BBE-0026	NBAII-CSG-BOM-0000026	NAN NAUNG 6D
27	BBE-0027	NBAII-CSG-BOM-0000027	CHINESE YELLOW
28	BBE-0028	NBAII-CSG-BOM-0000028	AZAD
29	BBE-0029	NBAII-CSG-BOM-0000029	AZERBAIJAN
30	BBE-0030	NBAII-CSG-BOM-0000030	SANISH E1(P)
31	BBE-0031	NBAII-CSG-BOM-0000031	SANISH-E1(M)
32	BBE-0032	NBAII-CSG-BOM-0000032	SANISH E2(M)
33	BBE-0033	NBAII-CSG-BOM-0000033	SANISH-8
34	BBE-0034	NBAII-CSG-BOM-0000034	SANISH-17
35	BBE-0035	NBAII-CSG-BOM-0000035	SANISH-18(M)
36	BBE-0036	NBAII-CSG-BOM-0000036	SANISH-18(P)
37	BBE-0037	NBAII-CSG-BOM-0000037	SANISH-30
38	BBE-0038	NBAII-CSG-BOM-0000038	SANISH-21
39	BBE-0039	NBAII-CSG-BOM-0000039	SHEIKHI-I
40	BBE-0040	NBAII-CSG-BOM-0000040	SHEIKHI-II
41	BBE-0041	NBAII-CSG-BOM-0000041	TASHKAHASHI-112
42	BBE-0042	NBAII-CSG-BOM-0000042	GYANDZA
43	BBE-0043	NBAII-CSG-BOM-0000043	BELKOKONA-II
44	BBI-0044	NBAII-CSG-BOM-0000044	NB4D2
45	BBI-0045	NBAII-CSG-BOM-0000045	SH-6
46	BBI-0046	NBAII-CSG-BOM-0000046	YS-3
47	BBI-0047	NBAII-CSG-BOM-0000047	SF-19
48	BBI-0048	NBAII-CSG-BOM-0000048	JD6
49	BBE-0049	NBAII-CSG-BOM-0000049	UKR-1
50	BBE-0050	NBAII-CSG-BOM-0000050	UKR-2
51	BBE-0051	NBAII-CSG-BOM-0000051	MEREFA-6
52	BBI-0052	NBAII-CSG-BOM-0000052	JAM-1
53	BBI-0053	NBAII-CSG-BOM-0000053	JAM-2
54	BBI-0054	NBAII-CSG-BOM-0000054	JAM-11
55	BBI-0055	NBAII-CSG-BOM-0000055	JAM-121

#	Acc. No.	National Accession No.	Race Name
56	BBI-0056	NBAII-CSG-BOM-0000056	JAM-21
57	BBI-0057	NBAII-CSG-BOM-0000057	JAM-23
58	BBI-0058	NBAII-CSG-BOM-0000058	JAM-27
59	BBI-0059	NBAII-CSG-BOM-0000059	JAM-103
60	BBI-0060	NBAII-CSG-BOM-0000060	JAM-110
61	BBI-0061	NBAII-CSG-BOM-0000061	JAM-118
62	BBI-0062	NBAII-CSG-BOM-0000062	JAM-119
63	BBI-0063	NBAII-CSG-BOM-0000063	JAM-122
64	BBI-0064	NBAII-CSG-BOM-0000064	JAM-124
65	BBI-0065	NBAII-CSG-BOM-0000065	JAM-125
66	BBI-0066	NBAII-CSG-BOM-0000066	PAM-101
67	BBI-0067	NBAII-CSG-BOM-0000067	PAM-102
68	BBI-0068	NBAII-CSG-BOM-0000068	PAM-103
69	BBI-0069	NBAII-CSG-BOM-0000069	PAM-104
70	BBI-0070	NBAII-CSG-BOM-0000070	PAM-105
71	BBI-0071	NBAII-CSG-BOM-0000071	PAM-106
72	BBI-0072	NBAII-CSG-BOM-0000072	PAM-107
73	BBI-0073	NBAII-CSG-BOM-0000073	PAM-108
74	BBI-0074	NBAII-CSG-BOM-0000074	PAM-109
75	BBI-0075	NBAII-CSG-BOM-0000075	PAM-110
76	BBI-0076	NBAII-CSG-BOM-0000076	PAM-111
77	BBI-0077	NBAII-CSG-BOM-0000077	PAM-112
78	BBI-0078	NBAII-CSG-BOM-0000078	PAM-113
79	BBI-0079	NBAII-CSG-BOM-0000079	P5
80	BBI-0080	NBAII-CSG-BOM-0000080	BL-1
81	BBI-0081	NBAII-CSG-BOM-0000081	NB-18
82	BBI-0082	NBAII-CSG-BOM-0000082	NB-7
83	BBI-0083	NBAII-CSG-BOM-0000083	CC-1
84	BBI-0084	NBAII-CSG-BOM-0000084	CA-2
85	BBI-0085	NBAII-CSG-BOM-0000085	KS (Oval)
86	BBI-0086	NBAII-CSG-BOM-0000086	KPG-A
87	BBI-0087	NBAII-CSG-BOM-0000087	KPG-B

#	Acc. No.	National Accession No.	Race Name
88	BBI-0088	NBAII-CSG-BOM-0000088	KPG-2
89	BBI-0089	NBAII-CSG-BOM-0000089	KPG-6
90	BBI-0090	NBAII-CSG-BOM-0000090	KPG-7
91	BBI-0091	NBAII-CSG-BOM-0000091	KPG-11
92	BBI-0092	NBAII-CSG-BOM-0000092	B.P (CHOCOLATE)
93	BBI-0093	NBAII-CSG-BOM-0000093	B.P (BLACK)
94	BBE-0094	NBAII-CSG-BOM-0000094	ZEBRA (SL)
95	BBI-0095	NBAII-CSG-BOM-0000095	KALIMPONG-A
96	BBI-0096	NBAII-CSG-BOM-0000096	HS6(SL)
97	BBI-0097	NBAII-CSG-BOM-0000097	HOSA MYSORE
98	BBI-0098	NBAII-CSG-BOM-0000098	S-36
99	BBI-0099	NBAII-CSG-BOM-0000099	R.P.-I
100	BBI-0100	NBAII-CSG-BOM-0000100	R.P-II
101	BBI-0101	NBAII-CSG-BOM-0000101	M-III
102	BBI-0102	NBAII-CSG-BOM-0000102	M-5
103	BBI-0103	NBAII-CSG-BOM-0000103	M-10
104	BBI-0104	NBAII-CSG-BOM-0000104	M-42
105	BBI-0105	NBAII-CSG-BOM-0000105	M-43
106	BBI-0106	NBAII-CSG-BOM-0000106	M-45
107	BBI-0107	NBAII-CSG-BOM-0000107	M-46
108	BBI-0108	NBAII-CSG-BOM-0000108	MJ-20
109	BBI-0109	NBAII-CSG-BOM-0000109	MJ-23
110	BBI-0110	NBAII-CSG-BOM-0000110	MJ-107
111	BBI-0111	NBAII-CSG-BOM-0000111	JAM-10
112	BBI-0112	NBAII-CSG-BOM-0000112	JAM-18 (P)
113	BBI-0113	NBAII-CSG-BOM-0000113	JAM-18 (M)
114	BBI-0114	NBAII-CSG-BOM-0000114	JAM-22 (P)
115	BBI-0115	NBAII-CSG-BOM-0000115	JAM-24
116	BBI-0116	NBAII-CSG-BOM-0000116	JAM-25
117	BBI-0117	NBAII-CSG-BOM-0000117	SF-17
118	BBI-0118	NBAII-CSG-BOM-0000118	PLF
119	BBI-0119	NBAII-CSG-BOM-0000119	SS

#	Acc. No.	National Accession No.	Race Name
120	BBI-0120	NBAII-CSG-BOM-0000120	SS-4A
121	BBI-0121	NBAII-CSG-BOM-0000121	SS-15A
122	BBI-0122	NBAII-CSG-BOM-0000122	SS-17
123	BBI-0123	NBAII-CSG-BOM-0000123	NB2-A (P)
124	BBI-0124	NBAII-CSG-BOM-0000124	NB2-A (M)
125	BBI-0125	NBAII-CSG-BOM-0000125	NB2D1 (P)
126	BBI-0126	NBAII-CSG-BOM-0000126	NB2D1 (M)
127	BBI-0127	NBAII-CSG-BOM-0000127	NB3C1 (M)
128	BBI-0128	NBAII-CSG-BOM-0000128	NB3D1 (P)
129	BBI-0129	NBAII-CSG-BOM-0000129	NB3D1 (M)
130	BBI-0130	NBAII-CSG-BOM-0000130	KPG-3
131	BBI-0131	NBAII-CSG-BOM-0000131	KPG-4
132	BBI-0132	NBAII-CSG-BOM-0000132	KPG-5
133	BBI-0133	NBAII-CSG-BOM-0000133	AT-4
134	BBI-0134	NBAII-CSG-BOM-0000134	AT-9
135	BBI-0135	NBAII-CSG-BOM-0000135	IB-2
136	BBI-0136	NBAII-CSG-BOM-0000136	IB-3
137	BBI-0137	NBAII-CSG-BOM-0000137	IB-9
138	BBI-0138	NBAII-CSG-BOM-0000138	IB-11
139	BBI-0139	NBAII-CSG-BOM-0000139	TA-3
140	BBI-0140	NBAII-CSG-BOM-0000140	BOROPOLU
141	BBI-0141	NBAII-CSG-BOM-0000141	BORPAT
142	BBE-0142	NBAII-CSG-BOM-0000142	C.NICHI(SL)
143	BBE-0143	NBAII-CSG-BOM-0000143	KY-1
144	BBE-0144	NBAII-CSG-BOM-0000144	KY-2
145	BBE-0145	NBAII-CSG-BOM-0000145	SHINREI SHENGETSU
146	BBE-0146	NBAII-CSG-BOM-0000146	JP1-A (P)
147	BBE-0147	NBAII-CSG-BOM-0000147	JP1-A (M)
148	BBE-0148	NBAII-CSG-BOM-0000148	JP1-B
149	BBE-0149	NBAII-CSG-BOM-0000149	SM-1
150	BBE-0150	NBAII-CSG-BOM-0000150	SM-2
151	BBE-0151	NBAII-CSG-BOM-0000151	SM-3

#	Acc. No.	National Accession No.	Race Name
152	BBE-0152	NBAII-CSG-BOM-0000152	CP1-B
153	BBE-0153	NBAII-CSG-BOM-0000153	J-PLAIN
154	BBE-0154	NBAII-CSG-BOM-0000154	J-MARKED
155	BBE-0155	NBAII-CSG-BOM-0000155	J-DEEP MARKED
156	BBE-0156	NBAII-CSG-BOM-0000156	KN-2
157	BBE-0157	NBAII-CSG-BOM-0000157	GUNKOMANRIE
158	BBE-0158	NBAII-CSG-BOM-0000158	THAICHOAN HOKOSHINYAKO
159	BBE-0159	NBAII-CSG-BOM-0000159	N124 C124
160	BBE-0160	NBAII-CSG-BOM-0000160	(N112.C110)(N124.C124)
161	BBE-0161	NBAII-CSG-BOM-0000161	SHOKOGINREI
162	BBE-0162	NBAII-CSG-BOM-0000162	FUJISAKURA
163	BBE-0163	NBAII-CSG-BOM-0000163	THAICHOAN
164	BBE-0164	NBAII-CSG-BOM-0000164	SHONGETSU HOSHO
165	BBE-0165	NBAII-CSG-BOM-0000165	TETRA HYBRID (M)
166	BBE-0166	NBAII-CSG-BOM-0000166	TETRA HYBRID (P)
167	BBE-0167	NBAII-CSG-BOM-0000167	KYORIESHIMPAKU (P)
168	BBE-0168	NBAII-CSG-BOM-0000168	KYORIESHIMPAKU (M)
169	BBE-0169	NBAII-CSG-BOM-0000169	SHINKI REYAKU (M)
170	BBE-0170	NBAII-CSG-BOM-0000170	Merefa-7
171	BBE-0171	NBAII-CSG-BOM-0000171	A25
172	BBI-0172	NBAII-CSG-BOM-0000172	Boropolu (Jammu)
173	BBE-0173	NBAII-CSG-BOM-0000173	CC (SL)
174	BBE-0174	NBAII-CSG-BOM-0000174	Feng shong
175	BBE-0175	NBAII-CSG-BOM-0000175	Hong zhou (G)
176	BBE-0176	NBAII-CSG-BOM-0000176	Hong zhou (R)
177	BBE-0177	NBAII-CSG-BOM-0000177	JPN5 x B25
178	BBE-0178	NBAII-CSG-BOM-0000178	JPN5 x NK25
179	BBE-0179	NBAII-CSG-BOM-0000179	JPN6 x A26
180	BBE-0180	NBAII-CSG-BOM-0000180	JPN6 x B25
181	BBE-0181	NBAII-CSG-BOM-0000181	JPN12D
182	BBE-0182	NBAII-CSG-BOM-0000182	CSGRC-12
183	BBE-0183	NBAII-CSG-BOM-0000183	CSGRC-1

#	Acc. No.	National Accession No.	Race Name
184	BBE-0184	NBAII-CSG-BOM-0000184	CSGRC-2
185	BBE-0185	NBAII-CSG-BOM-0000185	CSGRC-3
186	BBE-0186	NBAII-CSG-BOM-0000186	CSGRC-4
187	BBE-0187	NBAII-CSG-BOM-0000187	CSGRC-5
188	BBE-0188	NBAII-CSG-BOM-0000188	CSGRC-6
189	BBE-0189	NBAII-CSG-BOM-0000189	Zebra yellow
190	BBE-0190	NBAII-CSG-BOM-0000190	36 PC
191	BBE-0191	NBAII-CSG-BOM-0000191	39 P
192	BBE-0192	NBAII-CSG-BOM-0000192	44 B M
193	BBE-0193	NBAII-CSG-BOM-0000193	44 F (M)
194	BBE-0194	NBAII-CSG-BOM-0000194	644
195	BBE-0195	NBAII-CSG-BOM-0000195	6P
196	BBE-0196	NBAII-CSG-BOM-0000196	7042
197	BBE-0197	NBAII-CSG-BOM-0000197	A
198	BBE-0198	NBAII-CSG-BOM-0000198	AC (SL)
199	BBE-0199	NBAII-CSG-BOM-0000199	Auz-4
200	BBE-0200	NBAII-CSG-BOM-0000200	Auz-5
201	BBE-0201	NBAII-CSG-BOM-0000201	C124
202	BBE-0202	NBAII-CSG-BOM-0000202	C124 (SL)
203	BBI-0203	NBAII-CSG-BOM-0000203	CC1 (SL)
204	BBI-0204	NBAII-CSG-BOM-0000204	CCS
205	BBI-0205	NBAII-CSG-BOM-0000205	CDC2
206	BBE-0206	NBAII-CSG-BOM-0000206	CN X C140
207	BBI-0207	NBAII-CSG-BOM-0000207	CPP1
208	BBI-0208	NBAII-CSG-BOM-0000208	DD3
209	BBE-0209	NBAII-CSG-BOM-0000209	Daizo
210	BBE-0210	NBAII-CSG-BOM-0000210	Dong 306
211	BBE-0211	NBAII-CSG-BOM-0000211	European P
212	BBE-0212	NBAII-CSG-BOM-0000212	European M
213	BBE-0213	NBAII-CSG-BOM-0000213	FCC2(P)
214	BBE-0214	NBAII-CSG-BOM-0000214	FU247
215	BBI-0215	NBAII-CSG-BOM-0000215	G

#	Acc. No.	National Accession No.	Race Name
216	BBE-0216	NBAII-CSG-BOM-0000216	HO (SL)
217	BBE-0217	NBAII-CSG-BOM-0000217	HU 204
218	BBE-0218	NBAII-CSG-BOM-0000218	HUA1 X HUA2
219	BBE-0219	NBAII-CSG-BOM-0000219	I-1
220	BBE-0220	NBAII-CSG-BOM-0000220	I-15
221	BBE-0221	NBAII-CSG-BOM-0000221	JB1 (M)
222	BBE-0222	NBAII-CSG-BOM-0000222	JC2M
223	BBE-0223	NBAII-CSG-BOM-0000223	JC2P
224	BBE-0224	NBAII-CSG-BOM-0000224	JZH (MO)
225	BBE-0225	NBAII-CSG-BOM-0000225	JZH (PO)
226	BBE-0226	NBAII-CSG-BOM-0000226	M2
227	BBE-0227	NBAII-CSG-BOM-0000227	M56
228	BBE-0228	NBAII-CSG-BOM-0000228	M78
229	BBE-0229	NBAII-CSG-BOM-0000229	M910
230	BBE-0230	NBAII-CSG-BOM-0000230	N140 (SL)
231	BBE-0231	NBAII-CSG-BOM-0000231	N4
232	BBE-0232	NBAII-CSG-BOM-0000232	NB1
233	BBE-0233	NBAII-CSG-BOM-0000233	NB2
234	BBI-0234	NBAII-CSG-BOM-0000234	NB2A
235	BBI-0235	NBAII-CSG-BOM-0000235	NB2C2
236	BBE-0236	NBAII-CSG-BOM-0000236	NB3
237	BBI-0237	NBAII-CSG-BOM-0000237	NB4D2 (SL)
238	BBE-0238	NBAII-CSG-BOM-0000238	NBH (PO)
239	BBI-0239	NBAII-CSG-BOM-0000239	NCD
240	BBE-0240	NBAII-CSG-BOM-0000240	NJ1 (SL)
241	BBE-0241	NBAII-CSG-BOM-0000241	NJ3
242	BBE-0242	NBAII-CSG-BOM-0000242	NN6D
243	BBI-0243	NBAII-CSG-BOM-0000243	PBL1
244	BBE-0244	NBAII-CSG-BOM-0000244	SC1 (SL)
245	BBE-0245	NBAII-CSG-BOM-0000245	SH2
246	BBE-0246	NBAII-CSG-BOM-0000246	SPC2
247	BBE-0247	NBAII-CSG-BOM-0000247	SPJ2

#	Acc. No.	National Accession No.	Race Name
248	BBI-0248	NBAII-CSG-BOM-0000248	SS (OP)
249	BBI-0249	NBAII-CSG-BOM-0000249	TC1
250	BBE-0250	NBAII-CSG-BOM-0000250	Ud
251	BBE-0251	NBAII-CSG-BOM-0000251	Var-3
252	BBE-0252	NBAII-CSG-BOM-0000252	Wr
253	BBI-0253	NBAII-CSG-BOM-0000253	BL1
254	BBI-0254	NBAII-CSG-BOM-0000254	CC1
255	BBI-0255	NBAII-CSG-BOM-0000255	CP1B
256	BBI-0256	NBAII-CSG-BOM-0000256	KPGA
257	BBI-0257	NBAII-CSG-BOM-0000257	KPGB
258	BBI-0258	NBAII-CSG-BOM-0000258	P5
259	BBI-0259	NBAII-CSG-BOM-0000259	PLF
260	BBE-0260	NBAII-CSG-BOM-0000260	Thai-I
261	BBE-0261	NBAII-CSG-BOM-0000261	Thai-II
262	BBE-0262	NBAII-CSG-BOM-0000262	Anzali
263	BBE-0263	NBAII-CSG-BOM-0000263	101-D
264	BBE-0264	NBAII-CSG-BOM-0000264	31-D
265	BBE-0265	NBAII-CSG-BOM-0000265	CJ3P
266	BBE-0266	NBAII-CSG-BOM-0000266	J2P
267	BBE-0267	NBAII-CSG-BOM-0000267	14M
268	BBE-0268	NBAII-CSG-BOM-0000268	J1M
269	BBE-0269	NBAII-CSG-BOM-0000269	JZHMC
270	BBE-0270	NBAII-CSG-BOM-0000270	J2M
271	BBI-0271	NBAII-CSG-BOM-0000271	JA1
272	BBE-0272	NBAII-CSG-BOM-0000272	G146
273	BBI-0273	NBAII-CSG-BOM-0000273	NB1
274	BBI-0274	NBAII-CSG-BOM-0000274	1090A
275	BBI-0275	NBAII-CSG-BOM-0000275	690
276	BBI-0276	NBAII-CSG-BOM-0000276	990
277	BBI-0277	NBAII-CSG-BOM-0000277	890
278	BBI-0278	NBAII-CSG-BOM-0000278	KS-1
279	BBI-0279	NBAII-CSG-BOM-0000279	NMKC

#	Acc. No.	National Accession No.	Race Name
280	BBE-0280	NBAII-CSG-BOM-0000280	MIR-5
281	BBI-0281	NBAII-CSG-BOM-0000281	CBVC
282	BBI-0282	NBAII-CSG-BOM-0000282	DR. KANU
283	BBI-0283	NBAII-CSG-BOM-0000283	KS-4
284	BBI-0284	NBAII-CSG-BOM-0000284	NJ-1
285	BBI-0285	NBAII-CSG-BOM-0000285	SN-1
286	BBI-0286	NBAII-CSG-BOM-0000286	SPC-1
287	BBI-0287	NBAII-CSG-BOM-0000287	JB-2
288	BBE-0288	NBAII-CSG-BOM-0000288	EUROPEAN
289	BBI-0289	NBAII-CSG-BOM-0000289	SPJ-1
290	BBI-0290	NBAII-CSG-BOM-0000290	CSR-2
291	BBI-0291	NBAII-CSG-BOM-0000291	CSR-4
292	BBI-0292	NBAII-CSG-BOM-0000292	CSR-5
293	BBI-0293	NBAII-CSG-BOM-0000293	CSR-18
294	BBI-0294	NBAII-CSG-BOM-0000294	CSR-19
295	BBI-0295	NBAII-CSG-BOM-0000295	PY-5
296	BBI-0296	NBAII-CSG-BOM-0000296	SY-6
297	BBI-0297	NBAII-CSG-BOM-0000297	JJ-5
298	BBI-0298	NBAII-CSG-BOM-0000298	PY-1
299	BBI-0299	NBAII-CSG-BOM-0000299	NS-6
300	BBI-0300	NBAII-CSG-BOM-0000300	YS-5
301	BBI-0301	NBAII-CSG-BOM-0000301	YS-7
302	BBI-0302	NBAII-CSG-BOM-0000302	AF-2
303	BBI-0303	NBAII-CSG-BOM-0000303	KSO-1
304	BBI-0304	NBAII-CSG-BOM-0000304	SP-2
305	BBI-0305	NBAII-CSG-BOM-0000305	JAM-127
306	BBE-0306	NBAII-CSG-BOM-0000306	TMS-12
307	BBE-0307	NBAII-CSG-BOM-0000307	TMS-14
308	BBE-0308	NBAII-CSG-BOM-0000308	TMS-32
309	BBE-0309	NBAII-CSG-BOM-0000309	TMS-33
310	BBE-0310	NBAII-CSG-BOM-0000310	TMS-35
311	BBE-0311	NBAII-CSG-BOM-0000311	TMS-38

#	Acc. No.	National Accession No.	Race Name
312	BBE-0312	NBAII-CSG-BOM-0000312	TMS-61
313	BBE-0313	NBAII-CSG-BOM-0000313	TMS-62
314	BBE-0314	NBAII-CSG-BOM-0000314	TMS-64
315	BBE-0315	NBAII-CSG-BOM-0000315	TMS-65
316	BBE-0316	NBAII-CSG-BOM-0000316	TMS-66
317	BBE-0317	NBAII-CSG-BOM-0000317	TMS-67
318	BBE-0318	NBAII-CSG-BOM-0000318	TMS-75
319	BBE-0319	NBAII-CSG-BOM-0000319	TMS-82
320	BBE-0320	NBAII-CSG-BOM-0000320	TMS-2
321	BBE-0321	NBAII-CSG-BOM-0000321	TMS-17
322	BBE-0322	NBAII-CSG-BOM-0000322	TMS-31
323	BBE-0323	NBAII-CSG-BOM-0000323	TMS-69
324	BBI-0324	NBAII-CSG-BOM-0000324	CSR-3
325	BBI-0325	NBAII-CSG-BOM-0000325	CSR-6
326	BBI-0326	NBAII-CSG-BOM-0000326	CSR-12
327	BBI-0327	NBAII-CSG-BOM-0000327	CSR-16
328	BBI-0328	NBAII-CSG-BOM-0000328	CSR-17
329	BBE-0329	NBAII-CSG-BOM-0000329	MIR-4
330	BBI-0330	NBAII-CSG-BOM-0000330	RB-18
331	BBE-0331	NBAII-CSG-BOM-0000331	TMS-34
332	BBE-0332	NBAII-CSG-BOM-0000332	INDONESIA
333	BBE-0333	NBAII-CSG-BOM-0000333	OD-TRANSLUSCENT
334	BBI-0334	NBAII-CSG-BOM-0000334	APS-4
335	BBI-0335	NBAII-CSG-BOM-0000335	APS-5
336	BBI-0336	NBAII-CSG-BOM-0000336	APS-8
337	BBI-0337	NBAII-CSG-BOM-0000337	APS-9
338	BBI-0338	NBAII-CSG-BOM-0000338	DD-1
339	BBI-0339	NBAII-CSG-BOM-0000339	DD-2
340	BBI-0340	NBAII-CSG-BOM-0000340	DD-3
341	BBI-0341	NBAII-CSG-BOM-0000341	NK-1
342	BBI-0342	NBAII-CSG-BOM-0000342	NK-2
343	BBI-0343	NBAII-CSG-BOM-0000343	NK-3

#	Acc. No.	National Accession No.	Race Name
344	BBI-0344	NBAII-CSG-BOM-0000344	NP-4
345	BBI-0345	NBAII-CSG-BOM-0000345	NP-5
346	BBI-0346	NBAII-CSG-BOM-0000346	KSO-2
347	BBI-0347	NBAII-CSG-BOM-0000347	KSO-3
348	BBI-0348	NBAII-CSG-BOM-0000348	NP-2
349	BBI-0349	NBAII-CSG-BOM-0000349	HND
350	BBI-0350	NBAII-CSG-BOM-0000350	HDO
351	BBI-0351	NBAII-CSG-BOM-0000351	D4
352	BBI-0352	NBAII-CSG-BOM-0000352	D7
353	BBI-0353	NBAII-CSG-BOM-0000353	MC1
354	BBI-0354	NBAII-CSG-BOM-0000354	MC2
355	BBI-0355	NBAII-CSG-BOM-0000355	O1
356	BBI-0356	NBAII-CSG-BOM-0000356	O2
357	BBI-0357	NBAII-CSG-BOM-0000357	Borpat(White)
358	BBI-0358	NBAII-CSG-BOM-0000358	CSR-26
359	BBI-0359	NBAII-CSG-BOM-0000359	CSR-27
360	BBI-0360	NBAII-CSG-BOM-0000360	A-3
361	BBI-0361	NBAII-CSG-BOM-0000361	A-Chinese
362	BBI-0362	NBAII-CSG-BOM-0000362	AHT
363	BBI-0363	NBAII-CSG-BOM-0000363	BHT
364	BBI-0364	NBAII-CSG-BOM-0000364	GHT
365	BBI-0365	NBAII-CSG-BOM-0000365	FHT
366	BBI-0366	NBAII-CSG-BOM-0000366	J 2
367	BBI-0367	NBAII-CSG-BOM-0000367	H 281
368	BBI-0368	NBAII-CSG-BOM-0000368	916 B
369	BBI-0369	NBAII-CSG-BOM-0000369	935 E
370	BBI-0370	NBAII-CSG-BOM-0000370	SLWU-8
371	BBI-0371	NBAII-BBI-0371	SK-6
372	BBI-0372	NBAII-BBI-0372	SK-7
373	BBI-0373	NBAII-BBI-0373	DUN-6
374	BBI-0374	NBAII-BBI-0374	DUN-22
375	BBI-0375	NBAII-BBI-0375	PAM-114

#	Acc. No.	National Accession No.	Race Name
376	BBI-0376	NBAII-BBI-0376	PAM-117
377	BBI-0377	NBAII-BBI-0377	APS-12
378	BBI-0378	NBAII-BBI-0378	APS-45
379	BBI-0379	NBAII-BBI-0379	APDR-105
380	BBI-0380	NBAII-BBI-0380	APDR-115
381	BBI-0381	NBAII-BBI-0381	APDR-126
382	BBI-0382	NBAII-CSG-BBI-0382	Bcon-1
383	BBI-0383	NBAII-CSG-BBI-0383	Bcon-4
384	BBI-0384	NBAII-CSG-BBI-0384	Gen-2
385	BBI-0385	NBAII-CSG-BBI-0385	Gen-3
386	BBI-0386	NBAII-CSG-BBI-0386	CSR-50
387	BBI-0387	NBAII-CSG-BBI-0387	CSR-51
388	BBI-0388	NBAII-CSG-BBI-0388	CSR-52
389	BBI-0389	NBAII-CSG-BBI-0389	CSR-53

Multivoltine Accessions

1	BMI-0001	NBAII-CSG-BOM-0000001	PURE MYSORE
2	BMI-0002	NBAII-CSG-BOM-0000002	SARUPAT
3	BMI-0003	NBAII-CSG-BOM-0000003	MORIA
4	BMI-0004	NBAII-CSG-BOM-0000004	TAMILNADU WHITE
5	BME-0005	NBAII-CSG-BOM-0000005	C.NICHI
6	BMI-0006	NBAII-CSG-BOM-0000006	HOSA MYSORE
7	BMI-0007	NBAII-CSG-BOM-0000007	MYSORE PRINCESS
8	BMI-0008	NBAII-CSG-BOM-0000008	KOLAR GOLD
9	BMI-0009	NBAII-CSG-BOM-0000009	KOLLEGAL JAWAN
10	BMI-0010	NBAII-CSG-BOM-0000010	MY-1
11	BMI-0011	NBAII-CSG-BOM-0000011	P2D1
12	BME-0012	NBAII-CSG-BOM-0000012	RONG DAIZO
13	BME-0013	NBAII-CSG-BOM-0000013	GUANGNONG PLAIN
14	BMI-0014	NBAII-CSG-BOM-0000014	OS-616
15	BME-0015	NBAII-CSG-BOM-0000015	RAJ
16	BMI-0016	NBAII-CSG-BOM-0000016	G
17	BMI-0017	NBAII-CSG-BOM-0000017	NISTARI

#	Acc. No.	National Accession No.	Race Name
18	BMI-0018	NBAII-CSG-BOM-0000018	NISTARI(M)
19	BMI-0019	NBAII-CSG-BOM-0000019	NISTARI(P)
20	BMI-0020	NBAII-CSG-BOM-0000020	ZPN(SL)
21	BMI-0021	NBAII-CSG-BOM-0000021	CB5
22	BMI-0022	NBAII-CSG-BOM-0000022	KW2
23	BMI-0023	NBAII-CSG-BOM-0000023	M2
24	BMI-0024	NBAII-CSG-BOM-0000024	A23
25	BMI-0025	NBAII-CSG-BOM-0000025	A25
26	BMI-0026	NBAII-CSG-BOM-0000026	OVAL
27	BMI-0027	NBAII-CSG-BOM-0000027	O
28	BMI-0028	NBAII-CSG-BOM-0000028	M83(C)
29	BMI-0029	NBAII-CSG-BOM-0000029	B
30	BME-0030	NBAII-CSG-BOM-0000030	GNM
31	BMI-0031	NBAII-CSG-BOM-0000031	A14DY
32	BMI-0032	NBAII-CSG-BOM-0000032	A4e
33	BMI-0033	NBAII-CSG-BOM-0000033	PA12
34	BMI-0034	NBAII-CSG-BOM-0000034	AP12
35	BMI-0035	NBAII-CSG-BOM-0000035	A13
36	BMI-0036	NBAII-CSG-BOM-0000036	PMX
37	BMI-0037	NBAII-CSG-BOM-0000037	PMS2
38	BMI-0038	NBAII-CSG-BOM-0000038	MU-1
39	BMI-0039	NBAII-CSG-BOM-0000039	MU-11
40	BMI-0040	NBAII-CSG-BOM-0000040	WAI-1
41	BMI-0041	NBAII-CSG-BOM-0000041	WAI-4
42	BMI-0042	NBAII-CSG-BOM-0000042	MY23
43	BMI-0043	NBAII-CSG-BOM-0000043	MW13
44	BMI-0044	NBAII-CSG-BOM-0000044	MHMP(W)
45	BMI-0045	NBAII-CSG-BOM-0000045	MHMP(Y)
46	BMI-0046	NBAII-CSG-BOM-0000046	P4D3
47	BME-0047	NBAII-CSG-BOM-0000047	NISTID(Y)
48	BME-0048	NBAII-CSG-BOM-0000048	NISTID(W)
49	BME-0049	NBAII-CSG-BOM-0000049	NK4

#	Acc. No.	National Accession No.	Race Name
50	BME-0050	NBAII-CSG-BOM-0000050	CAMBODG
51	BME-0052	NBAII-CSG-BOM-0000052	DAIZO
52	BMI-0053	NBAII-CSG-BOM-0000053	LMP
53	BMI-0054	NBAII-CSG-BOM-0000054	DMR
54	BMI-0055	NBAII-CSG-BOM-0000055	LMO
55	BMI-0056	NBAII-CSG-BOM-0000056	MY1(SL)
56	BMI-0057	NBAII-CSG-BOM-0000057	PM(SL)
57	BMI-0058	NBAII-CSG-BOM-0000058	BL23
58	BMI-0059	NBAII-CSG-BOM-0000059	BL24
59	BMI-0060	NBAII-CSG-BOM-0000060	MU303
60	BMI-0061	NBAII-CSG-BOM-0000061	MU520
61	BMI-0062	NBAII-CSG-BOM-0000062	MU10
62	BMI-0063	NBAII-CSG-BOM-0000063	TW x SK6 x SK1
63	BMI-0064	NBAII-CSG-BOM-0000064	SK6 x SK1 x TW
64	BMI-0065	NBAII-CSG-BOM-0000065	BL43
65	BMI-0066	NBAII-CSG-BOM-0000066	APM-1
66	BMI-0067	NBAII-CSG-BOM-0000067	SLKSPM
67	BMI-0068	NBAII-CSG-BOM-0000068	M12(W)
68	BMI-0069	NBAII-CSG-BOM-0000069	M15
69	BMI-0070	NBAII-CSG-BOM-0000070	M6DP(C)
70	BMI-0071	NBAII-CSG-BOM-0000071	M6DP(C)Green
71	BMI-0072	NBAII-CSG-BOM-0000072	M6M81
72	BMI-0073	NBAII-CSG-BOM-0000073	BL-67
73	BMI-0074	NBAII-CSG-BOM-0000074	MH-1
74	BMI-0075	NBAII-CSG-BOM-0000075	PM (Mutant)
75	BMI-0076	NBAII-CSG-BOM-0000076	APM-2
76	BMI-0077	NBAII-CSG-BOM-0000077	APM-3
77	BMI-0078	NBAII-CSG-BOM-0000078	APDR-15
78	BMI-0079	NBAII-CSG-BMI-0079	Mcon-1
79	BMI-0080	NBAII-CSG-BMI-0080	Mcon-4
80	BMI-081	NBAII-CSG-BMI-081	L14
81	BMI-082	NBAII-CSG-BMI-082	L15