IDENTIFICATION OF WHITEFLY RESISTANCE IN MULBERRY GERMPLASM ACCESSIONS

BY

ENTOMOLOGY SECTION

Central Sericultural Research & Training Institute,
Central Silk Board,
Ministry Of Textiles, Govt. Of India
Berhampore – 742101, West Bengal

PART I: GENERAL INFORMATION

1.	Name of the Institute/University/ Organisation submitting the Project Proposal	:	Central Sericultural Research and Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, Berhampore 742 101, West Bengal
2.	State	:	West Bengal
3.	Status of the Institute	:	Research and Development
4.	Name and designation of the Executive Authority of the Institute /University forwarding the application	:	Dr. S. Nirmal Kumar, Director, Central Sericultural Research and Institute, Berhampore 742 101, West Bengal.
5.	Project Title	:	**Identification of whitefly resistance in Mulberry germplasm accessions**
6.	Category of the Project (Please tick)	:	R&D
7.	Specific Area	:	Crop Protection Entomology – Host plant resistance
8.	Duration Total Cost	:	3Years
9.	Total Cost	:	Rs. 23,15000/-
10.	Is the project Single Institutional or Multiple-Institutional (S/M) ?	:	Single institutional (S)
11.	If the project is multi-institutional, please furnish the following :	:	NA
	Name of Project Coordinator		-
	Affiliation		
	Address		

12. Project Summary (Not to exceed one page. Please use separate sheet).

Mulberry, *Morus alba* is the sole food plant of silkworms, *Bombyx mori*. Like most of the other economic plantations and field crops, mulberry is prone to the attack of a varied pest complex belonging to a large number of insect orders. Whitefly, a phytophagus insect showed varying degrees of association with particular plants species or a group of plants on which they feed. The change in the threshold of feeding stimuli plays a crucial role in host plant selection by insects. Whitefly, *Dialeuropora decempuncta* (Quaintance and Baker) and *Aleuroclava pentatuberculata* (Sundararaj & David) were observed infesting mulberry since 1994.

Whiteflies occur during monsoon and post monsoon periods regularly in West Bengal. The pest is of serious concern as it interferes with the leaf production of three seasons viz. Bhaduri (August), Aswina (September) and the most productive Agrahayani (November).

Scattered yellow specks or spots on young leaves are the first sign of the presence of the pest on the leaves. Due to excessive feeding, it drains out the nutrients causing drying of leaves and subsequently symptoms like leaf curl appear. Honey dew excreted by the nymphs of the whitefly causes development of sooty mould on the leaves. Development of sooty mould restricts the photosynthetic activity of the plants. The pest is reported to cause a leaf yield loss of 1630 kg/ha/season accounting to 24%. Thus, the damage inflicted makes the leaves unsuitable for silkworm rearing. This in turn affects the sericulture industry to a large extent (Bandopadhyay *et al*., 2000).

Host plant resistance (HPR) offers a low-cost, practical, long-term solution for maintaining lower whitefly populations and reducing crop losses. This is especially important for crops that are often grown by resource-limited smallholder farmers who cannot afford costly inputs. Mulberry is severely affected by white flies in three-crop season incurring a leaf yield loss to the tune of 24%. In order to identify promising sources of whitefly resistance the present study is proposed. In this study a preliminary screening trial to be undertaken on mulberry germplasm for identifying resistant and susceptible cultivars. Further identification of possible resistance mechanisms against the pest in the putative resistant sources is proposed. Finally the screened resistant and moderately resistant cultivars will be subjected to a no choice test simulation for confirming resistance against whitefly infestation.

PART II: PARTICULARS OF INVESTIGATORS

(*One or more co-investigators are preferred in every project. Inclusion of co-investigator(s) is mandatory for investigators retiring before completion of the project*)

13	Name: Date of birth Sex Indicate whether Principal Investigator / Co-investigator Designation Department Institute / University: Address		Dr. Manne Venkata Santha Kumar 01-07-1964 Male Principal Investigator Scientist C, Entomology CSR&TI,Berhampore
	Name: Date of birth Sex Indicate whether Principal Investigator / Co-investigator Designation Department Institute / University: Address		Mrs. Lalitha.N 26.11.1976 Female Co-Investigator Scientist B Entomology CSR&TI, Berhampore
	Name: Date of birth Sex Indicate whether Principal Investigator / Co-investigator Designation Department Institute / University: Address		Dr.S.Chattopadhyay 15-07-1961 Male Co-Investigator Scientist C Biotechnology CSR&TI, Berhampore
14.	No. of Projects being handled by each investigator at present: 1. Dr. Manne Venkata Santha Kumar 2. Mrs N.Lalitha 3. Dr. Soumen Chattopadhyay		 3 1 2
15	Proposed Research fellows		1

PART III: TECHNICAL DETAILS OF PROJECT

(Under the following heads on separate sheets)

16. Introduction (not to exceed 2 pages or 1000 words)

Mulberry eco - system of Eastern and North-eastern India is invaded by variety of polyphagus pests due to which crop losses are being recorded up to 25%. During outbreaks of these pests, due to non-availability of quality mulberry leaves farmers used to suffer total loss of silkworm crops. Chemical control measure is always not feasible, due to fixed silkworm crop schedule, which limits the time to spray and comply the safe periods for leaf harvest.

Whiteflies are considered one of the world's major agricultural pest groups, attacking a wide range of crop hosts and causing considerable crop loss. As active phloem feeders and as virus vectors, whiteflies cause major damage in several agroecosystems. The two important species infesting mulberry since 1994 include *Dialeuropora decempuncta* (Quaintance and Baker) and *Aleuroclava pentatuberculata* (Sundararaj & David). Whiteflies occur during monsoon and post monsoon periods regularly in West Bengal.

16.1 Origin of the proposal

Host plant resistance is one of the main basic components of IPM, and the utilization of resistant plants has been considered as one of the most effective components of insect control (Russell 1978). As a consequence, an increase in research aiming to favor the use of varieties resistant to pest organisms has been observed during recent years in many countries. HPR offers a low-cost, practical, long-term solution for maintaining lower whitefly populations and reducing crop losses. This is especially important for crops like mulberry that are often grown by resource-limited farmers with small land holdings who cannot afford costly inputs. Mulberry is severely affected by white flies in three-crop season incurring a leaf yield loss to the tune of 24%.

The large-scale screening or evaluation of an extensive collection of cultivars, breeding materials, hybrids or selected wild or cultivated species for whitefly resistance in mulberry offers enormous scope for identification of stable sources of resistance.

16.2 Rationale of the study

Repeated and indiscriminate use of pesticides has induced the development of insecticide resistance and devastated the crop ecosystem free of natural enemies. Whiteflies are known for developing rapid insecticide resistance. HPR holds a great promise for exploitation in IPM programmes due to unique features like ecofriendly, specificity, compatibility with other IPM tools, persistence etc. It offers better prospects in unique situations where chemical control measures are not always feasible or difficult to be executed as silkworm crop schedule is fixed, that limits the time to spray and comply with the recommended safe periods for leaf harvest. Identification of resistant sources and elucidation of resistance mechanisms like, antixenosis, antibiosis or tolerance against whitefly infestation can go a long way in suppressing the whitefly populations within the ETL and preventing economic damage.

16.3 Relevance to the current issues and expected outcome:

Host plant resistance offers a low-cost, practical, relatively long-term solution for maintaining lower whitefly populations and reducing crop/ foliage losses. There is practically little/no information on resistance mechanisms in mulberry against whitefly infestation. Hence the present study is proposed envisaging the identification of resistant and susceptible cultivars of mulberry by screening the available germplasm pool of CSR&TI, Berhampore. The screened resistant and moderately resistant cultivars subjected to no choice test simulation, confirming resistance against whitefly infestation would be a best source of breeding material with whitefly resistance. Further, basic information on the resistance mechanisms operating against the major pest whitefly in mulberry ecosystem can be elucidated. This information is useful in formulating low cost and effective ecofriendly management strategies.

16.4 OBJECTIVES

1. Screening of mulberry germplasm and identification of putative sources of resistance and susceptibility for whitefly infestation.
2. Evaluation of the possible resistance mechanisms in mulberry cultivars against whitefly infestation.

16.5 Current status of research and development in the subject (both international and national status):

International Status

Whitefly host plant resistance research has increased considerably since 1990, primarily due to the rise in importance and damage caused by the *B. tabaci* species complex. This species complex feeds on a wide range of crops, causing yield losses due to direct-feeding damage and as vectors of numerous Gemini viruses (De Ponti *et al.,* 1990; Brown *et al*., 1996, Drost *et al*., 1998). HPR studies with whitefly species including *Trialeurodes abutilonea* on soybeans (McPherson, 1996; Lambert *et al*., 1997); *Bemisia afer* on cassava in Malawi (Munthali, 1992); *T. vaporariorum* on tomatoes in the Netherlands (De Ponti *et al*., 1990, Van Giessen *et al*., 1995), *T. vaporariorum* on peppers in Europe (Laska *et al*., 1986); and *Aleurotrachelus socialis* on cassava in Colombia (Bellotti *et al*., 1999).

White fly resistance studies in cotton:

Diverse research has been carried out to compare the biotic potential of whiteflies in different cultivated plants such as sesame, beans, cucumber, cantaloupe, zucchini, cassava, corn, poinsettia, cabbage and tomato (Morales and Cermeli 2001; Villas-Bôas *et al.* 2002). However, most studies focused on testing different cultivars or accessions as potential sources of resistance to *B. tabaci* on a single crop. There have been many studies searching for host plant resistance on cotton because of the severity of whitefly problems in this crop, mostly investigating the relative resistance of different cotton genotypes (Meagher *et al.* 1997; Wahla *et al.* 1998; Chu *et al.* 2002; Syed *et al.* 2003; Ripple 2004). Recent field studies in California's Imperial Valley revealed consistent very high levels of resistance against the silverleaf whitefly in *Gossypium thurberi* Todaro, a wild cotton species native to Mexico and parts of the southwestern USA (Walker and Natwick 2006). However, the mechanisms of this resistance remains an enigma because both choice and no-choice experiments comparing oviposition and nymphal survival among *G. thurberi* and commercial cotton cultivars did not detect antibiosis or antixenosis (Walker and Natwick 2008).

White fly resistance studies in tomato:

Resistance to whiteflies and other insects found on the wild species of tomato *Solanum pennellii* (Corr.) D'Arcy were mostly attributed for the past to the presence of sugar esters in the glandular exudate of type IV trichomes (Gentile et al. 1968; Heinz and Zalom 1995). It was therefore concluded that selection for sugar ester accumulation should be an efficient technique in selecting for general insect resistance in *S. pennellii* (Goffreda *et al.* 1990) and other tomato plants (Kisha 1981; Berlinger 1986). Choice and non-choice assays revealed a correlation between resistance to *B. tabaci* (reported as *B. argentifolii*) and type IV trichome densities of six wild accessions of *Solanum habrochaites* (reported as *L. hirsutum*); the accessions included LA1777 (Snyder et al. 1998).

White fly resistance studies in peas and beans:

Elite germplasm from the peanut breeding program at the University of Florida (USA) and several commercial cultivars were evaluated for resistance to B. argentifolii, but only two genotypes supported fewer whiteflies (although not significantly) than the cultivar "Southern Runner" and no resistance was found in the peanut germplasm tested (McAuslane *et al.* 1995b). Another study was carried out to determine whether soybean could be used as a trap crop to reduce whitefly infestation in peanut and whiteflies preferred soybean (McAuslane *et al.* 1995a). Research to evaluate whitefly resistance in mungbean (*Vigna radiata* L.) has been conducted in the past, especially in Pakistan. The resistance level of 19 common bean (*Phaseolus vulgaris* L.) genotypes to whiteflies was studied for 2 years in Brazil, with variable results depending on the plant age and the rainy or dry season (Boiça *et al.* 2008). Thirty-eight plants of alfalfa (*Medicago sativa* L.) were evaluated for resistance to *B. argentifolii* in California, USA; 17 of them displayed low whitefly infestation and were categorized as potentially resistant. The plants were propagated vegetatively so that replicated measurements of whitefly performance could be made on each genotype. After different greenhouse and field assays, four genotypes demonstrated high whitefly resistance and three demonstrated moderate resistance (Teuber *et al.* 1999; Jiang *et al*. 2003).

White fly resistance studies in Cassava:

Much of the research on whitefly resistance in cassava (*Manihot esculenta* Crantz) in recent years has been carried out at the International Center for Tropical Agriculture, in Colombia, and several cultivars were identified with high levels of resistance to the cassava whitefly, *Aleurotrachelus socialis* Bondar (Bellotti and Arias 2001). However, research on cassava resistance to *B. tabaci* continues to be limited. Nevertheless, a range of new cassava elite clones were assessed in experimental fields of the International Institute of Tropical Agriculture, in Ibadan, Nigeria, and the researchers found that cassava genotypes 96/1089A and TMS 30572 supported the lowest whitefly infestation in all locations (Ariyo et al. 2005). Three genotypes (PN 2KS, SH 3322 and IBD-2KS) of sunflower (*Helianthus annuus* L.) were found to be resistant against *B. tabaci* among nine genotypes tested in Pakistan, with a negative correlation between pest population and yield of genotypes (Aslam and Misbah-ul-Haq 2003).

White fly resistance studies in Cucurbitaceous crops:

Research on host plant resistance to *B. tabaci* in melon (*Cucumis melo* L.) is relatively recent, with most of the results obtained during the last decade. Simmons and McCreight (1996) developed a method in a 2-week open-choice greenhouse test to screen germplasm of 31 selected melon entries based on whitefly immature density and changes in plant biomass and tolerance. Another screening of 8 cultivars of export cantaloupes by counts of eggs, live nymphs and pupae, showed that ‘Amarelo’ and ‘Concorde’ were the most resistant cultivars, with no correlation between whitefly densities and leaf pubescence (Morales 1997). This contradicted previous and later results which suggested the presence of the glabrous character of leaves as a resistance factor (Riley and Palumbo 1995a, b; Riley *et al.* 2001). Soria *et al.* (1999) reported the existence of genetic resistance to *B. tabaci* in genotypes, *C. melo* variety agrestis 87 and *C. melo* TGR-1551. In the French West Indies, field trials have been conducted since 1997 to test a number of genotypes from the germplasm collection of INRA-Avignon, France. Results indicated that 10 genotypes had potential partial resistance against the B-biotype of *B. tabaci* and that this resistance would be independent from resistance to *Aphis gossypii* (Boissot and Pavis 1999). Later assays indicated that three Indian accessions, PI 414723, PI 164723, and 90625, and one Korean accession, PI 161375, had partial resistance to *B. tabaci*. Although current levels of resistance in commercial watermelon is quite inadequate, some useful sources of germplasm have been identified that can be used for the improvement of this crop and to incorporate resistance from wild species such as *C. colocynthis* (L.), into advanced breeding lines (Simmons and Levi 2002; Simmons et al. 2006). Different breeding lines and varieties of *Cucurbita pepo* L. (zucchini and yellow crookneck squash) and accessions of two wild species, *C. ecuadorensis* Cutler and Whitaker and *C. martinezii* Bailey, were evaluated for resistance to *B. argentifolii* and for severity of silvering symptoms, but no clear relationship was found between both factors (McAuslane et al. 1996). Other screenings have been conducted to compare whitefly resistance in different genotypes of *C. moschata* and *C. maxima* (Wessel-Beaver 1997a; Baldin *et al.* 2000).

White fly resistance studies in Soybean:

In a study under greenhouse conditions, the main squash cultivars available in the Brazilian market were compared for resistance to the B-biotype of *B. tabaci* (Alves *et al.* 2005). Whitefly resistance in soybean (*Glycine max* L.) has been carried out during recent years mostly in the USA, Brazil, Pakistan and India. Significant differences in *B. argentifolii* densities were observed among 14 soybean genotypes in Georgia (USA), with Perrin, Cook and N88-91 harboring the lowest mean numbers of whiteflies (McPherson 1996; Lambert et al. 1997). Different field and controlled condition trials were conducted in Brazil to evaluate oviposition, non-preference and antibiosis of *B. tabaci* biotype B on different soybean genotypes (Lima *et al.* 2002; Lima and Lara 2004). The obtained results strongly suggest that the resistance observed in some of these genotypes was stable (Do Valle and Lourencao, 2002). In Pakistan, 23 varieties of soybean were tested; G-9956 and AGS-344 were the most resistant against B. tabaci (Khaliq *et al.* 2000).

Influence of morphological traits on resistance:

The structure of cotton leaves was suggested to have potential for breeding whitefly-resistant upland cotton cultivars (Chu *et al*. 1999). However, as occurred with tomato, the actual effect of this factor on *B. tabaci* populations has been broadly questioned. For example, whitefly adults and nymphs showed positive correlations with hair density and length of hair on leaf lamina, midrib and vein (Raza *et al*. 2000; Bashir *et al*. 2001; Aslam *et al*. 2004). However, while hair density on midrib and gossypol glands on veins was positive and high significant correlation to whitefly population, the length of hair on leaf lamina was negatively and highly significantly correlated (Sial *et al.* 2003). Research carried out in Arizona, USA (Chu *et al*. 1998, 1999, 2002) to identify cotton plant characteristics related to *B. tabaci* colonization agreed with previous results (Ellsworth *et al*. 1993; Norman and Sparks 1996, 1997) that hairy leaf cotton cultivars harbor higher populations compared with glabrous cultivars. More recently, Chu *et al*. (2000, 2001) reported that other factors, including leaf color, morphology and leaf-age related effects on lysigenous glands and leaf trichome densities, may affect *B. tabaci* biotype B oviposition and nymphal densities. In another screening for cotton resistance against B-biotype of *B. tabaci*, adults, eggs and nymphs were significantly correlated to leaf hairiness, with seasonal variability due to leaf color, shape, and hairiness types (Alexander *et al.* 2004). A brief review by Walker and Natwick (2006) pointed out the mixed and sometimes contradictory results presented in these and other previous studies which associate the two different traits in cotton (smooth-leaf and okra-leaf) with reduced whitefly susceptibility, while in other studies, a slight effect, no effect, or even the opposite effect occurred. All these results together indicate that many morphological plant traits cumulatively contribute to whitefly population fluctuation (Sial *et al*. 2003).

Glossy-leaf phenotypes (‘SC Glaze’, ‘SC Landrace’, ‘Green Glaze’) of collard (*Brassica oleraceae* L.) were found to be the most resistant to *B. tabaci* as compared with normal non-glossy cultivars and hybrids (Jackson *et al.* 2000). The glossy leaf characteristic results from phenotypes which express a reduced amount of leaf surface wax, and this gives the leaf a glossy appearance. Non preference appears to be the primary mode of resistance in certain collards. Studies on the relative resistance or susceptibility of four sesame cultivars (*Sesamum indicum* L.) were conducted in Pakistan, and ‘PR-14-2’ was found to be the least susceptible to *B. tabaci* (Wadhero *et al*. 1998). Additional relevant research was conducted in Venezuela to characterize six sesame genotypes in relation to foliar acidity and the presence or absence of certain secondary metabolites, by an analysis of principal components based on these features. This analysis separated two sesame genotypes with greater foliar acidity values, which harbored less eggs and nymphs of whiteflies (nearly 10 and 50% of the total incidence in the other genotypes), relating foliar acidity with resistance of sesame to whiteflies (Laurentin *et al.* 2003). Low abundance, parasitism and oviposition rates of *B. argentifolii* on certain soybean isolines in Florida (USA) were related to low trichome density (McAuslane et al. 1995a; McAuslane, 1996).

Influence of biochemical constituents on resistance:

Studies have focused on the relationship of biochemical constituents of cotton leaves with whitefly populations. Significant negative correlation for total phenols, o-dihydroxy phenols, gossypol and tannins with egg, nymph and adult was established. Reducing sugars showed a significant positive correlation for egg and nymph, but not to the colonization of adults. Total and nonreducing sugars did not show any significant correlation with insect population (Raghuraman *et al.* 2004).

Multigenic resistance to *B. tabaci* in tomato accession LA1777 was demonstrated to be correlated with the presence of type IV trichomes (Momotaz et al. 2005). Similarly, leaf-trichome densities and presence of acyl sugars in the exudate of glandular trichomes as well as type of trichomes were reported to be important factors affecting whitefly-tomato relationships (Williams et al. 1980; Kisha 1981; Berlinger 1986; Kishaba et al. 1992; Simmons 1994; Freitas et al. 2002; Baldin et al. 2005; Srinivasan and Uthamasamy 2005; Simmons and Gurr 2005; Sanchez-Pena et al.2006).

Nombela et al. 2000 studied two commercial cultivars of tomato, 'Alta' and 'Peto 95', the accession LA716 of *S. pennellii* and lines 94GH-006 and 94GH-033 (backcrosses between 'Peto 95' and LA716), with different leaf acyl sugar contents were screened for resistance to the Spanish B-biotype of *B. tabaci* in greenhouse and field no-choice experiments. There was no oviposition on LA716 (with the highest acyl sugar content) while the greatest fecundity and fertility values were observed on the cultivar Alta (no acyl sugar content). However, no clear relationship was found between the low acyl sugar content in the other tomato cultivars tested and whitefly reproduction. Resistance to *B. tabaci* appears to be independent of acyl sugar content below a threshold level of 37.8 µg cm–2 leaf.

Glabrousness, trichome density, latex, acylsugars and glossy foliage have also been linked to resistance. Glabrous cotton cultivars resulted in lower oviposition and few nymphs

(Butler *et al.*, 1992, Navon *et al.*, 1991), while glabrous-leafed melons (*Cucumis melo*) were found to reduce numbers of whitefly stages (adults and nymphs), when compared to commercial pubescent-leafed cultivars (Riley *et al.*, 2001). Higher phenolic and o- dihydroxy phenolic content of cotton cultivars resulted in fewer eggs oviposited by the Bemisia complex (Butler *et al.*, 1992), and vascular bundle depth was negatively related to *B. argentifolii* adult and nymph densities (Chu *et al.*, 1998).

NATIONAL STATUS

In India, soybean line DS 1016 was consistently found to be a promising source of resistance to *B. tabaci* (Sridhar and Siddiqui 2001; Sridhar *et al.* 2003). To highlight the results obtained during the past decade, out of 23 mungbean accessions, VC2755A was least susceptible while VCA 82 was the most susceptible accession (Arutkani and Ayyanathan 1999; Fargali *et al.* 1996). Moreover, NM-92 and NM-98 showed significantly lower mean whitefly population/leaf as compared with three other tested varieties (Khattak *et al.*2004). Wide screening assays were conducted in India on many genotypes of *Mentha arvensis*, *M. piperita*, *M. cardiaca* (*M. gracilis*), *M. citrata* (*M. piperita var. citrata*) and *M. spicata*, and their resistance potential against *B. tabaci* was compared (Singh and Singh 2004; Singh et al. 2004). Nine sweet-pepper (*Capsicum annuum* L.) genotypes obtained from the Asian Vegetable Research and Development Centre (Taiwan) and local cultivars were screened at the University of Agricultural Sciences, Bangalore (India); none were suitable for the development of *B. tabaci* (Maruthi *et al.* 2003). Tomato plants of the insect tolerant accession, Varushanadu Local mutagenised with EMS at 0.2 per cent had the minimum whitefly population (15.27 numbers/plant) and the maximum plant height (Selvanarayanan and Muthukumaran,2005).

Although HPR research on whiteflies is now being carried out in more than 15 countries, US researchers are the most active, followed by those in India, Israel and, to a lesser extent, Spain and Egypt. The range of agricultural crops being evaluated for whitefly resistance is increasing, primarily as a result of the wide host range of the *B. tabaci* species complex. Included in this list are numerous legumes [common beans (*Phaseolus vulgaris*), soybeans, mung beans, snap beans, groundnuts, alfalfa and cowpeas], cucurbits (melons, squash, cucumbers and zucchini), brassicas (cabbage, collards, broccoli), solanaceous crops (tomatoes, egg plant, tobacco and potatoes) and others (cotton and okra) (Tables 1 and 2). In several cases antixenosis (non preference for oviposition or feeding) or tolerance appears to be the resistance mechanism in operation (Table 2).

Table 1. Examples of HPR screening or evaluations of crop germplasm for resistance to *B. tabaci* species complex.

Crop	Country	Genotypes		Reference
		Evaluated	Selected	
Alfalfa	USA	73 plants from 10,000 1/2sib (F)	2 families with resistance	Teuber *et al*., 1996
Brassica oleraceae	USA	64 (F, C)	Glossy leaves associated with resistance	Farnham & Elsey, 1995
Common beans	Puerto Rico	41 (F)	?	Blair & Beaver, 1993
Common beans	Puerto Rico	4 (G)	2 genotypes less preferred	Peña Rojas *et al*., 1992
Cotton	Turkey	19 (F)	3	Ozgur & Sekeroglu, 1986
Cotton	Israel	3 (F)	1 (glabrous)	Navon *et al*., 1991
Cotton & wild relatives	USA	19 (F)	1 (wild species)	Wilson *et al*., 1993
Gossypium spp.	USA	24 (F, G)	4 genotypes low eggs/nymphs	Meagher *et al*., 1997
Groundnuts	USA	150 (F)	0 (no resistance)	McAuslane *et al*., 1995b
Melons	USA	31 (G)	8 (less damage)	Simmons & McCreight, 1996
Melons	Venezuela	8 (F)	2	Morales & Bastidas, 1997
Soybeans	USA	14 (F)	3	Lambert *et al*., 1997
Soybeans	USA	36 (F)	7	McPherson, 1996
Summer squash	USA	19 (F)	Differences in susceptibility	Paris *et al*., 1993
Tomatoes	India	1200 (F)	3	Channarayappa *et al*., 1992
Tomatoes-commercial	USA	20 (L)	(ovipositional differences)	Heinz & Zalom, 1995
Wild tomatoes	USA	7 (L)	2	Heinz & Zalom, 1995

(F) = field, (G) = greenhouse, (L) = laboratory, (C) = cages

Table 2: Crops with genotypes reported showing some resistance to the *B. tabaci* species complex.

Crop	County	Resistance Mechanism /Factor	Reference
Squash melon	USA	Tolerance	Cardoza *et al*., 1999
Melons	Venezuela	Antixenosis	Morales & Bastidas, 1997
Soybeans	USA	Antixenosis	Lambert *et al*., 1995
Tomatoes	India	Antixenosis (trichomes)	Channarayappa *et al*., 1992
Tomatoes	USA	Trichome density	Heinz & Zalom, 1995
Lettuce	USA	Latex (entrapment)	Dussourd, 1995
Tomatoes (wild)	USA	Acylsugars	Liedl *et al*., 1995
Cotton	USA	Not indicated	Smith *et al*., 1998
Cotton	Spain	Tolerance (varietal release)	Gutierrez, 1997
Soybeans	USA	Glabrousness	McAuslane, 1996
Broccoli	USA	Glossy foliage	Farnham & Elsey, 1995
Melons	USA	Glabrousness	Riley *et al*. 2001

16.4. Anticipated products, processes/technology packages, information or other outcome from the project and their expected utility:

There is practically little/no information on resistance mechanisms in mulberry against whitefly infestation. Hence the present study is proposed envisaging the identification of resistant and susceptible cultivars of mulberry by screening the available germplasm pool of CSR&TI, Berhampore. The screened resistant and moderately resistant cultivars subjected to no choice test simulation, confirming resistance against whitefly infestation would be a best source of breeding material with whitefly resistance. Additionally, resistance mechanism studies against the pest offer enormous scope for identification of stable sources of resistance. The generated basic information on the resistance mechanisms operating against the major pest whitefly in mulberry ecosystem can be utilized in the breeding programmes, formulating low cost and ecofriendly management strategies. Host plant resistance offers a low-cost, practical, relatively long-term solution for maintaining lower whitefly populations and reducing crop/ foliage losses.

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

Adequate expertise is available with proposed investigation group for carrying out the proposed project.

Name of the Scientists	Designation	Experience
Dr. M.V. Santha Kumar	Sc-D	Has been working since 22 years for Integrated management of major pests of mulberry with special emphasis on biological and botanical means. He has carried out 10 research projects pertaining to various aspects of Integrated pest Management.
Mrs. N.Lalitha	Sc.C	Having competence in agricultural entomology and host plant resistance studies with adequate knowledge and experience in the area of the proposed project
Dr. Soumen Chattopadhyay	Sc-D	Has been working in biotechnology section with special emphasis on biochemical investigations with respect to resistance.

Name and address of 5 experts in the field

Sl. No.	Name	Designation	Address
1.	Dr. K.C. Narayanaswamy	Professor	Department of Sericulture University of Agricultural Sciences, GKVK, Bangalore
2	Dr.Selvanarayanan	Professor	Faculty of Agriculture, Annamalai University
3.	Dr.A.M.Ranjith	Professor	Entomology Section, Kerala Agricultural University, Thrissur.
4.	Dr. U. Sreedhar	Principal scientist	Head, Division of Entomology, Central Tobacco Research Institute, ICAR, Rajahmundry, Andhra Pradesh
5.	Dr. N. Bakthavatsalam	Principal Scientist & Head	Division of Insect Ecology National Bureau of Agriculturally Important Insects, ICAR, H.A.Farm Post, Bellary Road, Bangalore.

17. Work Plan:

17.1 Work plan (methodology/experimental design to accomplish the stated aim)
Methodology:

E-01 Preliminary screening of germplasm for resistant and susceptible cultivars in the field (free choice test simulation) and bioassay studies to ascertain the impact of whitefly infested leaves on rearing.

Field screening of mulberry germplasm for resistance to whiteflies will be done when natural whitefly populations are high and damage levels are significant so as to distinguish susceptible cultivars. Whitefly (adult and nymph) feeding damages are most noticeable with the prominent symptoms of chlorosis, leaf curl, sooty mould and leaf fall. Feeding induces a yellow-to-green mottled appearance and twisted or curled leaves, eventually resulting in chlorosis and defoliation. Field evaluations of mulberry germplasm use a population (nymph and pupae) scale combined with a leaf-damage scale as in table1. Evaluations are done periodically throughout the growing cycle. From 4-5 evaluations are done, 1 ½ to 2 months apart. The purpose of the initial screening of accessions selected from the germplasm bank is to identify and discard susceptible genotypes. From 3 to 6 stem cuttings of each cultivar are planted in 2 replicates. Periodic evaluations were made during the peak infestation period of whitefly, record leaf damage and populations of immature whiteflies. Nymphs are usually observed on the mid-to-upper leaves, and pupae are found on the mid-to-lower leaves of the plant. This methodology makes it possible to compare plant (leaf) damage and corresponding whitefly populations.

Table1.Population and damage scales for evaluating resistance to whiteflies

Population scale (nymphs / adult)	Damage scale
1 = no whitefly stages present	1 = no leaf damage
2 = 1-10 individuals per leaf	2 = tender leaves showing mild chlorosis
3 = 11-20 per leaf	3 = leaf curling symptoms
4 = 21- 40 per leaf	4 = Initiation of sooty mould symptoms
5 = > 40 per leaf	5 = Total manifestation of sooty mould symptoms

The presence of high whitefly populations does not ensure uniform infestation in a mulberry field. Pockets of low populations and, therefore, low-selection pressure are often observed. Consequently some accessions showing little or no damage, or low populations may actually be "escapes." A common susceptible cultivar is planted strategically throughout a screening block to measure the whitefly population levels, distribution and damage. The yield depression caused by whitefly feeding is an additional indication of the levels of resistance present in a particular cultivar.

Whitefly incidence will be recorded by counting the number of life stages per top two leaves (adults), middle two leaves(early nymphs) and bottom two leaves (late nymphs) from a fixed plot for bio assay studies. Leaf yield data will be recorded after 45th day of pruning and calculated per hectare basis by multiplication with the conversion factors. To ascertain the harmful and deleterious effect of the whitefly infested leaves, bioassay will be conducted.

E-02 Confirmation studies for screened resistant and moderately resistant cultivars in a no choice test simulation.

Varietal resistance is further evaluated in the laboratory by studying the effect of resistance on whitefly biology and behaviour. The detailed biology of whitefly, i.e. developmental period, survival, fecundity, longevity and sex ratio were studied under 'no choice' conditions, by confining the whiteflies in leaf cages. The study will reveal the incubation period, nymphal developmental time, total developmental time, nymphal survival, total survival, fecundity and female longevity that are most important biological parameters for detecting the presence of antibiosis in resistant genotypes against whiteflies. On the other hand, pupal period and survival, male longevity and sex ratio do not seem to play important roles in antibiosis.

Selected resistant cultivars and susceptible controls are grown from stem cuttings in pots and infested with whiteflies (No-choice test). Infestations are made by attaching small (2.5 cm in diameter) clip cages to mulberry leaves, held in place with a rigid rod imbedded in the soil. Ten whitefly females are introduced into each cage and left to oviposit for 24 h, after which the cages and adults are removed. The whitefly-infested plants are maintained in a growth chamber, where temperature (average 27°C), humidity (68± % RH), and photoperiod (12:12 h day/night) are regulated (Arias, 1995). Those accessions selected as "promising" for resistance (i.e. low damage ratings and low whitefly populations) are reevaluated in subsequent cycles. It is common for an accession to be evaluated 6-7 times before it is considered resistant.

E03 Estimation of acyl sugars and trichomes in screened resistant and moderately resistant cultivars along with identified susceptible cultivars.

Leaf structure (primarily pubescence) and some specific constitutive chemical profiles are critically important determinants of whitefly varietal preference**.** The trichomes, or leaf hairs, have long been considered as a plants first line of defense against many herbivores. These epidermal protuberances are known to act as physical and/or chemical barriers against many pests including whiteflies. The leaf surface of mulberry species contains various types of glandular and non-glandular trichomes (Kesavacharyulu *et al* 2004; Kumar 2011). Glandular trichomes of different plants secrete various acyl-sugars, glucose and sucrose esters of fatty acids of different chain lengths. These acyl-sugars have been reported as an effective defense against white flies in various solanaceous plants (Chortyk *et al.* 1996). Many acyl-sugars, present in the leaf surface are often associated with moderate to high heritability values (Maluf *et al.,* 1997). Therefore, estimation of foliar acyl-sugars would therefore appear to be an effective indirect selection technique for white fly resistance (Nombela *et al.* 2000; Liedl *et al.* 1995). As glandular trichomes are quite abundant in most of the mulberry species, an effort would be made to identify resistant germplasm resources to white fly using acyl-sugar content as an additional selection tool. Acyl sugars in mulberry leaf discs will be estimated spectrophotometrically according to the method of Resende *et al.* (2002). Total two observations will be assured during peak season of whitefly incidences.

17.2: Organisation of work elements:

Name	Designation	Time	Work to be done
Dr. S.Nirmal Kumar, Executive Authority	Director		Over all coordination, guidance & periodical review of progress made.
Dr. A.K.Saha, Co-ordinator	Scientist- D		Coordination and guidance
Dr. M.V. Santha Kumar Principal Investigator	Scientist - D	45%	Data collection directly associated with E 01 and E 02, Compilation and interpretation of data, submission of report time to time and final report preparation.
Mrs. N. Lalitha Co-Investigator	Scientist- C	45%	Data collection, directly associated with E 01 and E 02, Compilation and interpretation of data,submission of report time to time .
Dr.S.Chattopadhyay Co-Investigator	Scientist - C	10%	Directly associated with E 03

17.3: Proprietary / patented items, if any, expected to be used for this project –
NA

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

The screened resistant and moderately resistant cultivars confirming resistance against whitefly infestation would be a best source of breeding material with whitefly resistance. The generated basic information on the resistance mechanisms operating against the major pest whitefly in mulberry ecosystem can be utilized in the breeding programmes, formulating low cost and eco friendly management strategies. Host plant resistance offers a low-cost, practical, relatively long-term solution for maintaining lower whitefly populations and reducing crop/ foliage losses in efficient and eco-friendly tool for conserving the ecosystem.

17.5. Time schedule of activities giving milestones

Sl.no	Activity	Symbol	Preceding Activity	Estimated time
1	Preliminary screening of germplasm for resistant and susceptible cultivars in the field (free choice test simulation)	A	-	2yrs
2	Confirmation studies for screened resistant and moderately resistant cultivars in a no choice test simulation.	B	-	1yr 6 m
3	Biochemical analysis	C	A	1yr
4	Data analysis, Compilation of results and outcome of the project work and submission of report.	D	ABC	6 m

Timelines:

Time scale	Year 1				Year 2				Year 3			
Quarter	1	2	3	4	1	2	3	4	1	2	3	4
Preliminary screening of germplasm for resistant Free choice test (Field)												
Screening of resistant and moderately resistant cultivars in a no choice test simulation (Field)												
Biochemical analysis (Laboratory)												
Data analysis,Compilation of results												

Activity table providing quantifiable outputs

Period of study	Achievable targets
1st year	Field screening of mulberry germplasm accessions for resistance to whiteflies will be done during peak seasons of whitefly infestation to distinguish susceptible cultivars. Scoring of whitefly population scale combined with a leaf-damage scale. Evaluations are done periodically throughout the growing cycle. From 4-5 evaluations are done per year. The purpose of the initial screening of accessions selected from the germplasm bank is to identify and discard susceptible genotypes. The selection of putative resistant genotypes completed. From the selected susceptible and resistant sources 3 to 6 stem cuttings of each cultivar are planted in 2 replicates. Periodic evaluations were made during the peak infestation period of whitefly, record leaf damage and populations of immature whiteflies. Nymphs are usually observed on the mid-to-upper leaves, and pupae are found on the mid-to-lower leaves of the plant. This methodology makes it possible to compare plant (leaf) damage and corresponding whitefly populations. Bioassay will be conducted with whitefly infested leaves with respect to control to ascertain the harmful effect of the whitefly infested leaves.
2nd year	Varietal resistance is further evaluated in the laboratory by studying the effect of resistance on whitefly biology and behaviour. The detailed biology of whitefly, i.e. developmental period, survival, fecundity, longevity and sex ratio were studied under 'no choice' conditions, by confining the whiteflies in leaf cages. Selected resistant cultivars and susceptible controls are grown from stem cuttings in pots and infested with whiteflies (No-choice test). Infestations are made by attaching small (2.5 cm in diam.) clip cages to mulberry leaves. Ten whitefly females are introduced into each cage and left to oviposit for 24 h, after which the cages and adults are removed. The whitefly-infested plants are maintained in a growth chamber, where temperature (average 27°C), humidity (68± % RH), and photoperiod (12:12 h day/night) are regulated. Acyl sugar estimation will be initiated in the selected genotypes screened in the field conditions as susceptible, moderately resistant and resistant sources.
3rd year	Those accessions selected as "promising" for resistance (i.e. low damage ratings and low whitefly populations) are reevaluated in subsequent cycles. Acyl sugar estimation and trichome observation will be continued in the selected genotypes screened in the field conditions as susceptible, moderately resistant and resistant sources. Data analysis, Compilation of results and outcome of the project work and submission of report.

17.6. Project Implementing Agency/ Agencies

Name of the Agency	Address of the Agency	Proposed Research Aspects	Proposed Amount	Cost Sharing %
Central Silk Board	BTM Layout, Madivala, Bangalore	Basic plus applied	13,15,000	100%

18. **PART IV: BUDGET PARTICULARS (in Lakhs) :** [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.)NA

Sl no.	Item	1st year	2nd year	3rd year	Total
A]	Land				
B]	Building				
C]	Vehicle				
D]	Equipments				
	1.Green House	1500000			1500000
E]	Furniture				
F]	Fan & fixtures				
G]	Tools, plant & machineries				
Total					1500000

Sub-Total (A) =1500000

B. Recurring

B.1 Manpower (See guidelines at Annexure-III)

S. No.	Position No.	Consolidated Emolument	Year 1	Year 2	Year 3	Total
1	JRF(CSR&TI, Berhampore)	16000/- pm for first 2 yrs and 18000/- pm afterwards	192000	192000	216000	600000
						600000

Sub-Total (B.1) = 600000

B.2 Consumables

S. No.	Item	1st Year	2nd Year	3rd year	Total
1.	Chemical	------	25,000	25,000	50,000
2.	Clip cages	2000	2000	2000	6,000
3.	Speciman jars	10000	10000	10000	30,000
4.	Specimen tubes	5000	2000	2000	9,000
5.	Test tubes, plastic containers	10000	10000	10000	30,000
6	Forceps, Needles, Brushes muslin cloth, Entomological pins, absolute alcohol and miscellaneous items	10000	10000	10000	30,000
	Sub-Total	37,000	59,000	59,000	155000

Sub-Total (B.2) =155000

Other Items:

S.No.	Item	1st Year	2nd Year	3rd Year	Total
B3	Travel	15000	15000	15000	45000
B4	Contingency	5000	5000	5000	15000
	Total	20000	20000	20000	60000
Subtotal (B1+B2+B3+B4etc.)					815000
Grand Total (A + B)					**2315000**

Note : Please give justification for each head and sub-head separately mentioned in the above table.

Financial Year : April - March

In case of multi-institutional project, the budget estimate to be given separately for each institution.

PART V : EXISTING FACILITIES

Resources and additional information

1. Laboratory:
   a. Manpower

   JRF: 1No. For collecting the extensive field data on incidence of whitefly on the available germplasm resources in free choice and no choice simulation conditions at CSR&TI, Berhampore and to conduct studies on developmental biology of the pest on putative resistant and susceptible sources.

   b. Equipments

   Green house:

   This facility is essential requirement for confirmation of resistance mechanism by no choice tests in E01. Further all simulation studies can be conducted with this facility and hence it will be a permanent asset to the Entomological Laboratory.

2. Other resources such as clinical material, animal house facility, glass house. Experimental garden, pilot plant facility etc. NA

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