PROFORMA FOR COLLECTION OF DATA OF RESEARCH PROJECTS IN SERICULTURE

PART- I: GENERAL INFORMATION

1	Name of the Institute / University / Organization submitting the Project proposal	:	Central Sericultural Research & Training Institute, Berhampore, West Bengal
2	Status of the Institute (s)	:	N.A.
3	Name (s) and designation (s) of the Executive Authority of the institute / University forwarding the application	:	Dr. S Nirmal Kumar, Director
4	Project Title	:	Assessment of promising powdery mildew resistant mulberry lines for prospective commercial use
5	Category of the Project	:	Applied
6	Specific Area	:	P – Plant; I – Improvement; T - Biotechnology
7	Duration	:	3 years
8	Total Cost	:	3.32Lakh
9	Is the Project single or multi-Institutional	:	Single Institutional
10	If the Project is multi-institutional, please furnish the following : Name, Designation and Address of the Project Coordinator.	:	N.A.

11. (a) Project summery:

Powdery mildew (PM) is a disease of great concern globally in sericulture world, and is responsible for valuable mulberry foliage loss up to 20%; besides silkworm cocoon loss due to the disease is enormous. However, commercially exploitable PM resistance cultivar is lacking in the country and little is known about the genetics of its host-pathogen interactions. In order to overcome above constrains, we have pursued two consecutive DBT supported projects in collaboration with CCMB, Hyderabad and obtained following leads:

1. Screened ~ 144 core germplasm, originated from various *Morus* species, multi-parametrically under natural and artificial epiphytotics, identified five useful sources of resistance for breeding utilization and some DNA tags (RAPD fragments) putatively associated with PM resistance.

2. Developed PM specific segregating populations and advance breeding lines utilizing crosses of identified resistant x susceptible resources and resistant x susceptible commercial cultivar (S-1), respectively and thoroughly evaluated the disease responsiveness.
3. Cloned 24 identified RAPD fragments, and developed/ converted 11 of them into SCAR markers. These markers along with some mulberry specific SSRs developed by CCMB were tested / validated on above PM specific progenies. Two each of SCARs and SSRs showed relatively good association with PM disease reaction.
4. Most importantly, identified ~11 transgressive progenies from the above crosses showed better yield potential than S-1 (20 – 32%; at least in minor scale evaluation of 5 plants/progeny) with resistance of varying degrees.

Though the identified markers were found positive in most of the resistant progenies, but validation was partial as PM resistance in mulberry is controlled by multiple gene(s)/QTLs (≥ 3 genes), seemingly by recessive alleles and additive gene action.

In present proposal the above identified promising lines will be thoroughly evaluated for important agronomical features associated with leaf biomass, rooting efficiency and assessed the present F-1 promising and F-2 derived from the sib mating of contrast responsive F-1 through the identified SCARs/SSR markers to generate authentic database for MAS based application. Finally, selected progenies will be subjected to silkworm bioassay prior to direct field utilization.

(b) Aims and Objectives

The availability of well-validated informative markers associated with PM resistance and suitable commercial cultivar with durable resistance to the disease is scant in mulberry. Therefore, thorough evaluation / in-depth characterization of above and below ground traits associated with leaf biomass of promising progenies resistant to PM followed by silkworm bioassay is the desirable logical steps before recommendation for field utilization. Moreover foolproof validation of identified SCAR / SSR markers developed in our previous projects, which seems to have good association with all promising progenies, as well as on the trait refined F-3 lines (generated by the cross of resistant x susceptible F1 [pseudo-F-2]) are essentially needed to generate valuable information on marker-trait association on a three generation pedigrees. If feasible, that would create the platform for MAS based selection possibility of PM resistant lines / clones in mulberry.

PART-II: PARTICULARS OF INVESTIGATORS

12	a) Name		**DR SOUMEN CHATTOPADHYAY**
	Date of Birth		
	Sex		M
	Indicate whether Principal Investigator/ Co-investigator		**PI**
	Designation		Scientist-D
	Department		Biotechnology
	Institute/University: Address		CSR&TI, Berhampore, West Bengal
	b) Name		**DR RITA BANERJEE**
	Date of Birth		
	Sex		F
	Indicate whether Principal Investigator/ Co-investigator		**CI-1**
	Designation		Scientist - D
	Department		Biotechnology
	Institute/University: Address		CSR&TI, Berhampore, West Bengal
	c) Name		**DR A K SAHA**
	Date of Birth		
	Sex		M
	Indicate whether Principal Investigator/ Co-investigator		**CI-2**
	Designation		Scientist-D
	Department		Sericulture Division
	Institute/University: Address		CSR&TI, Berhampore, West Bengal
	d) Name		**DR S NIRMAL KUMAR**
	Date of Birth		
	Sex		M
	Indicate whether Principal Investigator/ Co-investigator		**Executive Authority**
	Designation		Director
	Institute/University: Address		CSR&TI, Berhampore, West Bengal
13	No. of Projects being handled by each investigator at present		
a	Dr. S Chattopadhyay		PROJECTS: 1 (AS CO-I)
b	Dr R Banerjee		PROJECTS: 1 (AS PI-1), PILOT STUDY: 1(AS PI)
c	Dr A K Saha		PROJECTS: 1 (AS PI-1), PROGRAMS: 4 & PS: 3 NOS.

14. Proposed Research Fellows [justification with work sharing is a must]:

NIL

PART-III: TECHNICAL DETAILS OF THE PROJECT

15. **Introduction**

15.1 **Definition of the Problem**:

(a) Origin of the project:

Mulberry (*Morus* spp.) is a perennial tree and economically important for its foliage as nutrients to the domesticated silkworm (*Bombyx mori* L.). Though, India has contributed substantially to global silk generation (~18%), still our quality raw silk production is very limited (~2000kg of bivoltine silk). Inadequacy of suitable nutrient sufficient mulberry foliage is considered as one of the bottlenecks for international grade silk production (Datta and Nanavati 2005).PM, an obligate biotrophic ascomycete fungus *Phyllactinia corylea*, is one of the major diseases of mulberry throughout the world. The resulting foliage loss goes up to 20% and reduces substantially the yield of silkworm cocoons. It affects mulberry during two (out of five) commercial silkworm-rearing and one seed cocoon rearing seasons in West Bengal. None of the available commercial cultivars are resistance to PM.

In this pretext, two successive collaborative (with CCMB) projects were taken up with DBT supports. The detailed analysis of 147 potential mulberry germplasm, comprising of nine species, showed that five entries having useful (quantitative + qualitative) sources of resistance to PM, which may be exploitable in breeding program. The study also revealed a number of potential DNA variations (seen as specific RAPD fragments) that appear to be associated with mildew response of the mulberry genotypes.

These leads were intensified further collaboratively with another DBT project (2010 -2014) by transferring PM resistant trait of Vietnam-2 to three susceptible lines and commercial susceptible cultivar S-1. These segregating populations were evaluated phenotypically and some potential SCAR markers, developed from the PM associated putative DNA tags (RAPD fragments), as well as some mulberry specific SSRs selected from 80 in-house built SSRs by CCMB, were tested on it. The project emanates following leads and needed further work to achieve meaningful goals:

1. A few SCARs and two SSRs appear to be a good candidate for PM resistance response.
2. Though the assessment was conducted in minor scale (5 plants/progeny),~11 transgressive progenies from the above S-1 (a monoecious well accepted variety in Eastern &NE India) and three susceptible lines x resistant crosses showed better yield potential than S-1 (20 – 32%) with significant resistance to PM. *These lines need*

thorough evaluation under multiplied condition ('Field Yield Trial') for testing of their potential for direct field exploitation.

3. Interestingly, above markers were found positive in most of the promising resistant high yielding progenies but validation of these markers was partial association of marker –trait is 60 -70%. PM resistance in mulberry appears to be controlled by multiple gene(s)/QTLs (≥3 genes), seemingly by recessive alleles and additive gene action. *Therefore, attempt to pin-point PM responsive segregants by sib-mating of better performing resistant x susceptible progenies of the present F-1 population and further validation of them with identified markers to establish marker-trait association, which seems to be essential for MAS based selection of PM resistance.*

b) Expected outcome:

a. Identification of improved mulberry with PM resistance for commercial utilization.
b. Establishment of marker-trait association of powdery mildew resistance for MAS based selection in mulberry.

15.2 **Rationale of the Study:**

PM, the dreaded foliar disease is one of the major production constraints in mulberry cultivation worldwide. But true resistance against PM in presently using commercial mulberry cultivars is lacking. The situation calls for development/integration of modern efficient biotechnological tools/technologies such as those based on DNA markers to address the above problems, especially the identification/development of stable DNA markers that may be associated with disease resistance, horizontal transfer of the identified traits in a suitable cultivar that can be possible to use in direct field utilization and can make feasible the markers-based breeding for disease resistance in mulberry.

Our initiatives in this direction already generated some promising better foliage biomass producing lines with significant PM resistance on small scale testing, produced partially validated a few SCARs and SSRs, which warrants thorough assessment.

15.3 **Relevance to the current issues and expected outcome:**

In the study proposed here, above leads would be taken to their logical conclusion, by attempting: thorough evaluation of obtained promising PM resistant lines (~11 nos) for propagation efficiency, leaf biomass and associated ancillary traits, assess the potential of the selected lines for silkworm rearing through bioassay, comprehensive validation of the identified SCARs / SSRs on the present F-1 (pseudo-F2) and trait refined F-2 lines derived

from the sib mating of existing F-1 progenies most contrast responsive to PM. The expected anticipated deliverables of the project are:

- ✓ Availability of a PM resistant cultivar with significantly better yield potential than S-1 for direct commercial use.
- ✓ DNA markers-linked with PM resistance for early detection of PM resistance using MAS based approach.

15.4 Objective:

- *Evaluation of powdery mildew resistant promising lines for foliage biomass and associated ancillary traits.*
- *Assessment of propagation efficiency of powdery mildew resistant promising lines*
- *Evaluation of silkworm rearing efficiency of the selected line(s) through bio-assay.*
- *Crossing of promising lines contrast responsive to powdery mildew resistance for the assessment of marker-trait association.*
- *Testing of association of identified SCAR and SSR markers with the powdery mildew resistant for the use in MAS based selection.*

16. **Review of status of Research and Development on the subject**

16.1 **National Status**:

Mulberry sericulture

Mulberry (*Morus* spp.; Family: Moraceae) is one of the important commodity crops in all silk producing countries in the world. The plant is cultivated widely in geographical areas that include temperate and subtropical regions of the northern hemisphere and tropical parts of the southern hemisphere. Primarily its foliage is the sole feed for the domesticated silkworm *Bombyx mori* L.. In India, sericulture is a sustainable farm-based economic enterprise of the rural masses having the potential of long-term returns against lesser investments. Presently,~1.8 lakh hectare of land is under mulberry cultivation for the production of 18,715MT of raw silk with an employment generation of ~7.65 million persons per annum (Anonymous 2013). Profitability of the sericulture is directly correlated with production of high quality of mulberry leaves. Therefore, a continuous effort is necessary to improve the quality and quantity of the mulberry foliage.

Mulberry powdery mildew

PM, an obligate biotrophic ascomycete fungus [*Phyllactinia corylea* (syn. *P. guttata* syn. *P. moricola*), is one of the major diseases of mulberry throughout the world. The disease is characterized by white dust-like mycelia that develop over abaxial leaf surfaces. It prevents photosynthesis, causing leaf dryness, heavily infected tissues develop chlorosis and senesce prematurely (Gupta 2001).The disease appears in all stages of mulberry plant development

from 3-5 leaf stage seedling/ saplings to harvest maturity leaves for silkworm feeding. The disease is severe in the autumn and spring in most areas, extending into the monsoon in the hilly regions of India. The resulting foliage loss, typically 20%, reduces substantially the yield of silkworm cocoons (Manimegalai and Chandramohan 2007). Mulberry is generally cultivated in India as a monoculture by poor and marginal growers, usually with a very small landholding (Sahu et al.1997). PM control through inherent plant resistance is desirable since it would reduce dependence on costly fungicides that can damage both the environment and the silkworms themselves (Govindaiah and Gupta 2005). Therefore, from both economic and environmental points of view, the development of resistant cultivars is highly attractive.

As an initial step, we assembled and screened 147 core germplasm sources, representing 18 countries of origin for resistance to P. corylea in six seasonal fields and greenhouse trials. A relatively low disease reaction of 09 resources using different assessment scales after natural and artificial inoculations prove, for the first time, that they have potential in breeding for resistance in tropical mulberry to PM (Chattopadhyay et al 2010).

Powdery mildew resistance and associated micro-morphological traits

Unlike other PM pathogens, the genus *Phyllactinia* is partly (hemi) endoparasitic (Glawe 2008). The pathogen indirectly penetrates the mesophyll via stomata to form haustoria (Takamatsu et al. 2008).

Our finding also suggested that highly significant correlations between the prevalence of PM and micro-morphological features like stomata and trichome densities in mulberry. In fact PM-resistant germplasm group was distinguished by 17.4% lower stomatal density and 20.0% greater trichome density compared with the susceptible group. The links appeared causal and may be related to the level of successful spore penetration of the leaf, which indicated, for the first time, that an alternative 'avoidance' or pre-penetration mechanism, which operates after the contact of parasite on the host epidermal cell is apparent in mulberry–PM interaction (Chattopadhyay et al 2011).

Foliage biomass, agronomic traits associated with yield and powdery mildew resistance

In sericulture, the silkworm cocoon yield is considered an integral part of the foliage biomass of the host plant. However, leaf yield in mulberry is a complex trait and strongly correlated with shoot length, internodal distance, shoot number, petiole length, petiole width, and aboveground biomass in mulberry (Tikadar and Rao 2002; Banerjee et al. 2007; Doss et al 2011). Among the below-ground parameters root volume and fresh and dry root weight are important to assess quick multiplication of clonal cuttings for vegetative propagation

(Banerjee et al 2006). In mulberry longest shoot length, leaf area, intermodal distance, green and dry leaf weight, lamina length, lamina weight, root volume, and fresh and dry root weight have been recognized as important variables that had direct effect on silkworm cocoon yield (Banerjee et al. 2011).

PM reduces leaf yield potential which is due to leaf area destruction and premature defoliation (Govindaiah et al 1989). In addition disease also impairs the leaf quality, which adversely affects the silkworm rearing and cocoon production. Therefore, evaluation of identified promising lines for above/below ground traits and leaf quality are imperative prior to recommendation for commercial use.

Our findings of ~11 F-1 progenies derived from the horizontally transferred PM resistant trait(s) to commercial cultivar S-1 showed significantly higher leaf yield potential (≥ 21-32% than S-1) with considerable PM resistance under small scale evaluation (5plants / progeny). These promising lines now need to be thoroughly tested in large scale for direct field utilization.

16.2 **International Status**

Genetic basis of powdery mildew resistance

Both qualitative and quantitative resistances for PM from different host–parasite systems have been reported. The mechanisms and genetics of PM resistance have been extensively studied in some cereal and vegetable crops. Major types of PM resistance have been identified among these hosts including:

1) Pathogen race specific resistance that is usually short-term and determined by single dominant gene (Hsam and Zeller, 2002; Jorgensen, 1994),
2) Broad spectrum partial or adult plant resistance that is usually durable and inherited polygenically (Das and Griffey, 1995), and
3) Broad spectrum *mlo* resistance that is usually durable and controlled by recessive alleles (Collins et al 2002; Ramming et al 2011).

Compared with other crops, however, mulberry has little information available on sources of PM resistance. The need to locate resistance has instigated several germplasm-screening programs worldwide (Bubici and Cirulli 2008; Dreisitl and Bockelman 2003), and many workers have advocated multi-parametric assessments (Raghuchander et al. 2001; Gawande and Patil 2003).

Our findings on PM disease responsiveness of all three F-1 descendant showed that segregation was transgressive towards susceptibility with continuous distribution at varied degrees. The segregation genetics (based on X^2 –analysis) indicated that at least three

independent genes conferred the PM resistance in mulberry. Overall results suggest quantitative nature of resistance dominated by recessive alleles with possible involvement of multiple QTLs of additive gene action (Chattopadhyay et al unpublished).

Limitations of conventional disease resistance breeding and necessity of for implementation of Marker Assisted Section for powdery mildew resistance in mulberry

Targeted mulberry breeding is a slow and difficult process for reasons that include long juvenility periods, heterozygocity, laborious, depends upon environmental condition and complex nature of many economically important traits (Vijayan et al. 1997). Disease resistance breeding has traditionally been done by phenotypic selection alone. Efficiency of phenotypic selection is reduced by variability in the pathogen, infection, and disease progression. Development of a new disease resistant cultivar usually takes > 15 years from the original cross to the introduction into the market.

Early identification of individuals carrying the desired allele combinations allows breeders to grow larger effective populations, which results in decreasing maintenance and evaluation costs. Molecular markers linked to resistance genes can be employed as an alternative selection technique. Selection of seedlings based on the presence of markers without wasting time to develop characteristic symptom, or MAS is a relatively fast and not influenced by environmental factors (Arus and Moreno Gonzalez, 1993). In fact, the speed and precision of breeding has been improved in various plants by the development of genetic linkage maps based on molecular markers to locate discrete chromosomal regions (QTLs), which control a number of complex polygenic traits (Varshney et al 2007a) as appears in mulberry PM.

During the last two decades, there has been considerable progress in the development of molecular markers in mulberry. Indeed, the genetic characterization of available resources has proven worthwhile to allow a better understanding of the plant and indicated diversified gene pool. In most of the studies, RAPDs (Bhattacharya and Ranade 2001; Nayik et al 2002; Chatterjee et al 2004; Zhao and Pan 2004), ISSRs (Vijayan and Chatterjee, 2003; Awashti et al 2004; Vijayyan et al 2006) and AFLPs (Sharma et al 2000; Wang and Yu 2001; Kafkas et al 2008) have been the common marker systems used in the analysis of mulberry resources.

Sequence characterized amplified region (SCARs) and Microsatellites are marker of choice for directional improvement

All above mentioned markers are mostly random in nature, have poor repeatability between laboratories and lack of efficiency to differentiate heterozygous and homozygous loci (dominant). *Our study with 147 mulberry resource materials also revealed a number of*

potential DNA variations (seen as specific RAPD fragments) that appear to be associated with PM response of the mulberry genotypes. Short random primers used in RAPD analysis usually anneal with multiple sites in different regions of the genome, and thus they may amplify several genetic loci, some of which may represent repetitive DNA. Therefore, these markers may not be suitable for probing introgression of specific traits onto elite genetic background(s) to achieve various diversified goals of mulberry.

The conversion of RAPD and other molecular markers to SCARs based on sequence data significantly improves the reproducibility and reliability of PCR assays, and therefore their utility for many applications, such as MAS and cultivar identification (Paran and Michelmore 1993). After conversion it is necessary to examine and validate the markers by testing in genetically related segregating progeny for the maintenance of their correlation with the resistance traits (Scheef et al 2003; Busconi et al 2006) Indeed, SCAR markers are successfully utilized to linked with resistant trait(s) of many diseases like powdery mildew in grape (Akkurt et al 2007) and pea (Fondevilla et al 2008) and leaf spot in soybean (Filho et al.2002).

On the other hand, microsatellites or simple sequence repeats (SSRs) are stretches of genomic DNA, consisting of tandemly repeated short units of 1-6 base pairs in length. SSRs are the marker of choice because they have proven to be locus specific, co-dominant, highly polymorphic and highly reproducible. To date an evaluation of the amount of diversity detected with microsatellites has revealed more polymorphism compared to other assay procedures(Varshney et al 2005). Because of these useful properties, SSRs are the most popular markers in population genetics and have been used in many applications such as gene tagging, Quantitative Trait Locus (QTL) mapping, MAS, parentage analysis, fingerprinting, and phylogenetic and taxonomic studies. In mulberry, since the first report of SSR development in the year 2004, only a few microsatellite markers (~204 nos) have been available so far in mulberry (Aggrawal et al 2004; Zhao et al 2005; Balachandran et al 2013).

Accordingly, for quick and robust assessment of PM specific segregating progenies derived from resistant x susceptible crosses and advance breeding line derived from resistant x commercial susceptible cultivar, utilizing available putative PM resistant specific DNA tags, ~ 11 SCARs were developed. These SCARs along with ~ 20 mulberry specific SSRs developed by CCMB were tested / validated on PM specific segregating lines established at Berhampore. Some of the SCARs and two SSRs showed promising results with ~60-65% association with segregating population and advance breeding lines (Data unpublished). Most importantly, all promising advance breeding lines (~11nos) in respect of foliage

biomass with significant PM resistance showed a considerable association with the identified SCARs and SSRs, which needs further investigations to utilize the potential at the field.

16.3 **Importance of the proposed project in the context of current status**:

Though PM is responsible for significant foliage loss of mulberry globally, but true resistant variety for commercial exploitation is hitherto unavailable. The proposed study is based on the leads obtained in a recently concluded project on the development, validation and utilization of SCAR markers associated with PM resistance in mulberry. Under the project, PM resistant trait(s) of Vietnam-2 has been introgressed into commercial variety S-1 as well as in three other susceptible lines. The advance breeding lines (using S-1) and specific segregating progenies (using Philippines, Xuan-9, Kolitha-3) were evaluated for PM responsiveness and validated with 11 SCARs and a few mulberry specific SSR markers developed by CCMB. Some of the developed segregating pseudo-F_2 (F_1) progenies exhibited significant PM resistance with better foliage yield potential than S-1 in small scale evaluation. The developed SCARs and mulberry specific SSRs (two each) showed positive association with these promising high foliage yielding lines. In the study proposed here, these leads would be taken to their logical conclusion by attempting:

- Thorough evaluation of foliage biomass, ancillary above and below ground traits of promising lines through recommended field trails over the seasons and subsequent silkworm bioassay for commercial/ field use.
- Development and assessment of F-2 segregants using F-1 progeny most contrast responsive to PM, and comprehensive validation of the identified SCARs / SSRs on the present F-1 (pseudo-F2) and trait refined F-2 lines. It would provide valuable information on marker –trait (PM resistant) association / PM resistance inheritance pattern, which is essential for MAS based selection of PM resistance of mulberry.

16.4 **Anticipated Products, processes/Technology, Packages/ Information or other outcome from the project and their expected utility**:

Anticipated product: Developed new mulberry variety, durable (= quantitative) resistant to PM with ≥ 20% better yield potential of existing commercial cultivar S-1 for field utilization.

Generation of useful information: Valuable information on marker-trait association of PM resistance in mulberry, which is essential for MAS based selection of PM resistant lines in mulberry.

16.5 **Expertise available with proposed investigation group/ institution on the subject of the project*:**

Name of the Scientists	Design-ation	Experience
Dr S Chattopadhyay	Scientist-D	As a principal Investigator of the proposal, he has 21 years of research experience in various field of mulberry crop improvement. He has expertise (relevant to the proposal) in the areas of- a) screening of mulberry genetic resources using random molecular markers, b) analysis of various enzymes and cellular constituents of mulberry, and c) pathometrical analysis of various mulberry diseases. He has >43 publications in different International and National level peer review journals. He had concluded a DBT funded network project on-Identification of DNA markers associated with powdery mildew disease in mulberry as PI and instrumentally associated with two DBT supported projects (collaboratively with CCMB) to identify SCAR-markers associated with powdery mildew resistance and development of linkage map of mulberry.
Dr R Banerjee	Scientist-D	As a co-Investigator of the proposal, she has about 14 years research experience in the field of mulberry breeding and genetics. She has expertise in the areas of a) evaluation of mulberry germplasm materials using anatomical, morphological and molecular tools as well as b) various aspects of conventional/targeted breeding in crop plants and C) disease resistance breeding of mulberry in particular. She has more than 35 publications in International and National peer review journals. Presently She is working as PIs of two projects on "Development of DNA markers based genetic linkage map of mulberry and QTL analysis for agronomically important *planta* traits" and "Identification of DNA markers associated with bacterial leaf spot resistance in mulberry".
Dr A K Saha	Scientist-D	As a co-investigator of the proposal, he has 30 years of research experience in various fields of silkworm breeding genetics, physiology and rearing technology. He has expertise (relevant to the proposal) in the areas of- bioassay of various silkworm breeds / hybrids, He has >100 publications in different International and National level peer review journals.

** Dr R K Aggarwal, Chief scientist (Director grade), CCMB, collaborator of our previous two projects and instrumental behind the development of PM specific SCARs and SSRs, has agreed to provide all kind of technical supports / inputs for the work.*

17. **Work Plan**:

17.1 **Methodology**:

I) *Field evaluation of promising progenies for foliage biomass and other ancillary traits*:

a) Selected promising F-1 progenies (~11 nos) developed through introgression of PM resistant trait(s) to susceptible commercial cultivar S-1 and others would be established under field trail for 3 years keeping the current ruling variety S-1635, parental clones and S-1 as control. Recommended cultural practices will be followed (Ray et al 1973) and data were recorded from the middle 25 plants following five crop schedules for consecutive three years.

b) Crossing has already made between selected F-1 progenies contrast responsive to PM. About 100 randomly selected traits improved segregating full-sib progenies would be raised in ARBD and to be used for further studies on identified SCARs / SSRs validation for establishing marker-trait association / genetic analysis.

c) The evaluation of foliage biomass, aboveground ancillary traits and assessment of PM disease reaction will be done as follows:

- ✓ Foliage biomass and ancillary morphological traits will be measured according to the method of Matchii et al. (2001) with suitable modifications for tropical environment suggested in the mulberry descriptor (Thangavelu et al. 2000).
- ✓ The parameters like number of tillers, plant height, total shoot length, nodal distance, leaf fall percent, weight of 100 mature leaves, leaf biomass, shoot biomass, single leaf area and above ground biomass will be estimated after 60 ± 5 days of bottom pruning of the plants in each season (Banerjee et al 2014).
- ✓ Foliage constituents like chlorophyll, total soluble protein, total soluble sugar and total phenol will be estimated following standard procedures (Chattopadhyay et al.1992).

II) Disease assessment:

Disease reaction of segregating populations will be scored using Horsefall-Barratt (1 to 10 points) scale and disease severity index (DSI), accumulative area under disease progression curve (AUDPC) and apparent infection rate (r) will be estimated according to Chattopadhyay et al. (2010; 2014).

At least 04 times data collection / crop will be assured in two important seasons of PM occurrence in West Bengal in October-November and February-March of three consecutive years coinciding with 'Agrahayani' (October - November) and 'Falguni' (February - March) commercial silkworm crops.

III) Silkworm bioassay with identified promising line(s):

The silkworm bioassay of ruling multivoltine x bivoltine hybrid will be conducted with identified lines before recommendation for field use following the standard norms and methodology as suggested by Saha et al (2013a and 2013b).

IV) SCARs and SSRs based validation:

DNA isolation, purification and quantification

Genomic DNA will be isolated from the leaves of segregating progenies and clones following the method of Kang et al (1998). DNA concentration and quality will be evaluated by electrophoresis in 0.8% agarose gel before PCR amplification.

PCR amplification of promising SSR and SCAR markers

Identified / partially validated SCAR and SSR primer pairs (~6 nos) would be tested first for amplification on resistant and susceptible parental lines / clones for polymorphism. Afterwards, the markers would be validated on the segregating populations (Both F_1 and F_2) developed from the most contrasting genotypes. The SCAR(s) / SSRs inheritance would be compared with the disease screening/response of the progenies.

PCR amplifications of all primers will be performed on a Palmcycler Gradient System (Corbett Inc, Sydney, Australia) in 20µL reaction volume. The amplification products will be resolved initially on 3% agarose gel and analysed on Bio-Print Mega imaging system (Viber Lourmat, Cedex, France). The result will be confirmed with at least two replicated assayAll methods have already been standardized in our laboratory.

V) *Data analysis*

- ✓ Analyses of variance and frequency distribution of PM disease reaction of the mapping population will be determined using SPSS version 20 base.
- ✓ Polymorphic DNA markers will be scored for presence (1) or absence (0) of the amplified fragments and set in a binary matrix and analyzed following NTSYS pc version 2. The validated SCARs /SSRs would also be tried for foreground selection of segregating progenies developed for PM resistance breeding from parents comprising: widely cultivated susceptible genotypes with 1 or 2 resistance sources; and later on next generation progenies that may be developed from some of the selected promising progenies.
- ✓ The phenotypic data will also be analyzed using genetic model for 'Statistica' version 8.0 software (Statsoft Inc., Tulsa, OK, USA) for various attributes of genetic analysis including clustering to have an insight about the genetic basis of the trait. Pearson's correlation coefficients were calculated to compare disease ratings with agronomical characters for mean values of each germplasm line (Gomez and Gomez 1984).

17.2 **Organization of Work Elements**

Name of Scientists	Designation	Time	Organization of work elements
1. Dr S Chattopadhyay	PI	45%	• DNA isolation and SCAR / SSR analysis (a portion) • Phenotyping of progenies (above ground portion) • Analysis of data using Statistica & SPSS softwares • Compilation of analyzed data and all sorts of report writing.

2. Dr R Banerjee	CI	35%	• SCAR / SSR analysis (a portion) • Disease reaction study • Propagation efficiency and below ground agronomical traits • Analysis of data using NTSYSPc softwares.
3. Dr A K Saha	CI	5%	• Supervision of SW bioassay work.

17.3 **Proprietary / Patented items, if any, expected to be used for this Project**:

Nil

17.4 **Suggested plan of action for utilization of the expected outcome from the project:**

- Developed / evaluated PM resistant mulberry line will be given to the competent authority for immediate field utilization.
- Validated SCARs /SSRs primer sequences, information on marker-PM resistant trait relation and pattern of inheritance will be uploaded to the Institute's website for future uses of sericulture scientists, subject to clearance by the competent authority.

17.5 **Time Schedule of activities giving milestones**:

Sl. no	**Organization of work/ Milestone /Activity**	**Expected Date of**	
		Starting	**Completion**
1.	Establishment of plantation in RBD, *in planta* characterization and evaluation of promising F-1 lines	Beginning of 1st quarter	End of 11th quarter
2.	Disease scoring of promising lines	Beginning of 2rd quarter	End of 11th quarter
3.	Establishment of a clonal set of the identified promising lines in polybag and evaluation of propagation efficiency with other below ground traits	Beginning of 3rd quarter	End of 8th quarter
4	Silkworm bioassay of identified & evaluated 1-2 superior lines	Beginning of 6th quarter	End of 9th quarter
5	Establishment of trait refined F-2 progenies derived from the most contrast responsive F-1 progeny to PM	Beginning of 3rd quarter	End of 5th quarter
6	Genomic DNA isolation of progeny lines and quality assessment	Beginning of 2nd quarter	End of 5th quarter
7	Validation of identified SCARs and SSRs on promising F-1 lines and selected F-2 progenies	Beginning of 5th quarter	End of 11th quarter
8	Use of identified / validated marker(s) for foreground selection in segregating population(s)& F-2 progenies	Beginning of 7th quarter	End of 12th quarter
10	Data analysis, compilation of results, report preparation and submission of report	Beginning of12th quarter	End of 12th quarter

17.6 **Project Implementing Agency /Agencies**:

Name of the agency	Address of the agency	Proposed Research Aspects	Proposed Amount	Cost Sharing %
Central Silk Board	Central Silk Board, Ministry of Textiles, Govt. of India, Bangalore		2.95	100%

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PART-IV: BUDGET PARTICULARS

18. **BUDGET** (in Lakh): [In case of multi-institutional projects, the budget details should be provided separately for each of the Institute]

A) Non-Recurring (e.g. equipments, accessories, etc.): Nil

B) Recurring

B.1. Manpower: Nil

B.2. Consumables:

Sl. No.	Item	1st Year	2nd Year	3rd Year	Total (in lakh)
1.	Fine chemicals / chemicals / reagents/ others	1.00	0.80	0.70	2.50
	Sub Total B2:	**1.00**	**0.80**	**0.70**	**2.50**

Other Items:

Sl. No.	Item	1st Year	2nd Year	3rd Year	Total (in lakh)
B3	Travel	0.08	0.08	0.08	0.24
B4	Contingency	0.20	0.20	0.18	0.58
	Total	**0.28**	**0.28**	**0.26**	**0.82**
Sub-total (B1+B2+B3+B4 etc.)		**1.28**	**1.08**	**0.96**	**3.32**
Grand total (A+ B1+B2+B3+B4)		**1.28**	**1.08**	**0.96**	**3.32**

Justification:

Consumables

The proposed work is experiment-intensive involving huge numbers of PCR reactions, DNA-amplicon separations, therefore needs relatively high consumable support.

PART-V: EXISTING FACILITIES

19. Available equipment and accessories to be utilized for the project:

SI. No.	Name of the Equipment/ Accessory	Make	Funding Agency	Year of Procurement
1	PCR-Gradient Master Cycler	Eppendorf	CSB	2004
2	Uv-vis Spectrophometer	Eleco	DBT	2010
3	Ultra-freezer (-80°C)		CSIR	2006
4	Medium speed cold centrifuge	Remi	CSB	2002
5	Quick freezer	Remi	DBT	2011
6	Horizontal gel electrophoresis set with power pack	Atto Corporation	DBT	2007
7	Gel documentation system		CSB	2012
8	Gel electrophoresis unit	Tarson	DBT	2011
8	Experimental filed of ~2 acre containing mapping populations specific for various diseases, leaf biomass and a core germplasm of ~130 resources raised and during implementation of four DBT supported projects since 2006. A portion of the resources will be utilized in the proposed study			

PART-VI: DECLARATION / CERTIFICATION

It is certified that

a. The research work proposed in the project does not in any way duplicate the work already done or being carried out elsewhere on the subject.

b. The same project has not been submitted to any other agencies for financial support.

c. The emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute/ university or as per the Ministry of Science & technology guidelines (Annexure-III).

d. Necessary provision for the project will be made in the Institute in anticipation of the sanction of the scheme.

e. If the project involves the utilization of genetically engineered organism , it is agreed that we will ensure that an application will be submitted through our institutional bio-safety committee and we will declare that while conducting experiments, the bio-safety guidelines of the Department of Biotechnology would be followed in toto.

f. If the project involves field trials / experiments / exchange of specimens etc we will ensure that ethical clearances would be taken from the concerned ethical committees of Biotechnology before implementing the project.

g. It is agreed by us that any research outcome or intellectual property right(s)on the interven (s) arising out of the project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance . Department of Expenditure as contained in annexure-V

h.. We agree to accept the terms and conditions as enclosed in Annexure-IV. The same is signed and enclosed.

i. The institute agrees that the equipment, the basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended investigators through out the duration of the project .

j. The institute assumes to undertake the financial and other management responsibilities of the project.

Signature of Executive Authority of
Institute with Seal & date

Signature of Project Co-ordinator
[Applicable for inter-institutional Projects only]
Date:

Signature of Principal Investigator & date	**Signature of Co-Investigator-I & date**	**Signature of Co-Investigator-II & date**