DEVELOPMENT OF DROUGHT TOLERANT MULBERRY VARIETY FOR RAINFED SERICULTURE

[IN COLLABORATION WITH CSGRC, HOSUR]

PROJECT PROPOSAL
SUBMITTED TO

CENTRAL SILK BOARD
MINISTRY OF TEXTILES (GOVERNMENT OF INDIA)
BTM LAYOUT, MADIVALA
BANGALORE – 560 068

2014-2019

SUBMITTED BY

Dr. M.K. GHOSH
SCIENTIST-D

MULBERRY BREEDING & GENETICS SECTION
CENTRAL SERICULTURAL RESEARCH & TRAINING INSTITUTE,
CENTRAL SILK BOARD, MINISTRY OF TEXTILES: GOVT. OF INDIA,
BERHAMPORE - 742 101, WEST BENGAL, INDIA

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,** Central Silk Board, Ministry of Textiles: Govt. of India, Berhampore - 742 101, West Bengal, India
2.	Status of the Institute(s)	:	NA
3.	Name(s) and designation of the Executive Authority of the Institute / University forwarding the application	:	Dr. S. NIRMAL KUMAR DIRECTOR
4.	Project Title	:	PIB:3505: DEVELOPMENT OF DROUGHT TOLERANT MULBERRY VARIETY
5.	Category of the Project	:	Applied
6.	Specific Area	:	P – **P**lant I – **I**mprovement B - **B**reeding
7.	Duration	:	06 (Six) years from January, 2014 to December, 2019
8.	Total cost:	:	Rs. 11.818 Lakhs
9.	Is the Project is single institutional or multi-institutional	:	Multi-Institutional
10.	If the Project is multi-institutional, please furnish the following: Name, Designation and Address of the Project Co-ordinator	:	Director, CSGRC, Hosur, Tamil Nadu.

11a. PROJECT SUMMARY:

World wide, water deficit is the most important limiting factor for crop productivity (Jones and Corlett 1992). Additionally, natural water systems are under severe strain in many parts of the world at present and water table in parts of India declining by 1 m per year (Somerville and Briscoe 2001). Mulberry (*Morus spp*), the host plant of silkworm *Bombyx mori*, is predominantly cultivated as a rainfed crop in India. When the soil moisture is lost by more than one third of the field capacity mulberry shows bumpy growth, uneven yield, and the shoot elongation stops

completely (Ohyma, 1966). As there is no other scope for periodical irrigation to the growing mulberry in most of the rainfed areas, development of a mulberry genotype tolerant/resistant to moisture stress is a priority of Indian sericulture.

The idea of the present project was conceived from the project of CSGRC, Hosur, entitled "**PIG-3432: Physiological Characterization of Selected Mulberry Genetic Resources with reference to Water and Nitrogen use Efficiency**". To identify parents for directional breeding programme, experiment was initiated with 120 diversified selected mulberry accessions from mulberry field gene bank of CSGRC, Hosur. After the initial establishment period of one year, these accessions were characterized for different physiological characters that are associated with water use efficiency (WUE) and drought tolerance under water limited conditions. As an outcome of the project, 17 promising parents are identified having early vigour, low specific leaf area, less or no leaf senescence within 65-70 days of mulberry crop period, high Relative water content, showing constant growth during stress period and potential leaf yield.

In the present proposed project, strategic trait based crossing will be carried out at CSGRC, Hosur, then seeds will be brought to CSR&TI, Berhampore for raising the F1 progenies, preliminary screening and primary evaluation. Growth and morphological parameters will be evaluated at CSR&TI, Berhampore and the biochemical and anatomical investigations will be conducted at CSGRC, Hosur. From preliminary screening, about 25 -36 promising genotypes will be selected for Primary Yield Trial and will be evaluated for two years to select 7-8 superior genotypes for setting Final Yield Trial. The present project has been proposed up to the stage of completion of Primary Yield Trial.

Brief Description of the Previous Project

PIG-3432: Physiological characterization of selected mulberry genetic resources with reference to water and nitrogen use efficiency.

The project was initiated to identify parents for directional breeding aimed at drought tolerance with 120 diversified selected mulberry accessions from mulberry field gene bank, planted in ARBD at 90 cm x 90 cm spacing. After the initial establishment period of one year, these accessions were characterized for different physiological characters that are associated with water use efficiency (WUE) and drought tolerance for subsequent one year and observed wide variation for different physiological traits apart from yield differences under waterlimited conditions. Early vigour which reduces soil evaporation and make effective use of available soil moisture, low specific leaf area, surrogate trait for WUE , Relative water content which indicates plant water status, growth during stress period which indicates turgidity, osmotic adjustment and/or deep root system, staygreen (low leaf senescence) are reported as important physiological traits. High performing accessions for these traits were listed and identified 17 mulberry accessions viz., MI-0214, MI-0768, ME-0016, MI-0025, MI-0332, ME-0244, ME-0107, MI-0699, MI-0026, MI-0256, MI-0477, ME-0125, MI-0298, MI-0762, MI-0437, MI-0763, MI-0314 based on multiple traits.

11b. AIMS AND OBJECTIVES:

Development of drought tolerant mulberry variety.

PART II : PARTICULARS OF INVESTIGATORS

12. NAME, DESIGNATION AND ADDRESS OF THE PROJECT CO-ORDINATOR.

Dr. S. NIRMAL KUMAR
Director,
CSRTI, Berhampore – Executive authority

Director,
CSGRC, Hosur, Tamil Nadu – Co-ordinator

12 a) INVESTIGATORS

Name	: **PI**: Dr. M.K. Ghosh (upto 31.3.2016), Scientist -D
Date of birth	: 11.01.1959
Sex	: Male
Address	**: Central Sericultural Research & Training Institute,** Central Silk Board, Ministry of Textiles: Govt. of India, Berhampore - 742 101, West Bengal, India

Name	: **PI**: Mr. Suresh, K., (*w.e.f.* 1.4.2016), Scientist -B
Date of birth	: 13.07.1988
Sex	: Male
Address	**: Central Sericultural Research & Training Institute,** Central Silk Board, Ministry of Textiles: Govt. of India, Berhampore - 742 101, West Bengal, India

Name	: **CI:** Dr. P.K. Ghosh, Scientist –C
Date of birth	: 06.06.1957
Sex	: Male
Address	: CSR & TI, Berhampore, West Bengal

Name	: **CI:** Dr. S. K. Dutta, Scientist-C
Date of birth	: 20.04.1957
Sex	: Male
Address	: CSRTI, Berhampore, West Bengal

Name	: **CI:** Dr. M.V. Santhakumar, Scientist-C
Date of birth	: 01.07.1964
Sex	: Male
Address	: CSRTI, Berhampore, West Bengal

At CSGRC, Hosur

Name	: **CI:** Dr. K. Jhansi Lakshmi, Scientist-C
Date of birth	: 14.07.1965
Sex	: Female
Address	: CSGRC, Hosur, Tamil Nadu

Name	: **CI:** Sri M.M. Borpuzari, Scientist-D,
Date of birth	: 01.09.1955
Sex	: Male
Address	: CSGRC, Hosur, Tamil Nadu

13. NUMBER OF PROJECTS BEING HANDLED BY EACH INVESTIGATOR AT PRESENT

1. Dr. M.K. Ghosh : 02
2. Mr. Suresh, K. : 01
3. Dr. P.K. Ghosh : 01
4. Dr. S. K. Dutta : 01
5. Dr. M.V. Santhakumar : 01
6. Dr. K. Jhansi Lakshmi : 02 (will be completed in December,13)
7. Sri M.M. Borpuzari : 02 (one will be completed in December,13)

14. PROPOSED RESEARCH FELLOWS: nil

PART III: TECHNICAL DETAILS OF PROJECT

15. INTRODUCTION:

Drought is the most devastating abiotic stress affecting crop productivity, which is caused by in sufficient rainfall and / or altered precipitation patterns (Toker *et al*., 2007). The seriousness of drought depends on its timing, duration and intensity (Serraj *et al.*, 2005). Drought is often accompanied by relatively high temperature, which promotes evaporation and effects photosynthesis, thus intensifying the effects of drought further reducing the yield. It is anticipated that occurance of drought in many regions will increase in response to climate change (Collins et al., 2008; Renolds and Ortiz, 2010)

Tolerance to drought is a complex quantitative trait controlled by several small effects and often confounded by differences in phenology (Barnahas et al., 2008: Fleury *et al.*, 2010). To address this complexity of plant response to drought, it is vital to understand and use physiological traits for improving selection efficiency. In the past drought tolerance breeding has been hindered by quantitative inheritance of the trait and our poor understanding of physiological basis of yield under water stress (Sinclair, 2011). A physiological approach has an advantage over empirical breeding for yield per se as it increases the probability of crosses resulting in additive gene action for stress adaptation, provided the germplasm is characterized more thoroughly than for yield alone (Reynolds and Trethowan, 2007). This information can be used strategically in designing crosses, thereby increasing the likely hood of transgressive segregation events which bring together desirable traits.

The conceptual frame work for yield under drought adaptation by Passioura (1977) has three important drivers 1) water uptake (WU) 2) water use efficiency and 3) harvest index. These drivers stimulate trait-based breeding.

At present, though yield potentiality of different mulberry varieties under irrigated conditions is high, the potential yield under rainfed condition ranges from 11 to 13 mt/ha/yr with recommended varieties. Though mulberry grows predominantly under rainfed condition, common experience suggests that they fail miserably against prolonged stress of water at different growth stages. None of the existing selected genotypes show versatility against severe water stress at initial stage of establishment. Against this backdrop a project has been conceived to develop, screen and evaluate genotypes under moisture stress to develop high yielding mulberry variety with drought tolerance.

15.1: DEFINITION OF THE PROBLEM

Superior high yielding mulberry varieties with adaptive features for drought tolerance will be evolved through strategic trait based crossing followed by preliminary screening of F1 progenies and primary evaluation under water stress utilizing the available information on physiological traits and identified diversified germplasm for drought stress.

15.2. ORIGIN OF THE PROPOSAL/ RATIONALE OF THE STUDY

Presently in India 1,83,773 ha of land is under cultivation of mulberry. Of which, > 50% of the national acreage and 80% of the West Bengal plantation are maintained in rainfed condition (Anonymous, 2009). This area suffers from mild to moderate degree of water limitation for prolonged periods. Presently, available commercial cultivars are prone to moisture stress at different degrees. Therefore, low yields, poor leaf quality and hence low profitability are common in rainfed sericulture. Additionally, unsustainable use of ground aquifers and non-judicious water use from natural resources create a severe strain in water availability. Such strain is increasing in India and may adversely affect the mulberry cultivation in foreseeable future.

Most agricultural annuals may be replaced with another cultivar or species better adapted to contemporary circumstances (Veikko Koski, 1996). Whereas perennial crop like mulberry which will be maintained for 10-15 years selection of variety plays important role. In the present context of climate change there is a great need to develop mulberry varieties, which can withstand different abiotic stresses particularly moisture stress. Hence, future mulberry cultivars should have high yield potential, better yield stability and high input use efficiency. Only with such cultivars it will be possible to meet the production targets without harming the environment.

Therefore, development and cultivation of mulberry which can tolerate drought and high temperature is paramount important to improve the productivity, profitability, and sustainability in sericulture under rainfed condition.

15.3: RELEVANCE TO THE CURRENT ISSUES AND EXPECTED OUTCOME

With changing scenario of the present weather conditions and global warming, moisture stress is a major factor responsible for limiting mulberry silk production in India. Apart from quality, high yielding mulberry exhibits yield loss in stress soil due to lack of adequate soil moisture that increases with vagaries of weather in rain fed sericulture. Till now in mulberry breeding a few mulberry accessions were used as parents repeatedly in spite of a lagre number of diversified germplasm available at CSGRC, Hosur. In this proposed project new germplasm accessions which were characterized for drought and were not exploited will be utilized for developing broad based genotypes with drought tolerance for sustainable sericulture.

EXPECTED OUTCOME

The expected out come of the project will be identification of 7-8 promising genotypes under moisture stress condition that can yield better than the existing rainfed varieties for further evaluation through Final Yield Trial cum Multilocational trial.

15.4 OBJECTIVES

Development of drought tolerant mulberry variety.

16. REVIEW OF STATUS OF RESEARCH AND DEVELOPMENT OF THE SUBJECT.

16.1 INTERNATIONAL STATUS

Plant suffers from water deficit when the rate of water loss from the plant exceeds the rate of uptake of water from the soil (Passioura, 1988). Water deficit elicits a complex of responses beginning with stress perception (mostly on root), initiation of a signal transduction pathway(s) (main candidate is ABA) and manifestation through series of changes at cellular, physiological and developmental levels (Bray 1993). Main objective of all changes is the conservation of available soil water; while, photosynthetic CO_2 assimilation is associated with a large amount of water loss through transpiration. Thus, to prevent desiccation induced growth injury, most plant requires adequate soil moisture. As CO_2 entry and water loss are inseparably related via stomata, water-deficit limits assimilation, and growth and in turn economic yield (Boyer 1982, Kramer 1988 and Gasper et al.2002)

On the other hand, osmotic adjustment (OA) is one of the important mechanisms to tolerate water deficit in many crops (Bohnert et al 1995). OA could be advantageous, especially at intermittent shortage of water due to variable rainfall and/ or semi-arid climates (Turner and Jones, 1980). This adjustment is reportedly related to adaptation/ acclimatization of plant with mild to moderate drought (Ingram and Barlets 1996). Such adjustment can be done by accumulating compatible solutes (as they do not interfere with normal biochemical reactions). Basically four sources of solutes are reported- a) amino acid derivatives, b) polyols, c) betaines and d) sugars (Larher *et al*, 2003). Among these two, viz, proline and betaine, are almost universal (Larher *et al*, 2003). Proline as an osmoprotectant in the mechanism of stress adaptation in mulberry has already been established (Harinasut *et al*., 2003). Contribution of glycine betaine to osmotic adjustment in the leaves of mulberry in response to water stress has also been reported (Agastin and Vivekanandan, 2000).

Under normal condition, the production and destruction of the reactive oxygen species is well regulated in plant cells. However, under environmental stress, the balance between the production and the quenching activity of the antioxidant system is upset (Dhindsa and Matowe, 1981). A consistently high antioxidant capacity under stress condition can prevent and correlate with the plant's resistance to that particular stress. Hence, metabolic processes that reduce oxidative stress are expected to play an important role in imparting tolerance in plants under stress condition (Yu and Rengi, 1999).

The breeders have long desired for simple and rapid drought selection markers, governed by lesser amount of genes, and which are expected to be more stable in different degrees of water deficit environments. However, techniques of selection for drought resistance within large population should be rapid, early detectable and sensitive to cultivar variations (Bulm 1988 and Winter *et al., 1988).* Moreover, such marker must have high correlation with yield (Bulm, 1995).

Indeed techniques adopted for such water-deficit screening includes (Farquer and Snarkey, 1982, Bulm, 1996)- a) root length, 2) membrane stability index (Sairam *et al*., 1997 & 1998; Premchandra *et al*., 1990), d) chlorophyll stability index, e) photosynthetic water use efficiency (pWUE), f) stomatal conductance, g) leaf area duration, h) transpiration use efficiency (TE; Hubick and Farquer, 1989). Lower deduction in RWC, higher membrane stability, ABA content, root biomass and lower osmotic potential were showed by drought tolerant rice genotypes (Tyagi *et al*., 1999).

Though individually most of the parameters have been studied in various crops, but little work has been done to determine the usefulness of different drought related parameters as indices of selection against drought in mulberry. Moreover, at least a few of such markers need to be measured to conclusively ascertain a polygenic trait like drought.

Renolds *et al.*, 2008 detailed key steps in strategic trait based crossing as 1) Characterizing the parents 2) strategic crossing among parents with different but potentially complementary physiological trait expression, thus ensuring cumulative gene action in the progeny 3) Early generation testing. The main objective of strategic trait based crossing is to accumulate traits that will be complementary for a given target environment.

Stomatal conductance, root traits, WUE and osmotic adjustment are some important mechanisms allowing selection for drought avoidance (Updhyay *et. al*., 2012) while cell membrane stability test helps to identify genotypes with drought tolerance. Although, in general photosynthesis is markedly reduced under drought stress, many dicot species are depend on assimilates produced from current photosynthesis under drought and therefore, exploring genotype possing efficiency mechanisms of staygreen will be beneficial (Mir *et al.*, 2012). In foliage crop like mulberry the importance of this character need not be emphasized.

As these traits were not all present in single genotype, reflecting the complexity of drought tolerance and need to pyramid several beneficial traits through mulberry breeding.

16.2 NATIONAL STATUS

Due to limitation of silkworm rearing at severe drought prone (= arid) areas (as the insect is extremely vulnerable to high temperature), mulberry is mainly cultivated in semi-arid and/ or rain fed areas and the plantations face moisture stress. Effect of moisture stress on different high yielding genotypes of mulberry has been reported from different laboratories (Dorcus and Vivekanandan 1991, Esfandarani *et al*., 2002, Chaitanya *et al*. 2002).

Drought resistance in most of the crop is a measure of economic yield in a water deficit environment (Sinha *et al*., 1988). Leaf yield is a very complex trait directly or indirectly controlled by a number of component characters, which is again governed by a wide array of physio-biochemical events, which are precisely not known (Misra *et al*., 2002). Selection on the basis of leaf component characters lead to increased leaf productivity in some mulberry varieties (Bari *et al*., 1989; Rangaswamy *et al*., 1976 and Ogiere, 1977). The moisture stress is a perpetual problem in mulberry and most of the commercial fields experience water stress at different growth stages due to inadequate precipitation and/or non-availability of irrigation water, especially during the dry months. Effect of moisture stress has been examined in some mulberry genotypes on the basis of morpho-anatomical (Susheelamma and Jolly 1986; Susheelamma *et al* 1990, and Reddy, 1993), physio-biochemical (Susheelamma and Jolly, 1986; Das *et al*, 2000; Agastin *et al.*, 2000 and Das *et al*, 2001) as well as yield trial at rain fed areas (Susheelamma *et al*, 1992, Rahaman *et al*, 1999 and Das *et al*, 2000). As an outcome a few cultivars viz. S13, S34, RFS-175 and C1730 have shown higher survival, relatively better growth and yield than the existing variety in the stress areas of India. However, these varieties still have low yield potential i.e.11 to 13 mt/ha/yr. Therefore, increase of productivity is a limiting factor to make sericulture sustainable in those areas.

In order to identify a suitable genotype tolerant to drought stress it is necessary to screen a large population of mulberry on the basis of physio-biochemical parameters as well as their potentiality to produce more leaf under moisture stress condition

16.3 IMPORTANCE OF THE PROPOSED PROJECT IN THE CONTEXT OF CURRENT STATUS

Rainfed/ Moisture stress is a major factor responsible for limiting mulberry production in India. Apart from quality, high yielding mulberry exhibits yield loss in stress soil due to lack of adequate soil moisture that intensifies by the vagaries of weather in rain fed sericulture. At this juncture, development of moisture stress tolerant mulberry genotypes suitable for drought prone area under rain fed sericulture is the need of the hour.

16.4 ANTICIPATED PRODUCTS, PROCESSES/TECHNOLOGY, PACKAGES/INFORMATION OR OTHER OUTCOME FROM THE PROJECT AND THEIR EXPECTED UTILITY

The expected outcome of the project will be identification of 7-8 Nos. drought tolerance mulberry genotypes for further evaluation through FYT-cum-Multilocational Trial at hot spots areas.

Sl. No.	Region	Existing Varieties & Yield potential	Target yield
1	Rainfed areas of Malda and Mushirabad of West Bengal	S-1, 16 MT/ha/ yr	20 MT/ha/yr
2	Rainfed and drought areas of West Bengal, Orissa and Jharkhand	C-1730 and S-1 10-12 MT/ha/yr	15-16 MT/ha/yr
3	Rainfed areas of North Eastern states	S-1, S-1635, local 15-16 MT/ha/yr	18-20 MT/ha/yr

16.5EXPERTISE AVAILABLE WITH PROPOSED INVESTIGATION GROUP / INSTITUTION ON THE SUBJECT OF THE PROJECT:

Expertise on all the parameters envisaged to be studied is available within the investigating group and all the investigators having sufficient experience in mulberry breeding and physiology.

16. WORK PLAN

17.1 Methodology: The Project has **9** steps.

Step-01: Strategic trait based crossing and rising of progenies.

Step-02: **2a:** Evaluation of hybrid progenies for traits related to yield and drought resistance.
2b. Studies on propagation efficiency and root proliferation of the short listed genotypes.

Step-03: Studies on growth, yield, in different mulberry genotypes under PYT experiment

Step-04: Studies on Physio-biochemical parameters in different mulberry genotypes under PYT experiment

Step-05: Studies on disease and pest incidence in different mulberry genotypes under PYT experiment

Step-06: Bioassay of mulberry leaves of short listed genotypes by silkworm rearing

Details of different experiments

Step-01: Strategic trait based crossing and raising of progeny

The main objective of strategic trait based crossing is to accumulate traits that will be complementary for a given target environment.

The mulberry genetic resources that were characterized and identified for different physiological traits will be utilized in the crossing programme. This information will be used in designing crosses which involves parents with different but potential complementary physiological trait expression to increase the probability of transgressive segregation events which bring together desirable traits (Renolds *et al*., 2008).

Hybridization will be planned during flowering time i.e., in December-January. To synchronize flowering staggered pruning will be done and the young females will be bagged and when it will attain receptive condition will be pollinated with desired male parent. After maturity of the fruits, F1 seeds will be collected and will be sown in seed bed in 1st week of June **at CSR&TI, Berhampore**. Populations of hybrid mulberry seedlings to be raised in the nursery following standard methods (Sarkar and Fujita, 1993a, b, c, d)**.**

Initial screening and weeding of undesirable progenies to be made in the nursery beds on the basis of visible characters of leaves like rough, hairy, papery, dissected leaves. The progenies will not be transferred into separate plot and during sowing time it will be sown very thinly giving more spaces so that populations will not be thickly populated. The seedling progenies to be left in the nursery beds without irrigation for two (April to June) summer seasons and will be labeled those progenies which survive during the drought stress and will recover fast with the onset of rainy season during July.

To be identified one or very few distinctly dominating progenies and subject them for the preliminary trials in experimental plot.

Progenies from different parents to be tested together to select the best among them.

Details of hybridization programme:

List of crosses

Sl.No.	Cross
1	MI-0437 × ME-0125
2	MI-0437 × MI-0256
3	MI-0685 × MI-0314
4	MI-0685 × MI-0308
5	MI-0828 × ME-0125
6	MI-0827 × MI-0012
7	MI-0762 × ME-0065
8	MI-0763 × MI-0012
9	MI-0477 × ME-0016
10	MI-0437 × MI-0670

List of Recipient and donor varieties

Recipient Mulberry variety	Donor Mulberry variety	Remarks
S-1, S-30, Thailand lobed, T-15, T-16 [All the varieties are well acclimatized]	Baragarh-3, Mangari, Saranath-2, Chirayinkizh, Bundi-3, Khakad-2, UP-23, UP-27, ACC-8, Hosur C15, Hosur C16, Morus F6, Hosur C-8, Nagalur Estate, Lazuraso, Bir Baghera-2	Details of characters of all the parents presented in a separate Table just after page No. 14

Step 2: **Studies on propagation efficiency and root proliferation of the short listed genotypes.**

✓ ***Study of survival*:**

The selected progenies will be multiplied and cuttings will be planted in the nursery beds and simultaneously propagation efficiency will be studied.

✓ ***Study of propagation efficiency& root proliferation*:**

Saplings will be uprooted on 90th day of plantation and data will be recorded on the following parameters:

- Length of primary root
- Fresh and dry weight of roots
- Root volume
- Root-shoot ratio

Step 3: Studies on growth and yield in different mulberry genotypes under PYT experiment

Experimental details:

Design – Simple Lattice design with 4 replications with suitable no. of saplings of each genotype in each replication
Spacing – 90 × 90 cm
No. of leaf harvests – Three crops / year
Manure – 10 MT/ha/year
Fertilizer – N: P: K:: 150:50:50
No. of selected genotypes – 25 -30.
Check variety(s) – S1 and S_{1635}
Crop Protection measure – as per recommendation evolved by this Institute

PARAMETERS TO BE STUDIED

After initial period establishment, data recording will be initiated and following parameters will be recorded under water deficit conditions

Growth, yield attributing characters -

1. Days to sprouting
2. No. of shoot / plant
3. Total shoot length
4. Longest shoot length
5. Internodal distance

6. Leaf fall %
7. Leaf shoot Ratio
8. Leaf yield / plant
9. Drought resistance index(Fisher & Maurer, 1978)

Step 4: Studies on Physio-biochemical parameters in different mulberry genotypes under PYT experiment

PARAMETERS TO BE STUDIED

Physio-biochemical parameters

1. Stomatal frequency
2. Specific leaf area
3. Total Chlorophyll content(using CCI Meter)
4. Leaf moisture content
5. Stay green (leaf senescence)
6. Growth during stress period
7. Cell membrane stability through measuring electrolyte leakages.
8. Epicuticular wax content
9. Phenol, Protein and carbohydrate content of leaf
10. Cellular compatible solutes content (proline, sugars and K)

Step 5: Studies on disease and pest incidence in different mulberry genotypes under PYT experiment

Disease and Pest incidence recording (PDI)

Some of the major diseases (Fungal and bacterial leaf spots, powdery mildew etc.) and pest infestation (whitefly, thrips, tukra etc.) will be studied in each and every crop season. Studies will be concentrated on foliar and root zones only.

Step 6: Bioassay of mulberry leaves of short listed genotypes by silkworm rearing.

Genotypes found superior in leaf yield, shall be subjected for moulting test to assess the palatability and quality of leaves with the recommended Multi × Bi hybrid silkworm. Rearing will be conducted under standard rearing practices with 3 replications. The newly hatched larvae will be given for nine feedings in the first instar considering four feeding per day against thirteen feeding for normal rearing. The curtailment of around 28% of the normal feed is done as suggested for moulting test for variety screening (Bernchamin and Anantharaman, 1990). The control set of rearing with normal feeding will be compared with the treatments. The number of moult out larvae and also their weight in the treatment will be compared with control. Maximum number of worms moulted out will be considered for selection of the genotypes. Data will be recorded on No. of moult out larvae and weight of 100 larvae.

PARAMETERS TO BE STUDIED:

1. Moulting test during 1^{st} and 2^{nd} instar
2. Weight of 50 numbers 2^{nd} stage larvae (control & treatment).

Methodology for rearing:

No. of crops – 2 commercial crop seasons
Silkworm Breed – N×NB_4D_2
Silkworms to be reared – 1 dfl / replication
Replications – 3
Incubation:-As per recommended method, i.e., 25°C ± 1°C temperature, 75-80% RH, 16 h photoperiod per day followed by black boxing.
Rearing Method:- Wrap up method.
Feeding frequency:- 4 times (6.00 AM, 11.00 AM, 4.00 PM and 8.00 PM)/ day
Leaf harvesting:- 2 times a day.
Leaf preservation:- In wooden leaf chamber covered with wet gunny cloth.

Statistical Analysis – Data will be statistically analyzed as per design of the experiment (**S**imple **L**attice **D**esign).

17.2: ORGANISATION OF WORK ELEMENTS

Sl. No.	Name of the Scientist	Designation	Time	Organisation of work elements
1.	Dr. M.K. Ghosh (upto 31.3.2016)	Scientist – D CSR&TI, BHB	25%	**Step 1 :** Strategic trait based crossing and raising of progeny **Step 2 :** Studies on propagation efficiency and root proliferation of the short listed genotypes
2.	Mr. Suresh, K. (w.e.f. 1.4.2016)	Scientist – B CSR&TI, BHB	25%	**Step 2a:** evaluation of hybrid progenies for traits related to yield and drought resistance. **Step 2b.** Studies on propagation efficiency and root proliferation of the short listed genotypes. **Step 3 :** Studies on growth and yield in different mulberry genotypes under PYT experiment **Step 6 :** Bioassay of mulberry leaves of short listed genotypes by silkworm rearing
2.	Dr. P.K. Ghosh	Scientist – C CSR&TI, BHB	15%	**Step 1:** Strategic trait based crossing and raising of progeny.
3.	Dr. S. K. Dutta	Scientist – C CSR&TI, BHB	10%	**Step 5 :** Studies on **disease** incidence in different mulberry genotypes under PYT experiment **Step 6 :** Bioassay of mulberry leaves of short listed genotypes by silkworm rearing
4.	Dr.M.V. Santhakumar	Scientist – C CSR&TI, BHB	10%	**Step 5 :** Studies on **pest** incidence in different mulberry genotypes under PYT experiment
5.	Dr. K Jhansi Lakshmi	Scientist –C CSGRC, Hosur	15%	**Step 1 :** Strategic trait based crossing and raising of progeny **Step 4(A) :** Studies on Physio-biochemical parameters in different mulberry genotypes
6.	Sri M. M. Borpuzari	Scientist-D, CSGRC, Hosur	10%	**Step 4(B):** Studies on Physio-biochemical parameters in different mulberry genotypes

17.3 PROPRIETARY / PATENTED ITEMS, IF ANY, EXPECTED TO BE USED FOR THIS PROJECT:

Not applicable

17.4 SUGGESTED PLAN OF ACTION FOR UTILIZATION OF THE EXPECTED OUTCOME FROM THE PROJECT

The identified mulberry genotypes through PYT are to evaluated in multilocation traits in target environment.

TIME SCHEDULE OF ACTIVITIES GIVING MILESTONES

Sl. No.	Milestone / Activity	Expected Date		Expected Outcome / visible/ measurable indicator
		Starting	Completion	
1	**Step-1**: Strategic trait based crossing and raising of progeny Hybridization and collection of seeds Raising of progenies of different crosses.	Jan., 2014 Aug, 2014	July, 2014 Dec., 2014	Harvesting of seeds and raising of progenies
2	Observation of the progenies for two dry seasons in the nursery bed	Jan., 2015	June, 2016	Identification and tagging of plants with labels
3	**Step-2**: Multiplication cum studies on propagation efficiency and root proliferation of the short listed genotypes.	July, 2016	Sep, 2016	Identification of superior genotypes and multiplication for setting up of PYT
4	Plantation in PYT and its establishment	Oct, 2016	May 2017	PYT establishment
5	**Step-3**: Studies on growth, yield, in different mulberry genotypes under PYT experiment	June, 2017	Oct, 2019	Identification of superior genotypes under water stress (based on replication data on growth and yield parameters)
6	**Step-4:** Studies on Physio-biochemical parameters in different mulberry genotypes under PYT experiment	June, 2017	Oct, 2019	Identification of superior genotypes under water stress (based on replication data on physiological traits)
7	**Step-5:** Studies on disease and pest incidence in different mulberry genotypes under PYT experiment	June, 2017	Oct, 2019	Identification of superior genotypes based on reaction to disease and pest incidence.
8	**Step-6:** Bioassay of mulberry leaves of short listed genotypes by silkworm rearing	Nov, 2019	Nov, 2019	Identification of superior genotypes based on moulting test & Final identification genotypes for FYT
9	Data compilation, analysis and final report submissions	Dec, 2019	Dec. 2019	

17.5. PROJECT IMPLEMENTING AGENCY/ AGENCIES :

Name of the Agency	Address of the Agency	Proposed Research Aspects	Proposed Amount	Cost Sharing %
CSB	CSB, Bangalore			100 %

PART IV: BUDGET PARTICULARS

18. **BUDGET (in Rupees):** Rs.**11.818** lakh

[In case of multi-institutional projects, the budget details should be provided separately for each of the institute]

Budget for CSR&TI, Berhampore

A. Non-Recurring (e.g. equipments, accessories, etc.) [Rupees in Lakh]:

Sl.No.	Item	1st Yr	2nd Yr	3rd Yr	4th Yr	5th Yr	6th Yr	Total
	-	-	-	-	-	-	-	-

B. Recurring:

B1. Manpower: -

Sl. No.	Position	Nos.	Consolidated Emoluments	1st Yr	2nd Yr	3rd Yr	4th Yr	5th Yr	6th Yr	Total
	JRF	NA	-	-	-	-	-	-	-	-
	HRA	NA	-	-	-	-	-	-	-	-
	CCA	NA	-	-	-	-	-	-	-	-
	Sub-total B1:	-	-	-	-	-	-	-	-	-

B2. Consumables: [Rupees in Lakhs]

Sl. No.	Item	1st Yr	2nd Yr	3rd Yr	4th Yr	5th Yr	6th Yr	Total
1.	Stationeries/ Office Contingencies.	0.20	0.20	0.20	0.10	0.10	0.10	0.90
2.	Research Operations (FYM, Fertilizers) .	0.20	0.20	0.20	0.20	0.20	0.20	1.20
3.								
	Sub-total B2:	**0.40**	**0.40**	**0.40**	**0.30**	**0.30**	**0.30**	**2.10**

Sl.No.	Item	1st Yr	2nd Yr	3rd Yr	4th Yr	5th Yr	6th Yr	Total
B3	Travel	0.50	0.50	0.50	0.50	0.50	0.50	3.00
B4	Contingency	0.15	0.15	0.15	0.15	0.15	0.15	0.90
B5	Overhead charges	-	-	-	-	-	-	-
	Sub-total (B1+B2+B3+B4+B5):	**1.05**	**1.05**	**1.05**	**2.006**	**2.006**	**2.006**	**9.168**

Budget for CSGRC, Hosur

Sl. No.	Item	1st Yr	2nd Yr	3rd Yr	4th Yr	5th Yr	6th Yr	Total
1.	Stationeries	0.20	0.05	0.05	0.05	0.05	0.05	0.45
2	Chemicals	0.00	0.20	0.10	0.10	0.10	0.10	0.60
	Sub-total B2:	**0.20**	**0.25**	**0.15**	**0.15**	**0.15**	**0.15**	**1.05**

Sl.No.	Item	1st Yr	2nd Yr	3rd Yr	4th Yr	5th Yr	6th Yr	Total
B3	Travel	0.20	0.25	0.30	0.25	0.30	0.30	1.60
B4	Contingency	-	-	-	-	-	-	-
B5	Overhead charges	-	-	-	-	-	-	-
	Sub-total (B2+B3+B4+B5):	**0.40**	**0.50**	**0.45**	**0.40**	**0.45**	**0.45**	**2.65**

PART V: EXISTING FACILITIES

19. AVAILABLE EQUIPMENT AND ACCESSORIES TO BE UTILIZED FOR THE PROJECT:

[Should be provided separately for each of the Institution]

Sl. No.	Name of the Equipment / Accessory	Required or not	Make	Model	Funding Agency	Year of Procurement
1.	WORKSHOP FACILITIES	-				
2.	WATER & ELECTRICITY	✓				
3.	STAND-BY POWER SUPPLY	✓				
4.	LABORATORY SPACE & FURNITURE	✓				
5.	AIR CONDITION ROOM FOR EQUIP	-				
6.	TELECOMMUNICATION	-				
7.	TRANSPORTATION	-				
8.	ADMIN. & SECRETARIAL SUPPORT	✓				
9.	LIBRARY FACILITIES	✓				
10.	COMPUTATIONAL FACILITIES	✓				
11.	REARING / GLASS HOUSE	✓				
12.	MULBERRY GARDEN	✓				
13.	REARING EQUIPMENT	✓				
14.	LAND	✓				
15.	LABOUR	✓				
16	SPECTROPHOTOMETER	✓				
	HOT AIR OVEN	✓				
16.	ANY OTHER					

AT CSGRC, HOSUR

Sl. No.	Name Of Equipment/ Accessories	Make	Quantity	Remarks Year Of Purchase
1	Moisture analyzer	Mettler	One	1994
2	Centrifuge	Hermle	One	1995
3	Centrifuge	Remi	One	1996
4	Computers with internet	HCL and others	Nine	1998-2012
5	Diesel Generator 30 KVA	Kirloskar	One	2003
6	Hot air oven	S .K. Enterprises	Two	2003
7	Refrigerated High speed centrifuge	Sorvall	One	1992
8	Deep freezer –20 ºC	S.K. Enterprises Bangalore	One	1995
9	Binocular microscope with photo automat	Leica,	One	1993
10	Micro oven	LG, India	One	2003
11	Hot air oven	S.K.Enterprises Bangalore	One	2003
12	Analytical balance	Simadzu, Japan	One	2003
13	Rotary Shaker	Dutta scientific co, Bangalore	One	2002
14	Poly house with misting facilities	S.K. Enterprises Bangalore	One	2003
15	Laminar Air Flow with inbuilt gas bead sterilizer	Size 3’ × 2’× 2’	One	2009
16	Kjeldahl equipment	Pelican equipments	One	2010

PART VI: REFERENCES

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