CHARACTERIZATION OF *Bemisia tabaci* (Gennadius) (HEMIPTERA: ALEYRODIDAE), FOR GENETIC VARIABILITY, ENDOSYMBIONTS AND VECTOR-VIRUS INTERACTIONS IN CASSAVA

By

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(2013-21-106)

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2018

DECLARATION

I hereby declare that this thesis entitled **"Characterization of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), for genetic variability, endosymbionts and vector-virus interactions in cassava"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Characterization of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), for genetic variability, endosymbionts and vector-virus interactions in cassava"** is a bonafide record of research work done independently by **Mr. Harish E. R. (2013-21-106)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

Cassava whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most notorious invasive insect pests reported, infesting more than 900 species of plants and spreading more than 200 plant viral diseases.

Tuber crops, which include cassava, are the third most important group of food crops after cereals and grain legumes. Cassava Mosaic Disease (CMD) is a major limiting factor in cassava production, causing 30-40 per cent reduction in the yield (Malathi *et al.,* 1985). Cassava whitefly, *B. tabaci* is the vector responsible for transmission of *Cassava mosaic virus* in cassava.

The cassava mosaic disease (CMD) was first reported from East Africa in 1894 (Fauquet and Fargette, 1990). Since then, epidemics have occurred throughout the African continent, resulting in great economic loss and devastating famines. In Indian subcontinent, Abraham (1956) had reported the presence of CMD. It is also known as Indian cassava mosaic disease (ICMD) and is caused by *Indian cassava mosaic virus* (ICMV) or by *Sri Lankan cassava mosaic virus* (SLCMV; Patil *et al.*, 2005).

Polyphagous nature of *B. tabaci* makes it a complex species and genetic variation exists among the population in different cassava growing areas. Among various whitefly populations, some are more important than others, as far as disease transmission ability is concerned. However, no systematic study has been conducted so far on genetic variations among *B. tabaci*, which infests cassava from different agro-ecological zones of Kerala.

Recent developments in molecular biology have resulted in several tools and techniques to analyze genomic variation at both individual and population level (Black *et al.*, 2001) in *B. tabaci*. The DNA based marker system such as RFLP, RAPD, AFLP, SSRs, SNPs have profound uses in areas *viz.*, molecular ecology, molecular entomology, molecular systematics, population dynamics and diagnostics (Morin *et al.*, 2004).

This polyphagous agricultural pest is known to harbour diverse bacterial communities in its gut which are reported to perform many diverse functions in whiteflies that contribute to the polyphagous nature and general fitness of the host. Literature suggests endosymbionts present in the whitefly system could be a factor responsible for making them a successful sucking pest, virus transmitting agent and formation of different biotypes. A detailed study in these aspects could be useful in planning management strategies against the pest.

There are various kinds of interaction existing between whitefly and virus. The virus manipulates the behaviour of the vector to enhance the transmission efficiency and spread of its own kind. Studying these interactions precisely will help to understand the behavioural and physiological variations in whiteflies and this information could be a valuable tool to formulate management tactics against the cassava mosaic disease

Hence, the present investigation on "Characterization of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), for genetic variability, endosymbionts and vector-virus interactions in cassava" has been undertaken with the following objectives:

1. Analyzing the genetic variability in cassava whitefly
2. Characterization of endosymbionts in cassava whitefly
3. Elucidation of cassava whitefly - *Cassava mosaic virus* interactions

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Review of Literature

2. REVIEW OF LITERATURE

Tuber crops, which include cassava, are the third most important food crops after cereals and grain legumes. While considering the main limiting factor in cassava production, Cassava Mosaic Disease (CMD) stands out, causing on an average 30-40 per cent reduction in the yield. Cassava whitefly, *B. tabaci* is the vector responsible for transmission of *Cassava mosaic virus* in cassava. *B. tabaci* is one of the most important invasive insect pests reported, infesting more than 900 species of plants and spreading more than 200 plant viral diseases. Polyphagous nature of *B. tabaci* makes it as a complex species and genetic variation exists among the population in various cassava growing areas. Among various whitefly populations, some may be more important than others, as for as disease transmission ability is concerned. Only scanty information is available at present.

Literature suggests endosymbionts present in the whitefly system could be a factor responsible for making them as a successful sucking pest, virus transmitting agent and formation of different biotypes. A detailed study in these aspects could be useful in planning management strategies against the pest. There are various kinds of interaction existing between whitefly and virus. The virus manipulates the behaviour of the vector to enhance the transmission efficiency and spread of its own kind. Studying these interactions precisely will help to understand the behavioural and physiological variations in whiteflies and this information could be a valuable tool to formulate management tactics against the cassava mosaic disease. Hence the present study is proposed. The literature pertaining to the objectives of the study are presented hereunder.

2.1. Cassava

Manihot esculenta Crantz, commonly known as cassava is an important energy rich food for 450-500 million people in 26 tropical and subtropical countries. Cassava originated from the wild form *M. esculenta* of sub species *flabellifolia* from west central Brazil where it was domesticated for the last 10,000years. The plant is semi-perennial and normally matures in 10 months and is unique in tolerance to drought and establishment in poor soil. Cassava is unmatched for its capacity in carbohydrate production. i.e. 2,50,000 calories per hectare per day (Palaniswami *et al.*, 1996).

It is woody shrub belonging to family, *Euphorbiaceae* and its centre of origin is South America. Cassava is the third largest source of food carbohydrates in the tropics, after rice and maize (Fauquet and Fargette, 1990). In the developing world, cassava is a major staple food, providing a basic diet for over half a billion people (FAO, 1995).

The crop has been cultivated in India for more than a century. Portuguese introduced cassava into India, when they landed in Kerala state in 17th century, from Brazil. In India, the cultivation of cassava is mainly done in Kerala, Tamil Nadu, Andhra Pradesh, Nagaland, Meghalaya, Assam, etc. Tamil Nadu stands first both in area and production followed by Kerala and Andhra Pradesh (Edison *et al.*, 2006).

2.1.1. Cassava mosaic disease

The symptoms of the disease include chlorotic areas intermixing with normal green tissue gives mosaic pattern. In severe cases, leaves are reduced in size, twisted and distorted, reducing chlorophyll content and photosynthetic area. It causes 25-80% yield reduction (Edison *et al.*, 2006).

The first report of cassava mosaic disease (CMD) was from East Africa in 1894 (Fauquet and Fargette, 1990). Since then, epidemics have occurred throughout the African continent resulting in great economic loss and devastating famine. The first report of CMD in the Indian subcontinent was documented by Abraham (1956). It is known as Indian cassava mosaic disease (ICMD) and is caused by *Indian cassava mosaic virus* (ICMV) or by *Sri Lankan cassava mosaic virus* (SLCMV; Patil *et al.*, 2005).

There are three distinct species of circular single-stranded DNA viruses that are whitefly-transmitted and primarily infect cassava plants in Africa. They are, *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), and *South African cassava mosaic virus* (SACMV). Related species of viruses (*Indian cassava mosaic virus*, ICMV) are found in India and neighbouring islands (*Sri Lankan cassava mosaic virus*, SLCMV). Based on genomic sequencing and phylogenetic analysis, nine species of cassava-infecting *Geminiviruses* have been identified so far (Patil and Fauquet, 2010).

Cassava mosaic virus belongs to family *Geminiviridae* and the genus *Begomovirus*. Since the first report of Cassava mosaic disease (CMD) from East Africa in 1894, epidemics have occurred throughout the African continent. The result was great economic loss and devastating famine. In 1971 a resistant line of cassava, the predominant host of this plant pathogenic virus, was established and used by the International Institute of Tropical Agriculture in Nigeria. This resistance worked as an effective control for many years. However, in the late 20th century, a more virulent virus broke out in Uganda and quickly spread to East and Central Africa (Legg and Fauquet, 2004). This highly virulent virus was later discovered to be a chimaera of two distinct *Begomovirus* species (Patil, and Fauquet, 2010).

There has been fewer yield loss studies on CMD in India than in Africa and no estimates have been made of overall losses in the sub-continent. Reductions in weight of tuberous roots of 84% were reported in the first experiments with a susceptible local variety (Narasimhan and Arjunan, 1974). In other experiments losses were 42% in the variety Kalikalan, ranged from 17 to

36% in nine selected hybrids and were 17% in M4 which was at the time considered to be tolerant of infection (Malathi *et al.,* 1985). Losses were even less in a later trial with M4 (7-10%) and four hybrid varieties (9-21%) and there was a positive relationship between yield loss and symptom severity scores (Nair and Malathi, 1987). The disease is likely to have substantial effects in areas of India where CMD-sensitive varieties are grown and severe symptoms are prevalent.

2.2. Cassava whitefly, *Bemisia tabaci* (Gennadius)

This is one of the most notorious invaders in the world. It is a polyphagous sap sucking insect which feed more than 900 host plant species and also is a vector of 111 plant viruses according to Global Invasive Species Database (2017).

Whiteflies are insect pests of significant economic importance affecting agricultural crops such as tomatoes, cotton, cassava and beans, as well as ornamentals. Of importance is the fact that they have worldwide distribution and as such are commonly known insect pests and vectors to entomologists, virologists, agriculturists and growers. They are about one mm in length, and wings are present in the adult stage of both sexes. The wings are generally opaque and covered with a whitish powder or wax. Abdomen lacks cornicles (tubular structures located dorsally towards the posterior end of the abdomen), and the hind wings are nearly as long as the forewings (Borror *et al.*, 1989).

Unlike many Homopterans that undergo paurometabolous development (gradual metamorphosis), the metamorphosis of whiteflies is different showing apattern more towards complete metamorphosis (holometabolous development). Borror *et al*. (1989) describes the metamorphosis as “Intermediate”. There are five instars including the adult. The first instar is active, while the following three are inactive or sessile. During metamorphosis wing development is internal and the wing pads are averted at the end of the third instar, appearing in the fourth instar which resembles a pupa. Most whitefly species are oligophagous, but most whitefly pest species are polyphagous. There are however some oligophagous whitefly pest species such as *Aleurocybotus* spp. and *Aleurolobus* spp. that affect plants in the Family: *Gramineae*, and *Asterochiton* spp. affecting plants of *Acer* spp. (Byrne *et al.*, 1990).

Many whitefly pest species are multivoltine, producing several generations a year. They also tend to develop resistance rather quickly to a large number of pesticides (Byrne *et al.*, 1990). As vectors of pathogens, although bacteria and fungi can be transmitted by whiteflies (Costa, 1976) whitefly pest species are of greater economic importance as vectors of plant viruses. Some *Geminiviruses, Carlaviruses, Nepoviruses, Potyviruses* and *Closteroviruses* can be transmitted by this group of insects (Byrne *et al.*, 1990).

Brown (1994) reported 1,100 species of whiteflies worldwide, and only three are recognised as vectors of plant viruses. Of this number, *B. tabaci* is considered the most important of the whitefly vectors of plant viruses, and the only whitefly species transmitting *Geminiviruses* (Duffus, 1987; Harrison, 1985). Additionally, *B. tabaci* can also cause direct damage through sucking phloem sap and secretion of honeydew, which in particular, has caused serious problems in the cotton industry (Pollard, 1955). They produce different symptoms in its hosts; which include the "silverleaf" condition in squash, tomato irregular ripening and broccoli light stalk (Toscano *et al.*, 1994). The Indian subcontinent is the suspected centre of origin of *B. tabaci* based on the presence of a large number of its natural enemies in that region. It has been suggested that the spread of the species probably occurred from the Indian subcontinent to Africa, Europe and the Americas, through the movement of plant material by man (Cock, 1986).

Under favourable conditions *B. tabaci* can undergo 11–15 generations a year (Avidov, 1956), and a female can lay between 100 and 300 eggs in her lifetime, which varies from 3 to 6 weeks (Azab *et al.*, 1971; Bethke *et al.*, 1991). Early taxonomic separation of whitefly species was for the most part dependent on morphological characteristics of the pupal case (Gill, 1992). This approach however, had some weaknesses because morphological characteristics can be altered based on species adaptation to a specific host. For example Russel (1957) reported that the early literature identified several genera and species of whiteflies that are now grouped under the single species, *B. tabaci;* some 18 previously described *Bemisia* species are now classified as *B. tabaci.* International collections of *B. tabaci* were shown to be genetically variable (Costa *et al.*, 1993; Brown, 1994). Wool *et al.* (1993) reported that such populations differed in the ability to utilise specific host plants for feeding and reproductive purposes. These populations also showed differences in virus transmission characteristics (Bedford *et al.*, 1992 and 1994).

Out of many host plants, cassava is one of the most preferred hosts for the insect, as it was evident from higher survival and reproductive rates. According to Van Lenteren and Noldus (1990), shorter development time as well as greater total oviposition on a host reflects the suitability of the plant. Preferential feeding on cassava has been reported by several authors (Bethke *et al.,* 1991; Lisha *et al.,* 2003). Thompson (2000) studied the development and morphometrics of whitefly, *B. tabaci* on cassava and a description was made of the different nymphal instars and other biological characteristics.

Cassava mosaic disease spreads naturally in India and following earlier experience in Africa the main attention has been on *B. tabaci* in the search for an insect vector. Successful transmissions have been reported using whiteflies transferred from infected to healthy cassava, from infected cassava to herbaceous hosts and between herbaceous hosts. High rates of transmission were achieved in some experiments, as between cassava (19%) and from cassava to *Nicotiana tabacum* cv. Jayasri (100%), *N. rosulata* (67%) and 11 other *Nicotiana* spp. (20-50%) using 50 whiteflies per test plant (Mathew and Muniyappa, 1993). Through the application of DNA sequencing, Fauquet *et al.* (1998) were able to identify a distinctive population of *B. tabaci* suspected as the driving factor for the cassava mosaic disease (CMD) epidemic in Uganda.

Presence of biotypes in *B. tabaci* is reported for the first time in India from Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala (Lisha *et al.*, 2003). Later, Palaniswami and Henneberry (2011) reported *Bemisia* biotypes in India. Literature suggests various biotypes in cassava whitefly shows variation in their behavior (Carrillo *et al.*, 2007).

The existence of different *B. tabaci* whitefly biotypes was proposed in the 1950s, to describe distinct populations of insects based on host plant adaptation and the ability to transmit viruses (Brown *et al.*, 1995). This species is considered to be a species complex (Berry *et al.*, 2004), with a broad host spectrum.

The whitefly *B. tabaci* has become an important pest of field crop agriculture and horticulture throughout the world (Byrne *et al.*, 1992; Brown, 1994; Butler and Henneberry, 1994). It was collected and described from tobacco, *Nicotiana* spp., as *Aleurodes tabaci* in Greece in 1889 (Russell, 1957). The first record of occurrence of *B. tabaci* in India was from cotton in 1905 (Misra and Lambda, 1929; Reddy and Rao, 1989). It became an economic cotton pest in Punjab (now in Pakistan) by 1919. Different *B. tabaci* biotypes have been suspected in India (Palaniswami and Nair, 1995).

Presence of biotypes in *B. tabaci* is reported for the first time in India from Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala (Lisha *et al.*, 2003). Later, Palaniswami and Henneberry (2011) reported *Bemisia* biotypes in India. Literature suggests various biotypes in cassava whitefly shows variation in their behavior (Carrillo *et al.*, 2007). Comparative morphology, morphometrics and ISSR markers are used as tools to study the population genetic structure in *B. tabaci* (Dong *et al.*, 2008; Li *et al.*, 2013).

Recent studies have indicated that this complex is composed of at least 24 distinct and morphologically indistinguishable species that mainly differ in their ability to transmit *phytoviruses*, adapt to hosts, and induce physiological changes in certain hosts (do Valle *et al.*, 2013).

B. tabaci is widely distributed worldwide and is composed of a complex of more than 24 morphologically indistinguishable cryptic species (Dinsdale *et al.*, 2010; De Barro *et al.*, 2011). The 3.5% pair wise genetic divergence identified by Dinsdale *et al.* (2010) as being the boundary separating different species is further supported by evidence for either complete or partial mating isolation between a number of the putative *B. tabaci* "species" (Xu *et al.*, 2010; Wang *et al.*, 2011).

2.2.1. Morphological and molecular characterisation of cassava whitefly, *Bemisia tabaci*

So far only a single biotype/ genetic group of *B. tabaci* has been reported as a pest in cassava. It is known as cassava-strain (Lisha *et al.*, 2003) or Asia II-5 genetic group (Ellango *et al.*, 2015). Genetic variants (different genetic group) in cassava whitefly could be a warning sign and could lead to more difficulty in tackling the pest and virus it carries. It was known that genetic variations can happen in insects based on geographical variations (Yu *et al.*, 2012).

Morphometric variations found to be present among various biotypes and studies were conducted on this aspect by Li *et al.* (2013) and results indicated that differences of some morphological characters or morphometrics were significant among the six biotypes of *B. tabaci* in China. According to Gill and Brown (2010), in terms of taxonomy or systematics, morphology is considered the foremost basis for species separation, and it is convenient for identification as well (Yan, 2001).

According to Li *et al.* (2013) comparative morphometrics of puparium and adults of *B. tabaci* can be used to distinguish different biotypes. Morphological characters of whiteflies, *Bemisia* spp., from 17 populations from disparate locations worldwide were compared by Rosell *et al.* (1997) and the characters of 4th instars (pupae) are used for separating *Bemisia* spp. They assessed variability in the following characters of the 4th instar: anterior submarginal setae, anterior and posterior were fringes, dorsal setae, posterior submarginal setae, caudal setae, and tracheal folds. The study by Chaubey and his co-workers (2015a) evaluated the morphometric variations of developmental stages, puparia, and adults in three putative species of *B. tabaci* occurring in India. The genetic identity of these studied populations were confirmed by *mtCO1* analysis and revealed that the population from Amravati, Ludhiana, and Delhi were clustered with Asia I, Asia II1, and Asia II7 putative species, respectively. The morphological comparisons showed that fourth instar and adult of Asia-II1 was comparatively larger than Asia-I and Asia-II7.

Perring *et al.* (1993) conducted whitefly species identification by genomic and behavioral studies. Genetic identity of the 3 cryptic species of *B. tabaci,* Asia I, Asia II-1 and Asia II-7 is confirmed by studying their life history traits (Chaubey *et al.*, 2015b). Thomas and Gaur (2014) collected *B. tabaci* specimens from three cotton cultivars and were subjected to genetic characterization and morphometric studies. Genetic diversity in the populations using mitochondrial cytochrome oxidase 1 (mtCO-1) sequences revealed the presence of three genetic groups namely Asia 1, Asia II 1 and Asia II 7 groups. For morphometrics, 62 morphological characters on puparia were considered. The principal component analysis revealed a wide range of morphological variations in the critical taxonomic characters among the populations. Contrastingly, puparial length was more in Asia II 1 genetic group whereas breadth of puparia, length of antenna, lingula and vasiform orifice were more in Asia I genetic group.

2.2.1. 1. Molecular markers in the genetic variability study of *Bemisia tabaci*

Molecular markers are fragments of nuclear, mitochondrial or chloroplast DNA, which are linked with the gene of interest and hence acting as representative of the gene. Molecular marker analysis based on polymorphism in DNA, can be considered as objective measures of genetic variations and have catalyzed research in a variety of disciplines such as phylogeny, taxonomy, ecology, genetics and plant breeding. Markers are informative only if, they are polymorphic in populations. Level of polymorphism is an important determinant of what a marker is useful for. Different types of molecular markers with different properties exist, each with its own advantages and disadvantages (Karp *et al.,* 1997; Weising *et al.,* 2005).

However, it is extremely difficult to find molecular markers which could adequately meet all the ideal properties (Lowe *et al*., 2004). Depending upon the type of the study to be undertaken, one can identify between varieties of marker systems that could fulfil the objective of the study (Weising *et al*., 2005). Many authors also suggest the use of more than one type of molecular marker in a single experiment (Karp *et al.,* 1997). The DNA based marker systems are generally classified as hybridization-based (non-PCR) markers and PCR based markers (Joshi *et al*., 1999). The value of PCR based molecular markers is influenced by several consideration such as the speed, cost and technical simplicity, but must be sufficiently informative to distinguish between the most individuals (Charters and Wilkinsons, 2000).

2.2.1.1.1. Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction (PCR) technique has been the basis of a growing range of new techniques for genome analysis, based on the selective amplification of genomic DNA fragments (Saiki *et al*., 1988). Williams *et al.* (1990) reported the use of PCR with short oligonucleotide primers of arbitrary (random) sequence to generate markers, the basis of the Random Amplified Polymorphic DNA (RAPD). Welsh and McClelland (1990) also reported on Arbitrarily Primed Polymerase Chain Reaction (AP-PCR). DNA Amplification Fingerprinting (DAF) was also reported as another technique of PCR used in various experiments (Caetano-Anolles *et al*., 1991). The PCR reaction requires deoxynucleotides, DNA polymerase, primer, template and buffer containing magnesium (Taylor, 1991). Typical PCR amplification utilises oligonucleotide primers which hybridise to complementary strands. The product of DNA synthesis of one primer serves as template for another primer. The PCR process requires repeated cycles of DNA denaturation, annealing and extension with DNA polymerase enzyme, leading to amplification of the target sequence. This results in an exponential increase in the number of copies of the region amplified by the primer (Saiki *et al.*, 1988). The technique can be applied to detect polymorphism in various plants, animals, bacterial species and fungi.

The introduction of the PCR technique has revolutionized standard molecular techniques and was allowed for the proliferation of new tools for detecting DNA polymorphism (Hu and Quiros, 1991). The electrophoresis pattern of fragments generated by each primer for one isolate can be used as DNA fingerprints for assaying diversity (Tommerup *et al.*, 1998). Polymorphism between two individuals is generally scored as presence or absence (non-amplification) of a particular DNA fragment. The absence may result from deletion of a priming site or insertion rendering site too distant for successful amplification. Insertion can change the size of a DNA fragment without preventing its amplification (Williams *et al.*, 1990). PCR is simple, fast, specific, sensitive and the main advantage of this technique over others is its inherent simplistic analysis and the ability to amplify extremely, small quantities of DNA (Welsch *et al.*, 1991).

The limitation in using morphological, physiological and cytological markers for assessing genetic diversity and population dynamics have been largely circumvented by the developments in DNA based markers (Cruickshank, 2002). Molecular markers in nature are neutral to the stages of development, physiological status and environmental influences (Black *et al.*, 2001; Haeckel, 2003).

2.2.1.1.2. Analysis using dominant random primers

RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism) and ISSR (Inter Simple Sequence Repeats) primers are dominant random primers, used for the genetic variability studies of

B. tabaci. These primers are very useful in the genetic variability studies of closely related populations. Among them, RAPD primers are simple and rapid, but suffer from a certain lack of reproducibility. Even though, most labour intensive and time-consuming, RFLP provides the highest degree of polymorphism. ISSR primers provide highly discriminating information with better reproducibility, and are relatively abundant (Costa *et al.*, 2016).

2.2.1.1.2. 1. Analysis using RAPD primers

RAPD is a PCR based marker system; where genomic segments are amplified using oligo nucleotide primers. Generally random decamer primers are used to prime the synthesis of DNA from homologous sites on the test DNA in PCR. A diverse array of molecular technique is available for high resolution of genetic studies of population level processes. Among them, Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) using a single primer amplifies many regions of genomic DNA (Williams *et al.*, 1990).

RAPD markers were used (Perumal *et al.*, 2009; Lima *et al.*, 2002) in the genetic variability studies of *B. tabaci*. Qiu *et al.* (2009) identified three major *B. tabaci* biotypes in China based on morphological and DNA polymorphisms. In this study, these three biotypes were identified based on their morphological characteristics, RAPD–PCR analysis, and DNA sequences of the CO1 gene. The anterior and posterior wax fringes of the B, Q, and Cv biotypes significantly differed from each other. Based on this morphological characteristic, the three biotypes can easily be distinguished in greenhouses and fields. Genomic DNA RAPD–PCR band patterns also revealed differences between these biotypes using the H_{16} primer.

Abdullahi, *et al.* (2003), collected *B. tabaci* populations, from cassava and other plants in major cassava-cultivation areas of Sub-saharan Africa and from elsewhere around the world, and were studied to determine their biotype status and genetic variation. RAPD-PCR markers were used to examine the genetic structure of the populations. Analysis of the internally transcribed spacer region I (ITS 1) of the ribosomal DNA confirmed that the cassava populations of *B. tabaci* populations were distinct from non-cassava populations.

Bao-Li *et al.* (2003) collected twenty three *B. tabaci* populations on vegetables, ornamental plants and weeds from 23 different cities of 14 provinces in China. Biotypes of the different populations were identified and differentiated using RAPD-PCR method with a primer H_{16}. The results showed that 17 whitefly specimens from 11 provinces were B biotype. Sharma *et al.* (2008), analysed host associated genetic variations in whitefly, *B. tabaci* using RAPD-PCR and suggested that the whitefly types holding specificity for different host plants under study have evolved as three distinct genetic groups formed by cotton, sida and soyabean (Group 1); potato and brinjal (Group 2); and tomato (Group 3).

Khasdan *et al.* (2005) in their study, various DNA markers have been developed, applied and compared for studying genetic diversity and distribution of biotypes. For developing sequence characterized amplified regions (SCAR) and cleaved amplified polymorphic sequences (CAPS) techniques, single random amplified polymorphic DNA (RAPD) fragments of B and Q biotypes, respectively, were used.

2.2.1.1.2. 2. Analysis using AFLP primers

AFLP-PCR or just AFLP is a PCR-based tool used in genetics research, DNA fingerprinting, and in the practice of genetic engineering. AFLP uses restriction enzymes to digest genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments is then selected to be amplified. This selection is achieved by using primers complementary to the adaptor sequence, the restriction site sequence and a few nucleotides inside the restriction site fragments. The amplified fragments are separated and visualized on denaturing polyacrylamide gels, either through autoradiography or fluorescence methodologies, or via automated capillary sequencing instruments. The AFLP technology has the capability to detect various polymorphisms in different genomic regions simultaneously. It is also highly sensitive and reproducible. As a result, AFLP has become widely used for the identification of genetic variation in strains or closely related species of plants, fungi, animals, and bacteria (Vos *et al.*, 1995).

Çervera *et al.* (2000) studied genetic similarities between 13 samples belonging to nine reference biotypes and two field populations of *B. tabaci*, one field population of *B. medinae* Gómez-Menor and another of *B. afer* Priesner and Hosny, were evaluated using amplified fragment length polymorphism (AFLP) markers. *B. tabaci* biotypes were grouped in four clusters which comprised: (i) Near East and Indian subcontinent biotypes; (ii) B and Q biotypes plus a Nigerian population from cowpea; (iii) New World A biotype; and (iv) S biotype and a Nigerian population from cassava. *B. tabaci* biotypes were grouped in four clusters which comprised: (i) Near East and Indian subcontinent biotypes; (ii) B and Q biotypes plus a Nigerian population from cowpea; (iii) New World A biotype; and (iv) S biotype and a Nigerian population from cassava. The AFLP assay allowed the scoring of a total of 354 polymorphic bands in two reaction events with the use of two primer combinations

The diversity and genetic differentiation of a subset of 19 populations of *B. tabaci* were studied in China, using cDNA amplified fragment length polymorphism (AFLP) Guo *et al.* (2012). They determined that these Chinese haplotypes included 2 invasive species (MEAM1 and MED), and 4 indigenous cryptic species (Asia II 1, Asia II 3, China 3 and Asia II 7).

2.2.1.1.2. 3. Analysis using ISSR primers

ISSRs are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16-18 bp). About 10-60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of particular size. Because the analytical procedures include PCR, only low quantities of template DNA are required. Furthermore, ISSRs are randomly distributed throughout the genome. Because of the multilocus fingerprinting profiles obtained, ISSR analysis can be applied in studies involving genetic identity, parentage, clone and strain identification, and taxonomic studies of closely related species (Godwin *et al.*, 1997). Since an ISSR may be a conserved or non conserved region, this technique is not useful for distinguishing individuals, but rather for phylogeography analyses or maybe delimiting species (Fakruddin *et al.*, 2006).

When the analysis was conducted based on ISSR primers by Perring *et al.* (1993), about 10% genetic similarity was observed between biotype A and biotype B of *B. tabaci.* ISSR primers are used as tools to study the population genetic structure in *B. tabaci* (Dong *et al.*, 2008). *B. tabaci* biotypes B and Q are two invasive biotypes in the species complex. The comparison of the population genetic structure of the two biotypes is of significance to show their invasive mechanism and to their control. ISSR marker was used to analyze the 16 B-biotype populations and 4 Q-biotype populations worldwide with a *Trialeurodes vaporariorum* population in Shanxi Province, China, and a *B. tabaci* non-B/Q-biotype population in Zhejiang Province, China, was used as control populations. Cluster analysis suggested that it could easily distinguish the biotypes of *B. tabaci.* The difference of the population genetic structure between the biotype B and the biotype Q exists based on the ISSR marker.

Using ISSR marker technology, genetic diversity of 80 samples of 8 natural populations of *B. tabaci* collected from 7 regions of Fujian province, China was studied (Yu *et al.*, 2012). The 1776 bands were amplified with 4 ISSR primers from the DNA of 80 samples, and in that, 1728 bands were polymorphic. The results showed that genetic diversity of *B. tabaci* in natural populations were quite abundant in Fujian province. According to the UPGMA clustering based on the genetic distance between the populations, 8 populations were classified into 2 groups. In addition, a certain correlation was observed between the geographic distance and the genetic distance of the populations.

2.2.1.1.3. Analysis using mitochondrial cytochrome oxidase 1 primer and DNA barcoding

Mitochondrial genome is maternally inherited. Any changes in the mitochondrial DNA (mtDNA) are transmitted to the entire progeny. Evolutionary changes in the conserved regions of mtDNA spread rapidly within population. The Cytochrome Oxidase-I (CO-I) region of mtDNA is the most studied region of the insect mitochondrial genome. Analysis of this mtDNA provides insight to understand the natural genetic diversity and population structures in organisms (Avise, 1994).

Among the genetic markers *mtCO1* has gained widespread prominence during the past eight years (Dai *et al.*, 2012). The increasing number of species complexes or cryptic species is a main concern in this era. The selection of the genes and methods of analysis has been the central problem. The 5 prime segment of the *mtCO1* gene selected as the universal barcoding region is found less effective in some taxon groups (Hebert *et al.*, 2003; Elias *et al.*, 2007). Some studies have suggested the Internal Transcribed Spacerl region (ITS1-ribosomal DNA repeating unit) as a candidate DNA barcode (Gao, 2010; Chen *et al.*, 2010).

Many researchers (Frohlich *et al.*, 1999; Legg *et al.*, 2002; Berry *et al.*, 2004 and Hsieh *et al.*, 2006) used the primers for the genetic variability studies in *B. tabaci*. Dong *et al.* (2007) studied genetic differentiation of different geographical populations of *B. tabaci* complex. The genetic differentiation was further analyzed on the basis of the sequences of mtDNA CO1 and rDNA ITS1 recorded in the world's GenBank. Five groups are defined on the basis of mtDNA CO1 and rDNA ITS1, including the Asia group, America group, Africa group, Australia group, and Biotype B/Mediterranean/Middle East/Northern Africa/Biotype Ms group. Mitochondrial DNA variation of *B. tabaci* populations, infesting cassava in Kenya was studied by Atuncha and his co-workers (2011) and the results revealed two distinct haplotypes of cassava associated *B. tabaci* in Kenya.

The genetic variability of whitefly (*B. tabaci*) species, in cassava growing areas of Kenya, Tanzania, and Uganda, was investigated through comparison of partial sequences of the mitochondria cytochrome oxidase 1 (*mtCO1*) DNA. Two distinct species were obtained including sub-Saharan Africa 1 (SSA1), comprising of two sub-clades (I and II), and a South West Indian Ocean Islands (SWIO) species (Mugerwa, *et al.*, 2012). Seal *et al.* (2012) used Next Generation Transcriptome Sequencing (NGS) and Quantitative Real-Time PCR (RT-PCR) technologies for characterisation of the *B. tabaci* Asia 1 *mtCO1* phylogenetic clade. Shah *et al.* (2013) reported a new genetic variant of *B. tabaci*, Asia-II-7 (Cv biotype) from vegetable fields of Pakistan using *mtCO1* primer. Jahan *et al.* (2015) identified three genotypic cluster of indigenous whitefly BW1, BW2 and BW3 are from Bangladesh. Manani *et al.* (2017) carried out phylogenetic analysis of the two *B. tabaci* species present in Kenya, sub-Saharan Africa and it was comprising of five distinct clades (A–E) with percent sequence similarity ranging from 97.7 % to 99.5%.

Chowda-Reddy, *et al.* (2012) could identify genetic groups, Asia I, Asia II-5, Asia II-7and Asia II-8. The biotype-B of *B. tabaci* from India is found clustered into the Middle East-Asia Minor 1 group. A new group of *B. tabaci* from Coimbatore is also collected from pumpkin and was related most closely to the Asia I group (6.2% sequence divergence from the consensus Asia I sequence). A host specific molecular variation of *B. tabaci* is revealed, using mitochondrial and ribosomal marker by Ellango, *et al.* (2014) in Karnataka, India.

Thomas *et al.* (2014) confirmed the presence of three putative species Asia 1, Asia II 1, and Asia II 7 on the cultivars of cotton in India, using sequence analyses using mitochondrial cyctochrome oxidase I. Roopa, *et al.* (2015) investigated the prevalence of a new genetic group, MEAM-K, of the whitefly *B.*

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tabaci in Karnataka, India, as evident from *mtCO1* sequences. Ellango *et al.* (2015), surveyed *B. tabaci* populations across India and the blast analysis of sequenced 850 bp of the mitochondrial CO1 (*mtCO1*) gene, with sequences from the *mtCO1*dataset showed the presence of one invasive group, MEAM1, and eight other groups of *B. tabaci* in India. i.e., Asia I, Asia I-India, Asia II-1, Asia II-5, Asia II-7, Asia II-8, Asia II-11 and China-3. Also, found that more than one genetic group is coexisting in the same field.

2.2.2. Characterization of endosymbionts in *Bemisia tabaci*

Microorganisms and insects are the two most successful groups of organisms on planet earth. The ubiquitous microbes are also present inside the body of insects in close association and are known as endosymbionts. Out of the various endosymbionts present in insects, bacteria are found to be performing the most diverse roles and are the most studied ones (Engel and Moran, 2013). Only less than one per cent of insect endosymbionts are culturable and advances in modern analytical and laboratory techniques like next generation sequencing techniques (Illumina, Ion Torrent, Roche454 pyrosequencing *etc.*) are allowing researchers to peer ever further into the true complexity of insect-microbe associations, and it is apparent that the influence of microbial metabolic activity on the behavior of higher organisms is profound (Schloss and Handelsman, 2003).

Endosymbiotic theory, and was first articulated by the Russian botanist Konstantin Mereschkowski in 1910. Endosymbionts may be primary or secondary. Primary endosymbionts-(P-endosymbionts): associated with insect hosts for long period of time, they form obligate associations and display co speciation with their insect hosts. E.g: *Buchnera* in aphids. Secondary endosymbionts (S-endosymbionts): exhibit a more recently developed association, are sometimes horizontally transferred between hosts, live in the hemolymph of the insects and are not obligate. E.g: *Hamiltonella defense* in aphids. S-symbionts are not confined into specialized S-bacteriocytes found in gut tissues, glands, body fluids, cells surrounding the P-bacteriocytes or even invading the P-bacteriocytes themselves (Baumann *et al.*, 2006). S-symbionts seem to be the result of multiple independent infections and, although they are usually maternally inherited, their transmission may also occur horizontally from one host to another.

Endosymbionts help in a variety of ways; for the dominance of insects. S-symbionts can increase resistance to fungal pathogens. The *Regiella insecticola* in pea aphids protects the aphids from the lethal fungus *Pandora neoaphidis.* Therefore, they do not share a long evolutionary history with their hosts S-endosymbionts (Aksoy *et al.,* 1997). They provide various fitness advantages like increased fecundity, increased longevity, female-biased sex ratio as well as greater immunity against natural enemies and pathogens (Su *et al.*, 2013). Endosymbionts play various roles in their host insects like nutrition (*Buchnera* provides essential amino acids that are lacking in the plant sap diet of its aphid host, and *Portiera aleyrodidarum* its white fly host provides with B-complex vitamins and amino acids that are lacking in fly feeds), detoxification of toxins: plant allelochemicals and insecticides (Kikuchi *et al.*, 2012), a source of cues and signals (Dillon *et al.*, 2002), defense toward pathogens and parasites (Oliver *et al.* (2003), adaptation to environment shock (Montllor *et al.*, 2002), virus-vector interaction (Chiel *et al.*, 2007), impact of population dynamics (Kikuchi *et al.*, 2012), as hidden players in insect–plant interactions (Hosokawa *et al,.* 2007), biotype determination (Gueguen *et al.*, 2010) and protection against natural enemies (Oliver, *et al.*, 2003). Rosell *et al.* (2009) described about mutualistic and dependent relationships of endosymbionts with other organisms.

Gut microbiota of insects are composed of a wide variety of species, including bacteria, archaea, and eukaryea. Erstwhile, the gut bacterial community of termite (Warnecke *et al.*, 2007) have been studied in detail, similarly total bacterial community from intestinal tract of fruit fly, *Bactrocera minax* Enderlein, Diptera (Wang *et al.*, 2014) and red palm weevil, *Rhynchophorus ferrugineus* (Olivier) ,Coleoptera (Montagna *et al.*, 2015) have also been reported.

16S ribosomal RNA (rRNA) gene sequencing was the most popular method adopted earlier to identify bacteria (Petti *et al.*, 2005). But, it cannot be employed to reveal poly microbial specimen wherein multiple templates resulted uninterpretable Sanger reads (Drancourt, 2000). 1500 base pairs of 16S rDNA are large enough for bioinformatics' purposes (Patel, 2001). 16S rDNA sequences have been used routinely to study the bacterial taxonomy and phylogeny due to the fact that it is present in almost all bacteria and function of this gene has not changed over time (Janda and Abbott, 2007). However, the development of high throughput next generation sequencing with the primer spanning hyper variable regions (V1-V9) of 16S rRNA gene circumvent the limitations of earlier used methods and enlighten the way to identify total bacterial community and their subsequent classification. According to Thomas *et al.* (2012), metagenomics applies a suite of genomic technologies and bioinformatics tools to directly access the genetic content of entire communities of organisms.

Davidson *et al.* (2000) cultured different types of bacteria associated with the whitefly, *B. argentifolii* from surface-sterilized adults and nymphs, including *Bacillus* spp., Gram-variable pleomorphic rods and Gram-positive *Cocci*. Two of the isolates were capable of being ingested by adults and passed into the honeydew. One of these, *Enterobacter cloacae*, was found within the gut cells of adult whiteflies and was mildly pathogenic. This isolate represents the first bacterium with potential as a pathogen of whiteflies. According to Fauquet *et al.* (2008), *P. aleyrodidarum* is the only reported primary endosymiont in whitefly. Six secondary endosymbiotic genera present in them are *Rickettsia, Wolbachia, Arsenophonus, Cardinium, Fritschea* and *Hamiltonella.* Primary endosymbionts exclusively found in bacteriocytes (mycetocyte) throughout the life cycle of *B. tabaci*.

Ateyyat *et al.* (2010) used, both molecular- and culture-based methods to characterize the bacteria associated with the whitefly in Jordan, from different vegetable crops planted in different localities. The identities of the cultured bacteria were evaluated using PCR with sequencing of 16S rRNA gene fragments

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and fluorescence in situ hybridization. Three gram-negative bacteria were identified as *Erwinia persicinus*, *Pseudomonas plecoglossicida*, and *Pseudomonas putida*. The identified gram-positive bacteria included *Brevibacterium casei*, *Staphylococcus gallinarum*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Exiquobacterium acetylicum*, *Exiguobacterium undae*, and *Micrococcus caseolyticus*.

Biotype-dependent secondary symbiont communities in sympatric populations of *B. tabaci* were reported by Chiel *et al*., (2007). Chu *et al.* (2011) compared secondary symbionts from biotypes B and Q in China. Each of the SS, with the exception of *Fritschea*, was detected in both biotypes B and Q; *Fritschea* was found in none of the samples examined. For biotype B, the percentage infection of *Hamiltonella* was the highest (92.0%) followed by *Rickettsia* (70.2%). For biotype Q, the percentage infection of *Hamiltonella* was again the highest (73.3%). *Arsenophonus* was the least common of the SS observed in both biotypes B and Q. The percentage infection of *Wolbachia*, *Rickettsia* and *Hamiltonella* in biotype B was each significantly higher than in biotype Q, whereas the percentage infection of *Cardinium* in biotype B was significantly lower than in biotype Q. The percentage infection of SS in biotypes B and Q varied from year to year over the period 2005-2009. Furthermore, within biotype Q, two distinct subgroups were identified which differ from each other in terms of their SS complement.

In the study conducted by Singh and his co-workers (2012), using *B. tabaci* sampled from 14 different locations in North India, for the presence of bacterial endosymbionts associated with them, using 16S rDNA clone library sequences; they reported *Portiera*, the primary endosymbiont of *B. tabaci*, and other secondary endosymbionts like *Cardinium*, *Wolbachia*, *Rickettsia* and *Arsenophonus*. Along with these they also detected *Bacillus, Enterobacter, Paracoccus* and *Acinetobacter*. These secondary endosymbionts were not uniformly distributed in all the locations. Phylogenetic analysis of 16S rDNA sequences of *Cardinium, Wolbachia, Rickettsia* and *Arsenophonus* showed that each of these bacteria form a separate cluster when compared to their respective counterparts from other parts of the world.

Bing *et al.* (2013) studied diversity of secondary endosymbionts among different putative species of the whitefly *B. tabaci* in China. They tested five of the six S-endosymbiont lineages (excluding *Fritschea*) from 340 whitely individuals representing six putative species. Results demonstrate the variation and diversity of S-endosymbionts in different putative species of *B. tabaci*, especially between invasive and native whiteflies.

Tajebe *et al.* (2015) studied the diversity of symbiotic bacteria associated with *B. tabaci*, in cassava mosaic disease pandemic areas of Tanzania. All *B. tabaci* individuals harbour an obligate bacterial symbiont (*P. aleyrodidarum*), and also harbour non-essential facultative symbionts: *Rickettsia*, *Hamiltonella*, *Arsenophonus*, *Wolbachia, Cardinium* and *Fritschea*. Investigations were carried out by Hashmi *et al.* (2016), to record the spreading frequency of seven known endosymbionts between the field populations of *B. tabaci* collected from two host plants, *viz*., brinjal (*Solanum melongena*) and tomato (*S. lycopersicum*) in India. Based on the irregular distribution of the secondary endosymbionts, they came in to a conclusion that, this irregular distribution strengthens the hypothesis that each endosymbiotic bacterium not only has a role in the survival but may also have a part in the polyphagus nature of *B. tabaci*.

Karut *et al.* (2017) analyzed species, subgroups, and endosymbionts of *B. tabaci* from southwestern cotton fields in Turkey. The MED species (85.3%) were found to be more dominant than MEAM1. Secondary endosymbionts varied according to species and subgroups. *Arsenophonus* was found only from Q2, while *Hamiltonella* was detected in MEAM1 and Q1. In addition, high *Rickettsia* and low *Wolbachia* infections were detected in MEAM1 and Q1 populations, respectively. In conclusion, for the first time, they reported the presence and symbiotic communities of Q1 from Turkey and also found that the symbiont complement of the Q1 is more congruent with Q1 from Greece than other regions

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of the world, which may have some interesting implications for movement of this invasive subgroup.

P. aleyrodidarum in its white fly host provides with B-complex vitamins and amino acids that are lacking in fly feeds (Lai *et al.*, 1996). Sloan and Moran (2012) reported that endosymbiotic bacteria as a source of carotenoids in whiteflies. Unlike other sequenced insect endosymbionts, *Portiera* has bacterial homologues of the fungal carotenoid biosynthesis genes in aphids.

The potential of eleven whitefly-associated bacterial isolates as biological control agents was studied under lab conditions by Ateyyat and his co-workers (2009). They found that *Erwinia persicinus*, *Bacillus pumilus* and *Exiquobacterium acetylicum* were the most effective in reducing *B. tabaci* 2nd nymphal instar populations. *Erwinia persicinus* was the most promising bacterial isolate to be developed as a biological control agent. *Rickettsia* sp. in *B. tabaci* for instance, helps in their thermo tolerance (Brumin *et al.*, 2011).

The transmission of two plant viruses that belong to different virus genera was shown to be facilitated by a bacterial chaperone protein called GroEL. Gibbs (1999) suggested that the viral particles that reach the haemolymph interact with GroEL on their way to salivary glands forming a complex that protects virion from rapid proteolysis. Morin *et al.* (1999) implicated the role of a GroEL homologue from endosymbiotic bacteria of the whitefly *B. tabaci,* in the circulative transmission of *Tomato yellow leaf curl virus* and they proposed that GroEL of *B. tabaci* protects the virus from destruction during its passage through the haemolymph. According to Gottlieb *et al.* (2010), *Hamiltonella* sp. found in B-biotype of whitefly helps them to become successful vectors of *Tomato yellow leaf curl virus*. Rana *et al.* (2012) could find that, *Arsenophonus* GroEL, localized in midgut and salivary gland of whitefly *B. tabaci*, interacts with CLCuV (*Cotton leaf curl virus*) and helps its successful transmission in cotton plants of India.

According to Su *et al.* (2013), facultative symbiont *Hamiltonella* confers benefits to *B. tabaci. Hamiltonella* is found at high frequencies in the *B. tabaci*

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MED (type: Mediterranean-MED) in China. *Hamiltonella* infection substantially enhanced *B. tabaci* MED performance. This beneficial role may, at least partially, explain the high prevalence of *Hamiltonella* in *B. tabaci* MED populations and may also contribute to their effectiveness in spread of the plant pathogens *Tomato yellow leaf curl virus*. The role of bacterial chaperones in the circulative transmission of plant viruses by insect vectors was observed by Kliot and Ghanim (2013). This protein was shown to be implicated in the transmission of *Potato leafroll virus* (PLRV) by the green peach aphid *Myzus persicae* and the transmission of *Tomato yellow leaf curl virus* (TYLCV) by the sweet potato whitefly *B. tabaci*.

Raina *et al.* (2015) observed that the elimination of *Arsenophonus* and decrease in the bacterial symbionts diversity by antibiotic treatment leads to increase in fitness of whitefly, *B. tabaci*. In this study, tetracycline was used to eliminate *Arsenophonus* from *B. tabaci* to study its effects with regard to development and other fitness parameters. The results revealed that *Arsenophonus* negative (A(-)) whiteflies had more fecundity, increased juvenile developmental time, increased nymphal survival and increased adult life span as compared to control (A(+)) whiteflies.

2.2.3. Cassava whitefly - *Cassava mosaic virus* interactions

The relationships between plant viruses, their herbivore vectors and host plants will be beneficial, neutral, or antagonistic, depending on the species involved (Jiu *et al.*, 2007). This variation in relationships may affect the process of biological invasion and the displacement of indigenous species by invaders when the invasive and indigenous organisms occur with niche overlap but differ in the interactions (Min *et al.*, 2007). Coevolved adaptations are observed among virus and their vectors (Luan *et al.*, 2011). Similarly, plant mediated mutualism between a virus and its vector is also found (Tong *et al.*, 2012). Acquisition of plant viruses has various effects on physiological mechanisms in vector insects (Pusag *et al.*, 2012) *viz.*, increased feeding, crowding *etc.*

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Watson and Roberts (1939) gave the basic concept based on virus retention time by the vector. i.e., non-persistent and persistent and Sylvester (1958) introduced the term semi-persistent. *B. tabaci* adults from colonies reared on cassava or sweet potato plants were studied by Antony *et al.* (2006), to determine their ability to transmit *Indian cassava mosaic virus* (ICMV) (*Geminiviridae: Begomovirus*) from cassava to cassava. It was observed that virus acquisition access (feeding) periods (AAP) was 48 h on ICMV-infected cassava leaves and 48-h virus inoculation access (feeding) periods on virus-free cassava seedling leaves. They also provided evidences for transmission of *Indian cassava mosaic virus* through *B. tabaci* – cassava biotypes using a triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA), used to detect *Geminiviruses* in the purified cassava.

Begomovirus–whitefly interactions depend on the virus, the vector biotype and the endosymbionts of the vector (Gottlieb *et al.*, 2010). Whiteflies acquire virions during feeding on the phloem of an infected plant. The virions move through the alimentary canal into the whitefly midgut, where they enter the haemolymph and transit to the salivary glands for transmission during the next feeding cycle (Ghanim *et al.*, 2001). Endosymbiont is an important factor for the efficient virus transmission by *B. tabaci* (Chiel *et al.*, 2007). Viral coat proteins (CPs) bind to GroEL proteins encoded by endosymbionts in the whitefly gut (Rana *et al.*, 2012) and to the whitefly-encoded heat shock protein 16 (HSP16) (Ohnesorge and Bejarano, 2009). Interactions with GroEL and HSP16 might stabilize the virion during passage through the gut and/or facilitate its transfer across the gut epithelia into the haemolymph. Both possibilities are consistent with data showing that GroEL isoforms produced by different endosymbionts affect transmission efficiency (Gottlieb *et al.*, 2010). *Arsenophonus* GroEL interacts with CLCuV(*Cotton leaf curl virus*) and localized in midgut and salivary gland of whitefly *B. tabaci*, causes quick spread of the virus in cotton (Rana *et al.*, 2012).

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Narasimhan and Arjunan (1974), reported that CMD resistant cassava had low whitefly population compared to susceptible variety. According to Nair and Daniel (1983), *B. tabaci* have preference for certain varieties of cassava and found high population count on H-226 and H-165 and also adult flies prefer more cassava mosaic disease affected plants for feeding than un-affected ones. Cassava infested with EACMV-UG (*East African cassava mosaic virus*-Uganda) served as better host for *B. tabaci* than did by healthy plants (Colvin *et al.* 2006). Infection of susceptible cassava by EACMV-UG apparently alter the phloem sap content by increasing the concentrations of at least one amino acid and this is associated with an increased population growth rate of *B. tabaci* (Colvin *et al.* 2004).

Study conducted at International Center for Tropical Agriculture in Colombia by Bellotti and Arias (2001) for the host plant resistance of cassava to whiteflies shows that the nymphal mortality was highest on the CMD resistant cassava clone, M Ecu 72 (72.5%) and lowest on the susceptible clones CMC 40 (33%) and M Bra 12 (25.0%). When feeding on resistant genotypes, whiteflies had less oviposition, longer development periods, reduced size and higher mortality than when feeding on susceptible ones. Mortality is highest during the nymphal stages.

Vector-virus mutualism accelerates population increase of an invasive whitefly. The indirect mutualism between the B biotype whitefly and these viruses via their host plants, and the apparent lack of such mutualism for the indigenous whitefly, may contribute to the ability of the B whitefly biotype to invade, the displacement of indigenous whiteflies, and the disease pandemics of the viruses associated with this vector (Jiu *et al.*, 2007). Mann *et al.* (2008) studied the performance of *B. tabaci* on healthy and *Cotton leaf curl virus* infected cotton. Virus infection increased percent egg viability of *B. tabaci*. Whiteflies deposited significantly fewer eggs on virus infected plants compared to healthy plants. The development time of whiteflies from egg to adulthood was significantly reduced on virus infected plants with shorter nymphal and pupal duration. Male and

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female whiteflies had shorter longevity on CLCuV infected plants compared with healthy plants.

Fereres and Moreno (2009) reported that positive effects on the life cycle and population growth rate often occur when homopteran insects feed on plants infected on non-circulative or circulative nonpropagative viruses, while increased mortality and low population growth seems to be the rule when the vectors feed on plants infected with circulative propagative viruses.

Viral infection of tobacco plants improves performance of *B. tabaci* but more so for an invasive than for an indigenous biotype of the whitefly according to Liu *et al* (2010). They examined the transmission of *Tomato yellow leaf curl China virus* (TYLCCNV) by the invasive Q biotype and the indigenous ZHJ2 biotype of the whitefly *B. tabaci*, as well as the influence of TYLCCNV-infection of plants on the performance of the two whitefly biotypes. The data shown that the Q biotype acquired higher beneficial effects from TYLCCNV-infection of tobacco plants than the ZHJ2 biotype. According to Guo *et al.* (2010), the invasive B whitefly benefits from feeding on a *Begomovirus*-infected plant through increased egg production and realized fecundity.

Ingwell *et al.* (2012) proposed plant viruses alter insect behaviour to enhance their spread. They reported the first evidence that acquisition of a plant virus directly alters host selection behaviour by its insect vector. Results shown that the aphid *Rhopalosiphum padi*, after acquiring *Barley yellow dwarf virus* (BYDV) during in vitro feeding, prefers non infected wheat plants, while non infective aphids also fed in vitro prefer BYDV-infected plants. They proposed the ''Vector Manipulation Hypothesis'' to explain the evolution of strategies in plant pathogens to enhance their spread to new hosts. Liu *et al.* (2013) conducted two experiments testing the impact of TYLCV infection of the host plant (tomato) and vector (*B. tabaci* biotypes B and Q) on whitefly feeding behavior. Whiteflies of biotypes B and Q both appeared to find TYLCV-infected plants more attractive, probing them more quickly and having a greater number of feeding bouts; this did

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not, however, alter the total time spent for feeding. Viruliferous whiteflies fed more readily than uninfected whiteflies and spent more time salivating into sieve tube elements that provided TYLCV a direct fitness benefit for the virus.

A plant virus manipulates the behaviour of its whitefly vector to enhance its transmission efficiency and spread according to Moreno *et al.* (2013). Plant viruses can produce direct and plant-mediated indirect effects on their insect vectors, modifying their life cycle, fitness and behaviour. Viruses may benefit from such changes leading to enhanced transmission efficiency and spread. TYLCV directly manipulates the settling, probing and feeding behaviour of its vector *B. tabaci* in a way that enhances virus transmission efficiency and spread. TYLCV- *B. tabaci* interactions are mutually beneficial to both the virus and its vector because *B. tabaci* feeds more efficiently after acquisition of TYLCV. Fang *et al.* (2013), observed alteration of host preferences of *B. tabaci* by, *Tomato yellow leaf curl virus*; where TYLCV-free *B. tabaci* Q preferred to settle on TYLCV-infected tomato plants over healthy ones. TYLCV-free *B. tabaci* B, however, preferred healthy tomato plants to TYLCV-infected plants.

Shi *et al.* (2014) demonstrated that *B. tabaci* Q carrying *Tomato yellow leaf curl virus* strongly suppresses host plant defences and enhances the spread of Q and TYLCV in China. Shi *et al.* (2014) showed that feeding by viruliferous *B. tabaci* increases the longevity and fecundity of non viruliferous *B. tabaci* that subsequently feed on the same plant. Feeding by viruliferous *B. tabaci* increased the suppression of plant defenses involving JA and PI but did not increase responses involving SA. These results indicate that the interactive effects of *Tomato yellow leaf curl virus* and *B. tabaci* on plants increase vector fitness and virus transmission by reducing plant defense. Ning *et al.* (2015) compared the acquisition, retention, and transmission of TYLCV by B and Q females and males and found that Q females are more efficient than Q males, B females, and B males at TYLCV acquisition and transmission. Based on electrical penetration graphs determination of phloem sap ingestion parameters, females fed better than males, and Q females fed better than Q males, B females, or B males.

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Some negative effects of the association between the vector and virus are also reported. Luan *et al.* (2011) investigated the transcriptional response of the invasive *B. tabaci* Middle East-Asia Minor 1 species to *Tomato yellow leaf curl China virus* (TYLCCNV) using Illumina sequencing technology. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that TYLCCNV can perturb the cell cycle and primary metabolism in the whitefly, which explains the negative effect of this virus on the longevity and fecundity of *B. tabaci*. Study also demonstrated that TYLCCNV can activate whitefly immune responses, such as autophagy and antimicrobial peptide production, which might lead to a gradual decrease of viral particles within the body of the viruliferous whitefly.

Whiteflies mounting a defence against *Begomovirus* invasion, and the virus will counteract this activation of the immune response (Linda Hanley-Bowdoin *et al*., 2013). He *et al.* (2015) studied differential profiles of direct and indirect modification of vector feeding behaviour by a plant virus. They used electrical penetration graph recording to investigate the direct and indirect effects of TYLCCNV on the feeding behaviour of MEAM1. When feeding on cotton, a non-host of TYLCCNV, or uninfected tobacco, a host of TYLCCNV, virus-infection of the whiteflies impeded their feeding. They observed that, when viruliferous whiteflies fed on virus-infected tobacco, their feeding activities were no longer negatively affected; instead, the virus promoted whitefly behaviour related to rapid and effective sap ingestion.

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Materials and Methods

3. MATERIALS AND METHODS

The details of materials used and the methods adopted during the course of the investigation on "Characterization of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), for genetic variability, endosymbionts and vector-virus interactions in cassava" are as follows

3.1. Morphological characterization of cassava whitefly, *Bemisia tabaci*

3.1.1. Sampling of *Bemisia tabaci* population

Surveys were conducted in different agro-ecological zones of Kerala, where cassava is grown (Table 1, Plates 1 and 2), and whitefly samples were collected during the period from March, 2014 to April, 2016. Adult insects were collected using aspirator and other stages were collected by hand picking of whole leaves.

3.1.2. Rearing of *Bemisia tabaci*

Setts of cassava variety H-226 were planted in pots of 30 cm X 30 cm, filled with potting mixture. Whitefly rearing cages to cover the cassava plants were fabricated with plastic bottles cut at the top. Black and white coloured cloths with a mesh size of 50 were used to cover the bottles (Plate 3), since black coloured cloth used over white coloured one was found to provide a conducive environment for faster multiplication of whiteflies. The rearing conditions were 27-33 °C temperature and 65±5 per cent relative humidity. Collection and release of whiteflies were done using an aspirator.

3.1.3. Morphological characterization

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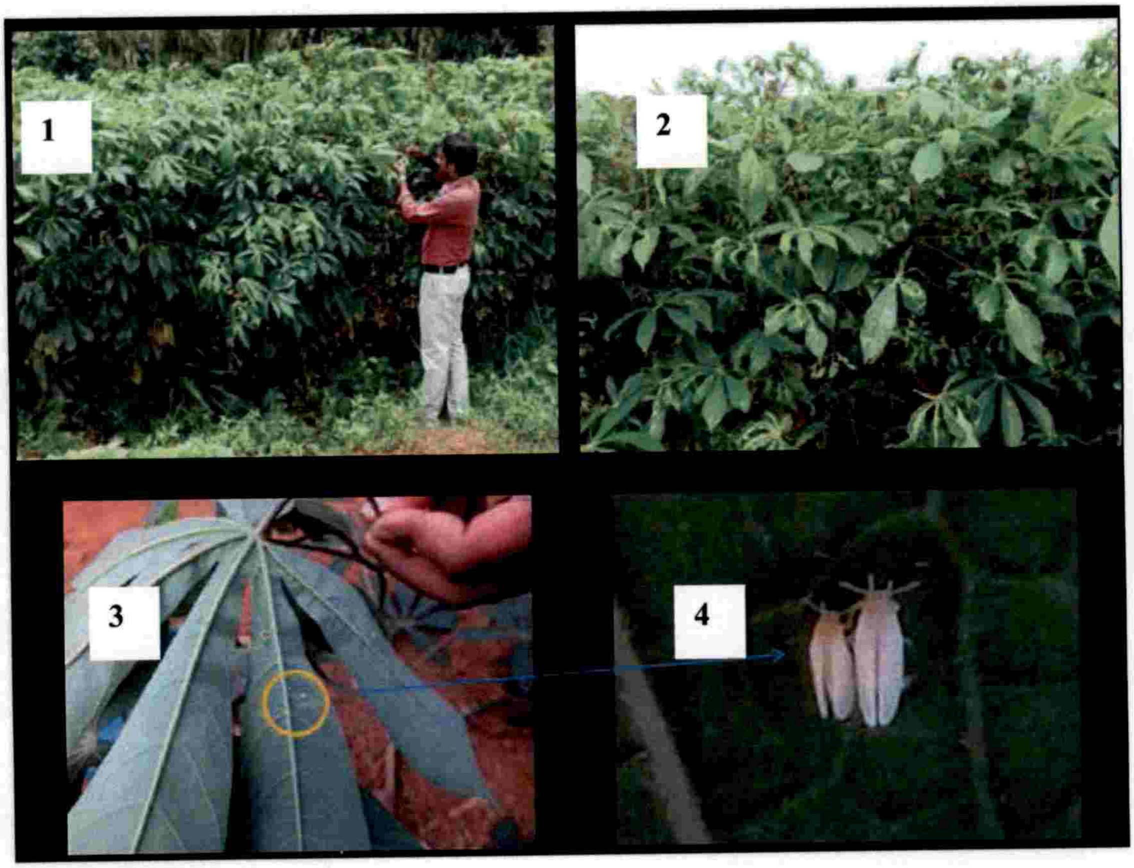

Plate 1. Survey and collection of *Bemisia tabaci* from different cassava growing areas of Kerala (1- Collection using aspirator, 2- Cassava leaves showing symptoms of cassava mosaic disease 3- *B. tabaci* adult pair on under surface of cassava leaves, 4- Magnified image of *B. tabaci* adult pair)

Plate 2. Healthy and cassava mosaic disease (CMD) affected cassava leaves

Plate 3. Whitefly rearing in laboratory on cassava plants

The pupal and adult samples drawn from these cultures were subjected to morphometric studies. Developmental stages were identified according to the methods described by Malumphy *et al.* (2009) and Chaubey *et al.* (2010). Puparia and adults were processed for mounting as recommended earlier (Malumphy, 2004). Specimens were placed in 70-90% ethanol in a watch glass; covered with a glass square and heated gently to around 80°C for 5-10 minutes. The alcohol was pipetted off. Specimens were simmered in 10% KOH for approximately 5-10 minutes or until the specimens lost most of their body colour. The body contents were expelled by making an incision and pumped the liquefied body contents out, using two fine spatulae. The excess KOH was pipetted off. The specimens were soaked in 70% ethanol for two minutes. Finally, the specimens were rinsed in fresh 70% ethanol for five minutes and mounted in Heinz on a glass microscope slide. The mounted specimens (n=10) were observed under the Leica DM100 phase contrast research microscope at 40x for studying the essential characters. Measurements and photographs were taken in Leica DM500 stereozoom microscope attached with DFC290 digital camera at 4x to 100x (n=10).

Pupal characters (male and female) studied were pupal length, pupal width, length and width of right and left anterior wax margin, vasiform orifice length, operculum length, operculum width, lingula length, lingula width, caudal furrow length, caudal seta length and distance between caudal setae. For male adult insects, nine characters *viz.*, antennal length, body length, body width, forewing length, forewing width, hind tarsal length, hind tarsal width, aedeagus length and clasper length, were compared. Above characters, except for length of aedeagus as well as clasper were compared for adult female insects. All these characters were compared from places in 13 different agroecological zones of Kerala (Table 1).

Significant characters were identified using univariate one way single factor ANOVA (Kalaisekar *et al.* 2012). After this, the pattern of clustering was analyzed using multivariate statistical approaches (Tabachnick and Fidell, 2007). Principal Component Analysis (PCA; SAS procedure; PRINCOMP; SAS version

9.1.3, SAS Institute Inc., Cary, NC, USA) was used without any prior assumption of groupings which assesses the components for total variation among the specimens by calculating linear combination of variables that explain the maximum of total variation.

3.2. Molecular characterization of *Bemisia tabaci* adults

3.2.1. Isolation of genomic DNA

Protocol for genomic DNA isolation from *B. tabaci* was optimized at the All India Network Project on Agricultural Ornithology (AINPAO) laboratory and at Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture, Vellanikkara, following the method developed by De Barro and Driver (1997).

Reagents

a. Lysis buffer

-50 mM KCl

-10 mM Tris (pH 8.4)

- 0.45 % Tween 20

- 0.2 % gelatin

- 0.45 % NP40

- 60 µg/ ml proteinase K

b. 70 per cent ethanol

c. Sterile distilled water

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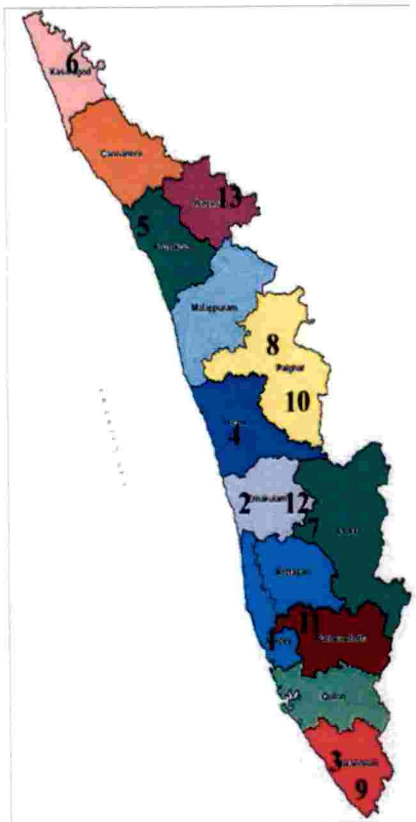
Table 1. Details of survey and collection of cassava whitefly, *Bemisia tabaci* from different agro-ecological zones of Kerala



No	Zones	Places	Code	Latitude	Longitude	Elevation (above MSL)
1	Onattukara	Kayamkulam	KYM	10.1722° N	76.500° E	8 m
2	Coastal Sandy	Palluruthy	PTY	9.9087° N	76.2730° E	0.5 m
3	Southern midlands	Sreekaryam	SKM	8.5241° N	76.9366° E	10 m
4	Central midlands	Vellanikkara	VKA	10.5452° N	76.2740° E	2.8 m
5	Northern midlands	Vadakara	VDA	11.6085° N	75.5917° E	21 m
6	Malappuram type	Kasargod	KGD	12.4387° N	75.2012° E	15 m
7	Malayoram	Thodupuzha	TPA	9.8930° N	76.7221° E	40 m
8	Palakkad plains	Palakkad	PKD	10.7867° N	76.6548° E	78 m
9	Red loam	Neyyattinkara	NYA	8.4016° N	77.0871° E	26 m
10	Chittoor black soil	Chittur	CTR	10.7003° N	76.7394° E	131 m
11	Kuttanad	Pulikeezhu	PKU	9.3581° N	76.5415° E	21 m
12	Riverbank alluvium	Muvattupuzha	MVA	9.9894° N	76.5790° E	15 m
13	High ranges	Sultan Bathery	SBY	11.6656° N	76.2627° E	1010 m

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3.2.1.1. Procedure

From the field, the adult whiteflies were collected with an aspirator and placed in 70 per cent ethanol. Samples were brought to the laboratory and stored at -20°C. Prior to homogenisation for DNA extraction, adults were washed in sterile distilled water to remove alcohol. Various techniques for the extraction of DNA were attempted. The method suggested by Walsh *et al.* (1991), Ahmed *et al.* (2009), Frohlich *et al.* (1999), De Barro and Driver (1997) and also a self standardized method using distilled water and without using any chemical were followed. The method suggested by De Barro and Driver (1997) has given better results. In brief, individual whiteflies were homogenised in 10 µL of the lysis buffer (50 mM KCl, 10 mM Tris pH 8.4, 0.45% Tween 20, 0.2% gelatin, 0.45% NP40, 60 µg/ ml proteinase K) using a 1.5 ml microcentrifuge tube and a micropestle that fits well with the bottom of microcentrifuge tube. After homogenisation a further 15 µl of lysis buffer was added. The homogenate was then incubated at 65 °C for 30 min in a water bath with intermittent shaking. After incubation, the sample was boiled for 10 min to inactivate the proteinase K. Sterile distilled water was then added to yield a final homogenate volume of 50 µL. Samples were then stored at -20°C. DNeasy blood and tissue kit (Qiagen®) was also used for whitefly DNA extraction and the procedure was followed according to user's manual.

3.2.1.2. Quality checking of DNA

3.2.1.2.1. Agarose gel electrophoresis

Agarose gel electrophoresis was performed based on the method described by Sambrook *et al.* (1989) to check the quality of DNA.

Reagents

1. Agarose - 0.8 per cent (Sigma-Aldrich™)

2. 50X TAE buffer (pH8.0) (prepared by dissolving 242g Tris base in water, adding 57.1 ml glacial acetic acid, and 100 ml of 500mM EDTA (pH 8.0) solution, and bringing the final volume up to 1 liter)
3. Tracking/loading dye (6X) (Thermo Scientific™)
4. Ethidium bromide (stock 10 mg/ ml; working concentration 0.5 μg/ ml) (Thermo Scientific™)

Procedure

Agarose gel electrophoresis was carried out in electrophoresis unit (BioRad®) and the steps followed are given below.

Agarose (0.8% - 0.8 g in 100 ml) was taken in a conical flask and 100 ml of 1X TAE buffer was added. It was heated in microwave oven for 60 seconds until agarose was completely dissolved and the solution was clear. The solution was allowed to cool to about 45 °C and ethidium bromide (2 μl) was added and mixed well. The solution was poured into the gel casting tray (which was placed on a horizontal surface, comb was placed properly in gel caster and both the open sides sealed with cellotape) and allowed to solidify for about 30 min. at room temperature. The comb was removed gently and the gel casting tray placed in the buffer tank, and submerged (just until wells were submerged) with electrophoresis buffer (1X TAE).

DNA samples were prepared by mixing 1 μl of tracking dye with 5 μl of DNA solution. Using a micropipette, each sample was mixed well with the tracking dye and 6 μl was loaded per well. λDNA marker (*Eco*RI+*Hind* III double digest) 1 kb plus ladders was used as molecular weight size marker for each gel along with DNA samples. The electrophoresis was performed at 70 volts until the dye migrated to two third the length of the gel.

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3.2.1.2.2. NanoDrop spectrophotometry

The purity of DNA was checked using NanoDrop spectrophotometer (model-NanoDrop-1000, Thermo Scientific™). Nucleic acid shows absorption maxima at 260 nm whereas proteins show peak absorbance at 280 nm. Absorbance has been recorded at both wavelengths and the purity was indicated by the ratio OD_{260}/OD_{280}. A value between 1.8 and 2.0 indicated that the DNA was pure and free from proteins and RNA. When the ratio is <1.8, the sample is contaminated with protein and the ratio is >2.0 the sample is RNA contaminated.

3.2.2. Molecular marker analysis

3.2.2.1. Inter Simple Sequence Repeats (ISSR) marker analysis

ISSR is a general term for a genome region between microsatellite loci. The complementary sequences to two neighboring microsatellites are used as PCR primers; the variable region between them gets amplified. The genomic DNA isolated from different *B. tabaci* were pooled to get a master pool of whitefly DNA and was amplified with 35 ISSR primers to screen the best performing ones (Table 2). The amplification patterns for all the whiteflies with individual primers were used to assess the genetic variability existing among them.

Table 2. Name and sequence of ISSR primers used in the study

Sl. No.	Primer	Nucleotide Sequence
1	F1	5'-GAGAGAGAGAGACC-3'
2	F2	5'-CACCACCACGC-3'
3	F3	5'-ACACACACACACACACC-3'
4	F4	5'-CTCTCTCTCTCTCTTG-3'
5	F5	5'-GAGAGAGAGAGAGAGAC-3'
6	F6	5'-CTCTCTCTCTCTCTTG-3'

7	I4	5'-GAGAGAGAGAGAGAGAT-3'
8	ISSR 2	5'-ATTATTATTATTATTCAT-3'
9	ISSR 3	5'-TTATTATTATTATTACTT-3'
10	ISSR 4	5'-ATTATTATTATTATTGTT-3'
11	ISSR 5	5'-ATTATTGTTGTTGTTTTC-3'
12	ISSR 6	5'-TTATTATTATTATTATAA-3'
13	ISSR 7	5'-ATTATTGTTGTTGTTGTA-3'
14	ISSR 8	5'-ATTATTATTATTATTGTA-3'
15	ISSR 9	5'-TTATTATTATTATTATTACT-3'
16	UBC 808	5'-AGAGAGAGAGAGAGAGC-3'
17	UBC 810	5'- GAGAGAGAGAGAGAGAT-3'
18	UBC 811	5'-GAGAGAGAGAGAGAGAC-3'
19	UBC 815	5'-CTCTCTCTCTCTCTCTG-3'
20	UBC 818	5'-CACACACACACACACAG-3'
21	UBC 826	5'-ACACACACACACACACC-3'
22	UBC 827	5'-ACACACACACACACACG-3'
23	UBC 835	5'-AGAGAGAGAGAGAGAGYC-3'
24	UBC 841	5'-GAGAGAGAGAGAGAGAY-3'
25	UBC 844	5'-CTCTCTCTCTCTCTCTRC- 3'
26	UBC 846	5'-CACACACACACACACART-3'
27	UBC 847	5'-CACACACACACACACARC-3'
28	UBC 848	5'-CACACACACACACACARG-3'
29	UBC 850	5'-GTGTGTGTGTGTGTGTYC-3'
30	UBC 854	5'-TCTCTCTCTCTCTCTCRG-3'
31	UBC 855	5'-ACACACACACACACACYT-3'
32	UBC 857	5'-ACACACACACACACACYG-3'
33	UBC 865	5'-CCGCCGCCGCCGCCGCCG-3'

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34	UBC 866	5'-CTCCTCCTCCTCCTCCTC-3'
35	UBC 873	5'-GACAGACAGACAGACA-3'

3.2.2.1.1. Standardization of polymerase chain reaction (PCR) conditions

The PCR conditions required for effective amplification of ISSR markers included appropriate proportions of the components of the reaction mixture and the thermal profiles. The reaction mixture included template DNA, Taq assay buffer B (10X), $MgCl_2$, dNTP mix (10Mm), Taq DNA polymerase enzyme, ISSR primer and autoclaved distilled water. The aliquot of this master mix were dispensed into 0.2 ml PCR tubes. The PCR was carried out in Veriti Thermal Cycler (Applied Biosystems^{TM}).

The temperature gradients were set to find out the optimum annealing temperature for the PCR. Annealing temperatures in the range of 32 °C to 65 °C were tested for 35 ISSR primers. Different template concentrations of 10 ng/µl, 20 ng/µl, and 50 ng/µl were used for the standardization of PCR. The thermal cycler was programmed for desired number of cycles and temperatures for denaturation, annealing and polymerization. The amplicons were electrophoresed in two per cent agarose gel, documented and compared with 100 bp DNA ladder (Genei^{TM}, Bangalore).

3.2.2.1.2. Thermal cycling

Good quality genomic DNA (20 ng/µl) isolated from adult *B. tabaci* was used in the ISSR analysis. PCR amplification was performed in a 20 µl reaction mixture which consisted of,

a) Genomic DNA (20 ng) - 1.0 µl

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b) 10X Taq assay buffer B	-	2.0 μl
c) $MgCl_2$	-	2.0 μl
d) dNTPs mix (10mm each)	-	1.5 μl
e) Taq DNA polymerase (1U)	-	0.4 μl
f) Primer	-	1.5 μl
g) Autoclaved Distilled Water	-	11.6 μl
Total volume	-	20.0μl

The thermal cycling was carried out with the following programme

Initial denaturation	-	94 °C for 4 minute	
Denaturation	-	94 °C for 45 seconds	36 cycles
Primer annealing	-	32 °C to 65 °C for 1 minute	36 cycles
Primer extension	-	72 °C for 2 minutes	36 cycles
Final extension	-	72 °C for 8 minutes	
Incubation	-	4 °C for infinity to hold the sample	

3.2.2.1.3. Screening of ISSR primers and analysis

Thirty five ISSR primers (Sigma-Aldrisch, India) were used for screening of the best performing ones (Table 2). ISSR primers were selected based on PIC values as reported in the study of Xie *et al.* (2014). The amplified products were run along with marker (100bp ladder) on two per cent agarose gel using 1X TAE buffer and stained with ethidium bromide. The image was documented on gel documentation system (BioRad Gel DOC-It™ imaging system). Software 'GelQuant Express' is utilised for counting bands in agarose gel (GelQuant Express Report is the report which shows the banding pattern of amplicons). The documented ISSR profiles were carefully examined for the polymorphism in banding pattern, among whiteflies collected from different agro-ecological zones of Kerala.

3.2.2.1.4. Data analysis

Scoring of bands was done with the 'GelQuant Express' software. The bands were scored as one and zero for the presence and absence respectively and their size recorded in relation to the molecular weight marker used and with the software Quantity One. The scored marker data matrix was analyzed using the standard procedure in NTsys Pc 2.0 package (Rholf, 1998) and the genetic distance or similarity was determined using the Dice coefficient (Dice, 1945). A dendrogram was constructed after cluster analysis of the similarity coefficients by the un-weighted pair-group method analysis, UPGMA (Sneath and Sokal, 1973).

3.2.2.2. Analysis using mitochondrial cytochrome oxidase 1 primer (DNA barcode primer)

Good quality total genomic DNA (20 ng/μl) isolated from adult *B. tabaci* was used for the analysis. The barcode primer specific to the larger unit of mitochondrial cytochrome oxidase I (*mtCO1*) of *B. tabaci* (Frohlich *et al*., 1999) was used for PCR amplification. The *mtCO1* region was amplified by polymerase chain reaction from total genomic DNA using the barcode primers (F: C1-J2195 COI: 5′-TTGATTTTTTGGTCATCCAGAAGT-3′, R: L2-N-3014 COI: 5′-TCCAATGCACTAATCTGCCATATTA-3′) in Veriti Thermal Cycler (Applied Biosystems®). The PCR reaction was performed using 1 μl template DNA (20 ng), 0.8 μl each of the forward and reverse primers, 1.8 μl of 10 mM dNTP (Genei®), 0.4 μl of Taq DNA polymerase (Genei®), 2 μl of Taq DNA buffer B (Genei®), 1.8 μl of $MgCl_2$ and 11.4 μl of Millipore® water. The PCR conditions were programmed as, initial denaturation 94 °C for 4 min., 35 cycles each of denaturation 94 °C for 30 seconds, primer annealing 45 °C for 1 min. and primer extension 72 °C for 2 min., followed by 10 min. extension at 72 °C and incubation at 4 °C. The PCR product was electrophoresed on 1 % agarose gel and the product was sequenced (SciGenom labs, Cochin).

3.2.2.2. Sequence analysis and submission to GenBank, NCBI

The sequence generated from this study was analyzed for sequence homology using the nucleotide BLAST at NCBI, submitted to GenBank and the accession numbers were generated. Further, dendrogram showing sequence variability was generated using MEGA version 6.0 software (Neighbour joining method). A per cent identity matrix was generated using multiple sequence alignment programme 'Clustal Omega'.

3.2.2.2.1. Barcoding

The sequences generated using DNA bar coding primer and specimen details were submitted to Barcode of Life Database (BOLD system v3). An account was opened in workbench session of BOLD systems v3 database and a new project was created. Specimen data *viz*., specimen identifiers, specimen taxonomy, specimen details, collection details was submitted and an auto generated process ID was obtained. Further, primer details, high resolution specimen images, mitochondrial DNA sequences (fasta) and the trace files (.ab1) obtained from sequencer were uploaded to the database and the corresponding barcode of *B. tabaci* was generated from 13 agro-ecological zones of Kerala.

3.3. Metagenomic study of adult *Bemisia tabaci*

3.3.1. Isolation of metagenomic DNA from adult *Bemisia tabaci*

Direct method of isolation of metagenomic DNA (Zhou *et al.*, 1996) was modified and adopted to isolate metagenomic DNA from two combined samples based on differences in elevation. From the cassava plants surveyed for severity of cassava mosaic disease symptoms, the cassava plants present in high elevations of Sultan Bathery (> 900 m from mean sea level) shown very less severity score of 1 compared to plants from other areas (with severity score of 3-5) (Ikotun and Hahn, 1991). Based on the information, the first sample (P) was made and consisted of a group of 12 adult whiteflies, which included one each from the respective agro-ecological zone of plains (<150 m) from mean sea level). The second sample (H) consisted of 12 adult whiteflies from different locations of Sultan Bathery. The insects were homogenized in 400 µl of extraction buffer [200 mM Tris-HCl (pH-8.0), 25 mM EDTA (pH-8.0), and 250 mM NaCl, SDS (0.5%)] in a 1.5 ml Eppendorf tube and spun at 6000 rpm for 10-15 min. The homogenized sample was incubated at room temperature for 1 h. The samples were centrifuged at 12,000 rpm for 5 min. and the supernatants were collected in a fresh Eppendorf tube. An equal volume of phenol: chloroform: isoamyl alcohol (24:25:1) was added to the supernatant and centrifuged at 10,000 rpm for 20 min. at 4 °C. The aqueous phase was pipetted out into a fresh Eppendorf tube; an equal volume of iso propanol was added, the mixture was incubated at room temperature for 15 min., centrifuged at 13,000 rpm for 5 min. at room temperature and the metagenomic DNA pellet was precipitated out. The DNA pellet was washed with ethanol (95 %) by centrifugation at 10,000 rpm for 10 min. The DNA pellet was air dried, dissolved in 25 µl of autoclaved distilled water and stored in deep freezer (-80 °C) for future use.

3.3.1.1. Quality checking of metagenomic DNA

The 16S rDNA fragment was amplified by polymerase chain reaction from the metagenomic DNA using the universal 16S rDNA primers (**F**- 5'-GAGTTTGATCCTGGCTCAG-3', **R**-5'-ACGGCTACCTTGTTACGACTT-3') in

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Veriti thermal cycler (Applied Biosystems®) (Haris *et al.*, 2014). The PCR reaction was performed using 0.2 µl template DNA (1:50 diluted sample), 0.1 µl each of the forward and reverse primers, 1 µl of 10 mM dNTP (Genei®), 0.2 µl of Taq DNA polymerase (Genei®), 2.5 µl of Taq DNA buffer (Genei®) and 15.9 µl of molecular biology grade water. The PCR conditions were, initial denaturation 94 °C for 2 min., 29 cycles each of denaturation 94 °C for 45 seconds, primer annealing 55 °C for 1 min. and primer extension 72 °C for 2 min., followed by 10 min. extension at 72 °C and incubation at 4 °C. The reaction product was separated on agarose (0.8 %) gel to check the quality of bands.

3.3.2. 16S ribosomal RNA amplicon sequencing using Next Generation Illumina MiSeq™

The metagenomic DNA isolated from *B. tabaci* adults were sequenced (SciGenom Lab, Cochin). Amplicon library was prepared with specific primers spanning the hypervariable V3 region of 16S rRNA gene (Fig. 1) and used for sequencing and subsequent classification.

Amplicon PCR

The extracted whitefly metagenomic DNA were normalized to 5 ng/µl each (purified DNA, 10 mM, Tris pH-8.5) and amplicon PCR was carried out using V3 primers (341F 5'CCTACGGGAGGCAGCAG 3', 518R 5'ATTACCGCGGCTGCTGG 3') (Bartram *et al.*, 2011). The PCR master mix consisted of 2 µl each 10 pmol/ µl forward and reverse primers, 0.5 µl 40 mM dNTP, 5 µl 5X Phusion HF reaction buffer, 0.2 µl 2U/ul or µl F-540 Special Phusion HS DNA polymerase, 5ng input DNA and water to make up the total volume to 25 µl. PCR reaction was programmed, initial denaturation of 98 °C for 30 sec., 30 cycles of denaturation at 98 °C for 10 sec., primer extension of 72 °C for 30 sec. and final extension at 72 °C for 5 min. followed by 4 °C hold. The PCR product was quantified

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using the fluorescence quantitative (Qubit 2.0®) fluorometer with the Qubit dsDNA HS assay kit (Invitrogen,USA).

3.3.2.1. 16S rRNA amplicon library preparation

PCR clean-up

PCR clean up was carried out using AMPure XP beads to purify the 16S V3 amplicon away from free primers and primer dimer species. The reagents consisted of 10 mM Tris pH 8.5 (52.5 µl per sample), AMPure XP beads (20 µl per sample), freshly prepared ethanol (80%) (400 µl per sample). Standard protocol was followed and the cleaned up PCR product was stored at -20°C.

Index PCR

Illumina™ Truseq adapters and indices were added to the cleaned PCR products. PCR master mix consisted of 2 µl each 10 pmol/ul forward and reverse primers, 1 µl 40 mM dNTP, 10 µl 5 X Phusion HF reaction buffers, 0.4 µl 2U/ul F-540 special Phusion HS DNA polymerase, 10 µl (minimum 5 ng) PCR1 amplicon and water to make up the total volume to 50 µl. PCR reaction was programmed with initial denaturation at 98 °C for 30 sec., 15 cycles of denaturation at 98 °C for 10 sec., and primer extension at 72 °C for 30 sec. and final extension at 72 °C for 5 min. followed by 4 °C hold.

PCR clean-up 2

AMPure XP beads were used to clean up the final library before quantification. The reagents consisted of 10 mM Tris pH 8.5 (27.5 µl per sample), AMPure XP beads (56 µl per sample), freshly prepared 80 % ethanol (400 µl per sample). Standard protocol was followed and the PCR product was stored at -20°C.

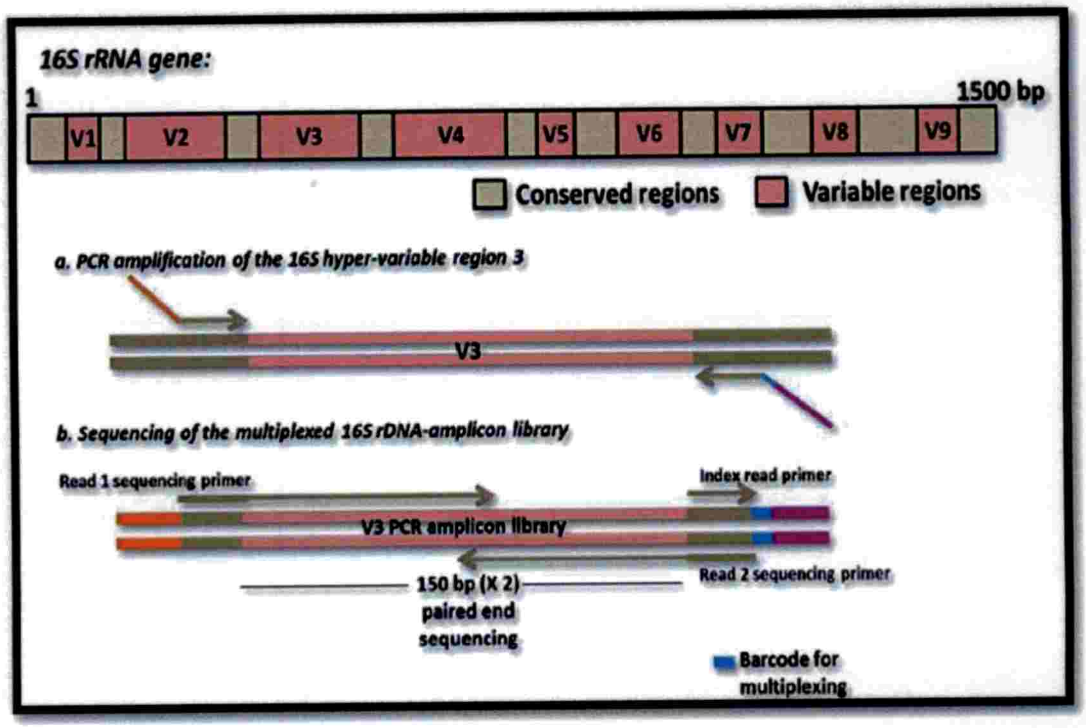


Fig. 1. Multiplexed 16 S rRNA –amplicon sequencing on Illumina MiSeq system

3.3.2.2. Library quantification, normalization, and pooling

Libraries were quantified using a fluorometric quantification method and concentrated final library was diluted using distilled water. Diluted DNA (5 μl) from each library was pooled with unique indices.

3.3.2.3. Library denaturing and MiSeq sample loading

In preparation for cluster generation and sequencing, pooled libraries were denatured with NaOH, diluted with hybridization buffer, and then heat denatured before MiSeq® sequencing. Each run included a minimum of PhiX (5%) to serve as an internal control for these low diversity libraries. Denatured library was loaded into the reagent cartridge of Illumina MiSeq™ sequencer. The output files (fastq) generated from the sequencer were used for analysis.

3.3.3. Analysis of NGS data

Total raw sequencing reads obtained from sequencer were checked for quality parameters *viz.*, base quality parameters, base composition distribution and GC distribution. After trimming the unwanted sequences from original paired-end data, a consensus V3 region sequence was constructed using Clustal Omega program. Subsequently, multiple filters *viz.*, conserved region filter, spacer filter and mismatch filter were applied and the highest quality V3 region sequences were taken for various downstream analyses.

As a part of pre-processing of sequence reads, singletons were removed that were likely due to the sequencing errors and could result in spurious operational taxonomic units (OTUs). This step was achieved by removing the reads that did not cluster with other sequences (abundances <2). Chimeras were also removed using the *de-novo* chimera removal method UCHIME implemented in the tool USEARCH.

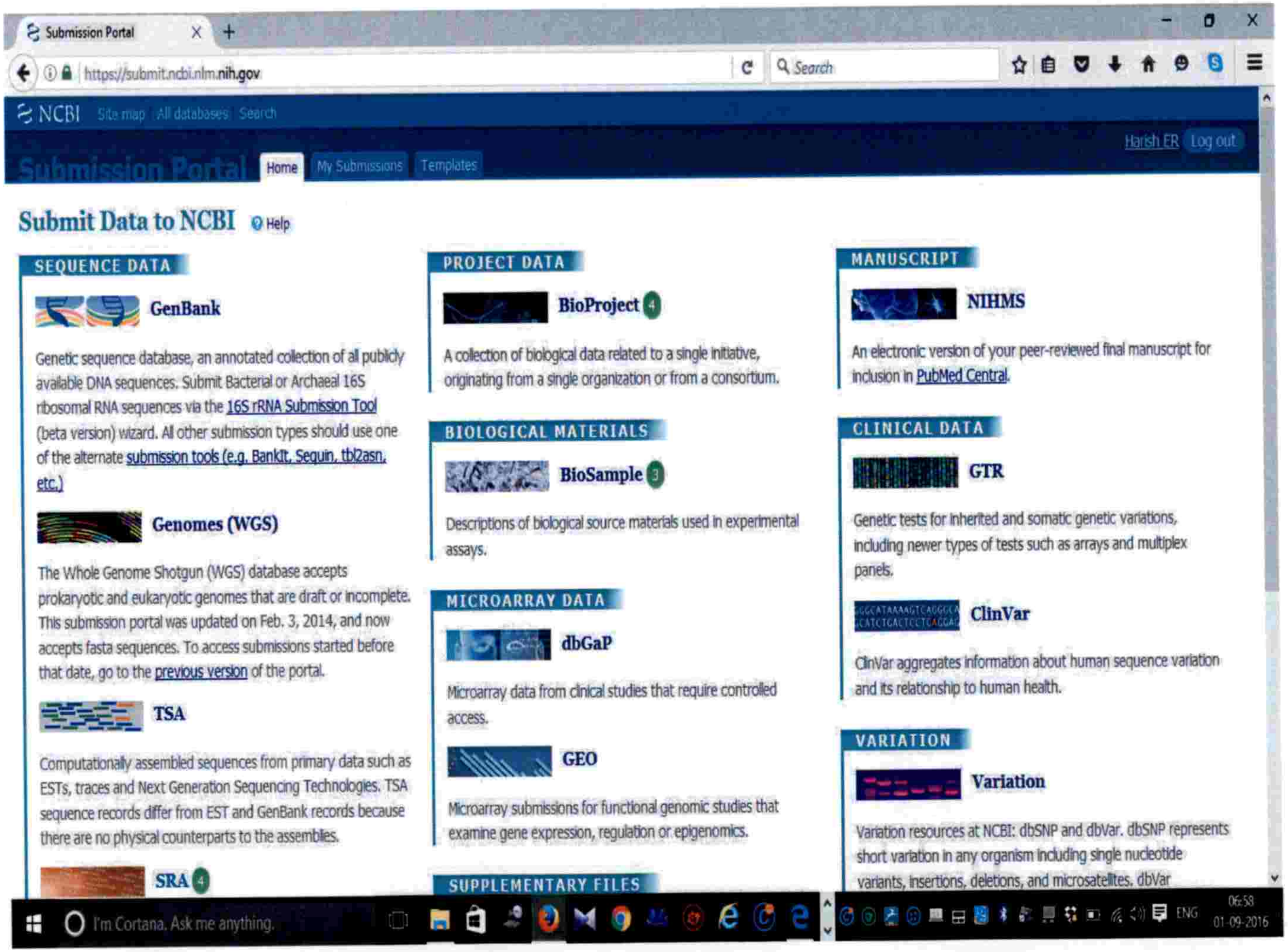


Plate 4. Screenshot of SRA submission portal

Pre-processed reads from all samples were pooled and clustered into OTUs based on their sequence similarity using Uclust program (similarity cutoff at 0.97). QIIME (Caporaso *et al.*, 2010) and MG-RAST (Meyer *et al.*, 2008) programmes were used for downstream analysis. Representative sequences were identified for each OTU and aligned against Greengenes core set of sequences using PyNAST program (DeSantis *et al.*, 2006a; DeSantis *et al.*, 2006b). Further this representative sequences were aligned against reference chimeric datasets. Then, taxonomic classification was performed using RDP classifier and Greengenes OTUs database.

3.3.4. Sequence Read Archive (SRA) submission

Metagenomic sequences were submitted to Sequence Read Archive (SRA) site of NCBI (Plate 4). The steps were, creation of bioproject, biosample submission, gathering of sequence data files, entering metadata on SRA website (a.create sra submission, b.create experiment(s) and link to bioproject and biosample, c.create run(s)), transfer data files to SRA and update submission with PubMed links, release date or metadata changes. The successful submission in SRA provided an Experiment ID and Run ID.

3.4. Study of *Bemisia tabaci – Cassava mosaic virus* interactions in cassava

3.4.1. Cassava genotypes with varying degree of responses to virus infection

Six genotypes of cassava were used for studying different kinds of interaction existing between the whitefly and virus. The genotypes used were, known resistant genotypes (CMR-9 and CMR-128), infected but recovering types of genotypes (CMR-1 and CMR-102) and known susceptible genotypes (H-226 and H-165) (Plates 5, 6 and 7). They were used for studying the interactions, as normal plants and also as purposefully virus infected plants. Three replications were used for all the different category of genotypes in the study. All the studies were conducted in chambers made with plastic bottles with top portion and side were properly cut (Plate 8). Muslin cloths were used to cover the cut-portions (pasted with gum); which provided aeration. Aspirator was used for the release and collection of whitefly from the chamber.

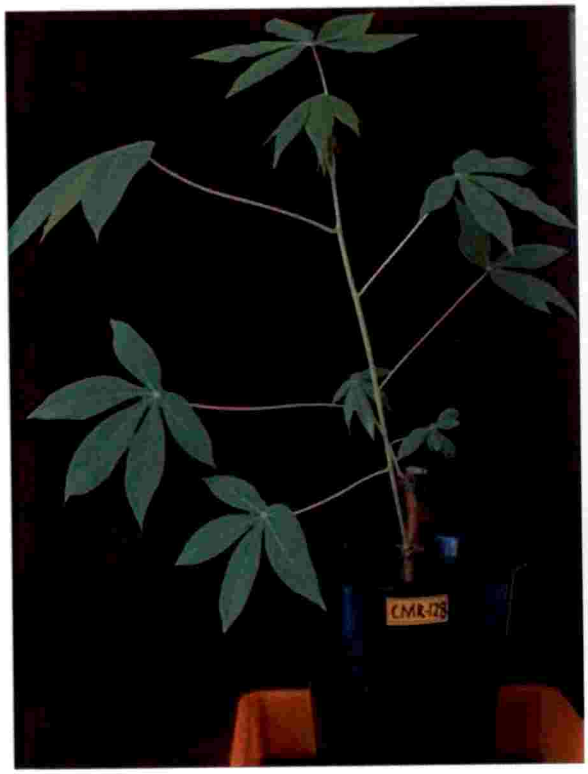
For the entire study, virus free *B. tabaci* culture was maintained separately in cassava plants raised out of meristem culture (Plate 9) and virus infected whitefly were maintained in diseased cassava genotypes H-226 and H-165.

3.4.2. Study on interaction between *Bemisia tabaci* and *Cassava mosaic virus*

Behavioural responses of virulent and non-virulent *B. tabaci* on the cassava genotypes were studied for its dispersal, feeding, fecundity, longevity and life cycle.

- For studying the dispersal and settling pattern (cm/s) of *B. tabaci* on different cassava genotypes, tappings were given outside study chambers at uniform intervals (10 seconds), after the release of male and female whiteflies using aspirator. Average of one week observations were taken using ten male and ten female insects.
- Feeding patterns of *B. tabaci* were studied on different cassava genotypes, by counting the number of stylet sheaths on cassava leaves; one week after the release of male and female whiteflies. Staining technique suggested by Backus *et al.* (1988), was used for studying the number of stylet sheaths present in leaves.
- For studying the influence of cassava genotype variation on fecundity of *B. tabaci*, number of eggs laid for three generations by the insects (One pair used/ plant) was counted.
- Influence of cassava genotype variation on adult longevity of *B. tabaci* was studied by releasing ten male and female pupae per plant.

CMR 9



CMR 128

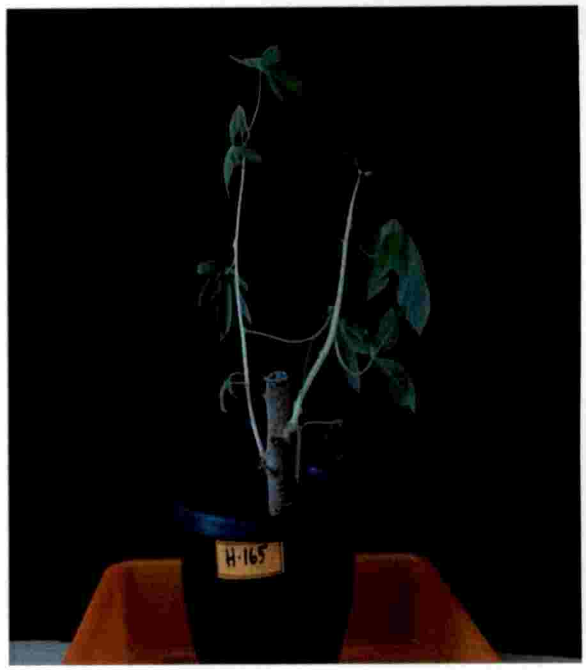
Plate 5. Resistant cassava genotypes (R)

CMR 102

CMR 1

Plate 6. Infected but recovering type of cassava genotypes (IR)

H 226



H 165

Plate 7. Susceptible cassava genotypes (S)

Plate 8. Study chamber for *B. tabaci* (vector) - *Cassava mosaic virus* interactions

Plate 9. Tissue cultured cassava seedlings (H-226)

- For studying the influence of cassava genotype variation on life cycle of *B. tabaci*, average life cycle of male and female insects was taken for three generations.

Statistical analyses were performed using OPSTAT software (three factorial CRD), of CCS Haryana Agricultural University, Hisar (Sheoran *et al.*, 1998).

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Results

4. RESULTS

The results of the experiments carried out in the study entitled "Characterization of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), for genetic variability, endosymbionts and vector-virus interactions in cassava" during 2013-2016 at various fields and facilities are presented in this chapter.

4. 1. Morphological characterisation of cassava whitefly, *Bemisia tabaci*

The samples of pupal and adult insects drawn from the cultures were subjected to morphometric studies. Developmental stages (Plate 10) were identified according to the methods described by Malumphy *et al.* (2009) and Chaubey *et al.* (2010). Puparia and adults were processed for mounting as recommended earlier (Malumphy, 2004).

A total of 21 characters of females and 23 characters of males were studied. Pupal characters (male and female) studied included pupal length, pupal width, length and width of right and left anterior wax margin, vasiform orifice length, operculum length, operculum width, lingula length, lingula width, caudal furrow length, caudal seta length and distance between caudal setae. For male adult insects, nine characters were compared and the characters were antennal length, body length, body width, forewing length, forewing width, hind tarsal length, hind tarsal width, aedeagus length and clasper length. Seven characters except aedeagus length and clasper length were compared for adult female insects. All these characters were compared for places belonging to 13 different agroecological zones of Kerala, *viz.*, Neyyattinkara, Sreekaryam, Vellanikkara, Vadakara, Sultan Bathery, Kasargod, Thodupuzha, Palluruthy, Pulikeezhu, Muvattupuzha, Kayamkulam, Palakkad and Chittur.

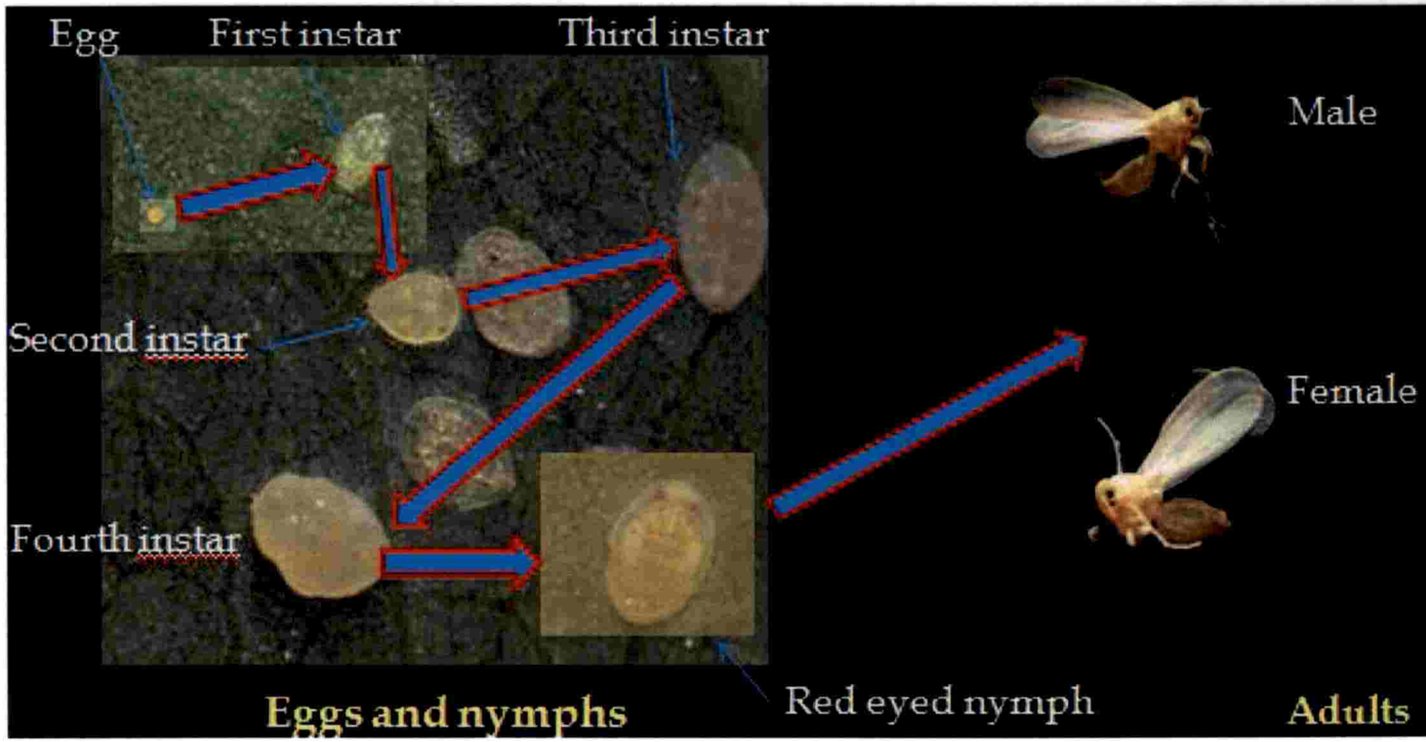


Plate 10. Developmental stages of *Bemisia tabaci*

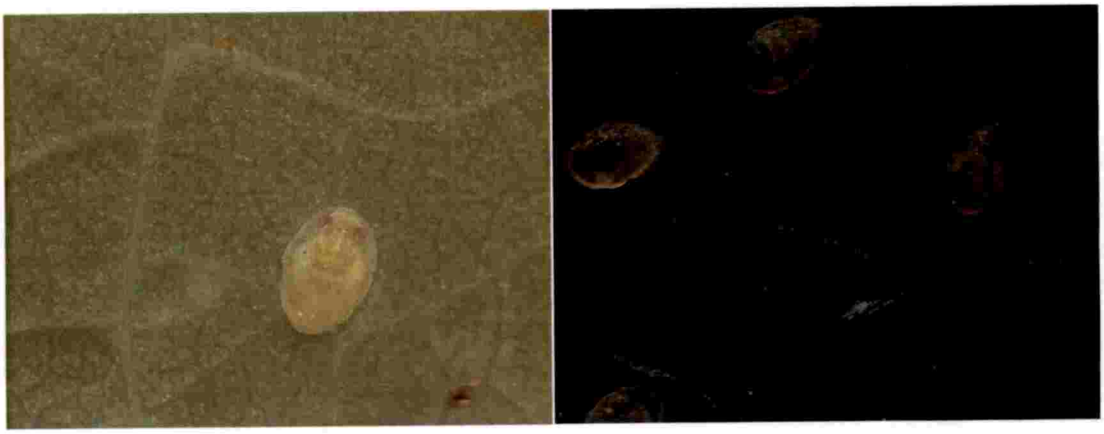
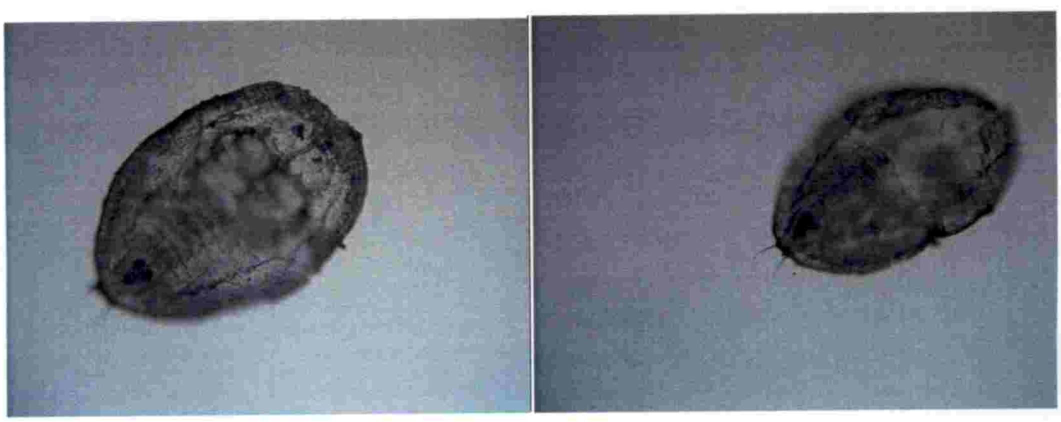


Plate 11. Red eyed nymph of *Bemisia tabaci*

Plate 12. Puparium of *Bemisia tabaci*



Female Male

Plate 13. Female and male pupariae of *Bemisia tabaci*

4.1.1. Morphometric variations in *Bemisia tabaci* pupa from different agro-ecological zones of Kerala

For the study of pupa, 'puparium' (pupal case of late fourth nymphal stage with red eyes, known as red eyed nymph, (Plate 11) was used (Plates 12, 13). Pupal characters (male and female) studied included pupal length, pupal width, length and width of right and left anterior wax margin, vasiform orifice length, operculum length, operculum width, lingula length, lingula width, caudal furrow length, caudal seta length and distance between caudal setae.

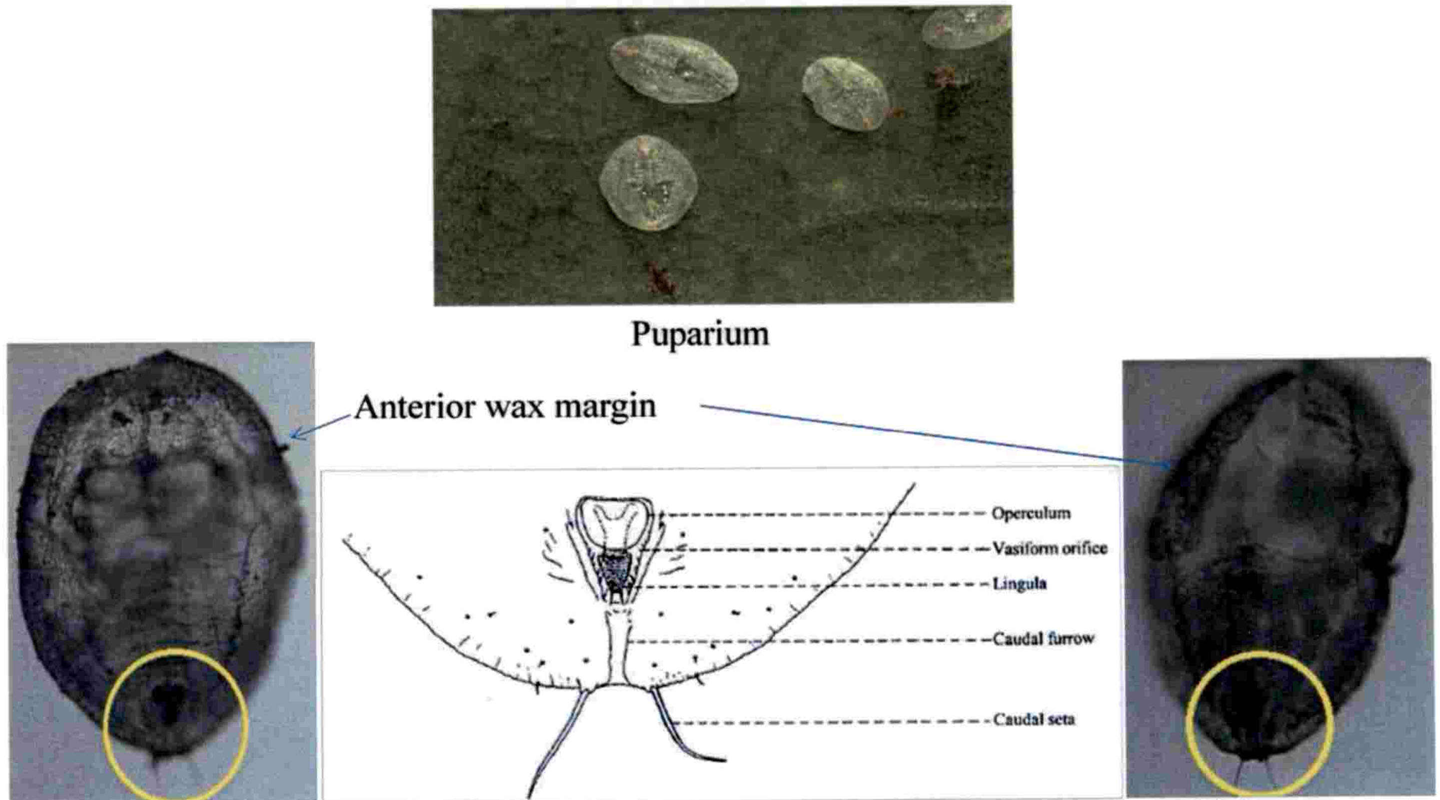
4.1.1.1. Morphometric variations in female pupa of cassava whitefly, *Bemisia tabaci*

Among female pupal characters (Plate 13, 14, Table 3), mean pupal length was highest in population from Palluruthy (0.746 mm); followed by Vadakara and Pulikeezhu (0.724 mm), Chittur (0.720 mm), Muvattupuzha (0.715 mm), Palakkad, Thodupuzha and Sreekaryam (0.714 mm), Neyyattinkara (0.713 mm), Kasargod (0.712 mm), Vellanikkara and Kayamkulam (0.706 mm) and the lowest was found to be in the population from Sultan Bathery (0.668 mm). Whereas, pupal width was highest in populations from Sultan Bathery (0.539 mm); followed by Muvattupuzha (0.523 mm), Palluruthy (0.520 mm), Thodupuzha (0.512 mm), Pulikeezhu (0.497 mm), Vadakara (0.495 mm), Neyyattinkara (0.487 mm), Vellanikkara (0.476 mm), Kayamkulam (0.474 mm), Kasargod and Sreekaryam (0.471 mm), Palakkad and Chittur (0.468 mm). Most of the other characters like width of anterior wax margin, vasiform orifice length, length and width of operculum and lingula, caudal furrow and caudal seta length and distance between caudal setae were showing no significant difference between various agro-ecological zones.

Length and width of right anterior wax margin were highest in populations from Sreekaryam (0.086 mm) and Palakkad, Vadakara, Vellanikkara, Thodupuzha, Pulikeezhu (0.024 mm), respectively; lowest were from Sultan Bathery (0.056 mm) and Muvattupuzha (0.019 mm), respectively. Length and width of left anterior wax margin were highest in populations from Sreekaryam (0.084 mm) and Palakkad, Vellanikkara, Thodupuzha, Pulikeezhu (0.024 mm), respectively; lowest was from Sultan Bathery (0.055 mm) and Muvattupuzha (0.019 mm) respectively. Sultan Bathery population had shown 0.070 mm vasiform orifice length, which was the highest among all the populations and the lowest was shown by populations from Chittur, Palluruthy and Kayamkulam (0.062 mm). Operculum length was highest in Sultan Bathery population. (0.068 mm) and lowest was in populations from Chittur, Palluruthy and Kayamkulam (0.062 mm). Operculum width was highest in population from Neyyattinkara (0.036 mm) and lowest was in population from Kasargod (0.025 mm). Lingula length (0.059 mm) and width (0.030 mm) were highest in Sultan Bathery population; lowest were in populations from Palakkad, Chittur, Muvattupuzha (0.038 mm) and Palakkad, Vadakara, Muvattupuzha (0.021 mm) respectively. Caudal furrow length was highest in populations from Kasargod and Palluruthy (0.054 mm) and lowest was from population of Palakkad (0.042 mm). For caudal seta length, highest value was seen in population from Thodupuzha (0.096 mm).and lowest was seen in population from Sultan Bathery (0.086 mm). Sultan Bathery population found to have highest distance between caudal setae (0.049 mm) among the populations. Lowest distance between caudal setae was found to be present in populations of Chittur and Kayamkulam (0.041 mm).

4.1.1.2. Morphometric variations in male pupae of cassava whitefly, *Bemisia tabaci*

Among male pupal characters (Plate 13, 14, Table 4), pupal length was highest in population from Muvattupuzha (0.688 mm); followed by Kasargod (0.685 mm), Kayamkulam (0.679 mm), Vadakara (0.668 mm), Vellanikkara (0.636 mm), Neyyattinkara (0.633 mm), Palluruthy (0.630 mm), Sreekaryam (0.629 mm), Chittur (0.628 mm), Pulikeezhu (0.627 mm), Thodupuzha (0.626 mm), Palakkad (0.624 mm) and the lowest was found to be in the population from Sultan Bathery (0.582 mm). Pupal width was highest in population from Sultan Bathery (0.422 mm); followed by Neyyattinkara (0.413 mm), Sreekaryam (0.412 mm), Kasargod (0.410 mm), Palakkad (0.404 mm), Pulikeezhu (0.402 mm), Chittur (0.394 mm), Kayamkulam (0.391 mm), Vadakara (0.385 mm), Muvattupuzha (0.381 mm), Vellanikkara (0.379 mm), Palluruthy (0.376 mm) and Thodupuzha (0.366 mm). Other characters were showing no significant difference between various agro-ecological zones.



Female pupa | Graphical representation | Male pupa

Plate 14. Female and male puparial characters of *Bemisia tabaci*

Table 3. Characters of female pupae*of cassava whitefly, *Bemisia tabaci* from different agro-ecological zones of Kerala

Place	Pupal length	Pupal width (unit)	Anterior wax margin (R-l)[1]	Anterior wax margin (R-w)[2]	Anterior wax margin (L-l)[3]	Anterior wax margin (L-w)[4]	Vasiform orifice length (unit?)	Oper culum length	Oper culum width	Lingula length	Lingula width	Caudal furrow length	Caudal seta length	Distance between caudal setae
PKD	0.714	0.468	0.070	0.024	0.071	0.024	0.065	0.065	0.030	0.038	0.021	0.042	0.095	0.047
CTR	0.720	0.468	0.072	0.021	0.072	0.022	0.062	0.062	0.031	0.038	0.022	0.045	0.093	0.041
KGD	0.712	0.471	0.082	0.020	0.082	0.020	0.066	0.066	0.025	0.044	0.024	0.054	0.088	0.042
TPA	0.714	0.512	0.082	0.024	0.081	0.024	0.065	0.065	0.034	0.048	0.023	0.052	0.096	0.046
SBY	0.668	0.539	0.056	0.019	0.055	0.020	0.070	0.068	0.035	0.059	0.030	0.048	0.086	0.049
PTY	0.746	0.520	0.072	0.021	0.072	0.021	0.062	0.062	0.029	0.048	0.029	0.054	0.091	0.046
VKA	0.724	0.476	0.076	0.024	0.076	0.024	0.066	0.066	0.035	0.039	0.026	0.049	0.090	0.043
VDA	0.706	0.495	0.074	0.024	0.075	0.022	0.066	0.066	0.034	0.040	0.021	0.051	0.088	0.047
NYA	0.713	0.487	0.081	0.022	0.081	0.022	0.063	0.063	0.036	0.046	0.024	0.049	0.091	0.042
KYM	0.706	0.474	0.077	0.022	0.077	0.023	0.062	0.062	0.034	0.045	0.023	0.052	0.087	0.041
SKM	0.714	0.471	0.086	0.021	0.084	0.021	0.067	0.067	0.030	0.050	0.028	0.053	0.090	0.045
MVA	0.715	0.523	0.071	0.019	0.071	0.019	0.065	0.065	0.031	0.038	0.021	0.050	0.090	0.046
PKU	0.724	0.497	0.082	0.024	0.082	0.024	0.063	0.063	0.028	0.049	0.023	0.046	0.092	0.047
C.D. (p=0.05)	0.022	0.020	0.014	N/S	0.014	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
SE (m)	0.007	0.007	0.005	0.005	0.005	0.005	0.007	0.007	0.007	0.006	0.005	0.005	0.007	0.005
SE (d)	0.010	0.009	0.006	0.008	0.007	0.007	0.010	0.010	0.009	0.008	0.007	0.008	0.011	0.007
C.V.	1.375	1.885	8.497	36.142	8.670	33.803	14.799	15.921	30.271	18.357	27.723	15.441	11.677	16.457

*Mean of 10 observations (mm) 1. R-l: Right-length 2. R-w: Right-width 3. L-l: Left-length 4. L-w: Left-width

NB: NYA-Neyyattinkara, SKM-Sreekaryam, VKA-Vellanikkara, VDA-Vadakara, SBY-Sultan Bathery, KGD-Kasargod, TDA-Thodupuzha, PTY-Palluruthy, PKU-Pulikeezhu, MVA-Muvattupuzha, KYM- Kayamkulam, PKD-Palakkad, CTR-Chittur

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Length of right anterior wax margin was highest in population from Sreekaryam (0.079 mm) and and width was highest in Palakkad, Vadakara, Vellanikkara, Thodupuzha, and Pulikeezhu (0.024 mm); lowest was from Sultan Bathery (0.051 mm) and Sultan Bathery (0.017 mm) respectively. Length and width of left anterior wax margin were highest in populations from Sreekaryam, Thodupuzha, Pulikeezhu (0.078 mm) and Vellanikkara, Thodupuzha, Pulikeezhu (0.024 mm) respectively; lowest was from Sultan Bathery (0.051 mm) and Sultan Bathery (0.017 mm) respectively. Sultan Bathery populations shown 0.068 mm vasiform orifice length, which was the highest among all the populations and the lowest was shown by populations from Chittur, Palluruthy and Kayamkulam (0.062 mm).

Operculum length was highest in Sultan Bathery population (0.066 mm) and lowest was in populations from Chittur, Palluruthy, Thodupuzha, Vellanikkara and Kayamkulam (0.062 mm). Operculum width was highest in populations from Neyyattinkara, Vellanikkara, Vadakara, Kayamkulam (0.034 mm) and lowest was in population from Kasargod (0.025 mm). Lingula length (0.049 mm) and width (0.027 mm) were highest in Sultan Bathery population; lowest were in populations from Palakkad, Chittur, Kayamkulam (0.038 mm) and Palakkad, Vadakara, Muvattupuzha (0.021 mm) respectively. Caudal furrow length was highest in populations from Sreekaryam (0.052 mm) and lowest was from population of Palakkad (0.042 mm). For caudal seta length, highest observation seen on population from Palakkad (0.091 mm) and lowest was seen in population from Sultan Bathery (0.084 mm). Sultan Bathery population found to have highest distance between caudal setae (0.046 mm) among the populations. Lowest distance between caudal setae was found to be present in population of Vadakara (0.040 mm).

Principal component analysis (PCA) methods for the estimated variables of whitefly pupa

The results of principal component analysis, based on 14 morphological characters of female and male pupae collected from cassava plants of various agro-ecological zones of Kerala, are presented in Tables 5 and 6. The principal component analysis divided these 14 traits into major principal components. The first principal component (PC1) accounts for maximum variability in the data.

The scree plot of the principal components (PC) showed that the first five (female pupae) and four (male pupae) eigen values corresponded most to the variances in the dataset (Fig.2 and 3). The major principal components were extracted, the total cumulative variance of these principal components amounted to 86.3 per cent and 80.6 per cent respective variations (in female and male pupa) and these principal components had eigen values more than one. The principal component analysis grouped the estimated variables into five major groups in case of female pupa; in which PC1 accounted for 37.4 per cent, PC2 for 17.5 per cent, PC3 for 12.3 per cent, PC4 for 10.4 per cent and PC5 for 8.8 per cent (Table 5) of the total variation. For male pupa, the variables are grouped into four major principal components; in which PC1 accounted for 45.3 per cent, PC2 for 15.1 per cent, PC3 for 12.2 per cent and PC4 for 8 per cent (Table 6) of the total variation.

For female pupae, among the five principal components analysed, PC1 which made the largest contribution of 37.4 per cent of the total variation, had shown highest positive contribution for pupal width, vasiform orifice length, lingula length and lingula width. In case of PC2 positive contributions came from length of right and left anterior wax margin and caudal furrow length. Caudal seta length had highest positive contribution in PC3. PC4 had width of right and left anterior wax margin, operculum length and operculum width as positively contributing characters. For

PC5, pupal length and distance between caudal setae had given positive contributions (Table 5).

In case of male pupae, four principal components had eigen values more than one. The largest contributor for variation PC1 had shown highest positive contribution for width of right anterior wax margin, length and width of left anterior wax margin and operculum width. Pupal length and caudal furrow length had shown highest positive contributions in PC2.

For PC3, vasiform orifice length, operculum length and caudal seta length were given highest positive contributions for variations. Pupal width, length of right as well as left anterior wax margin, lingula length, lingula width and distance between caudal setae were given largest share of diversity in PC4 (Table 6).

The first two principal components contributing the major share of variances and were plotted to observe the relationships between measured whitefly traits/variables (Figs. 4 and 5). In first and second principal components, all the characters under consideration contributing diversity positively and up to second principal component 54.9 per cent (female pupae) and 60.4 per cent (male pupae) diversities were observed. So other principal components were not considered as major contributing principal components (even though their eigen values were more than one and some characters were positive) but they were already considered and measured in first two principal components.

Table 4. Characters of male pupae* of cassava whitefly, *Bemisia tabaci* from different agro-ecological zones of Kerala

Place	Pupal length	Pupal width	Anterior wax margin (R-l)[1]	Anterior wax margin (R-w)[2]	Anterior wax margin (L-l)[3]	Anterior wax margin (L-w)[4]	Vasiform orifice length	Oper culum length	Oper culum width	Lingula length	Lingula width	Caudal furrow length	Caudal seta length	Distance between caudal setae
PKD	0.624	0.404	0.067	0.024	0.068	0.022	0.065	0.064	0.030	0.038	0.021	0.042	0.091	0.044
CTR	0.628	0.394	0.072	0.021	0.072	0.021	0.062	0.062	0.031	0.038	0.022	0.045	0.088	0.041
KGD	0.685	0.41	0.077	0.020	0.076	0.021	0.064	0.063	0.025	0.039	0.024	0.051	0.088	0.042
TPA	0.626	0.366	0.078	0.024	0.078	0.024	0.064	0.062	0.033	0.044	0.023	0.051	0.088	0.044
SBY	0.582	0.422	0.051	0.017	0.051	0.017	0.068	0.066	0.030	0.049	0.027	0.048	0.084	0.046
PTY	0.63	0.376	0.070	0.021	0.070	0.021	0.062	0.062	0.029	0.040	0.024	0.051	0.090	0.043
VKA	0.636	0.379	0.076	0.024	0.077	0.024	0.064	0.062	0.034	0.039	0.025	0.049	0.090	0.043
VDA	0.668	0.385	0.069	0.024	0.069	0.023	0.066	0.063	0.034	0.042	0.021	0.051	0.088	0.040
NYA	0.633	0.413	0.076	0.022	0.075	0.022	0.063	0.063	0.034	0.041	0.024	0.049	0.089	0.042
KYM	0.679	0.391	0.077	0.022	0.077	0.022	0.062	0.062	0.034	0.038	0.023	0.050	0.086	0.041
SKM	0.629	0.412	0.079	0.021	0.078	0.022	0.065	0.064	0.030	0.042	0.024	0.052	0.089	0.042
MVA	0.688	0.381	0.071	0.019	0.071	0.019	0.063	0.064	0.026	0.039	0.021	0.050	0.089	0.042
PKU	0.627	0.402	0.078	0.024	0.078	0.024	0.063	0.063	0.028	0.044	0.023	0.046	0.090	0.042
C.D. (p=0.05)	0.046	0.032	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
SE (m)	0.016	0.011	0.006	0.005	0.007	0.005	0.008	0.007	0.008	0.006	0.005	0.009	0.008	0.006
SE (d)	0.022	0.016	0.009	0.007	0.009	0.007	0.011	0.010	0.011	0.009	0.007	0.013	0.011	0.009
C.V.	4.206	4.835	12.592	30.651	13.037	31.162	17.626	15.235	36.198	21.610	32.503	27.044	12.875	21.087

*Mean of 10 observations (mm) 1. R-l: Right-length 2. R-w: Right-width 3. L-l: Left-length 4. L-w: Left-width

NB: NYA-Neyyattinkara, SKM-Sreekaryam, VKA-Vellanikkara, VDA-Vadakara, SBY-Sultan Bathery, KGD-Kasargod, TDA-Thodupuzha, PTY-Palluruthy, PKU-Pulikeezhu, MVA-Muvattupuzha, KYM- Kayamkulam, PKD-Palakkad, CTR-Chittur

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Table 5. PCA/ Eigen analysis of the correlation matrix (*Bemisia tabaci* female pupa)

Eigen value	5.2292	2.4506	1.7193	1.4523	1.2337
Proportion	0.374	0.175	0.123	0.104	0.088
Cumulative	0.374	0.549	0.671	0.775	0.863
Characters	**Principal components**				
	PC1	**PC2**	**PC3**	**PC4**	**PC5**
Pupal length	-0.32	0.16	0.104	-0.357	0.164
Pupal width	0.295	-0.055	0.103	-0.468	-0.006
Anterior wax margin(R-L)	-0.305	0.289	-0.384	0.007	0.030
Anterior wax margin(R-W)	-0.265	-0.342	-0.369	-0.087	-0.108
Anterior wax margin(L-L)	-0.326	0.267	-0.356	0.024	0.041
Anterior wax margin(L-W)	-0.267	-0.363	-0.287	-0.115	-0.236
Vasiform orifice length	0.338	-0.077	-0.361	0.245	0.212
Operculum length	0.284	-0.052	-0.424	0.292	0.286
Operculum width	0.095	-0.276	-0.081	0.104	-0.675
Lingula length	0.271	0.122	-0.264	-0.370	-0.226
Lingula width	0.255	0.223	-0.164	-0.316	-0.215
Caudal furrow length	0.058	0.517	-0.198	-0.107	-0.145
Caudal seta length	-0.257	-0.271	-0.051	-0.309	0.262
Distance between caudal setae	0.241	-0.279	-0.179	-0.365	0.366

Table 6. PCA/ Eigen analysis of the correlation matrix (*Bemisia tabaci* male pupae)

Eigen value	6.3473	2.1146	1.7096	1.1157
Proportion	0.453	0.151	0.122	0.080
Cumulative	0.453	0.604	0.727	0.806
Characters	**Principal components**			
	PC1	**PC2**	**PC3**	**PC4**
Pupal length	0.228	0.466	-0.105	-0.192
Pupal width	-0.230	0.076	0.145	0.341
Anterior wax margin(R-L)	0.345	0.013	-0.152	0.364
Anterior wax margin(R-W)	0.280	-0.431	0.121	-0.099
Anterior wax margin(L-L)	0.355	-0.012	-0.126	0.339
Anterior wax margin(L-W)	0.310	-0.383	-0.050	0.102
Vasiform orifice length	-0.291	-0.190	-0.037	-0.146

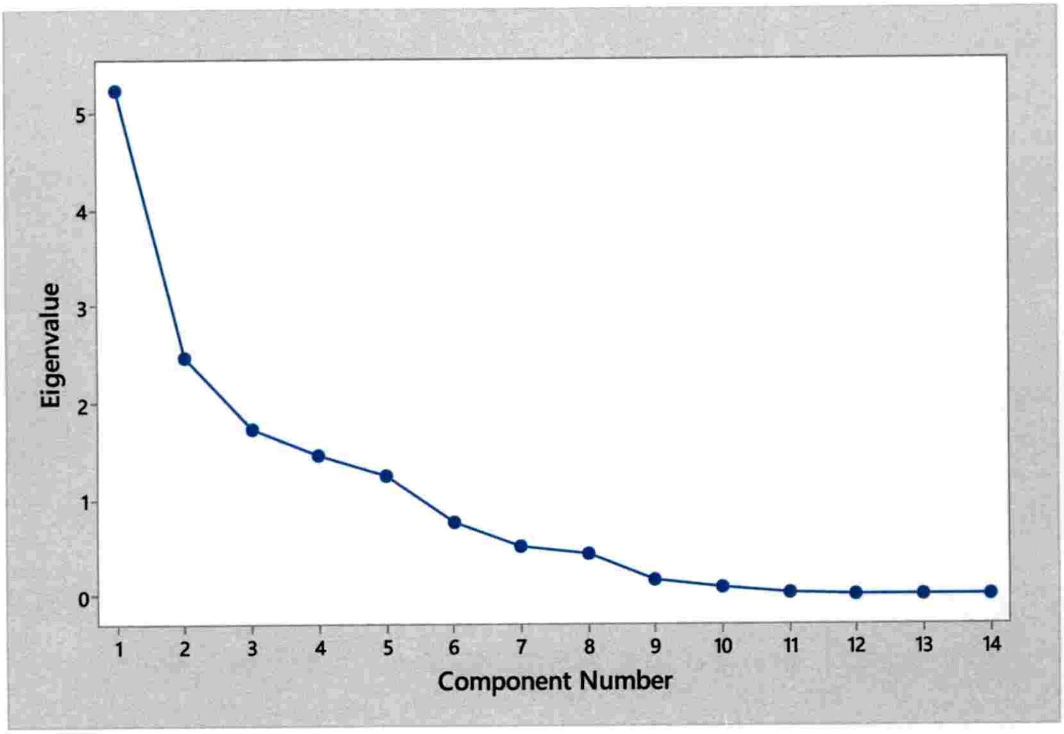

Fig. 2. Scree plot showing eigen values in response to principal components in *Bemisia tabaci* female pupae

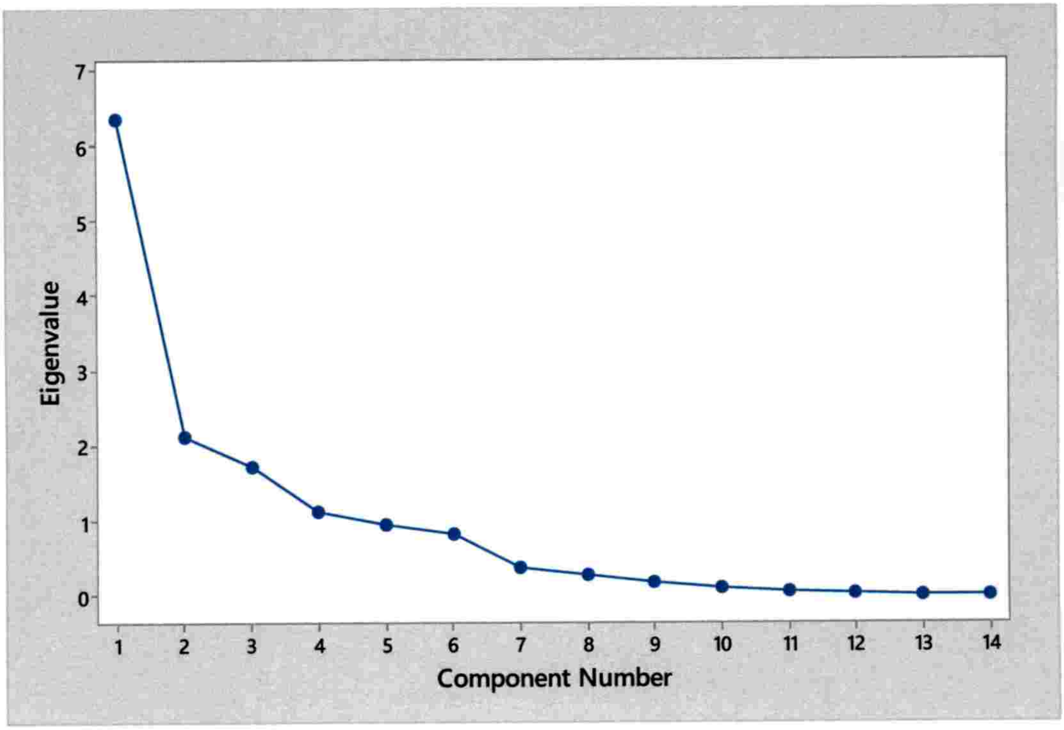

Fig. 3. Scree plot showing eigen values in response to principal components in *Bemisia tabaci* male pupae

Operculum length	-0.341	0.086	0.164	0.055
Operculum width	0.085	-0.440	-0.262	-0.457
Lingula length	-0.278	-0.262	-0.251	0.100
Lingula width	-0.229	-0.156	-0.380	0.427
Caudal furrow length	0.047	0.190	-0.661	0.067
Caudal seta length	0.242	-0.111	0.407	0.307
Distance between caudal setae	-0.276	-0.256	0.076	0.234

Plot of the first two principal components showing relation among various whitefly pupal characters

The correlation coefficient (r) between any two characters is approximated by the cosine of the angle between their vectors. The correlation coefficients among the traits indicated that the plot currently shows the relationship among the traits based on plot that had relatively large loading on both PC1 and PC2 axes (Fig. 4 and 5).

In case of biplot for female pupae (Fig. 4), there were near zero (angle 0-25°) angle between many characters (small obtuse/ acute angles between their vectors) and as correlation coefficient (r=cos0=+1) between any two characters is approximated by the cosine of the angle between their vectors, they have strong positive correlations. The characters with strong positive correlations were operculum length, pupal width and vasiform orifice length; lingula length and lingula width; operculum width and distance between caudal setae; length of right and left anterior wax margin and pupal length; width of right and left anterior wax margin and caudal seta length. Pupal length and caudal furrow length; caudal seta length and operculum width; lingula width and operculum width were mutually near perpendicular vectors (r=cos90=0).

There were negative correlations between length of left anterior wax margin and distance between caudal setae; caudal seta length and lingula width, as indicated by the angle of approximately 180° (150-160°) (r=cos180= -1) between their vectors. In case of biplot for male pupa (Fig. 5), strong positive correlations observed between length of right and left anterior wax margins and caudal seta length; pupal width and operculum length; lingula length, lingula width, vasiform orifice length and distance between caudal setae; width of right and left anterior wax margins and caudal furrow length and pupal length.

Mutually near perpendicular vectors (r=cos90=0), were formed between operculum length and caudal furrow length; caudal furrow length and width of right anterior wax margin; distance between caudal setae, lingula length and operculum width. Negative correlations were observed between pupal width and caudal seta length; length of right and left anterior wax margins and operculum length. Some discrepancies of the plot predictions and original data were expected because the first two principal components accounted for less than 100 per cent of the total variation.

Score plot of first two principal components for whitefly pupae collected from various agro-ecological zones

Scores for whitefly pupae collected from different agro-ecological zones of Kerala, based on PC1 and PC2 are plotted in Fig. 6 and Fig. 7. The 13 zones were grouped into four major distinct clusters/quarters. In case of female pupa, the distribution pattern revealed that maximum number of entries (5) were included in quarter III, namely SKM, CTR, TPA, VDA, NYA whereas, quarter II, included the minimum number of entries (2) i.e. PTY and SBY. Quarter I included entries like KYM, KGD, PKD and Quarter IV included VKA, MVA and PKU. i.e. three number of entries in each cluster (Fig. 6).

The distribution pattern in case of male pupa, revealed that maximum number of entries (5 each) were included in quarters I and II, namely MVA, KGD, KYM, CTR, PTY (quarter I) and VDA, NYA, PKU, VKA, TPA (quarter II). Quarter III, included only two entries PKD, SBY and quarter IV had only one entry SKM (Fig. 7). Score plot analysis of female and male pupa did not provide any clear cut idea

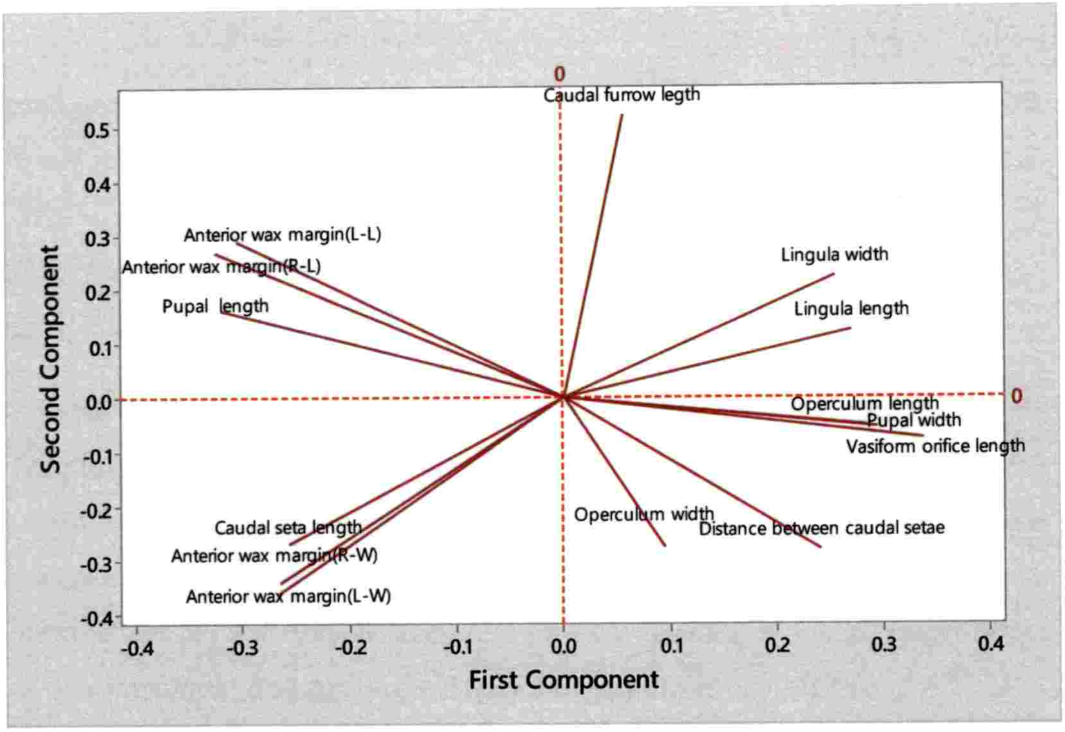

Fig. 4. Biplot/loading plot of the first two principal components showing relation among various *Bemisia tabaci* female pupal characters

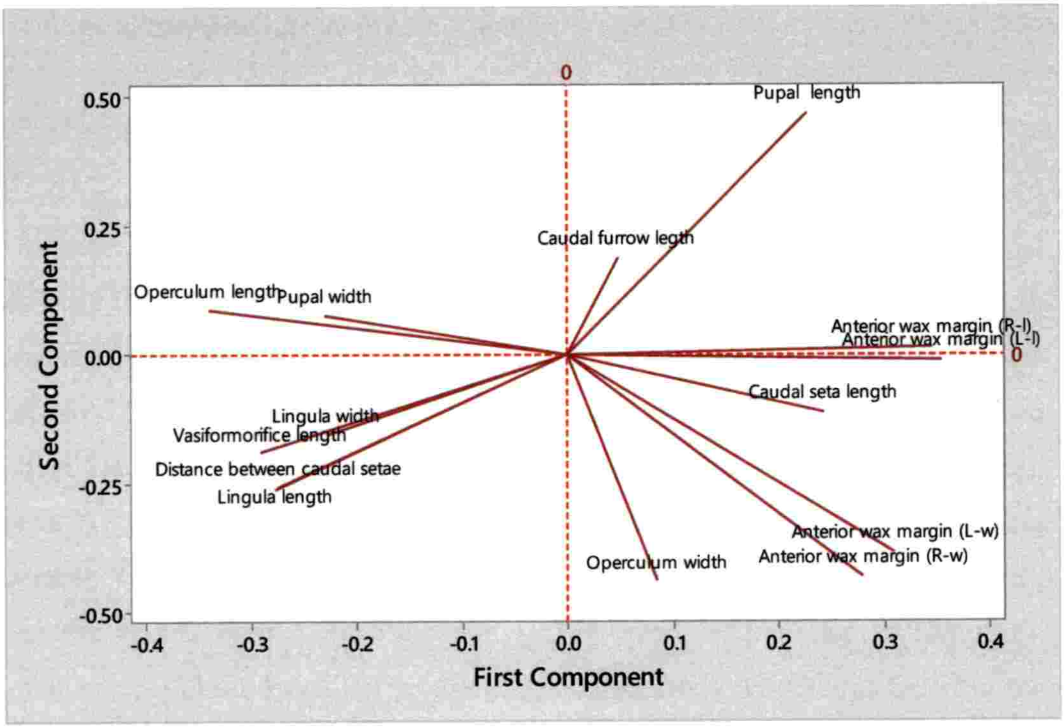

Fig. 5. Biplot/loading plot of the first two principal components showing relation among various *Bemisia tabaci* male pupal characters

about the presence of different genetic groups. But it was found that in case of both female and male pupae, SBY population is in a separate group.

4.1.2. Morphometric variations in adult *Bemisia tabaci* from different agro-ecological zones of Kerala

Different female and male adult (Plate 15, 16) characters like antennal length, body length, body width, forewing length, forewing width, hind tarsal length and hind tarsal width were studied. Additionally, for adult males, aedeagus length and clasper length were also studied. Whitefly antenna found to have seven segments (Plate 17). Hind tarsa (Plate 18) and genital characters of male (Aedeagus and claspers) (Plate 19) are very important in comparing whitefly biotypes (Li *et al.*, 2013).

Among the different characters studied for female and male adults, variations in antennal length, body length, body width, forewing length and forewing width were found to be significantly different among the populations collected from different agro-ecological zones of Kerala (Table 7 and 8).

4.1.2.1. Morphometric variations in adult female of cassava whitefly, *Bemisia tabaci*

In case of characters of adult female cassava whitefly (Table 7), antennal length, body length, body width, forewing length and forewing width were found to be significantly different between the populations; whereas, no significant difference was observed for hind tarsal length and width. Highest antennal length was observed for Sultan Bathery population (0.376 mm). It was followed by populations from Palakkad (0.352 mm), Neyyattinkara (0.351 mm), Chittur (0.346 mm), Sreekaryam (0.343 mm), Vellanikkara (0.338 mm), Vadakara (0.336 mm), Kasargod (0.333 mm), Thodupuzha (0.331 mm), Muvattupuzha (0.329 mm), Pulikeezhu (0.326 mm), Kayamkulam (0.325 mm) and Palluruthy (0.320 mm). Body length was found in the order, Pulikeezhu (0.835 mm) > Kayamkulam (0.834 mm) > Thodupuzha (0.831 mm) > Neyyattinkara (0.828 mm) > Kasargod (0.823 mm) > Sreekaryam (0.822 mm) > Palluruthy (0.821 mm) > Palakkad (0.819 mm) > Muvattupuzha (0.818 mm) > Vadakara (0.817 mm) > Chittur (0.816 mm) > Vellanikkara (0.811 mm) > Sultan Bathery (0.742 mm).

Body width was highest in Sultan Bathery population (0.288 mm), followed by Pulikeezhu (0.264 mm), Palluruthy (0.258 mm), Kasargod (0.257 mm), Kayamkulam, Sreekaryam (0.256 mm), Palakkad, Vadakara (0.255 mm), Chittur, Vellanikkara, Muvattupuzha (0. 254 mm), Thodupuzha (0.253 mm) and Neyyattinkara (0.251 mm). Forewing length was in the order, Chittur (0.933 mm), Palakkad (0.931 mm), Muvattupuzha (0. 928 mm), Vellanikkara (0.923 mm), Sreekaryam, Palluruthy (0.920 mm), Vadakara (0.919 mm), Kayamkulam (0.912 mm), Kasargod (0.911 mm), Neyyattinkara (0.904 mm), Thodupuzha (0.903 mm), Pulikeezhu (0.901 mm) and Sultan Bathery (0.839 mm). Forewing width was found to be highest in Sreekaryam population (0.341 mm). The others were in the order, Neyyattinkara (0.338 mm) > Palakkad, Thodupuzha (0.336 mm) > Palluruthy, Muvattupuzha (0. 330 mm) > Vadakara, Kayamkulam (0.328 mm) > Pulikeezhu (0.317 mm) > Kasargod (0.314 mm) > Vellanikkara (0.311 mm) > Chittur (0.310 mm) > Sultan Bathery (0.253 mm). Highest hind tarsal length was present in populations from Kasargod and Vadakara (0.206 mm) and lowest was present in population from Sultan Bathery (0.180 mm). Kasargod population was found to have the highest hind tarsal width (0.037 mm) and Sultan Bathery population had the lowest (0.018 mm).

4.1.2.2. Morphometric variations in adult male of cassava whitefly

Antennal length, body length, body width, forewing length and forewing width were found to be significantly different between the populations; whereas, no significant difference was observed for hind tarsal length, hind tarsal width, aedeagus length and clasper length (Table 8). Highest antennal length was observed for Sultan Bathery population (0.339 mm). It was followed by populations from Palakkad (0.310 mm), Pulikeezhu (0.308 mm), Muvattupuzha (0.305 mm), Vellanikkara (0.304 mm), Thodupuzha (0.303 mm), Sreekaryam (0.302 mm), Kayamkulam (0.300 mm), Vadakara (0.298 mm), Palluruthy (0.296 mm), Chittur, Kasargod (0.294 mm) and Neyyattinkara (0.293 mm). Body length was found in the order, Vellanikkara (0.783 mm) > Pulikeezhu (0.781 mm) > Palakkad (0.780 mm) > Thodupuzha, Kayamkulam (0.778 mm) > Muvattupuzha (0.777 mm) > Sreekaryam (0.775 mm) > Vadakara (0.769 mm) > Neyyattinkara (0.767 mm) > Palluruthy (0.765 mm) > Kasargod (0.764 mm) > Chittur (0.759 mm) > Sultan Bathery (0.735 mm). Body width was highest in Sultan Bathery population (0.253 mm) and was followed by Pulikeezhu (0.238 mm), Palluruthy (0.237 mm), Chittur (0.233 mm), Muvattupuzha (0.231 mm), Thodupuzha (0.230 mm), Neyyattinkara (0.228 mm), Vellanikkara, Sreekaryam (0.225 mm), Kasargod (0.224 mm), Palakkad, Vadakara (0.222 mm) and Kayamkulam (0.221 mm).

Table 7. Characters of adult female*of cassava whitefly, *Bemisia tabaci* from different agro-ecological zones of Kerala

Place	Antennal length	Body length	Body width	Forewing length	Forewing width	Hind tarsal length	Hind tarsal width
PKD	0.352	0.819	0.255	0.931	0.336	0.196	0.028
CTR	0.346	0.816	0.254	0.933	0.310	0.195	0.030
KGD	0.333	0.823	0.257	0.911	0.314	0.206	0.037
TPA	0.331	0.831	0.253	0.903	0.336	0.204	0.029
SBY	0.376	0.742	0.288	0.839	0.253	0.180	0.018
PTY	0.320	0.821	0.258	0.920	0.337	0.203	0.035
VKA	0.338	0.811	0.254	0.923	0.311	0.204	0.029
VDA	0.336	0.817	0.255	0.919	0.328	0.206	0.030
NYA	0.351	0.828	0.251	0.904	0.338	0.201	0.033
KYM	0.325	0.834	0.256	0.912	0.328	0.203	0.031

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SKM	0.343	0.822	0.256	0.920	0.341	0.201	0.029
MVA	0.329	0.818	0.254	0.928	0.330	0.193	0.034
PKU	0.326	0.835	0.264	0.901	0.317	0.205	0.032
C.D. (p=0.05)	0.022	0.021	0.017	0.020	0.016	N/S	N/S
SE (m)	0.007	0.007	0.006	0.006	0.005	0.007	0.006
SE (d)	0.010	0.010	0.008	0.009	0.007	0.010	0.008
C.V.	3.003	1.178	3.061	0.973	2.322	5.134	27.323

*Mean of 10 observations (mm)

(NYA-Neyyattinkara, SKM-Sreekaryam, VKA-Vellanikkara, VDA-Vadakara, SBY-Sultan Bathery, KGD-Kasargod, TDA-Thodupuzha, PTY-Palluruthy, PKU-Pulikeezhu, MVA-Muvattupuzha, KYM- Kayamkulam, PKD-Palakkad, CTR-Chittur)

Forewing length was in the order, Muvattupuzha (0.886 mm), Palakkad, Sreekaryam (0.881 mm), Chittur (0.876 mm), Vellanikkara (0.874 mm), Kasargod (0.871 mm), Thodupuzha, Vadakara (0.869 mm), Kayamkulam (0.866 mm), Neyyattinkara (0.860 mm), Pulikeezhu (0.858 mm), Palluruthy (0.857 mm) and Sultan Bathery (0.829 mm). Forewing width was found to be highest in Thodupuzha populations (0.293 mm). The others were in the order, Kayamkulam (0.289 mm) > Muvattupuzha (0.288 mm) > Kasargod, Sreekaryam (0. 286 mm) > Pulikeezhu (0.278 mm) > Vadakara (0.277 mm) > Palakkad, Neyyattinkara (0. 274 mm) > Vellanikkara (0.272 mm) > Chittur (0.271 mm) > Palluruthy (0.268 mm) > Sultan Bathery (0.244 mm). Highest hind tarsal length was present in populations from Neyyattinkara (0.193 mm) and lowest was present in populations from Sultan Bathery (0.170 mm). Vadakara, Kayamkulam, Sreekaryam (0.028 mm) populations found to have the highest hind tarsal width (0.028 mm) and Sultan Bathery populations found to have the lowest (0.019 mm). Aedeagus length was highest in Kayamkulam populations (0.105 mm) and lowest was in Sultan Bathery populations (0.074 mm). Chittur and Kayamkulam populations had the highest Clasper length (0.106 mm) and Sultan Bathery populations found to have the lowest clasper length (0.081 mm).

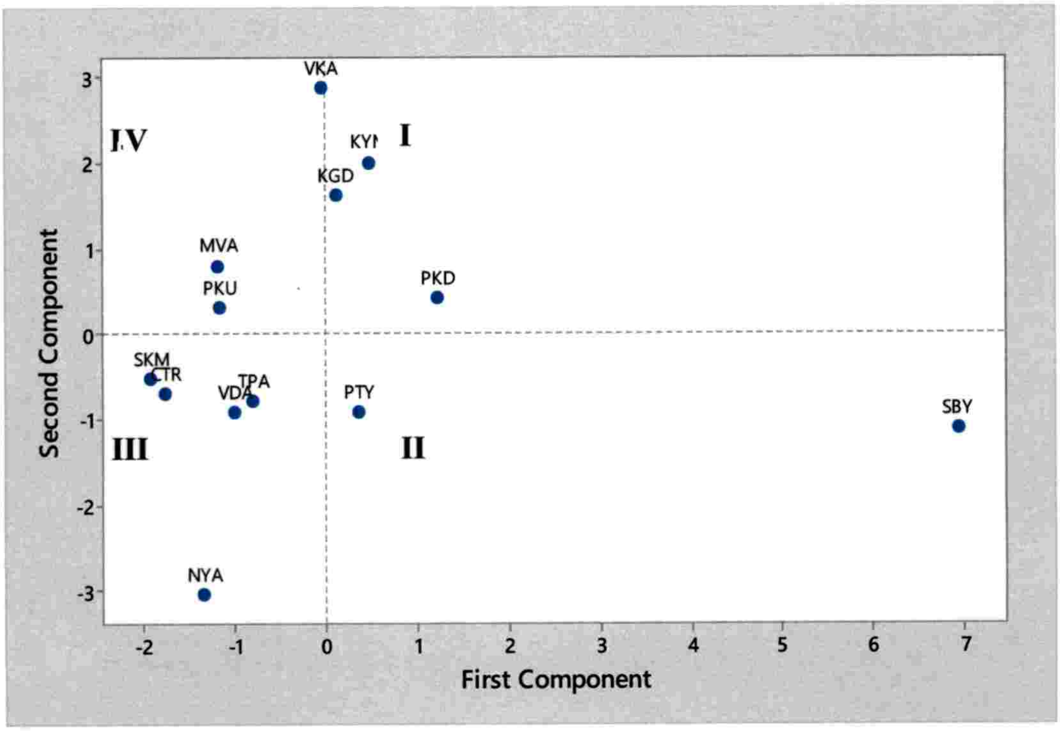


Fig. 6. Score plot of first two components for *Bemisia tabaci* female pupa collected from various locations

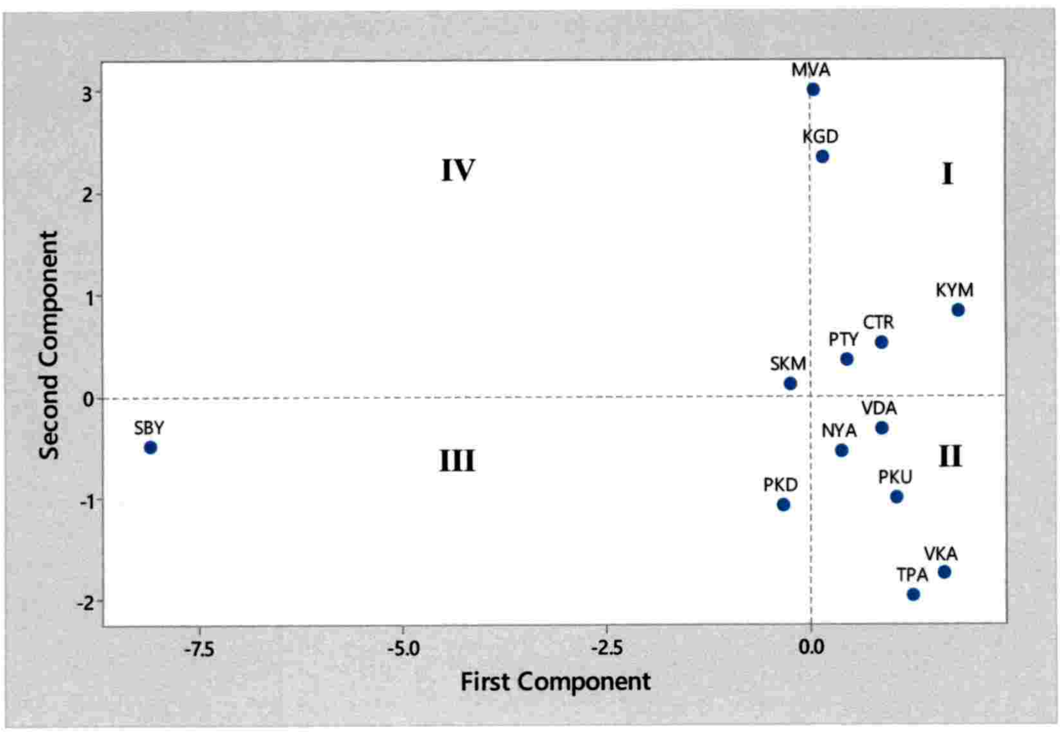


Fig. 7. Score plot of first two components for *Bemisia tabaci* male pupa collected from various locations

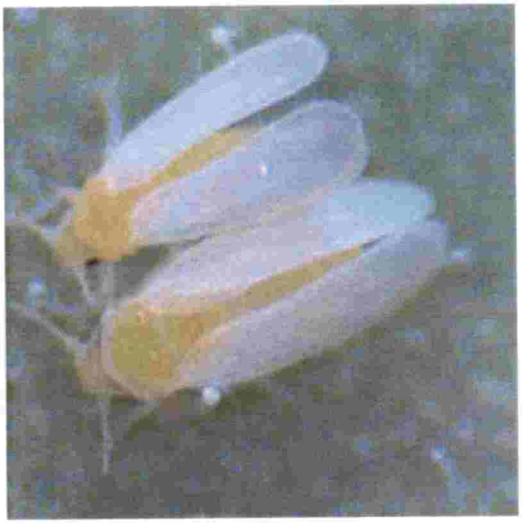
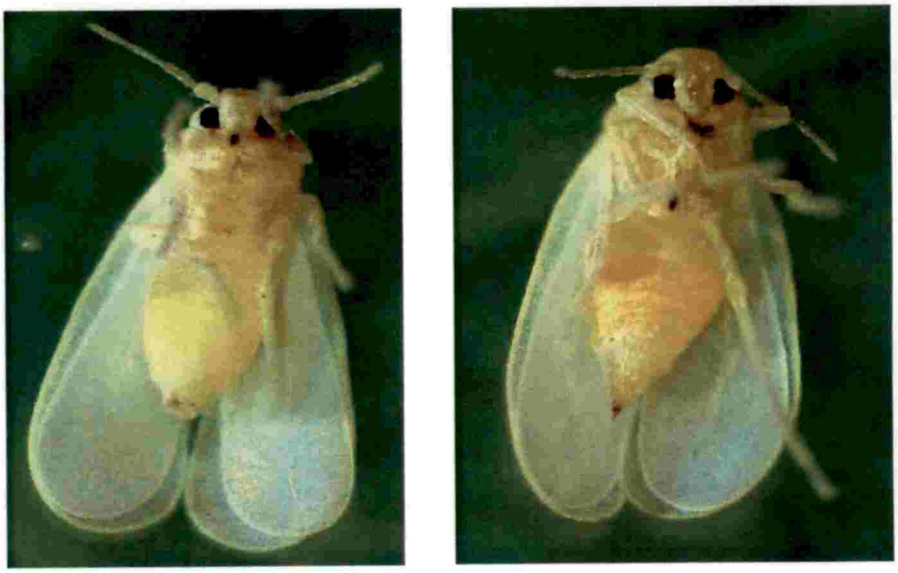


Plate 15. Adult pair of *Bemisia tabaci* (Dorsal view)



Female Male

Plate 16. *Bemisia tabaci* adults (Ventral view)

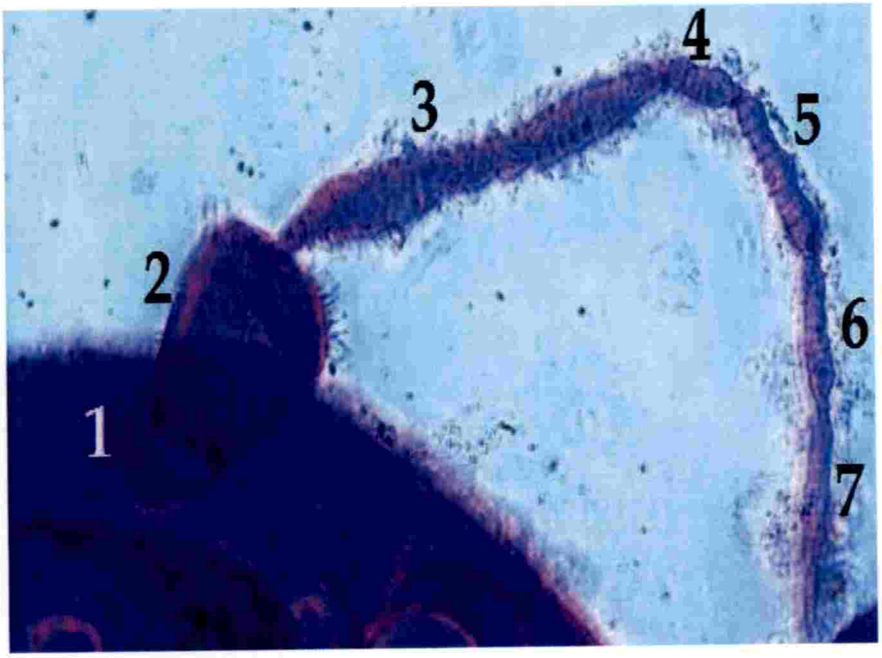
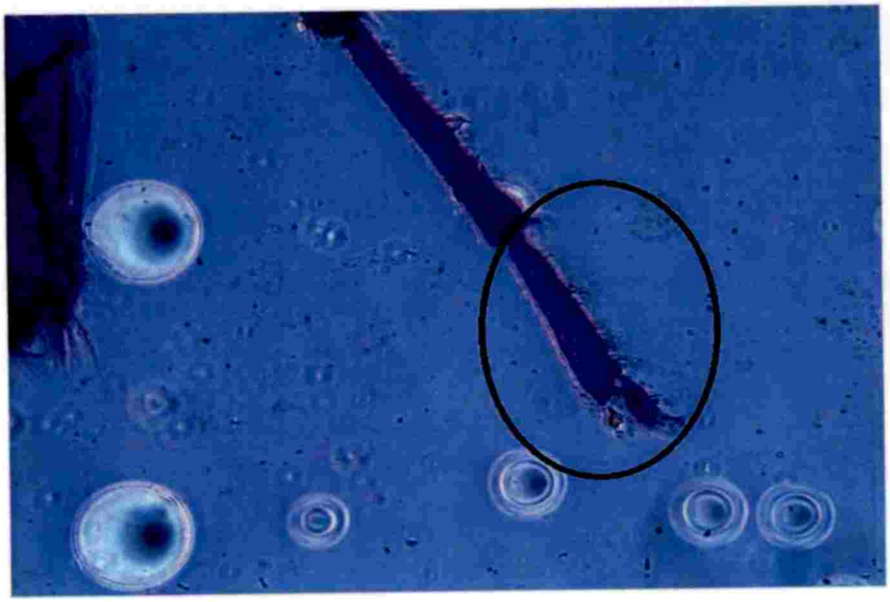


Plate 17. Antenna of *Bemisi tabaci*



Hind tarsus

Plate 18. Hind tarsus of *Bemisi tabaci*

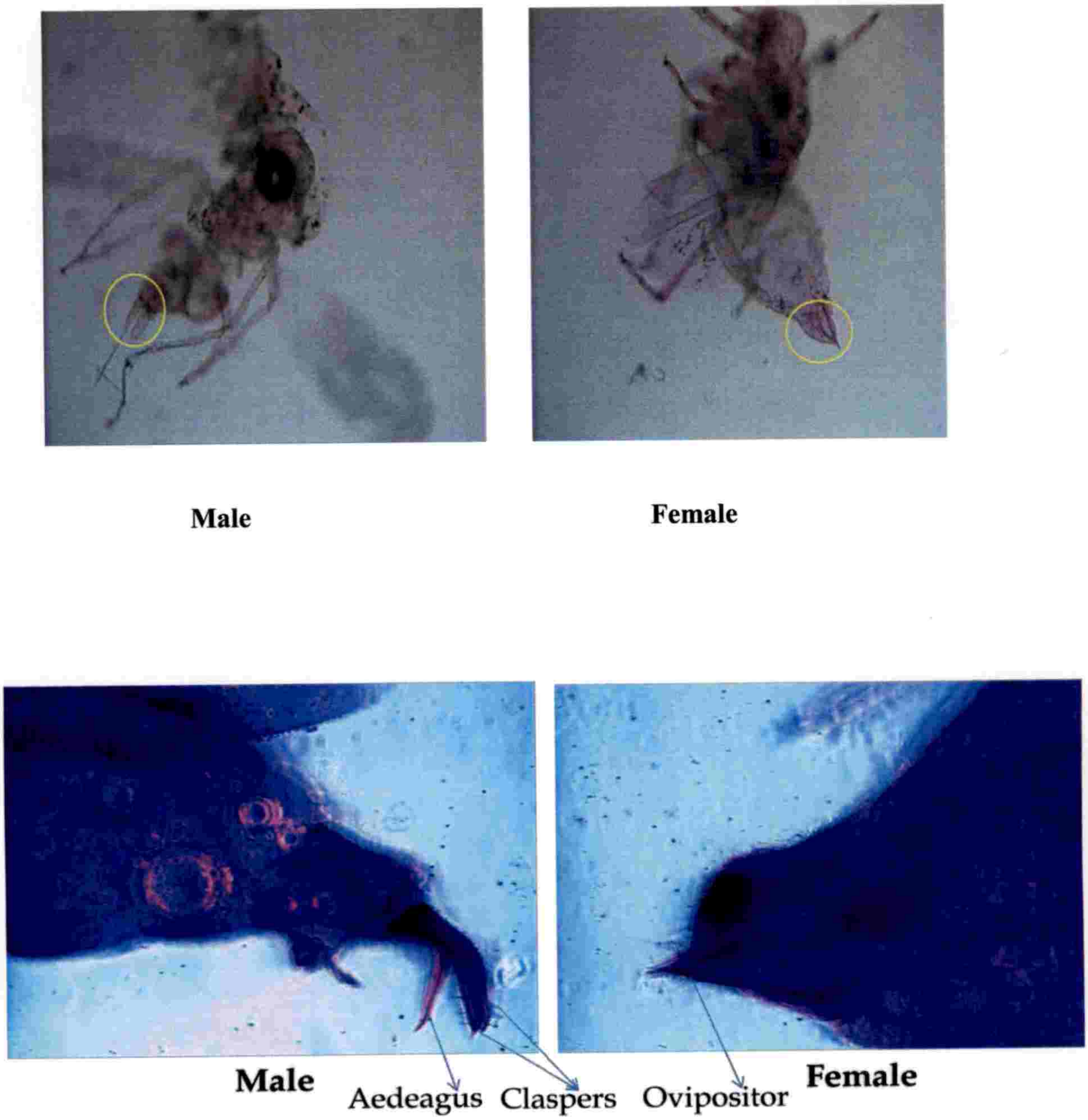


Plate 19. Male and female genitalia of *Bemisia tabaci*

Table 8. Characters of adult male*of cassava whiteflyfrom different agro-ecological zones of Kerala

Place	Antennal length	Body length	Body width	Forewing length	Forewing width	Hind tarsal length	Hind tarsal width	Aedeagus length	Clasper length
PKD	0.310	0.780	0.222	0.881	0.274	0.179	0.025	0.091	0.092
CTR	0.294	0.759	0.233	0.876	0.271	0.188	0.026	0.104	0.106
KGD	0.294	0.764	0.224	0.871	0.286	0.179	0.027	0.089	0.090
TPA	0.303	0.778	0.230	0.869	0.293	0.178	0.026	0.080	0.085
SBY	0.339	0.735	0.253	0.829	0.244	0.170	0.019	0.074	0.081
PTY	0.296	0.765	0.237	0.857	0.268	0.190	0.025	0.097	0.098
VKA	0.304	0.783	0.225	0.874	0.272	0.185	0.026	0.094	0.094
VDA	0.298	0.769	0.222	0.869	0.277	0.192	0.028	0.083	0.084
NYA	0.293	0.767	0.228	0.860	0.274	0.193	0.025	0.101	0.102
KYM	0.300	0.778	0.221	0.866	0.289	0.187	0.028	0.105	0.106
SKM	0.302	0.775	0.225	0.881	0.286	0.181	0.028	0.087	0.093
MVA	0.305	0.777	0.231	0.886	0.288	0.192	0.026	0.088	0.089
CTR	0.308	0.781	0.238	0.858	0.278	0.183	0.025	0.085	0.086
C.D. (p=0.05)	0.015	0.018	0.016	0.025	0.020	N/S	N/S	N/S	N/S
SE (m)	0.005	0.006	0.005	0.008	0.007	0.007	0.006	0.008	0.007
SE (d)	0.007	0.008	0.007	0.011	0.009	0.010	0.009	0.011	0.011
C.V.	2.291	1.052	3.258	1.322	3.332	5.236	34.203	11.844	11.484

*Mean of 10 observations (mm)

(NB: NYA-Neyyattinkara, SKM-Sreekaryam, VKA-Vellanikkara, VDA-Vadakara, SBY-Sultan Bathery, KGD-Kasargod, TDA-Thodupuzha, PTY-Palluruthy, PKU-Pulikeezhu, MVA-Muvattupuzha, KYM- Kayamkulam, PKD-Palakkad, CTR-Chittur)

In case of both adult female and adult male, whitefly populations from Sultan Bathery found to have the highest antennal length (0.376 mm and 0.339 mm respectively) and highest body width (0.288 mm and 0.253 mm respectively). For other morphometric characters studied, SBY populations had shown the lowest observations when compared with other 12 zones.

Principal component analysis (PCA) for the variables of adult whitefly

The results of principal component analysis of adult female and adult male (based on seven and nine morphological characters of respectively), collected from cassava plants of various agro-ecological zones of Kerala, are presented in Tables 9 and 10.

Table 9. PCA/ Eigen analysis of the correlation matrix (Adult female *Bemisia tabaci*)

Eigen value	5.4448	0.6844
Proportion	0.778	0.098
Cumulative	0.778	0.876
Characters	**Principal components**	
	PC1	**PC2**
Antennal length	-0.354	0.543
Body length	0.411	-0.043
Body width	-0.393	-0.407
Forewing length	0.362	0.492
Forewing width	0.386	0.284
Hind tarsal length	0.365	-0.384
Hind tarsal width	0.372	-0.262

The scree plot of the principal components (PC) showed that the first one (adult female) and two (adult male) eigen values correspond to most of the variances in the dataset (Figs. 8 and 9). The major principal components were extracted, the total cumulative variance of these principal components amounted to 77.8 per cent and 81.3 per cent respective variations (in female and male adults respectively) and these principal components had eigen values more than one. The principal component analysis divided these seven and nine traits into major principal components. The first principal component (PC1) accounts for maximum variability in the data with respect to succeeding components. The principal component analysis grouped the estimated

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variables into a single major group in case of adult female; which accounted for 77.8 per cent (Table 9) of the total variation. For adult male, the variables are grouped into two major principal components; in which PC1 accounted for 60.8 per cent and PC2 accounted for 20.5 per cent (Table 10) of the total variation.

Table 10. PCA/ Eigen analysis of the correlation matrix (Adult male *Bemisia tabaci*)

Eigen value	5.4751	1.8463
Proportion	0.608	0.205
Cumulative	0.608	0.813
Characters	**Principal components**	
	PC1	**PC2**
Antennal length	-0.379	0.141
Body length	0.321	0.307
Body width	-0.372	-0.170
Forewing length	0.349	0.233
Forewing width	0.339	0.337
Hind tarsal length	0.288	-0.287
Hind tarsal width	0.395	0.159
Aedeagus length	0.280	-0.527
Clasper length	0.247	-0.551

For adult female, the only principal component with a contribution of 77.8 per cent of the total variation, had shown highest positive contribution for body length (0.411); followed by forewing width (0.386), hind tarsal width (0.372), hind tarsal length (0.365), forewing length (0.362), antennal length (-0.354) and body width (-0.393) (Table 9). In case of adult male, two principal components had eigen values more than one. The largest contributor for variation, PC1 had shown highest positive contributions for body length, forewing length, forewing width, hind tarsal length, hind tarsal width, aedeagus length and clasper length. PC2 had shown more positive contributions for antennal length and body width (Table 9). The first two principal components contributing the major share of variances and were plotted to observe the relationships between measured whitefly traits/variables (Figs. 10 and 11). The first two principal components contributing 87.6 per cent of variation in adult female and for adult male, the first two principal components contributing 81.3 per cent diversity. So other principal components were not considered as major contributing principal components but they were already considered and measured in first two principal components.

Plot of the first two principal components showing relation among various adult whitefly characters

The correlation coefficient (r) between any two characters is approximated by the cosine of the angle between their vectors. The correlation coefficients among the traits indicated that the plot currently shows the relationship among the traits based on plot that had relatively large loading on both PC1 and PC2 axes (Fig. 10 and 11).

In case of biplot for adult female (Fig. 10), there were near zero (angle 0-25°) angle between many characters (small obtuse/ acute angles between their vectors) and as correlation coefficient (r=cos0=+1) between any two characters is approximated by the cosine of the angle between their vectors, they have strong positive correlations. The characters with strong positive correlations are forewing length and forewing width; hind tarsal length and hind tarsal width. Antennal length and forewing length were mutually near perpendicular vectors (r=cos90=0). There were negative correlations between body width and forewing length; antennal length and hind tarsal length as indicated by the angle of approximately 180° (150-160°) (r=cos180= -1) between their vectors.

In case of biplot for adult male (Fig. 11), strong positive correlations observed between forewing width, body length, forewing length and hind tarsal width; aedeagus length and clasper length. Mutually near perpendicular vectors (r=cos90=0), were formed between forewing width and clasper length. Negative correlations were observed between body width and hind tarsal width. Some discrepancies of the plot predictions and original data were expected because the first two principal components accounted for less than 100 per cent of the total variation.

Score plot of first two principal components for adult whitefly collected from various agro-ecological zones

Scores for adult whitefly collected from different agro-ecological zones of Kerala, based on PC1 and PC2 are plotted in Fig. 12 and Fig. 13. The 13 zones were grouped into four major distinct clusters/quarters. In case of adult female, the distribution pattern revealed that maximum number of entries (6) were included in quarter II, namely PTY, VDA, MVA,KGD, VKA and CTR. It was followed by quarter I, with 5 entries; namely NYA, KYM, PKU, PKD and TPA. The other two quarters had single entries each. *viz*., SKM (quarter IV) and SBY (quarter III) (Fig. 12). The distribution pattern in case of adult male, revealed that maximum number of entries (6) were included in quarters I, namely KYM, PTY, PKD, NYA, VKA and TPA. Quarter II had three entries (MVA, PKU and SKM) and quarter III and IV had two entries each (SBY and KGD for quarter III and VDA and CTR for quarter IV) (Fig. 13).

Score plot analysis of adult female and adult male did not provide any clear cut idea about the presence of different genetic groups. But for both adult females and males, SBY population was present in a separate group.

4. 2. Molecular characterization of *Bemisia tabaci*

4. 2. 1. Isolation of genomic DNA from *Bemisia tabaci* adults and quality checking

Genomic DNA of *B. tabaci* adults collected from cassava plants of 13-agro ecological zones of Kerala; *viz*. Neyyattinkara, Sreekaryam, Vellanikkara, Vadakara, Sultan Bathery, Kasargod, Thodupuzha, Palluruthy, Pulikeezhu, Muvattupuzha, Kayamkulam, Palakkad and Chittur were isolated using standard procedures, mentioned earlier.

Agarose gel electrophoresis was carried out to check the quality and found to have good quality bands at 0.8 per cent agarose gel. NanoDrop spectrophotometer method was also used for the purpose and gave ratio of UV absorbance -A260/280 value (quality) in the range of 1.78 to 1.92 and quantity (ng/µl) in the range of 11.43 to 29.26 (Table 11).

Table 11. Quality and quantity of DNA extracted from cassava whitefly, *Bemisia tabaci* determined using NanoDrop spectrophotometer (ND-1000)

Collection location	UV absorbance at 260 nm (A260)	UV absorbance at 280 nm (A280)	A260/280	Quantity (ng/µl)
(1) NYA	0.229	0.122	1.87	11.43
(2) SKM	0.417	0.225	1.85	20.84
(3) VKA	0.330	0.173	1.90	16.51
(4) VDA	0.585	0.308	1.90	29.26
(5) SBY	0.450	0.246	1.83	22.52
(6) KGD	0.391	0.208	1.88	19.55
(7) TDA	0.374	0.208	1.80	18.70
(8) PTY	0.407	0.229	1.78	20.35
(9) PKU	0.357	0.192	1.86	17.83
(10) MVA	0.487	0.256	1.90	24.37
(11) KYM	0.414	0.216	1.92	20.69
(12) PKD	0.350	0.187	1.87	17.50
(13) CTR	0.294	0.161	1.82	14.68

(NB: NYA-Neyyattinkara, SKM-Sreekaryam, VKA-Vellanikkara, VDA-Vadakara, SBY-Sultan Bathery, KGD-Kasargod, TDA-Thodupuzha, PTY-Palluruthy, PKU-Pulikeezhu, MVA-Muvattupuzha, KYM- Kayamkulam, PKD-Palakkad, CTR-Chittur)

4.2.2. Molecular marker analysis

4.2.2.1. Analysis using Inter Simple Sequence Repeats (ISSR) primers

The genomic DNA isolated from different *B. tabaci* were pooled to get a master pool of whitefly DNA and was amplified with 35 ISSR primers to screen the best performing ones. The amplification pattern for all the whiteflies with a specific primer was used to assess the genetic variability existing among them. The primers with good banding pattern with minimum of 10 bands were selected for further analysis. The primers selected were UBC 808, UBC 811, UBC 827, UBC 846, UBC 855, ISSR 3, ISSR 8, I 4, F1 and F2. UBC 808 had an amplification pattern with 20 bands, out of which 8 bands were distinct and 12 were faint. For UBC 811, out 26 bands 9 were distinct and 17 were faint. UBC 827 produced 9 distinct and 13 faint bands, out of total 22 bands. UBC 846 produced, total 19 bands, in which 8 and 11 were distinct and faint respectively. Total 21 bands were produced by UBC 855, out of which 8 were distinct. ISSR 3 produced 5 distinct and 6 faint bands. ISSR 8 produced 13 bands and 6 bands were distinct in that. Out of 23 bands in I 4, 8 were distinct. F1 and F2, produced 17 and 14 total bands respectively; out of F1 has got 7 distinct bands and F2 has got 6 distinct bands (Table 12)

Table 12. Amplification pattern of ISSR primers in *Bemisia tabaci* adults

Sl. No.	Primers	Amplification pattern			
		No. of Bands	Type of bands		Remarks
			Distinct	Faint	
1	UBC 808	20	8	12	**Selected**
2	UBC 810	0	0	0	Not selected
3	UBC 811	26	9	17	**Selected**

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4	UBC 815	7	1	6	Not selected
5	UBC 818	5	2	3	Not selected
6	UBC 826	0	0	0	Not selected
7	UBC 827	22	9	13	**Selected**
8	UBC 835	1	0	1	Not selected
9	UBC 841	6	2	4	Not selected
10	UBC 844	4	2	2	Not selected
11	UBC 846	19	8	11	**Selected**
12	UBC 847	5	2	3	Not selected
13	UBC 848	8	1	7	Not selected
14	UBC 850	4	2	2	Not selected
15	UBC 854	5	1	4	Not selected
16	UBC 855	21	8	13	**Selected**
17	UBC 857	0	0	0	Not selected
18	UBC 865	1	0	1	Not selected
19	UBC 866	3	0	3	Not selected
20	UBC 873	7	4	3	Not selected
21	ISSR 2	3	0	3	Not selected
22	ISSR 3	11	5	6	**Selected**
23	ISSR 4	2	1	1	Not selected
24	ISSR 5	9	2	7	Not selected
25	ISSR 6	4	0	4	Not selected
26	ISSR 7	6	0	6	Not selected
27	ISSR 8	13	6	7	**Selected**
28	ISSR 9	8	2	6	Not selected
29	I 4	23	8	15	**Selected**
30	F 1	17	7	10	**Selected**

31	F 2	14	6	8	**Selected**
32	F 3	4	1	3	Not selected
33	F 4	8	0	8	Not selected
34	F 5	6	1	5	Not selected
35	F 6	7	0	7	Not selected

The ten selected primers were used for the amplification of ISSR region in genomic DNA of *B.tabaci*, with thermal cycling programmed with primer specific annealing temperatures (Table 13). DNA extracted from thirteen *B.tabaci* adults were amplified with ten selected ISSR primers, the amplified products were resolved in 2 per cent agarose gel and docked. Software 'GelQuant Express' was used for counting bands in agarose gel (GelQuant Express Report is the report which shows the banding pattern of amplicons). Details on amplification of ISSR region in genomic DNA of cassava whiteflies from different agro-ecological zones are given below.

Table 13. Details of ISSR primers selected

Sl. No.	Primer	Annealing temperature (°C)	Nucleotide Sequence
1	F1	54.0	5'- GACAC GACACGACAC -3'
2	I 4	53.7	5'- ACACACACACACACACAG-3'
3	UBC 846	53.9	5'- CACACACACACACACART-3'
4	UBC 855	51.9	5'- ACACACACACACACACYT-3'
5	F 2	54.0	5'- GACA GACAGACA GACA-3'
6	UBC 827	54.9	5' -ACACACACACACACACG-3'
7	ISSR 8	54.9	5'-ACACACACACACACACG-3'
8	UBC 808	56.0	5'- AGAGAGAGAGAGAGAGC-3'
9	UBC 811	58.0	5'- GAGAGAGAGAGAGAGAC-3'
10	ISSR 3	37.1	5'-AGAGAGAGAGAGAGT-3'

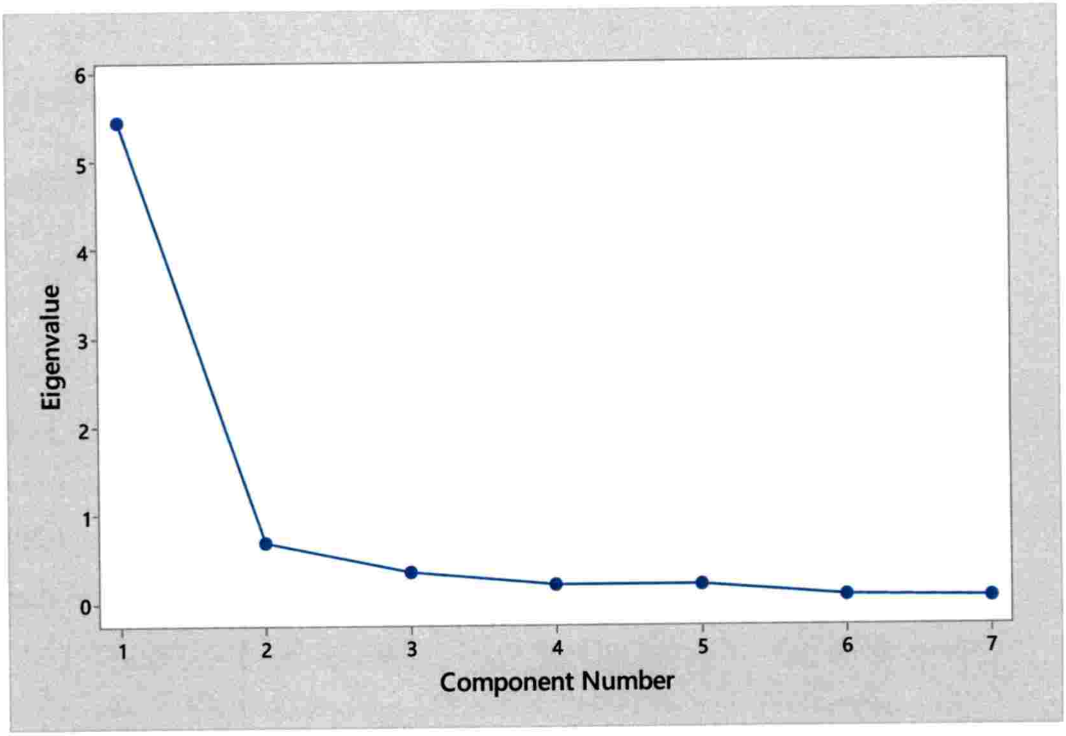

Fig. 8. Scree plot showing eigen values in response to principal components in adult female *Bemisia tabaci*

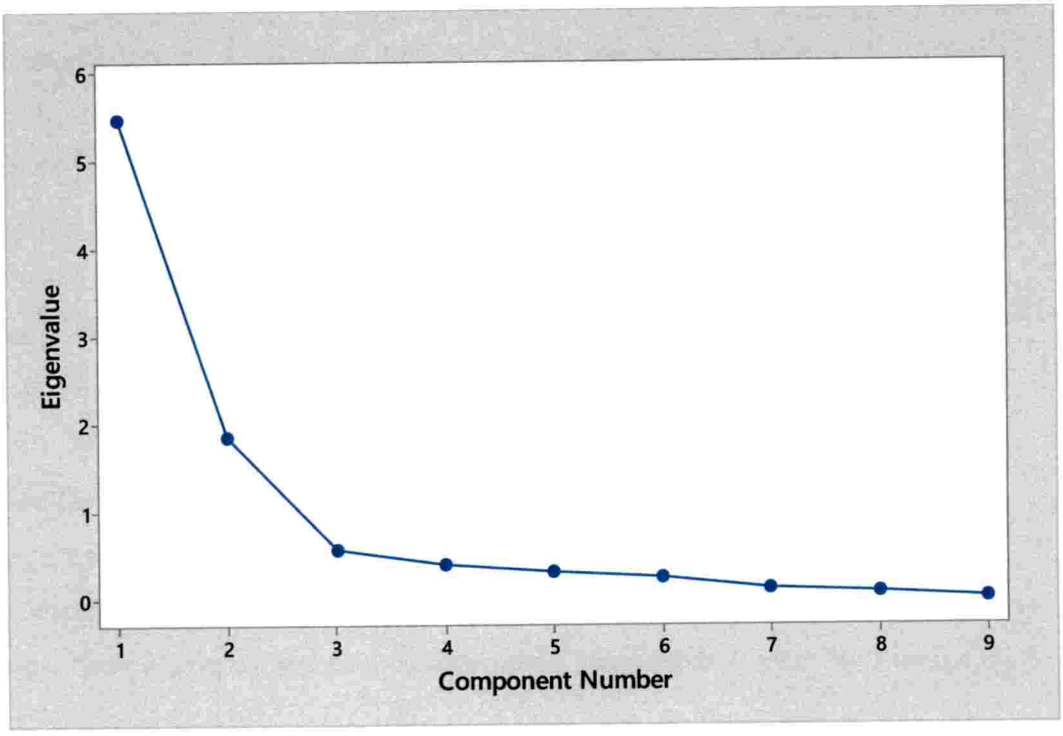

Fig. 9. Scree plot showing eigen values in response to principal components in adult male *Bemisia tabaci*

4.2.2.1.1. Analysis using ISSR primer F1

The amplification pattern of primer F1 (5'- GACAC GACACGACAC -3'), in ISSR region in genomic DNA of cassava whiteflies is presented in Plates 20a and 20b.

In the whitefly genomic DNA samples, primer F1 formed 17 bands with amplicon size ranged from 600 bp to 2500 bp. At 600 bp amplicon size, bands are formed by samples from Thodupuzha (4), Palluruthy (6), Vellanikkara (7), Vadakara (8), Neyyattinkara (9), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13). At 650 bp, bands are formed by samples from Chittur (2), Palluruthy (6), Muvattupuzha (12) and Pulikeezhu (13). At 700 bp, bands are formed by samples from Thodupuzha (4), Palluruthy (6), Vellanikkara (7), Muvattupuzha (12) and Pulikeezhu (13). Kasargod (3), Thodupuzha (4), Palluruthy (6), Vadakara (8) and Muvattupuzha (12) samples formed bands at at 800 bp. At 900 bp, bands are formed by samples from Kasargod (3), Vellanikkara (7) and Sreekaryam (11). At 950 bp, only samples from Neyyattinkara (9) formed bands. At 1000 bp, bands are formed by samples from Palakkad (1), Chittur (2), Sultan Bathery (5), Palluruthy (6), Vellanikkara (7), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13). Only Sreekaryam (11) samples formed bands at 1050 bp. At 1200 bp, all the 13 samples formed bands. Except Neyyattinkara (9) samples, all others formed bands at 1300 bp and 1600 bp; whereas, only Neyyattinkara (9) samples could form band at 1400 bp. At 1500 bp, samples from Chittur (2), Kayamkulam (10), Muvattupuzha (12) and Pulikeezhu (13) formed bands. At 1900 bp, bands are formed by samples from Palakkad (1), Chittur (2), Kasargod (3), Thodupuzha (4), Palluruthy (6), Vellanikkara (7) and Vadakara (8). At 2000 bp, only samples from Chittur (2) and Kayamkulam (10) could form bands. At 2100 bp, except samples from Sultan Bathery (5) and Kayamkulam (10), all others formed bands. Only Kayamkulam (10) sample could form band at 2500 bp. Overall, four unique bands (highlighted with arrow) are produced by the primer at 950 bp,

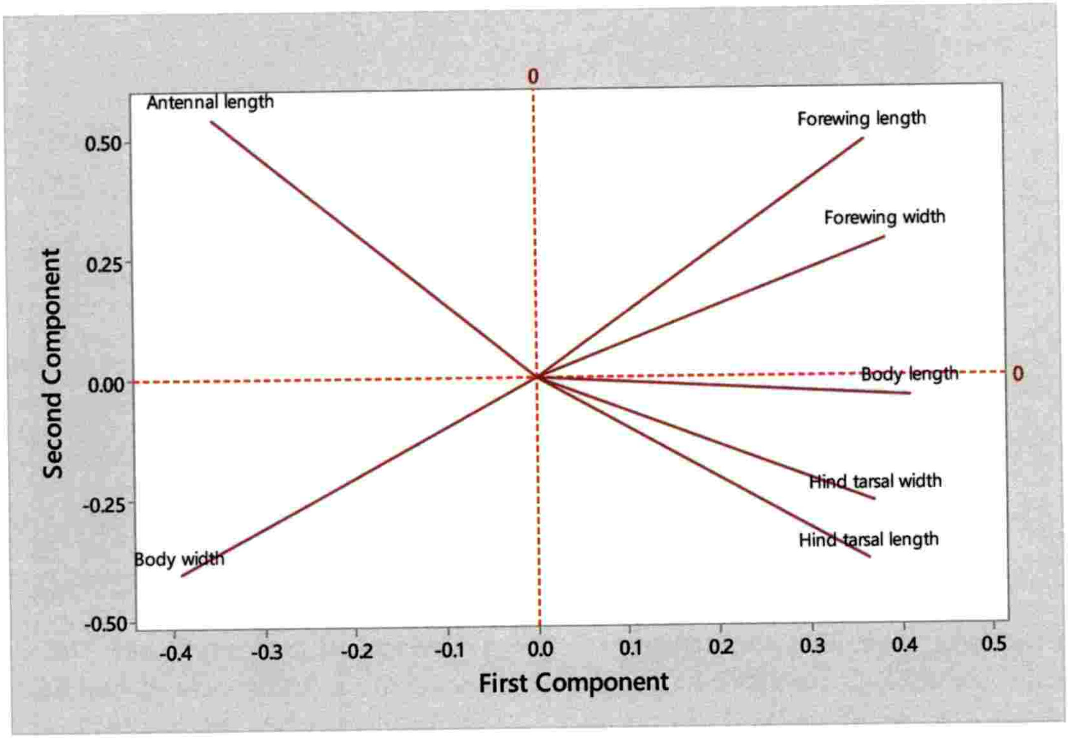

Fig. 10. Biplot/loading plot of the first two principal components showing relations among various *Bemisia tabaci* adult female characters

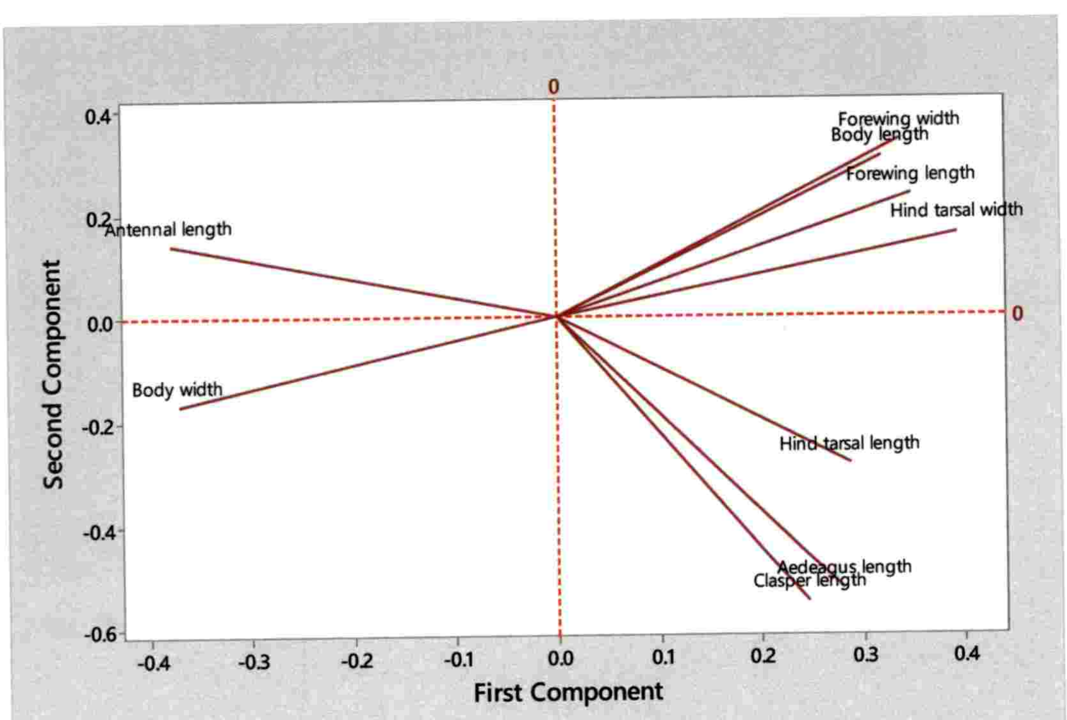

Fig. 11. Biplot/loading plot of the first two principal components showing relations among various *Bemisia tabaci* adult male characters

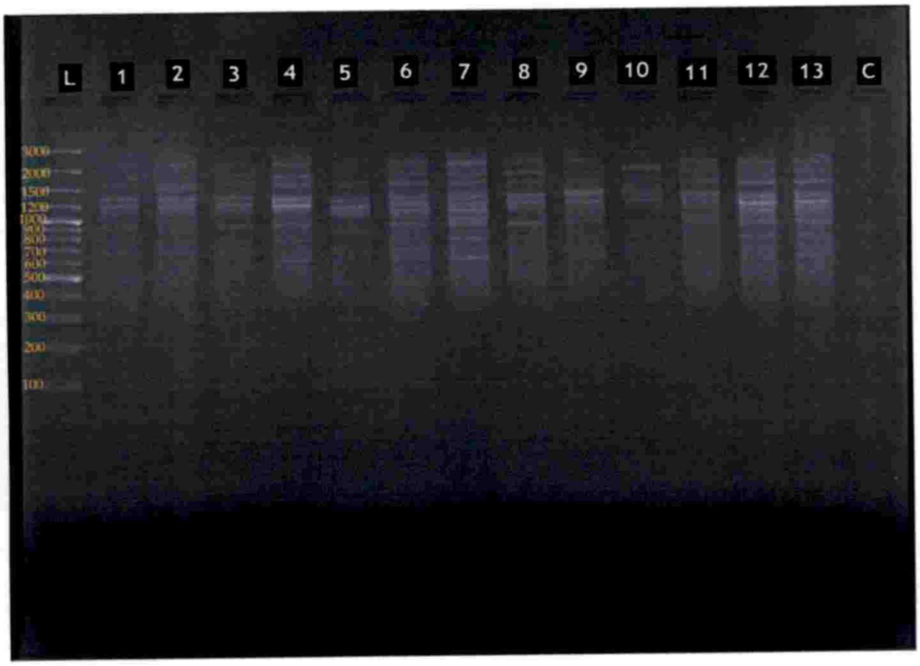

Plate 20a. **Inter Simple sequence repeat fragments generated from genomic DNA of thirteen *Bemisia tabaci* adults (lane 1to lane 13, *viz.*, 1-Palakkad, 2- Chittur, 3- Kasargod, 4- Thodupuzha, 5-Sultan Bathery, 6- Palluruthy, 7-Vellanikkara, 8- Vadakara, 9- Neyyattinkara, 10-Kayamkulam, 11-Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu) occuring on cassava upon amplification with F1 when resolved in agarose gel (2%). C- control, L: 100 bp plus ladder**

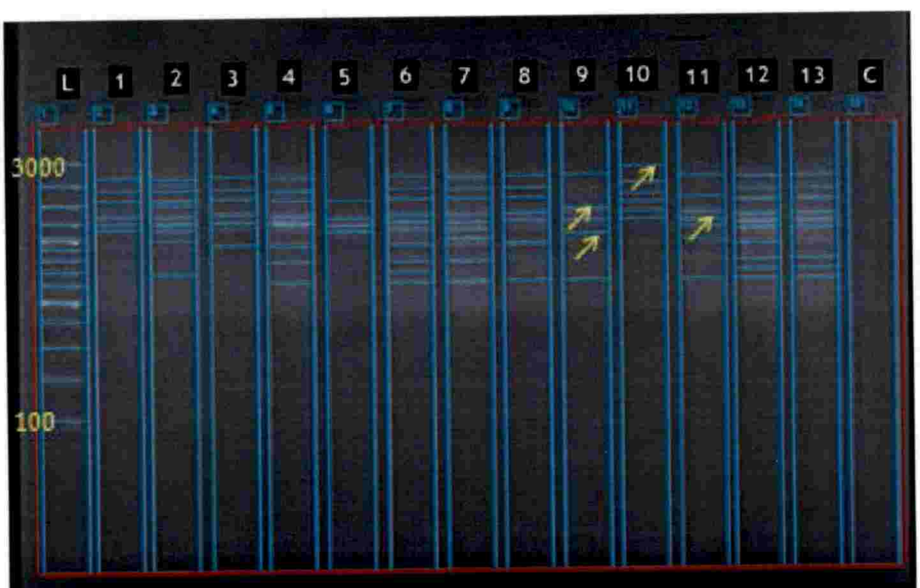

Plate 20b. Image generated using GelQuant Express for Plate 20a.

1400 bp (Neyyattinkara (9), 1050 bp (Sreekaryam (11), and 2500 bp (Kayamkulam (10).

4.2.2.1.2. Analysis using ISSR primer I4

The amplification pattern of primer I4 is shown below (Plate21). In the whitefly genomic DNA samples, primer I4 formed 23 bands with amplicon size ranging from 450 bp to 2900 bp. At 450 bp amplicon size, only Sultan Bathery (5) population had formed the band. Only Neyyattinkara (9) and Muvattupuzha (12) populations formed bands at 500 bp. Except Vellanikkara (7) population, no other population formed bands at 520 bp. Palluruthy (6) and Muvattupuzha (12) populations formed bands at 550 bp. At 600 bp, bands are formed by samples from Palakkad (1), Chittur (2), Kasargod (3), Kayamkulam (10), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13). At 620 bp, bands are formed by samples from Sultan Bathery (5), Vadakara (8) and Muvattupuzha.

At 650 bp, bands are formed by samples from Chittur (2), Kasargod (3), Thodupuzha (4), Palluruthy (6) and Neyyattinkara (9). At 700 bp, samples from Kayamkulam (10), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13) formed bands. Samples from Palakkad (1), Chittur (2), Kasargod (3), Palluruthy (6), Vellanikkara (7) and Neyyattinkara (9) formed bands at 750 bp. At 800 bp, bands are formed by samples from Thodupuzha (4), Sultan Bathery (5), Vadakara (8), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13). Only sample from Kayamkulam (10) formed band at 850 bp. At 900 bp, bands are formed by samples from Palakkad (1), Chittur (2), Kasargod (3), Thodupuzha (4), Palluruthy (6) and Neyyattinkara (9). Samples from Chittur (2), Muvattupuzha (12) and Pulikeezhu (13) formed bands at 950 bp. At 1000 bp, bands are formed by samples Vellanikkara (7), Vadakara (8) and Sreekaryam (11). Samples from Palakkad (1), Chittur (2), Kasargod (3), Thodupuzha (4), Vellanikkara (7), Vadakara (8) and Sreekaryam (11) formed

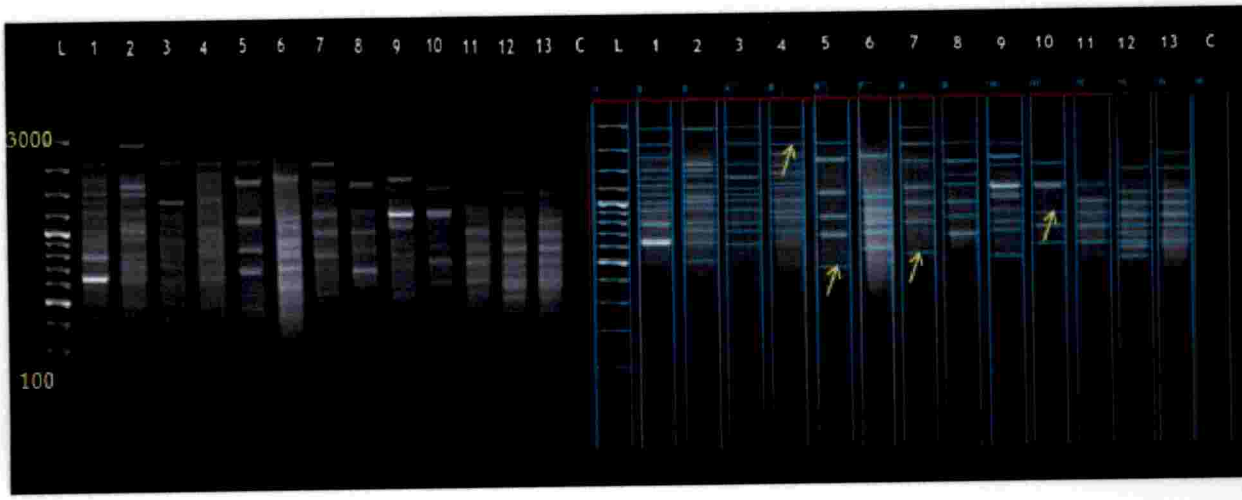

Plate 21. Inter Simple sequence repeat fragments amplified from genomic DNA of thirteen *Bemisia tabaci* adults and banding pattern generated using GelQuant Express (lane 1to lane 13, *viz.*, 1-Palakkad, 2- Chittur, 3- Kasargod, 4- Thodupuzha, 5-Sultan Bathery, 6-Palluruthy, 7-Vellanikkara, 8- Vadakara, 9-Neyyattinkara, 10-Kayamkulam, 11-Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu) occuring on cassava upon amplification with I4 when resolved in agarose gel (2%). C-control, L: 100 bp plus ladder

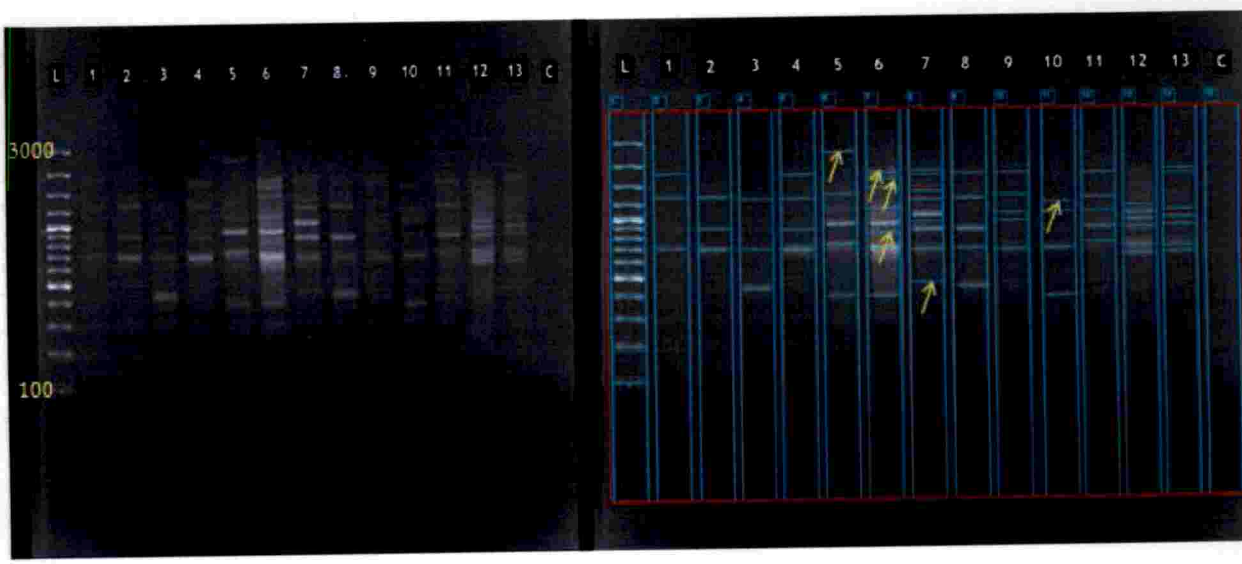

Plate 22. Inter Simple sequence repeat fragments amplified from genomic DNA of thirteen *Bemisia tabaci* adults and banding pattern generated using GelQuant Express (lane 1to lane 13, *viz.*, 1-Palakkad, 2- Chittur, 3- Kasargod, 4- Thodupuzha, 5-Sultan Bathery, 6-Palluruthy, 7-Vellanikkara, 8- Vadakara, 9-Neyyattinkara, 10-Kayamkulam, 11-Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu) occuring on cassava upon amplification with UBC 846 when resolved in agarose gel (2%). C-control, L: 100 bp plus ladder

bands at 1050 bp. At 1100 bp, bands are formed by samples from Palakkad (1), Chittur (2), Kasargod (3), Thodupuzha (4), Palluruthy (6), Neyyattinkara (9), Muvattupuzha (12) and Pulikeezhu (13) and at 1200 bp it was from Palakkad (1), Chittur (2), Kasargod (3) and Vadakara (8). At 1300 bp, bands are formed by samples from Palakkad (1), Chittur (2), Kayamkulam (10), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13). Chittur (2), Kasargod (3), Vellanikkara (7), Vadakara (8) and Pulikeezhu (13) samples formed bands at 1500 bp. At 1600 bp, samples from Sultan Bathery (5), Palluruthy (6), Neyyattinkara (9) and Pulikeezhu (13) formed bands. Only sample from Thodupuzha (4) formed band at 1800 bp. At 2100 bp, bands are formed by samples from Palakkad (1), Kasargod (3), Thodupuzha (4), Sultan Bathery (5), Vellanikkara (7), Vadakara (8), Neyyattinkara (9) and Muvattupuzha (12); whereas, at 2900 bp, bands are formed by samples from Palakkad (1), Chittur (2), Kasargod (3), Thodupuzha (4) and Vellanikkara (7). Overall, four unique bands (highlighted with arrow) are produced by the primer at 450 bp (Sultan Bathery (5)), 520 bp (Vellanikkara (7)), 850 bp (Kayamkulam (10)) and 1800 bp (Thodupuzha (4).

4.2.2.1.3. Analysis using ISSR primer UBC 846

The amplification pattern of primer UBC 846 is shown below (Plate 22). In the whitefly genomic DNA samples, primer UBC 846 formed 19 bands with amplicon size ranged from 400 bp to 2600 bp. At 400 bp, bands are formed by samples from Sultan Bathery (5), Palluruthy (6) and Kayamkulam (10). Kasargod (3) and Vadakara (8) samples formed bands at 420 bp. Only sample from Vellanikkara (7) formed band at 450 bp. Muvattupuzha (12) and Pulikeezhu (13) samples formed bands at 650 bp. Except samples from Sultan Bathery (5) and Sreekaryam (11), all others formed bands at 700 bp; whereas, only these samples formed bands at 720 bp. Only sample from Palluruthy (6) formed band at 800 bp. Samples from Chittur (2), Thodupuzha (4), Vellanikkara (7), Vadakara (8), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13) formed bands at 850 bp. At 900 bp, samples from Sultan

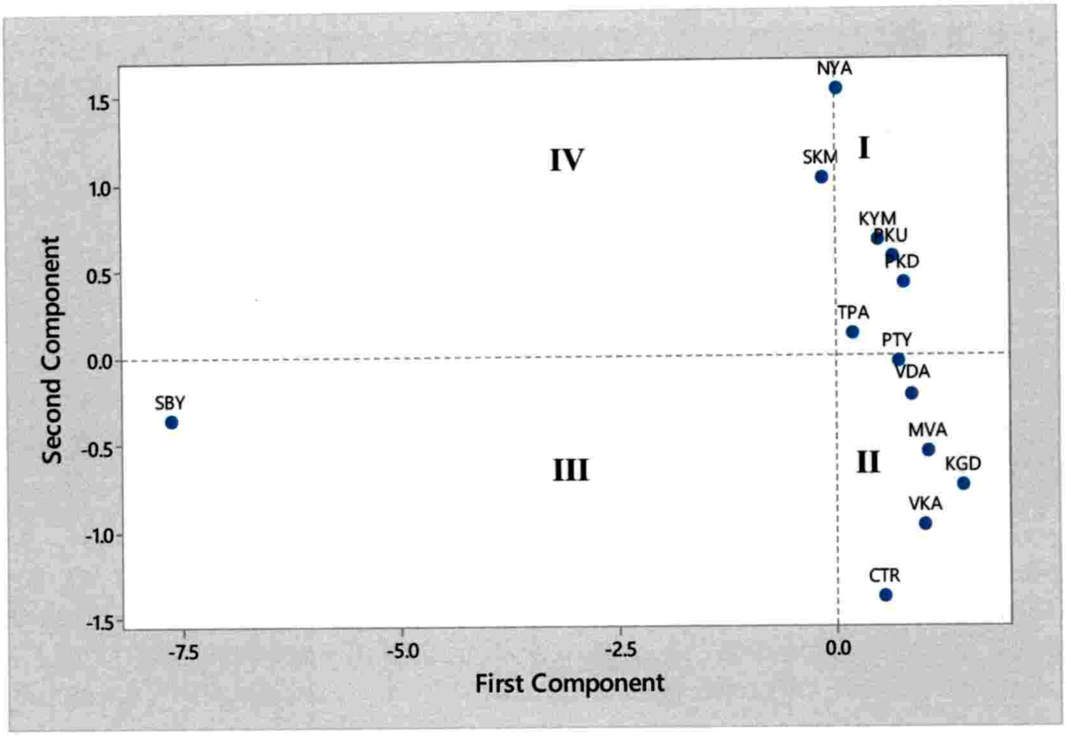

Fig. 12. Score plot of first two components for adult female *Bemisia tabaci* collected from various locations

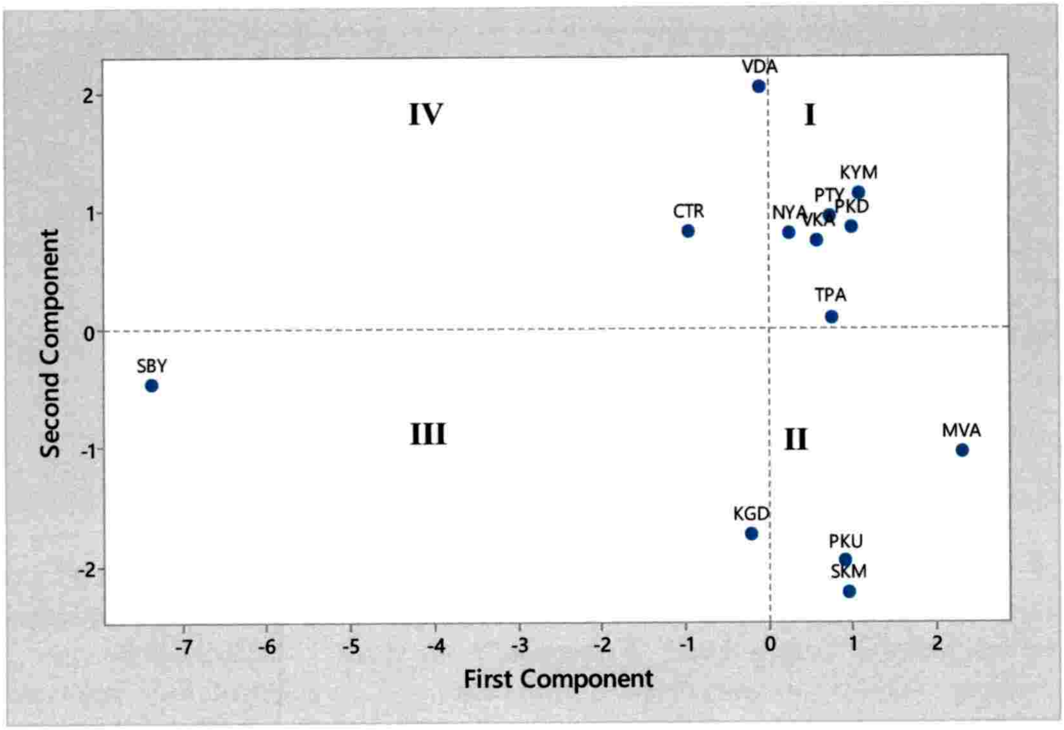

Fig. 13. Score plot of first two components for adult male *Bemisia tabaci* collected from various locations

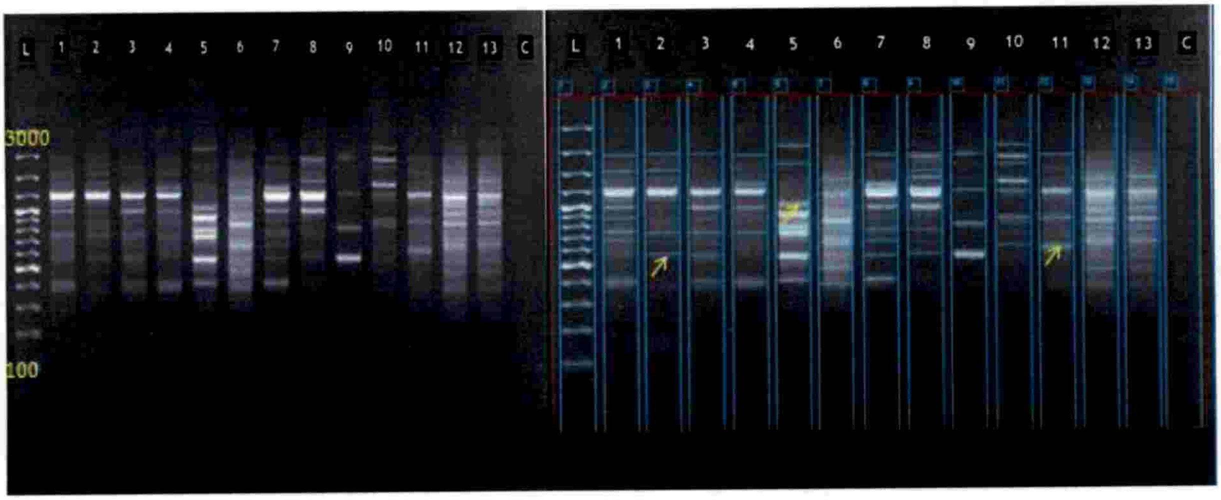

Plate 23. Inter Simple sequence repeat fragments amplifieded from genomic DNA of thirteen *Bemisia tabaci* adults and banding pattern generated using GelQuant Express (lane 1to lane 13, *viz.*, 1-Palakkad, 2- Chittur, 3- Kasargod, 4- Thodupuzha, 5-Sultan Bathery, 6-Palluruthy, 7-Vellanikkara, 8- Vadakara, 9-Neyyattinkara, 10-Kayamkulam, 11-Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu) occuring on cassava upon amplification with UBC 855 when resolved in agarose gel (2%). C-control, L: 100 bp plus ladder

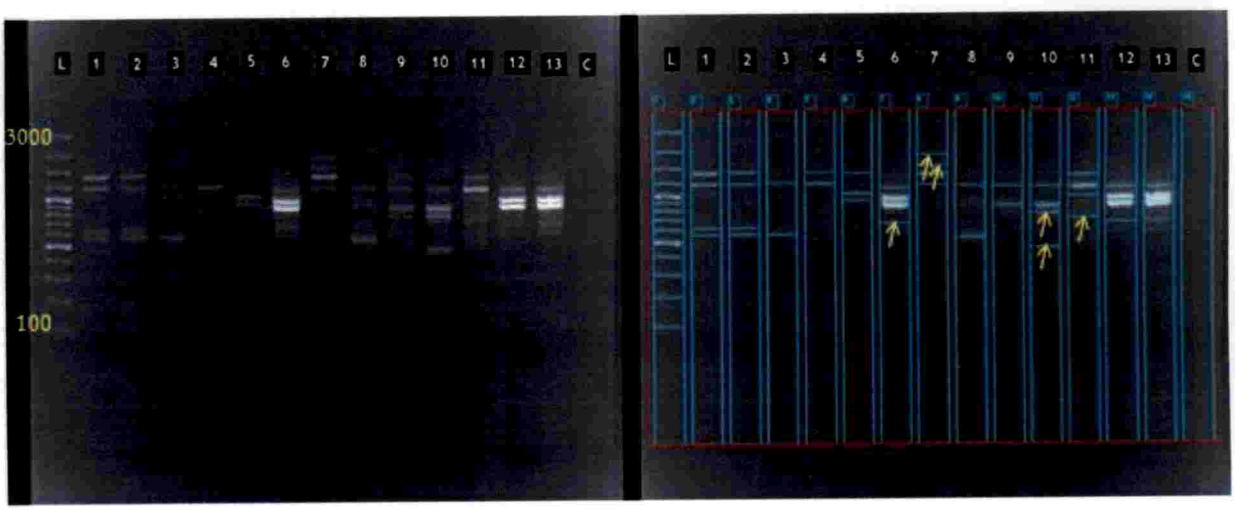

Plate 24. Inter Simple sequence repeat fragments generated from genomic DNA of thirteen *Bemisia tabaci* adults and banding pattern generated using GelQuant Express (lane 1to lane 13, *viz.*, 1-Palakkad, 2- Chittur, 3- Kasargod, 4- Thodupuzha, 5-Sultan Bathery, 6-Palluruthy, 7-Vellanikkara, 8- Vadakara, 9-Neyyattinkara, 10-Kayamkulam, 11-Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu) occuring on cassava upon amplification with F2 when resolved in agarose gel (2%). C-control, L: 100 bp plus ladder

Bathery (5), Palluruthy (6), Vellanikkara (7), Neyyattinkara (9), Muvattupuzha (12) and Pulikeezhu (13) formed bands. Samples from Vellanikkara (7), Neyyattinkara (9), Kayamkulam (10), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13) formed bands at 950 bp. At 1000 bp, only samples from Palluruthy (6) and Muvattupuzha (12) formed bands. Only samples from Kayamkulam (10) formed band at 1100 bp. Samples from Palakkad (1), Chittur (2), Kasargod (3), Thodupuzha (4), Vellanikkara (7), Vadakara (8), Neyyattinkara (9) and Sreekaryam (11) formed bands at 1200 bp. Only samples from Sultan Bathery (5) and Vellanikkara (7) formed bands at 1300 bp. At 1500 bp and 1600 bp, only sample from Palluruthy (6) formed bands. At 1700 bp, bands are formed by samples from Thodupuzha (4), Sultan Bathery (5), Vadakara (8), Neyyattinkara (9) and Sreekaryam (11). Samples from Palakkad (1), Vellanikkara (7) and Pulikeezhu (13) formed bands at 1900 bp; whereas, Sultan Bathery (5) sample only could form band at 2600 bp. Overall, six unique bands (highlighted with arrow) are produced by the primer at 450 bp (Vellanikkara (7), 800 bp (Palluruthy (6), 1100 bp (Kayamkulam (10), 1500 bp, 1600 bp (Palluruthy (6)) and 2600 bp (Sultan Bathery (5).

4.2.2.1.4. Analysis using ISSR primer UBC 855

The amplification pattern of primer UBC 855 (5'-ACACACACACACACACYT-3'), in ISSR region in genomic DNA of cassava whitefly, is shown below (Plate 23).

In the whitefly genomic DNA samples, primer UBC 855 formed 21 bands with amplicon size ranged from 400 bp to 2100 bp. At 400 bp, bands are formed by samples from Palakkad (1), Kasargod (3), Thodupuzha (4) and Palluruthy (6). Only samples from Sultan Bathery (5) and Vellanikkara (7) formed bands at 420 bp; whereas, samples from Palluruthy (6) and Muvattupuzha (12) formed bands at 450 bp. At 550 bp, samples from Kasargod (3), Sultan Bathery (5), Vellanikkara (7), Vadakara (8), Neyyattinkara (9) and Kayamkulam (10) formed bands. Only sample from Chittur (2) formed band at 600 bp; whereas, only sample from Sreekaryam (11) formed band at 650 bp. Samples from Palakkad (1), Palluruthy (6), Vellanikkara (7), Muvattupuzha (12) and Pulikeezhu (13) formed bands at 700 bp. Except samples from Neyyattinkara (9), Kayamkulam (10) and Sreekaryam (11), all others formed bands at 750 bp. At 800 bp, bands are formed by samples only from Sultan Bathery (5) and Neyyattinkara (9). At 850 bp, samples from Palluruthy (6), Kayamkulam (10), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13) formed bands. Samples from Palakkad (1), Sultan Bathery (5), Muvattupuzha (12) and Pulikeezhu (13) formed bands at 900 bp. Only Sultan Bathery (5) sample could form band at 950 bp and 1050 bp. At 1000 bp, bands are formed by samples from Palakkad (1), Kasargod (3), Thodupuzha (4), Vellanikkara (7) and Vadakara (8). Except sample from Sultan Bathery (5), all others could form bands at 1200 bp. Only samples from Thodupuzha (4) and Vellanikkara (7) could form bands at 1300 bp; whereas, samples from Palluruthy (6) and Vadakara (8) formed bands at 1400 bp. Samples from Palakkad (1), Chittur (2) and Vadakara (8) formed bands at 1500 bp. At 1800 bp and 2100 bp, samples from Sultan Bathery (5) and Kayamkulam (10) formed bands. Except samples from Sultan Bathery (5), Palluruthy (6), Muvattupuzha (12) and Pulikeezhu (13), all others formed bands at 2000 bp. Overall; three unique bands (highlighted with arrow) are produced by the primer at 600 bp (Chittur (2)), 650 bp (Sreekaryam (11) and 1050 bp (Sultan Bathery (5).

4.2.2.1.5. Analysis using ISSR primer F2

The amplification pattern of primer F2 (5'- GACA GACAGACA GACA-3'), in ISSR region in genomic DNA of cassava whitefly, is shown below (Plate 24).

In the whitefly genomic DNA samples, primer F2 formed 14 bands with amplicon size ranged from 500 bp to 2000 bp. At 500 bp, only sample from Kayamkulam (10) could form band. Palakkad (1), Chittur (2), Kasargod (3) and Vadakara (8) samples formed bands at 550 bp. At 600 bp, samples from Palakkad (1)

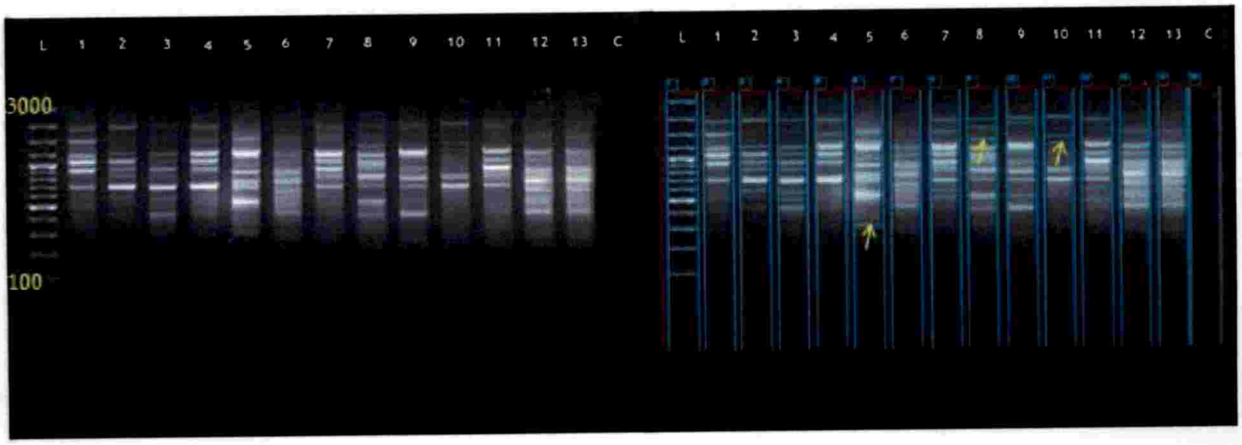

Plate 25. **Inter Simple sequence repeat fragments amplified from genomic DNA of thirteen *Bemisia tabaci* adults and banding pattern generated using GelQuant Express (lane 1to lane 13, *viz.*, 1-Palakkad, 2- Chittur, 3- Kasargod, 4- Thodupuzha, 5-Sultan Bathery, 6-Palluruthy, 7-Vellanikkara, 8- Vadakara, 9-Neyyattinkara, 10-Kayamkulam, 11-Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu) occuring on cassava upon amplification with UBC 827 when resolved in agarose gel (2%). C-control, L: 100 bp plus ladder**

Plate 26. **Inter Simple sequence repeat fragments amplified from genomic DNA of thirteen *Bemisia tabaci* adults and banding pattern generated using GelQuant Express (lane 1to lane 13, *viz.*, 1-Palakkad, 2- Chittur, 3- Kasargod, 4- Thodupuzha, 5-Sultan Bathery, 6-Palluruthy, 7-Vellanikkara, 8- Vadakara, 9-Neyyattinkara, 10-Kayamkulam, 11-Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu) occuring on cassava upon amplification with ISSR 8 when resolved in agarose gel (2%). C-control, L: 100 bp plus ladder**

and Chittur (2) formed bands. Only Palluruthy (6) sample could form band at 650 bp. At 710 bp, samples from Muvattupuzha (12) and Pulikeezhu (13) formed bands. Samples from Sreekaryam (11) and Kayamkulam (10) formed bands at 750bp and 800 bp, respectively. At 850 bp, samples from Palluruthy (6), Neyyattinkara (9), Kayamkulam (10), Muvattupuzha (12) and Pulikeezhu (13) formed bands. Samples from Sultan Bathery (5), Palluruthy (6), Muvattupuzha (12) and Pulikeezhu (13) formed bands at 900 bp. Only samples from Sultan Bathery (5) and Sreekaryam (11), formed bands at 1000 bp. Except sample from Sultan Bathery (5), all others formed bands at 1200 bp. At 1400 bp, samples from Palakkad (1), Chittur (2), Thodupuzha (4), Sultan Bathery (5), Vellanikkara (7) and Sreekaryam (11) formed bands. At 1700 bp and 2000 bp, only sample from Vellanikkara (7) could form band. Overall, six unique bands (highlighted with arrow) are produced by the primer at 500 bp, 800 bp (Kayamkulam (10)), 650 bp (Palluruthy (6)), 750 bp (Sreekaryam (11)), 1700 bp and 2000 bp (Vellanikkara (7).

4.2.2.1.6. Analysis using ISSR primer UBC 827

The amplification pattern of primer UBC 827 (5'-ACACACACACACACACG -3'), in ISSR region in genomic DNA of cassava whitefly, is shown below (Plate 25). In the whitefly genomic DNA samples, primer UBC 827 formed 22 bands with amplicon size ranged from 300 bp to 2000 bp. Single sample only could form bands at 300 bp (Sultan Bathery (5), 1150 bp (Kayamkulam (10) and 1300 bp (Vadakara (8). Two samples formed bands at 500 bp (Kasargod (3) and Sreekaryam (11), 580 bp (Kasargod (3) and Pulikeezhu (13) and 1190 bp (Vadakara (8) and Kayamkulam (10); whereas, three samples formed bands at 600 bp (Chittur (2), Vellanikkara (7) and Sreekaryam (11), 650 bp (Palluruthy (6), Muvattupuzha (12) and Pulikeezhu (13)) and 1500 bp (Palakkad (1), Thodupuzha (4) and Kayamkulam (10). At 750 bp and 850 bp four samples formed bands; and were Sultan Bathery (5), Palluruthy (6), Muvattupuzha (12) and Pulikeezhu (13). Five samples formed bands at 550 bp (Sultan Bathery (5), Vellanikkara (7), Vadakara (8), Neyyattinkara (9) and Muvattupuzha (12) and at 1700 bp (Palakkad (1), Sultan Bathery (5), Vellanikkara (7), Muvattupuzha (12) and Pulikeezhu (13)). At 450 bp, bands are formed by samples from Kasargod (3), Palluruthy (6), Vadakara (8), Neyyattinkara (9), Muvattupuzha (12) and Pulikeezhu (13). Except samples from Palluruthy (6), Muvattupuzha (12) and Pulikeezhu (13), all others formed bands at 700 bp; whereas, except sample from Thodupuzha (4), all others formed bands at 800 bp. At 900 bp, samples from Chittur (2), Kasargod (3), Vadakara (8), Neyyattinkara (9), Kayamkulam (10), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13) formed bands. At 950 bp, except samples from Kayamkulam (10), Muvattupuzha (12) and Pulikeezhu (13), all others formed bands. At 1000 bp and 1100 bp, banding pattern looked similar; and bands were produced by samples except Sultan Bathery (5), Vadakara (8) and Kayamkulam (10). At 1200 bp, all samples except Palluruthy (6), Vellanikkara (7), Vadakara (8), Muvattupuzha (12) and Pulikeezhu (13) formed bands. Except samples from Sultan Bathery (5), Palluruthy (6), Muvattupuzha (12) and Pulikeezhu (13), all others formed bands at 2000 bp. Overall, three unique bands (highlighted with arrow) are produced by the primer at 300 bp (Sultan Bathery (5), 1150 bp (Kayamkulam (10) and 1300 bp (Vadakara (8).

4.2.2.1.7. Analysis using ISSR primer ISSR 8

The amplification pattern of primer ISSR 8 (5'-ACACACACACACACACG-3'), in ISSR region in genomic DNA of cassava whitefly, is shown below (Plate 26).

In the whitefly genomic DNA samples, primer ISSR 8 formed 13 bands with amplicon size ranged from 400 bp to 1900 bp. At 400 bp, bands are formed by samples from Kasargod (3), Palluruthy (6), Neyyattinkara (9), Muvattupuzha (12) and Pulikeezhu (13). At 550 bp, only samples from Sultan Bathery (5) and Vadakara (8) formed bands. Bands are formed by samples from Chittur (2), Kasargod (3), Palluruthy (6), Muvattupuzha (12) and Pulikeezhu (13) at 600 bp. Except Palluruthy

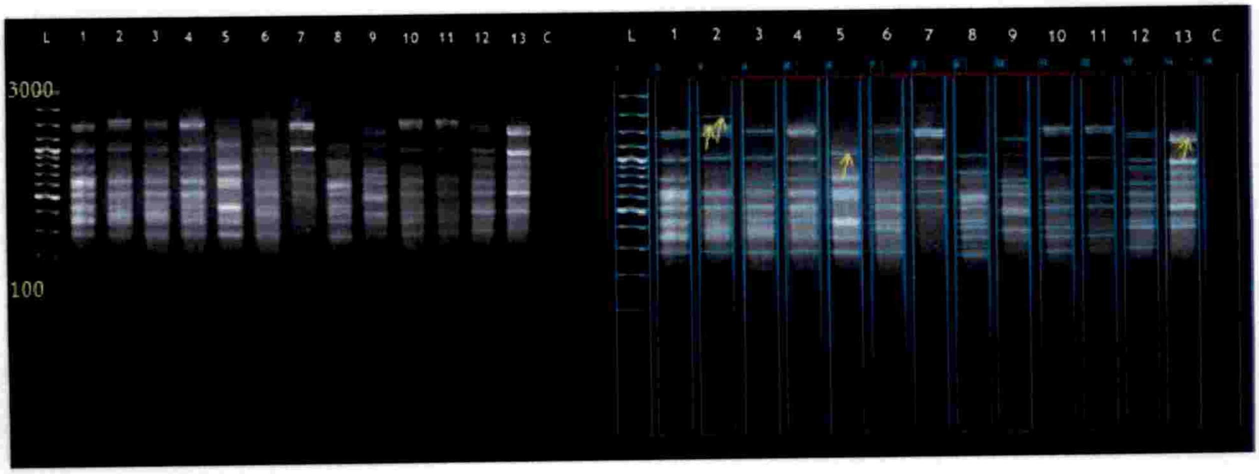

Plate 27. Inter Simple sequence repeat fragments amplified from genomic DNA of thirteen *Bemisia tabaci* adults and banding pattern generated using GelQuant Express (lane 1to lane 13, *viz.*, 1-Palakkad, 2- Chittur, 3- Kasargod, 4- Thodupuzha, 5-Sultan Bathery, 6-Palluruthy, 7-Vellanikkara, 8- Vadakara, 9-Neyyattinkara, 10-Kayamkulam, 11-Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu) occuring on cassava upon amplification with UBC 808 when resolved in agarose gel (2%). C-control, L: 100 bp plus ladder

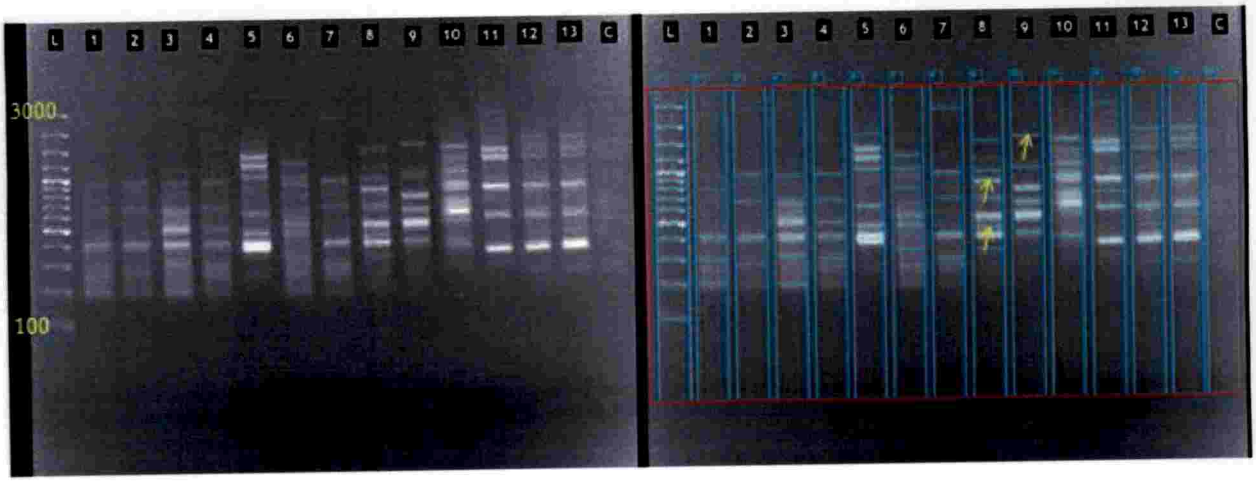

Plate 28. Inter Simple sequence repeat fragments amplified from genomic DNA of thirteen *Bemisia tabaci* adults and banding pattern generated using GelQuant Express (lane 1to lane 13, *viz.*, 1-Palakkad, 2- Chittur, 3- Kasargod, 4- Thodupuzha, 5-Sultan Bathery, 6-Palluruthy, 7-Vellanikkara, 8- Vadakara, 9-Neyyattinkara, 10-Kayamkulam, 11-Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu) occuring on cassava upon amplification with UBC 811 when resolved in agarose gel (2%). C-control, L: 100 bp plus ladder

(6), all other samples formed bands at 700 bp. At 750 bp, bands are formed by samples from Sultan Bathery (5), Palluruthy (6), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13). Only samples from Kasargod (3) and Kayamkulam (10) formed bands at 800 bp. At 850 bp, samples from Vadakara (8), Neyyattinkara (9), Muvattupuzha (12) and Pulikeezhu (13) formed bands. All samples formed bands at 900 bp. Except samples from Sultan Bathery (5), Neyyattinkara (9) and Kayamkulam (10) all others formed bands at 1000 bp. At 1200 bp, all samples except Kayamkulam (10) formed bands. Only samples from Chittur (2) and Vadakara (8) formed bands at 1300 bp. At 1500 bp, samples from Palakkad (1), Chittur (2), Kasargod (3), Thodupuzha (4), Sultan Bathery (5), Vellanikkara (7) and Vadakara (8) formed bands. Samples from Palakkad (1), Chittur (2), Thodupuzha (4), Vellanikkara (7), Vadakara (8) and Kayamkulam (10) formed bands at 1900 bp. There were no unique bands produced by the primer.

4.2.2.1.8. Analysis using ISSR primer UBC 808

The amplification pattern of primer UBC 808 (5'-AGAGAGAGAGAGAGAGC-3'), in ISSR region in genomic DNA of cassava whitefly, is shown below (Plate 27).

In the whitefly genomic DNA samples, primer UBC 808 formed 20 bands with amplicon size ranged from 290 bp to 2000 bp. At 290 bp, bands are formed by samples from Palakkad (1), Chittur (2), Kasargod (3), Thodupuzha (4), Sultan Bathery (5), Kayamkulam (10) and Muvattupuzha (12). Only samples from Vadakara (8) and Muvattupuzha (12) formed bands at 320 bp. At 350 bp, bands are formed by samples from Palakkad (1), Sultan Bathery (5), Vadakara (8), Kayamkulam (10) and Sreekaryam (11). Samples from Chittur (2), Kasargod (3), Palluruthy (6), Vellanikkara (7), Muvattupuzha (12) and Pulikeezhu (13) formed bands at 390 bp. At 430 bp, bands are formed by samples from Palakkad (1), Kasargod (3), Thodupuzha (4), Sultan Bathery (5), Palluruthy (6), Vadakara (8), Muvattupuzha (12) and

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Pulikeezhu (13). Except Sultan Bathery (5), all other samples formed bands at 500 bp; whereas, only samples from Sultan Bathery (5) and Vadakara (8) formed bands at 600 bp. Except Vadakara (8) all other samples formed bands at 620 bp. At 700 bp, samples from Kasargod (3), Neyyattinkara (9), Muvattupuzha (12) and Pulikeezhu (13) formed bands. Samples from Palakkad (1), Sultan Bathery (5), Vellanikkara (7) and Neyyattinkara (9) formed bands at 750 bp. At 800 bp, bands are formed by samples from Thodupuzha (4) and Sultan Bathery (5). Palakkad (1), Vadakara (8) and Muvattupuzha (12) samples formed bands at 850 bp. At 1000 bp, except samples from Sultan Bathery (5) and Neyyattinkara (9) all others formed bands. Band is formed only by Sultan Bathery (5) sample at 1050 bp. Only a single band is found to be formed at 1200 bp, by Pulikeezhu (13) sample. At 1300 bp, only samples from Neyyattinkara (9) and Muvattupuzha (12) formed bands. Samples from Palakkad (1), Chittur (2), Vellanikkara (7), Sreekaryam (11) and Muvattupuzha (12) formed bands at 1400 bp. At 1500 bp, bands are formed by samples from Palakkad (1), Chittur (2), Kasargod (3), Thodupuzha (4), Palluruthy (6), Vellanikkara (7), Kayamkulam (10) and Sreekaryam (11). At 1600 bp and 2000 bp, only a single band was found to be formed and was by the sample from Chittur (2). Overall, four unique bands (highlighted with arrow) are produced by the primer at 1050 bp (Sultan Bathery (5), 1200 bp (Pulikeezhu (13), 1600 bp and 2000 bp (Chittur (2).

4.2.2.1.9. Analysis using ISSR primer UBC 811

The amplification pattern of primer UBC 811 (5'-GAGAGAGAGAGAGAGAC-3'), in ISSR region in genomic DNA of cassava whitefly, is shown below (Plate 28).

In the whitefly genomic DNA samples, primer UBC 811 formed 26 bands with amplicon size ranged from 200 bp to 2900 bp. At 200 bp, bands are formed only by samples from Palakkad (1) and Kasargod (3); whereas, at 290 bp, bands are formed by samples from Chittur (2), Vellanikkara (7) and Pulikeezhu (13). Samples from Palakkad (1), Kasargod (3), Thodupuzha (4) and Vellanikkara (7) formed bands at 300 bp. At 320 bp, bands are formed by samples from Palluruthy (6), Vellanikkara (7) and Vadakara (8). Only Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13) samples formed bands at 390 bp. At 400 bp, bands are formed by samples from Palakkad (1), Chittur (2), Kasargod (3), Thodupuzha (4), Sultan Bathery (5) and Kayamkulam (10). At 420 bp, only samples from Vellanikkara (7), Vadakara (8) and Neyyattinkara (9) formed bands. Only a single sample could form band at 450 bp, 820 bp (Vadakara (8) and 1600 bp (Neyyattinkara (9). At 490 bp, samples from Sultan Bathery (5) and Palluruthy (6) formed bands; whereas, at 500 bp, samples from Kasargod (3), Thodupuzha (4) and Sreekaryam (11) formed bands. Samples from Palluruthy (6), Vadakara (8) and Neyyattinkara (9) formed bands at 550 bp.

At 600 bp, bands are formed by samples from Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13). Only Sultan Bathery (5) and Kayamkulam (10) samples formed bands at 620 bp; whereas, at 650 bp bands are formed by samples from Kasargod (3) and Neyyattinkara (9). At 690 bp, samples from Chittur (2), Thodupuzha (4), Vellanikkara (7) and Vadakara (8) formed bands. At 800 bp, bands are formed by samples from Palakkad (1), Palluruthy (6), Neyyattinkara (9) and Sreekaryam (11). Kayamkulam (10), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13) samples formed bands at 900 bp. At 950 bp, bands are formed by samples from Palakkad (1), Chittur (2), Kasargod (3), Vellanikkara (7) and Vadakara (8). Samples from Sultan Bathery (5), Palluruthy (6) and Kayamkulam (10) formed bands at 1000 bp. At 1200 bp, bands are formed by samples from Sultan Bathery (5) and Palluruthy (6). Samples from Sultan Bathery (5), Sreekaryam (11), Muvattupuzha (12) and Pulikeezhu (13) formed bands at 1300 bp. At 1500 bp, bands are formed by samples from Vadakara (8), Sreekaryam (11) and Pulikeezhu (13) and at 1800 bp, bands are formed by samples from Sultan Bathery (5), Muvattupuzha (12) and Pulikeezhu (13). Samples from Vellanikkara (7) and Sreekaryam (11) formed

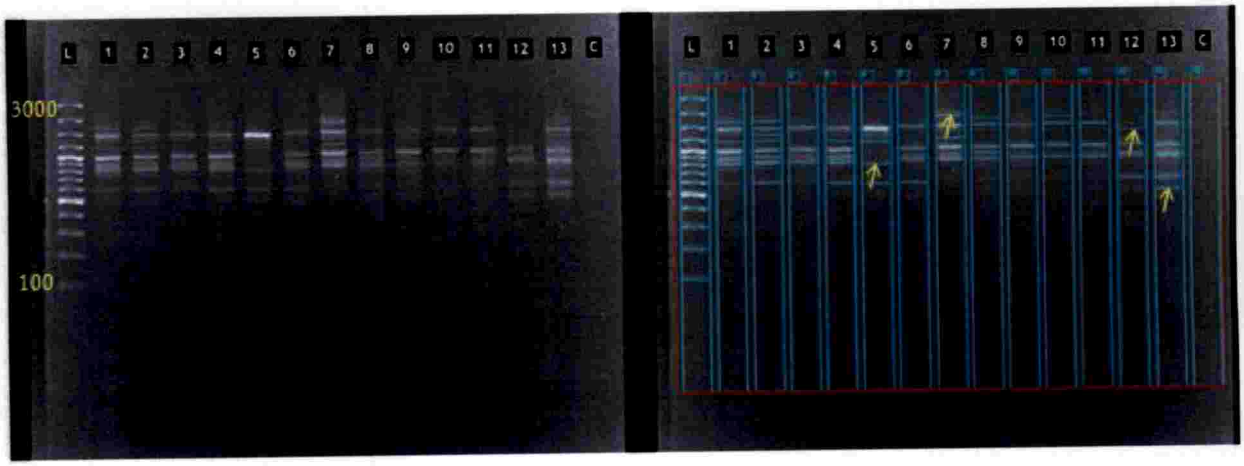

Plate 29. Inter Simple sequence repeat fragments amplified from genomic DNA of thirteen *Bemisia tabaci* adults and banding pattern generated using GelQuant Express (lane 1to lane 13, *viz.*, 1-Palakkad, 2- Chittur, 3- Kasargod, 4- Thodupuzha, 5-Sultan Bathery, 6- Palluruthy, 7-Vellanikkara, 8- Vadakara, 9-Neyyattinkara, 10-Kayamkulam, 11- Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu) occuring on cassava upon amplification with ISSR 3 when resolved in agarose gel (2%). C-control, L: 100 bp plus ladder

bands at 2900 bp. Over all, three unique bands (highlighted with arrow) are produced by the primer at 450 bp, 820 bp (Vadakara (8) and 1600 bp (Neyyattinkara (9).

4.2.2.1.10. Analysis using ISSR primer ISSR 3

The amplification pattern of primer ISSR 3 (5'-AGAGAGAGAGAGAGT-3'), in ISSR region in genomic DNA of cassava whitefly, is shown below (Plate 29).

In the whitefly genomic DNA samples, primer ISSR 3 formed 11 bands with amplicon size ranged from 550 bp to 2000 bp. Only a single sample found to form band at 550 bp (Pulikeezhu (13), 790 bp (Sultan Bathery (5), 1300 bp (Muvattupuzha (12) and 2000 bp (Vellanikkara (7). At 600 bp, samples from Chittur (2), Thodupuzha (4), Sultan Bathery (5), Palluruthy (6), Muvattupuzha (12) and Pulikeezhu (13) formed bands. Except Sultan Bathery (5) sample, all others formed bands at 800 bp and 1000 bp. At 900 bp, bands are formed by samples from Palakkad (1), Chittur (2), Thodupuzha (4) and Vadakara (8). Samples from Palakkad (1), Chittur (2), Thodupuzha (4), Sultan Bathery (5) and Vellanikkara (7) formed bands at 1200 bp. Except Muvattupuzha (12) sample; all others formed bands at 1500 bp. At 1700 bp, bands are formed by samples from Palakkad (1), Chittur (2), Vadakara (8) and Kayamkulam (10). Overall, four unique bands (highlighted with arrow) are produced by the primer at 550 bp (Pulikeezhu (13), 790 bp (Sultan Bathery (5), 1300 bp (Muvattupuzha (12) and 2000 bp (Vellanikkara (7).

Genetic variability of cassava whitefly, *Bemisia tabaci* from different agro-ecological zones of Kerala

Dice coefficient values were calculated based on presence or absence of ISSR bands. The calculated value ranged from 0.51 to 1.00 (Table 14). The samples from Muvattupuzha (12, MVA) and Pulikeezhu (13, PKU) were found to be the most closely related with similarity coefficient of 0.85. The samples from Palakkad (1, PKD), Chittur (2, CTR) and Thodupuzha (4, TPA), Kasargod (3, KGD) were shown the next closest association with similarity coefficient of 0.81 and in between samples of Palakkad (1, PKD) and Thodupuzha (4, TPA), a similarity coefficient of 0.80 was found. Least similarity was observed between samples from Sultan Bathery (5, SBY) and Chittur (2, CTR) (similarity coefficient of 0.51). In general, the sample from Sultan Bathery (5, SBY) had shown the least similarity with all other samples (similarity coefficients in between 0.51-0.60).

Table 14. Dice coefficient matrix for *Bemisia tabaci* from different agro-ecological zones of

Kerala

	PKD	CTR	KGD	TPA	SBY	PTY	VKA	VDA	NYA	KYM	SKM	MVA	PKU
PKD	1.0000												
CTR	0.8152	1.0000											
KGD	0.7989	0.7880	1.0000										
TPA	0.8097	0.7880	0.8152	1.0000									
SBY	0.5760	0.5108	0.5380	0.6032	1.0000								
PTY	0.6250	0.6358	0.6739	0.6739	0.5815	1.0000							
VKA	0.7445	0.7119	0.7065	0.7500	0.5597	0.6086	1.0000						
VDA	0.6902	0.6902	0.7173	0.7391	0.5489	0.5869	0.6847	1.0000					
NYA	0.6793	0.6467	0.7391	0.7065	0.5923	0.6847	0.6630	0.6739	1.0000				
KYM	0.6630	0.6630	0.6684	0.6576	0.5978	0.6032	0.5923	0.6250	0.6684	1.0000			
SKM	0.6847	0.6847	0.6793	0.7010	0.5869	0.6141	0.7010	0.6576	0.6902	0.7065	1.0000		
MVA	0.5978	0.5978	0.6032	0.6032	0.5652	0.7228	0.5706	0.5706	0.6358	0.5978	0.6630	1.0000	
PKU	0.5869	0.6304	0.6358	0.6250	0.5543	0.7228	0.5923	0.5597	0.6358	0.6195	0.6847	0.8586	1.0000

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(NB: PKD-Palakkad, CTR-Chittur, KGD-Kasargod, TPA-Thodupuzha, SBY-Sultan Bathery, PTY-Palluruthy, VKA-Vellanikkara, VDA-Vadakara, NYA-Neyyattinkara, KYM-Kayamkulam, SKM-Sreekaryam, MVA-Muvattupuzha, PKU-Pulikeezhu)

Dice coefficient values were used to construct dendrogram by un-weighted pair-group method analysis, UPGMA. Dendrogram analysis revealed the occurrence of two major clusters and an out group (Fig. 14). The out group is formed by Sultan Bathery (SBY) sample. The biggest cluster formed is having samples from Palakkad

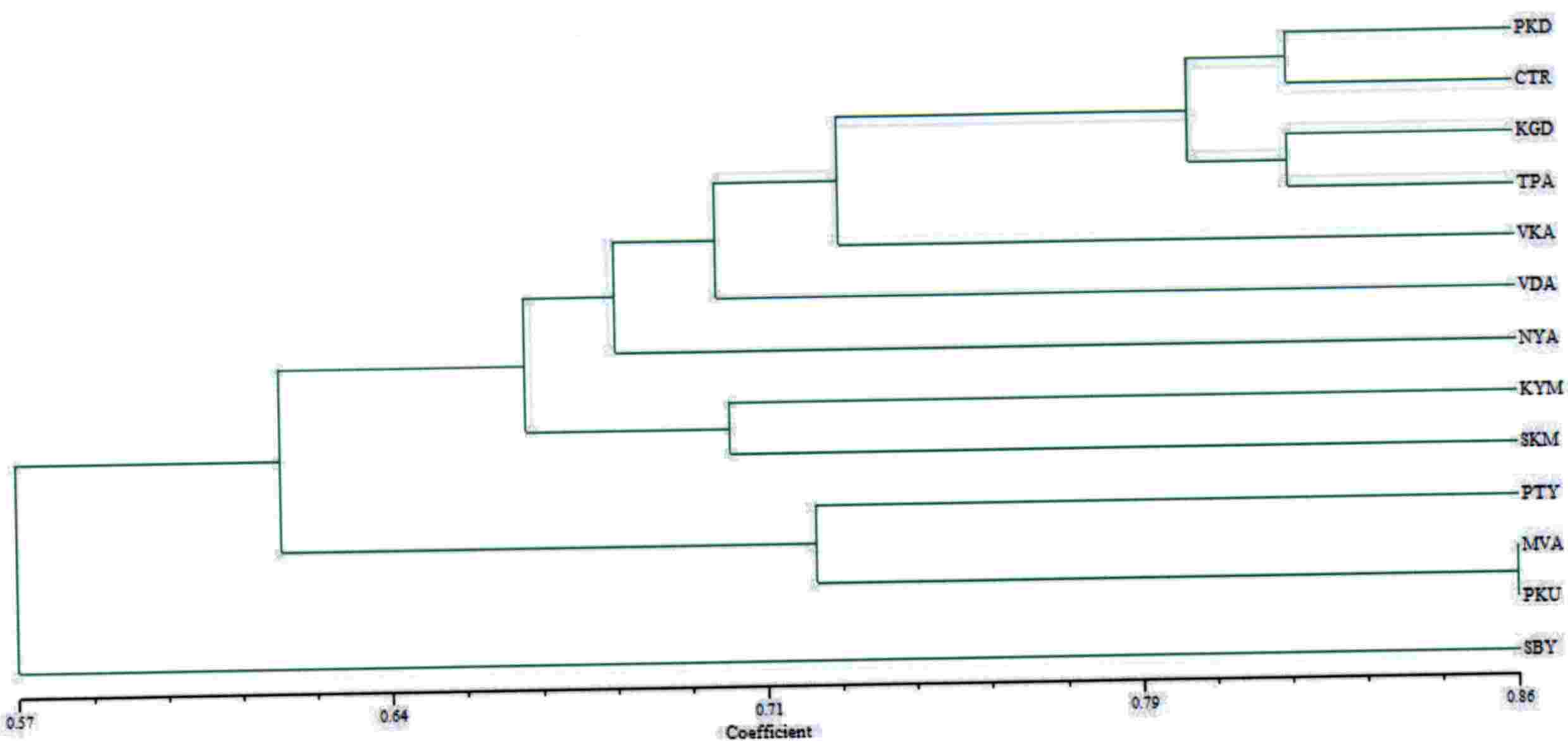

Fig. 14. Dendrogram deduced from matrix of pair wise similarities in ISSR analysis between *Bemisia tabaci* from 13-agro-ecological zones of Kerala using the un-weighted pair-group method analysis, UPGMA (PKD-Palakkad, CTR-Chittur KGD-Kasargod, TPA-Thodupuzha, SBY-Sultan Bathery, PTY-Palluruthy, VKA-Vellanikkara, VDA-Vadakara, NYA-Neyyattinkara, KYM-Kayamkulam, SKM-Sreekaryam, MVA-Muvattupuzha, PKU-Pulikeezhu)

(PKD), Chittur (CTR), Kasargod (KGD), Thodupuzha (TPA), Vellanikkara (VKA), Vadakara (VDA), Neyyattinkara (NYA), Kayamkulam (KYM) and Sreekaryam (SKM); the order in which the samples are closely related based on ISSR analysis. The second cluster is having samples from Palluruthy (PTY), Muvattupuzha (MVA) and Pulikeezhu (PKU). The samples from Muvattupuzha (MVA) and Pulikeezhu (PKU) are very closely related.

4.2.2.2. Analysis using mitochondrial cytochrome oxidase 1 primer and DNA barcoding

The *mtCO1* region was amplified using the barcode primers (F: C1-J2195 COI: 5′-TTGATTTTTTGGTCATCCAGAAGT-3′, R: L2-N-3014 COI: 5′-TCCAATGCACTAATCTGCCATATTA-3′) and the PCR products gave intact bands at 850 bp when resolved at 1 per cent agarose gel (Plate 30). The *mtCO1*sequences generated from whiteflies consisted of 765 bp to 795 bp. The homology of sequences with the reported sequences was analysed. The sequence showed significant homology to *B.tabaci* mitochondrial Cytochrome Oxidase CO1 gene already deposited in the public domain database using ‘BLASTn’ search tool. The blast results showed 100 per cent query coverage and 84 per cent to 100 per cent identity to *B.tabaci mtCO1*gene. Then the sequence was aligned and annotated using bioinformatics tools, BioEdit and MEGA6. The sequences thus obtained were submitted to Banklt, NCBI under the accession numbers KX574324, KX580954, KX668396, KX668397, KX668398, KX668399, KX668400, KX668401, KX668402, KX668403, KX668404, KX668405 and KX668406.

An account was opened in workbench session of BOLD systems v3 database and new projects were created for all the 13 samples. Eg: for the sample from Chittur ‘BTCTR’ was created. Specimen data *viz.*, specimen identifiers, specimen taxonomy, specimen details, collection details was submitted and an auto generated process ID

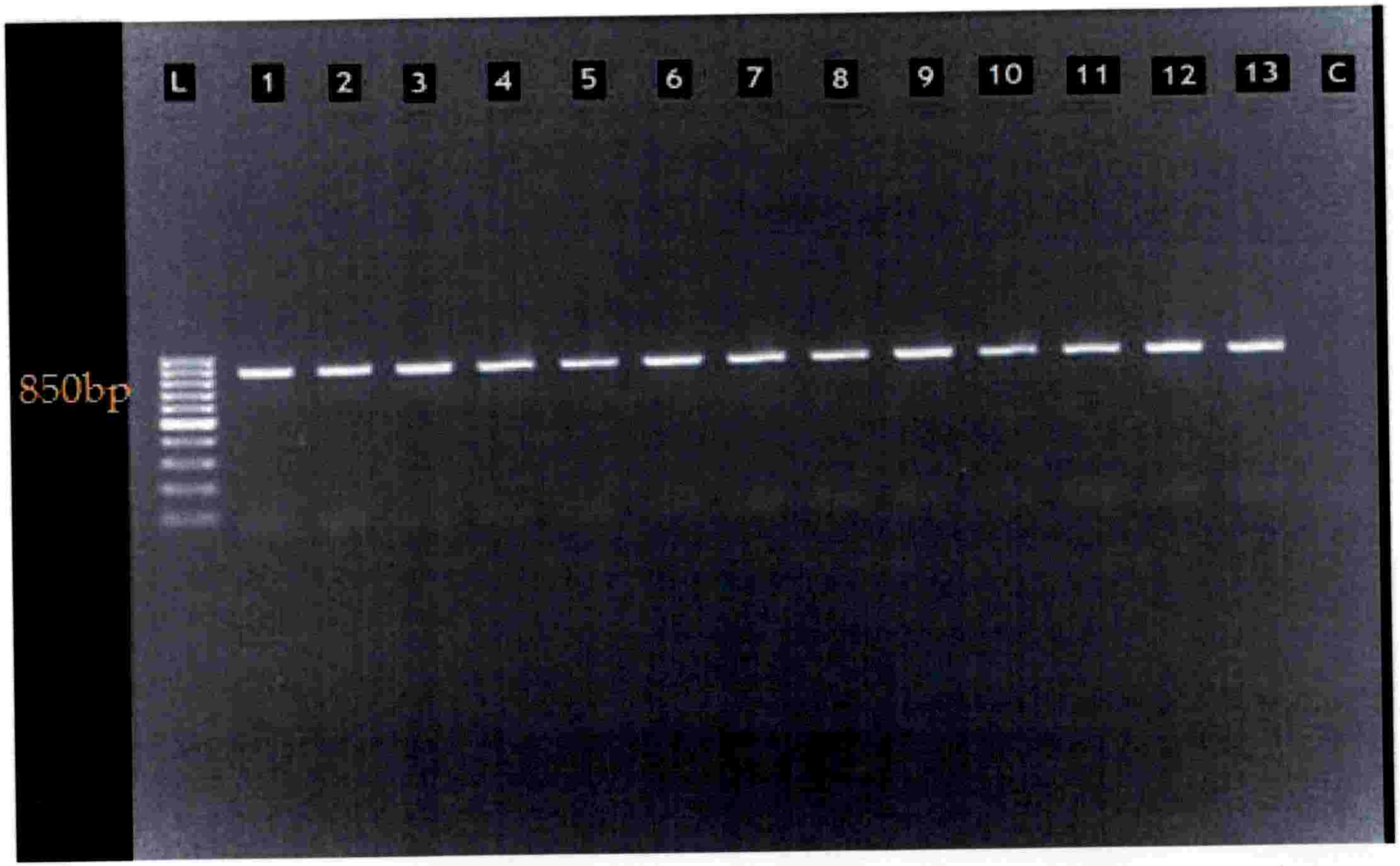

Plate 30. Agarose gel electrophoresis of genomic DNA isolated from *Bemisia tabaci* upon amplification with *mtCO1* primer (L: 50 bp ladder, C-control, 1-13: *Bemisia* DNA samples) (NB: 1-Palakkad, 2- Chittur, 3-Kasargod, 4-Thodupuzha, 5-Sultan Bathery, 6- Palluruthy, 7-Vellanikkara, 8-Vadakara, 9-Neyyattinkara, 10-Kayamkulam, 11-Sreekaryam, 12-Muvattupuzha, 13-Pulikeezhu)

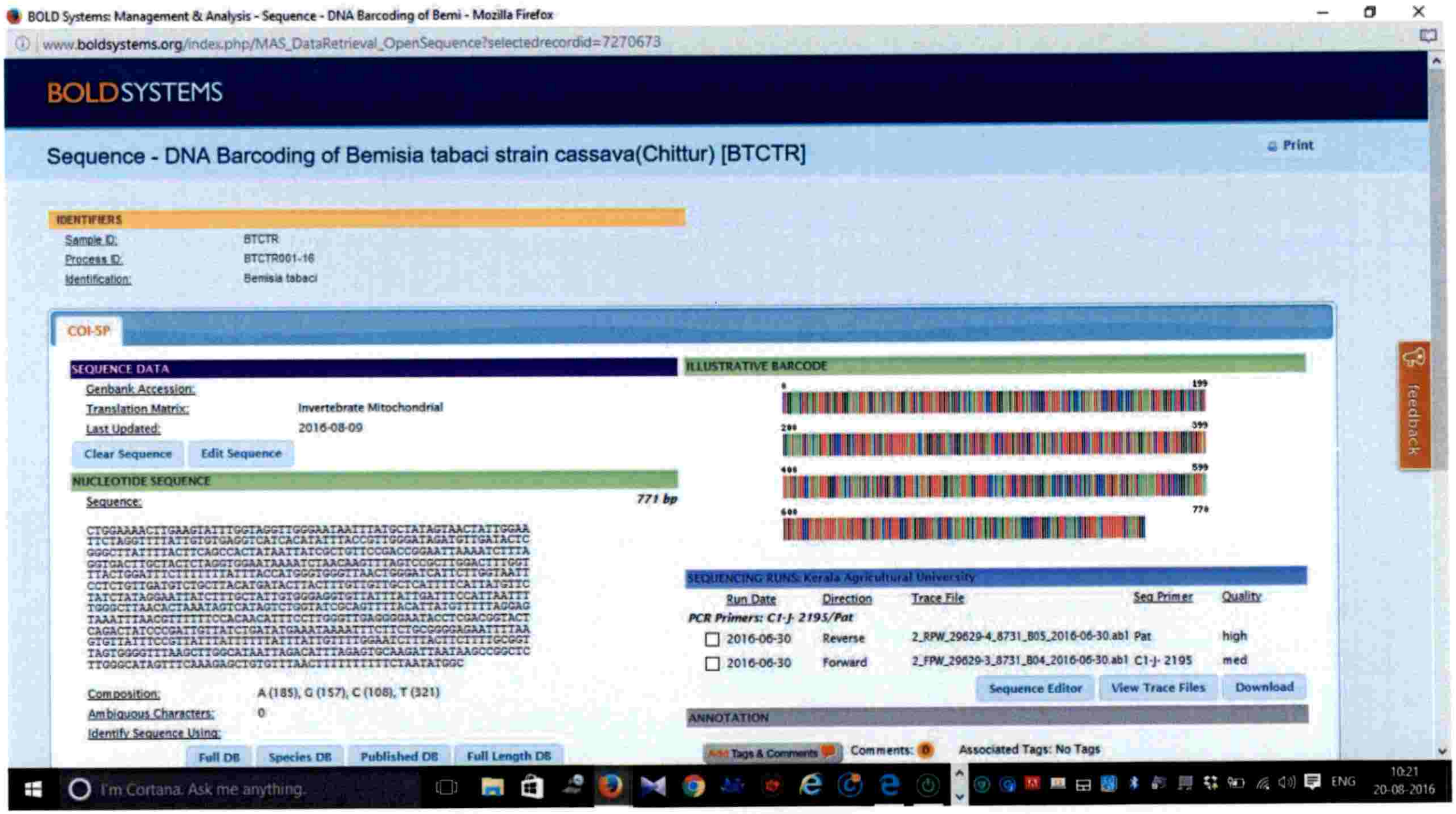

Plate 31. DNA barcode of *Bemisia tabaci* generated by BOLD systems v3

'BTCTR001-16' was obtained. Further, primer details, high resolution specimen images, mitochondrial DNA sequences (fasta) and the trace files (.ab1) obtained from sequencer were uploaded to the database and the corresponding barcode of *B.tabaci* (Plate 31) was generated. Per cent identity matrix values were calculated based on the similarity in their *mtCO1* sequences. All the 13 sequences were aligned using online software 'Clustal 2.1' and the respective values for their similarity were obtained by pair wise arrangement. The calculated values ranged from 83.50 to 100 (Table 15). Among all, the sample from Sultan Bathery (SBY) shows least similarity (83.50 - 85.63) in the matrix; while the others were in the range of 96.64 per cent to 100 per cent similarity.

Table 15. Per cent identity matrix (Clustal 2.1) for *Bemisia tabaci* (*mtCO1* sequences) from different agro-ecological zones of Kerala

1: SBY	100.00	84.97	85.15	85.04	85.08	84.55	84.58	84.70	84.72	83.50	84.38	85.63	84.38
2: TPA	84.97	100.00	99.87	99.87	99.87	99.35	99.62	99.49	99.49	97.96	99.00	99.85	98.72
3: NYA	85.15	99.87	100.00	100.00	100.00	99.48	99.49	99.62	99.62	97.95	98.86	100.00	98.86
4: VDA	85.04	99.87	100.00	100.00	100.00	99.61	99.62	99.75	99.75	97.81	98.86	100.00	98.86
5: SKM	85.08	99.87	100.00	100.00	100.00	99.48	99.49	99.62	99.62	97.81	98.86	100.00	98.86
6: CTR	84.55	99.35	99.48	99.61	99.48	100.00	99.74	99.87	99.87	99.85	98.81	100.00	98.81
7: PTY	84.58	99.62	99.49	99.62	99.49	99.74	100.00	99.88	99.88	97.96	99.00	99.85	98.72
8: VKA	84.70	99.49	99.62	99.75	99.62	99.87	99.88	100.00	100.00	97.81	98.86	100.00	98.86
9: PKU	84.72	99.49	99.62	99.75	99.62	99.87	99.88	100.00	100.00	97.81	98.86	100.00	98.86
10: KYM	83.50	97.96	97.95	97.81	97.81	99.85	97.96	97.81	97.81	100.00	97.11	97.72	96.64
11: MVA	84.38	99.00	98.86	98.86	98.86	98.81	99.00	98.86	98.86	97.11	100.00	99.85	99.86
12: PKD	85.63	99.85	100.00	100.00	100.00	100.00	99.85	100.00	100.00	97.72	99.85	100.00	100.00
13: KGD	84.38	98.72	98.86	98.86	98.86	98.81	98.72	98.86	98.86	96.64	99.86	100.00	100.00

(NB: PKD (1) -Palakkad, CTR (2) -Chittur, KGD (3) -Kasargod, TPA (4) -Thodupuzha, SBY (5) -Sultan Bathery, PTY (6) -Palluruthy, VKA (7) -Vellanikkara, VDA (8) -Vadakara, NYA (9) -Neyyattinkara, KYM (10) -Kayamkulam, SKM (11) -Sreekaryam, MVA (12) -Muvattupuzha, PKU (13) -Pulikeezhu)

Per cent identity matrix values were utilized to construct dendrogram by pair wise comparisons in *mtCO1* sequences of *B. tabaci* from 13-agro-ecological zones of Kerala using MEGA-6 software (Neighbour joining method). Dendrogram analysis revealed the occurrence of two major clusters and an out group (Fig. 15). The out group is Sultan Bathery (SBY) sample. The members of the first cluster include samples from Kayamkulam (KYM), Muvattupuzha (MVA), Palluruthy (PTY) and Thodupuzha (TPA) in the order of their similarity. The order of similarity in the members of second cluster is Kasargod (KGD), Palakkad (PKD), Vellanikkara (VKA), Chittur (CTR), Sreekaryam (SKM), Vadakara (VDA), Neyyattinkara (NYA) and Pulikeezhu (PKU).

Table 16. Similarity check of *mtCO1* sequences (*Bemisia tabaci*) in NCBI database

Sl. No.	Places of collection of *Bemisia tabaci*	GenBank Accession No. obtained	Similarity (per cent)
1	Palakkad	KX574324	Asia II-5 (99)
2	Chittur	KX580954	Asia II-5 (100)
3	Kasargod	KX668396	Asia II-5 (99)
4	Kayamkulam	KX668397	Asia II-5 (99)
5	Sultan Bathery	KX668398	Asia I (98)
6	Thodupuzha	KX668399	Asia II-5 (97)
7	Palluruthy	KX668400	Asia II-5 (99
8	Vellanikkara	KX668401	Asia II-5 (99)
9	Vadakara	KX668402	Asia II-5 (99)
10	Neyyattinkara	KX668403	Asia II-5 (99)
11	Sreekaryam	KX668404	Asia II-5 (99)
12	Muvattupuzha	KX668405	Asia II-5 (99)
13	Pulikeezhu	KX668406	Asia II-5 (99)

The different *B. tabaci* sequences were submitted to BankIt, NCBI under the accession numbers KX574324, KX580954, KX668396, KX668397, KX668398, KX668399, KX668400, KX668401, KX668402, KX668403, KX668404, KX668405 and KX668406. Their similarity was compared with the reference sequences of *B. tabaci* available in the NCBI data base. It was observed that all the samples except Sultan Bathery recorded 97 to 100 per cent similarity to Asia II-5 genetic group of *B. tabaci*. While, the sample from Sultan Bathery found to belong to

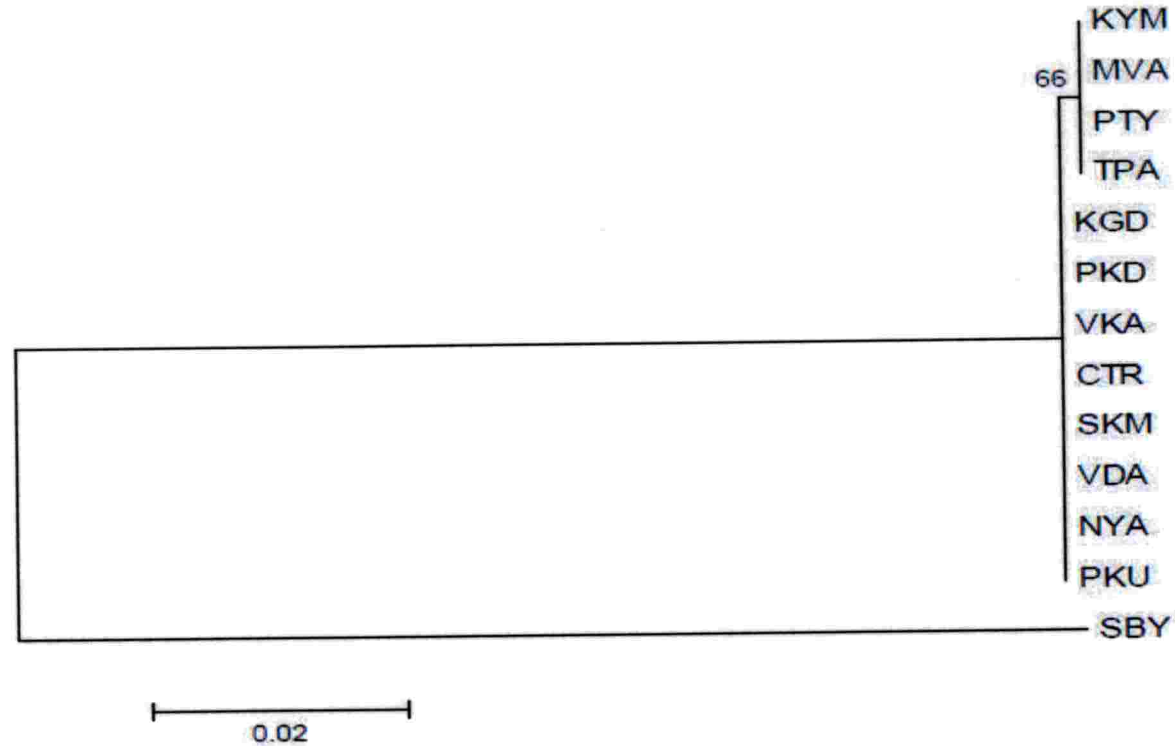

Fig. 15. Dendrogram deduced from matrix of pair wise comparisons in *mtCO1* sequences of *Bemisia tabaci* from 13-agro-ecological zones of Kerala (MEGA-6, Neighbour joining method)

(PKD-Palakkad, CTR-Chittur, KGD-Kasargod, TPA-Thodupuzha, SBY-Sultan Bathery, PTY-Palluruthy, VKA-Vellanikkara, VDA-Vadakara, NYA-Neyyattinkara, KYM-Kayamkulam, SKM-Sreekaryam, MVA-Muvattupuzha, PKU-Pulikeezhu)

Asia I genetic group with 98 per cent similarity to the reference sequence (Table 16). The results were re-confirmed by repeating the experiment.

4. 3. Metagenomic study of adult *Bemisia tabaci*

4. 3. 1. Isolation and quality checking of metagenomic DNA from adult *Bemisia tabaci*

Whiteflies collected from hilly areas (H = >900 m from MSL) and Plains (P = <150 m from MSL) were used in the study. Metagenomic DNA isolated from the two samples of *B. tabaci* were confirmed with the presence of 16S rRNA fragment in the isolated products by amplification with universal 16S rDNA primers. An intact band at 1500 bp was obtained when resolved at 0.8 per cent agarose gel (Plate 32). The metagenomic DNA was quantified with fluorometer (Qubit 2.0) and the concentrations were 30.6 ng/ µl and 30.4 ng/ µl. The hypervariable V3 region of 16S rRNA was amplified with specific primers (Fig. 16) and proceded for 16S rRNA library preparations.

4. 3. 2. 16S rRNA library preparation and sample loading

Metagenomic DNA (5 ng) was taken and standard protocol was followed for 16S rRNA library preparation and sample loading to the Illumina MiSeq^{TM} sequencer.

4. 3. 3. Illumina sequencing data

Total raw sequencing reads (paired end) of 1,321,906 and 690,661 with average sequence length of 150 bp each was obtained from Illumina MiSeq^{TM} sequencer. The following quality parameters were checked. The quality of left and right end of the paired-end read sequences of the sample was shown in the fig. 17 and

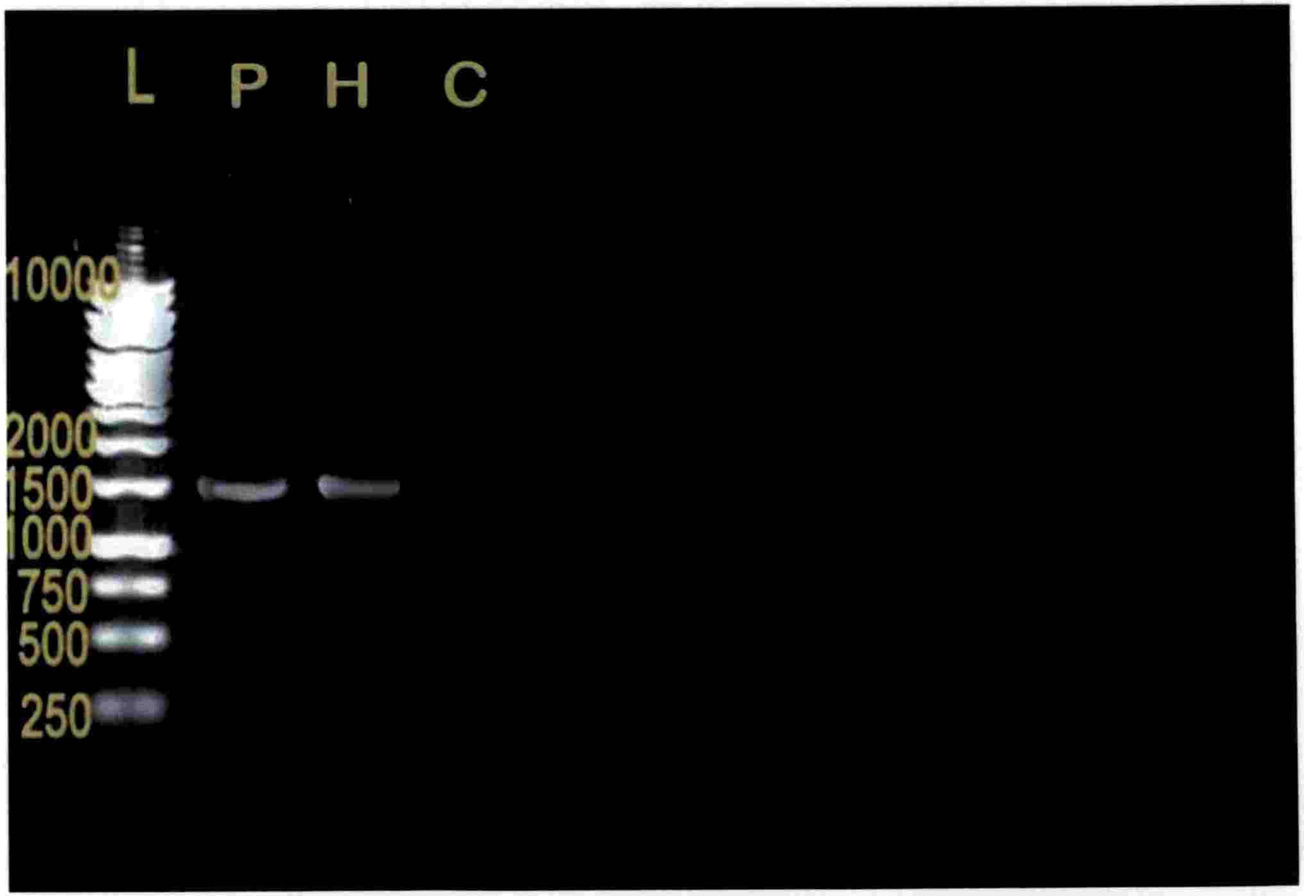

Plate 32. Agarose gel electrophoresis of metagenomic DNA isolated from *Bemisia tabaci* upon amplification with I6S rDNA primers (L: 1 kb ladder, P- metagenomic DNA of *B. tabaci* from plains , H- metagenomic DNA of *B. tabaci* from highranges)

18. Nearly 90 per cent of the total reads had phred score greater than 30 (>Q30; error-probability >= 0.001).

The base composition distribution of two samples were adenine (24.05 %, 23.63 %), cytosine (24.06 %, 24.64 %), guanine (27.46 %, 27.78 %) and thiamine (24.43%, 23.95 %) and the average GC content in the range 40-50 per cent was observed in each sequence reads. Application of multiple filters such as conserved region filter, spacer filter, quality filter and mismatch filter had resulted 1,240,613, 1,240,116, 1,239,993 and 640,996 reads respectively for first sample (P) and for the second sample (H), the values were 640,923, 640,500, 640,450 and 341,937 respectively. While making consensus V3 sequence, more than 48 per cent of the paired-end reads were aligned to each other with zero mismatches with an average contig length of 135 to 165 bp (Fig. 19).

From the 640,996 and 341,937 consensus reads, of two whitefly samples, singletons and chimeric sequences were removed and thus we obtained 611,218 and 334,634 high quality pre-processed reads. The pre-processed reads from all samples were pooled and clustered into OTUs based on their sequence similarity (similarity cutoff = 0.97) and a total of 3,513 OTUs were identified from 945,852 reads (Fig. 20). The total number of bacteria species detected in the sample was strongly affected by number of sequence analyzed (Shi *et al.*, 2012). We carried out rarefaction analysis to verify the amount of sequencing reflected in the diversity of original microbial community and the analysis revealed that the species count increased sharply before attaining a plateau (Fig. 21). The alpha diversity (6.22 and 3.82 for P and H populations respectively) obtained from rarefaction analysis indicated the extent of bacteria species diversity present in *B. tabaci*.

4. 3. 3. 1. Composition of bacterial community of adult *Bemisia tabaci* identified by Illumina sequencing

We analyzed the composition of bacteria present in adult *B. tabaci* and grouped them into each taxonomic category from phyla to species level. The abundance of 10 major bacterial groups in each taxonomic category is given in Table 17 and 18, for whitefly samples P and H. Altogether, 16 bacterial phyla were detected both in P and H samples. Among the phyla for P populations of *B. tabaci*, *Proteobacteria* was the most dominant which consisted of 87.57 per cent of total bacterial community and for H populations; *Firmicutes* was the most dominant with 82.67 per cent. It was followed by *Firmicutes* (9.29 %) for P populations and *Proteobacteria* (13.40 %) for H populations. Bacteria belongs to *Bacteroidetes* consisted of 2.91 per cent for P populations. Reads belongs to *Chlorobi, Actinobacteria, Planctomycetes, Verrucomicrobia, Spirochaetae, Tenericutes* and *Acidobacteria* were found to be the other phyla with only a few reads (<1%) for P; and for H the smaller populations belongs to *Bacteroidetes, Actinobacteria, Chlorobi, Planctomycetes, Verrucomicrobia, Spirochaetae,Tenericutes* and *Acidobacteria.*

A total of 27 and 31 bacterial classes were identified for P and H populations respectively and among them *Gammaproteobacteria* [class] was the most dominant group (86.47 %) for P and for H the most dominant was *Bacilli* (82.65 %). It was followed by *Flavobacteriia* (1.70 %), *Betaproteobacteria* (0.94 %), *Bacteroidia* (0.59 %), *Sphingobacteriia* (0.59 %), *Chlorobia* (0.16 %), *Deltaproteobacteria* (0.16 %), *Negativicutes* (0.15 %) and *Cytophagia* (0.03 %) for P and for H populations, *Bacilli* was followed by *Gammaproteobacteria* (16.28 %), *Bacteroidia* (0.71 %), *Flavobacteriia* (0.12 %), *Deltaproteobacteria* (0.09 %), *Actinobacteria* [class] (0.05 %), *Betaproteobacteria* (0.03 %), *Chlorobia* (0.03 %), *Negativicutes* (0.02 %) and *Cytophagia* (0.01 %). When we analyzed the reads at order level altogether, 56 and

60 bacterial orders for P and H populations respectively were detected. The most dominant group was *Enterobacteriales* (85 %), followed by *Bacillales* (8.70 %), *Flavobacteriales* (1.71 %), *Vibrionales* (1.30 %), *Burkholderiales* (0.69 %), *Bacteroidales* (0.60 %), *Sphingobacteriales* (0.59 %), *Lactobacillales* (0.47 %), *Pseudomonadales* (0.38 %) and *Chlorobiales* (0.17 %) for P populations and for H populations, the order of dominance was *Bacillales* (82.58 %), *Enterobacteriales* (8.34 %), *Oceanospirillales* (7.62 %), *Bacteroidales* (0.71 %), *Vibrionale*s (0.23 %), *Lactobacillales* (0.16 %), *Flavobacteriales* (0.12 %), *Actinomycetales* (0.05 %), *Chlorobiales* (0.03 %) and *Pseudomonadales* (0.03 %).

Analyses at family level revealed a total of 91 and 88 bacterial families (P and H respectively) were present in the samples. Major 10 bacterial families present in P samples were *Enterobacteriaceae* (85.01 %), followed by *Bacillaceae* (8.25 %), *Flavobacteriaceae* (1.71 %), *Vibrionaceae* (1.30 %), *Oxalobacteraceae* (0.65 %), *Sphingobacteriaceae* (0.58 %), *Bacteroidaceae* (0.39 %), *Enterococcaceae* (0.38 %), *Pseudomonadaceae* (0.37 %) and *Staphylococcaceae* (0.24 %). The major 10 bacterial families present in H samples include, *Bacillaceae* (77.42 %), *Enterobacteriaceae* (8.34 %), *Alcanivoracaceae* (7.62 %), *Staphylococcaceae* (5.19 %), *Bacteroidaceae* (0.41 %), *Vibrionaceae* (0.23 %), *Rikenellaceae* (0.20 %), *Enterococcaceae* (0.09 %), *Prevotellaceae* (0.07 %) and *Paenibacillaceae* (0.04 %) (Fig. 22).

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Table 17. Abundance of major 10 taxonomic category from phyla to species level of bacteria occur in the gut of *Bemisia tabaci* P population

Sl.no	Phylum	Class	Order	Family	Genus	Species
1	Proteobacteria (87.57)	Gammaproteobacteria (86.47)	Enterobacteriales (85.00)	Enterobacteriaceae (85.01)	*Bacillus* (35.57)	Secondary endosymbiont of *Bemisia tabaci* [un-specified] (70.38)
2	Firmicutes (9.29)	Bacilli (9.14)	Bacillales (8.70)	Bacillaceae (8.25)	*Arsenophonus* (24.69)	*Arsenophonus* endosymbiont of *Trialeurodes vaporariorum* (7.19)
3	Bacteroidetes (2.91)	Flavobacteriia (1.70)	Flavobacteriales (1.71)	Flavobacteriaceae (1.71)	*Vibrio* (5.83)	*Bacillus cereus* (4.07)
4	Chlorobi (0.16)	Betaproteobacteria (0.94)	Vibrionales (1.30)	Vibrionaceae (1.30)	*Riemerella* (4.53)	*Bacillus megaterium* (3.78)
5	Actinobacteria (0.02)	Bacteroidia (0.59)	Burkholderiales (0.69)	OxaloBacteraceae (0.65)	*Lysinibacillus* (4.20)	*Bacillus flexus* (1.70)
6	Planctomycetes (0.01)	Sphingobacteriia (0.59)	Bacteroidales (0.60)	Sphingobacteriaceae (0.58)	*Flavobacterium* (2.87)	*Riemerella anatipestifer* (1.32)
7	Verrucomicrobia (0.007)	Chlorobia (0.16)	Sphingobacteriales (0.59)	Bacteroidaceae (0.39)	*Janthinobacterium* (2.94)	*Vibrio harveyi* (1.30)
8	Spirochaetae (0.005)	Deltaproteobacteria (0.16)	Lactobacillales (0.47)	Enterococcaceae (0.38)	*Sphingobacterium* (2.79)	*Lysinibacillus sphaericus* (1.22)
9	Tenericutes (0.004)	Negativicutes (0.15)	Pseudomonadales (0.38)	Pseudomonadaceae (0.37)	*Bacteroides* (1.90)	*Janthinobacterium sp.* J3 (0.86)
10	Acidobacteria (0.002)	Cytophagia (0.03)	Chlorobiales (0.17)	Staphylococcaceae (0.24)	*Enterococcus* (1.83)	*Bacillus pumilus* (0.69)

Proportion [%] of each category is given in parenthesis

Table 18. Abundance of major 10 taxonomic category from phyla to species level of bacteria occur in the gut of *Bemisia tabaci* H population

Sl. no	Phylum	Class	Order	Family	Genus	Species
1	Firmicutes (82.67)	Bacilli (82.65)	Bacillales (82.58)	Bacillaceae (77.42)	*Bacillus* (82.27)	*Bacillus thuringiensis* (72.62)
2	Proteobacteria (13.40)	Gammaproteobacteria (16.28)	Enterobacteriales (8.34)	Enterobacteriaceae (8.34)	*Alcanivorax* (8.19)	*Alcanivorax sp. EPR 6* (7.58)
3	Bacteroidetes (0.84)	Bacteroidia (0.71)	Oceanospirillales (7.62)	Alcanivoracaceae (7.62)	*Staphylococcus* (5.58)	*SBR proteobacterium* (6.97)
4	Actinobacteria (0.07)	Flavobacteriia (0.12)	Bacteroidales (0.71)	Staphylococcaceae (5.19)	*Pantoea* (1.13)	*Staphylococcus pasteuri* (3.31)
5	Chlorobi (0.05)	Deltaproteobacteria (0.09)	Vibrionales (0.23)	Bacteroidaceae (0.41)	*Lysinibacillus* (0.76)	*Bacillus amyloliquefaciens* (1.75)
6	Planctomycetes (0.04)	Actinobacteria (class) (0.05)	Lactobacillales (0.16)	Vibrionaceae (0.23)	*Bacteroides* (0.44)	*Staphylococcus sciuri* (1.63)
7	Verrucomicrobia (0.04)	Betaproteobacteria (0.03)	Flavobacteriales (0.12)	Rikenellaceae (0.20)	*Alistipes* (0.22)	*Bacillus megaterium* (1.10)
8	Spirochaetae (0.02)	Chlorobia (0.03)	Actinomycetales (0.05)	Enterococcaceae (0.09)	*Photorhabdus* (0.21)	*Pantoea dispersa* (1.02)
9	Tenericutes (0.01)	Negativicutes (0.02)	Chlorobiales (0.03)	Prevotellaceae (0.07)	*Terribacillus* (0.16)	*Lysinibacillus sphaericus* (0.70)
10	Acidobacteria (0.01)	Cytophagia (0.01)	Pseudomonadales (0.03)	Paenibacillaceae (0.04)	*Enterococcus* (0.10)	*Bacillus pumilus* (0.34)

Proportion [%] of each category is given in parenthesis

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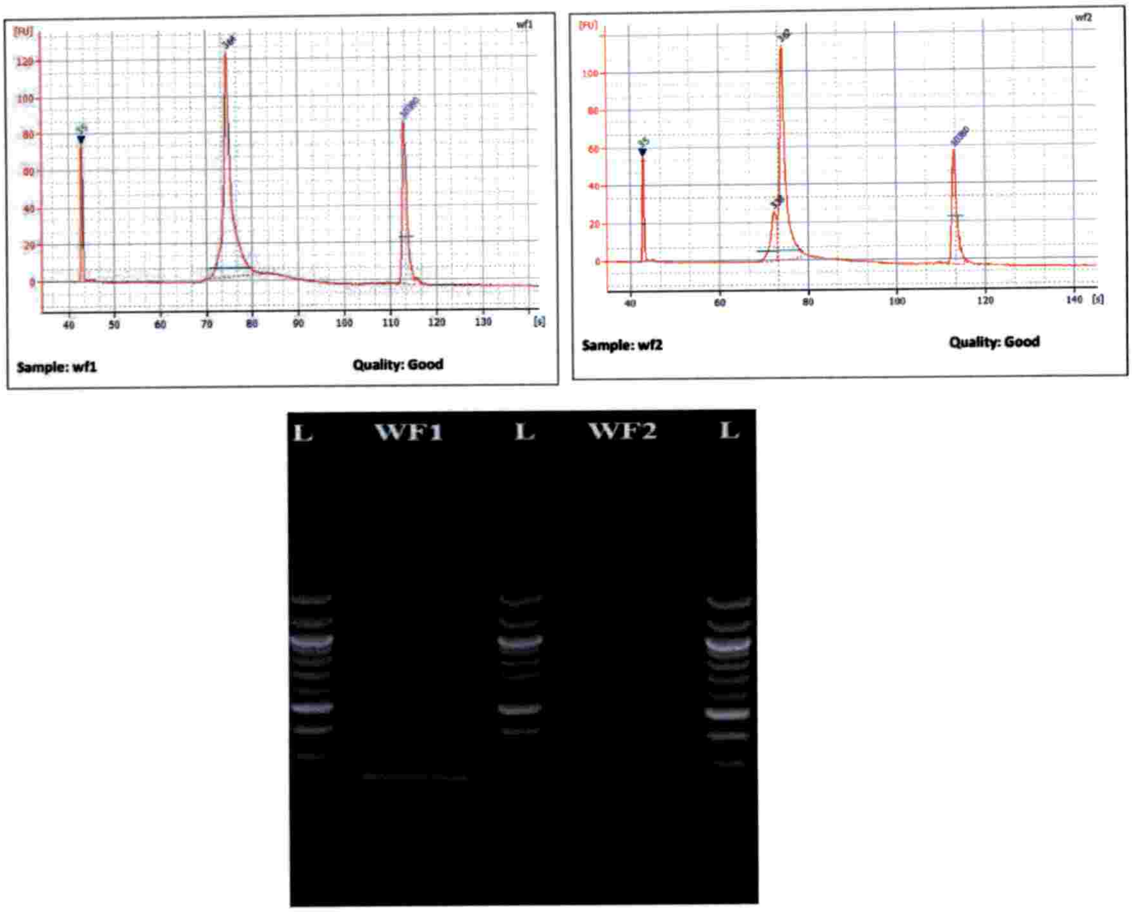


Fig 16. Amplification of V3 region shown by fluorometer (Qubit 2.0)

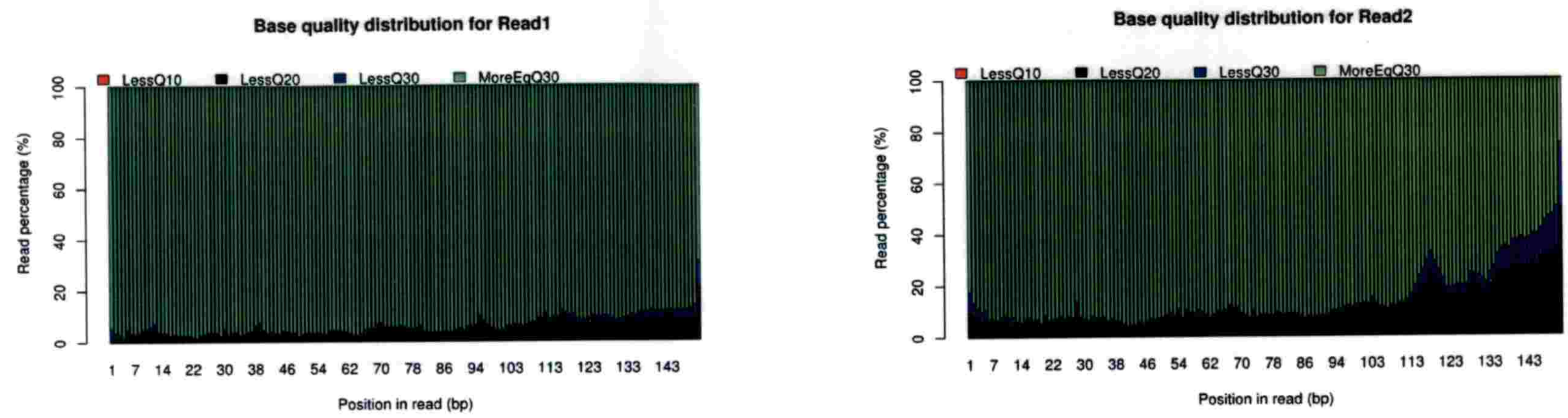


Fig. 17. Base quality distribution of paired end sequences (read 1 and read 2) in whitefly sample P

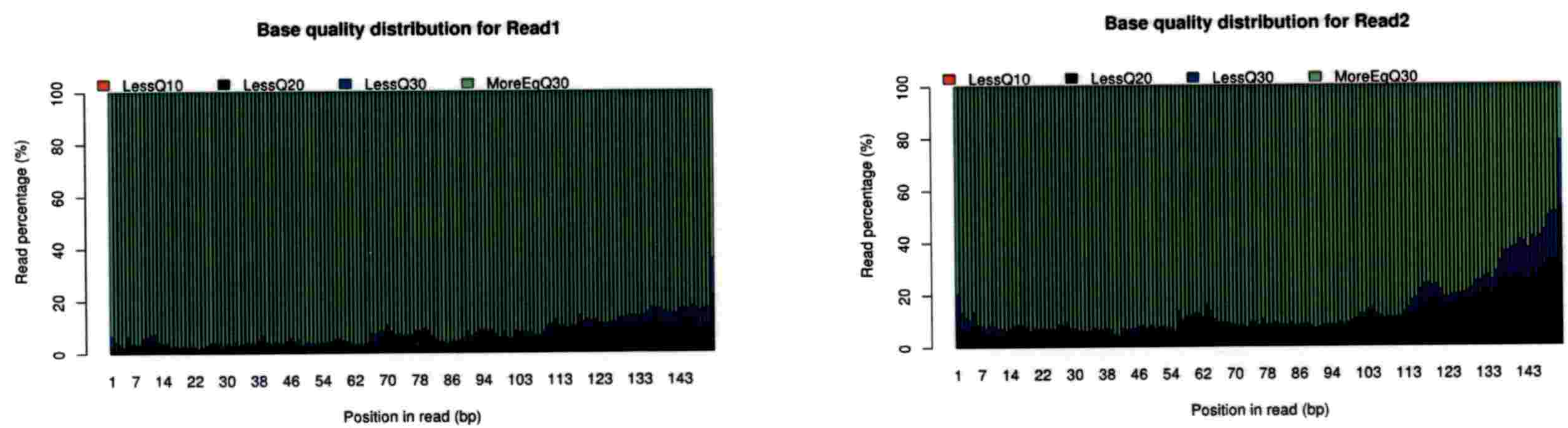
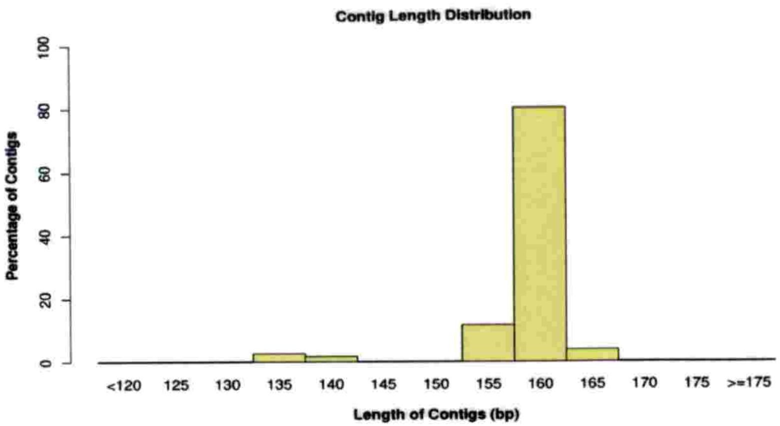
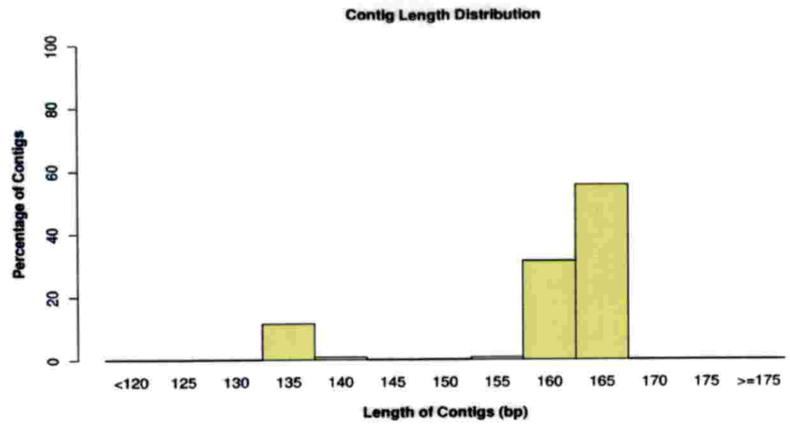


Fig. 18. Base quality distribution of paired end sequences (read 1 and read 2) in whitefly sample H



Sample P



Sample H

Fig. 19. Contig Length distribution of V3 sequences versus percentage of contigs

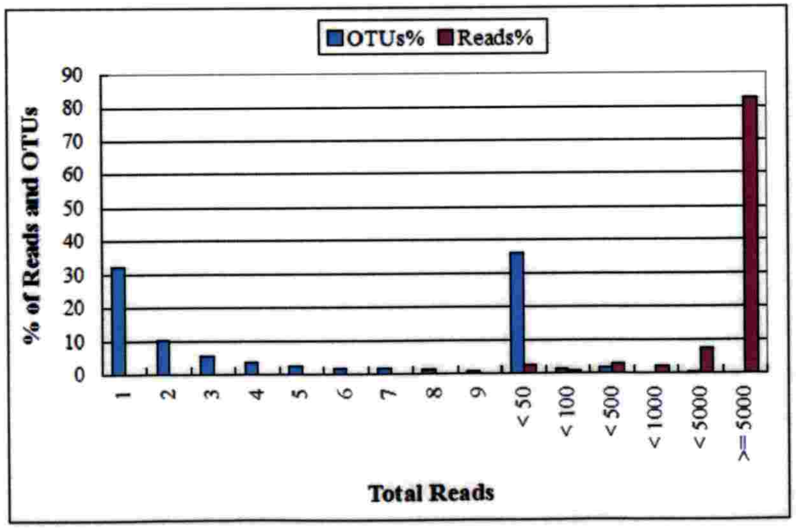


Fig. 20. The percentage of total OTUs and percentage of total read contributed by OTUs

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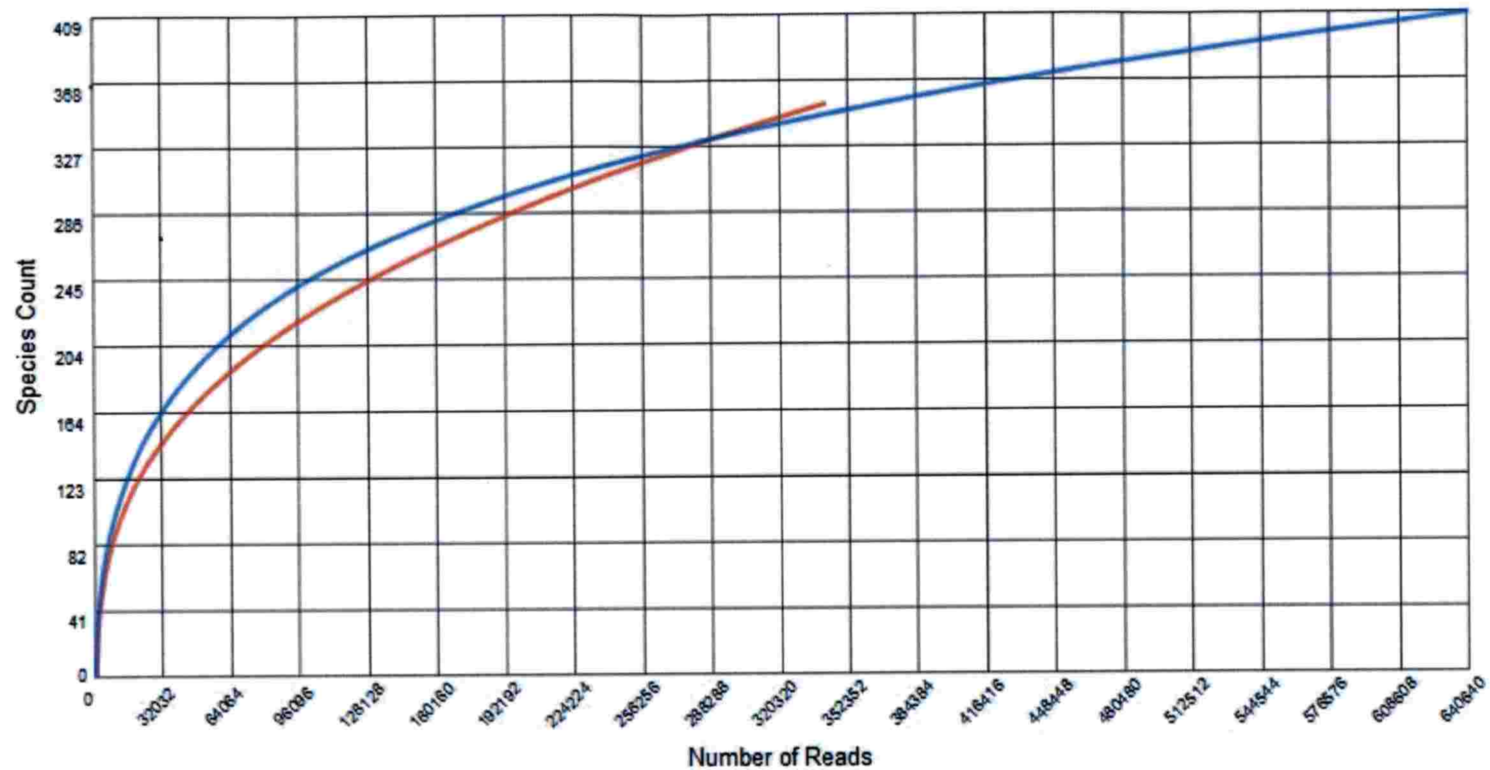

rarefaction curve	metagenome	alpha diversity
	4679021.3	3.82
	4679433.3	6.22

Fig. 21. Rarefaction analyses of *Bemisia tabaci* bacterial communities

(NB: P-poulation: indicated by blue line, H-population: indicated by red line)

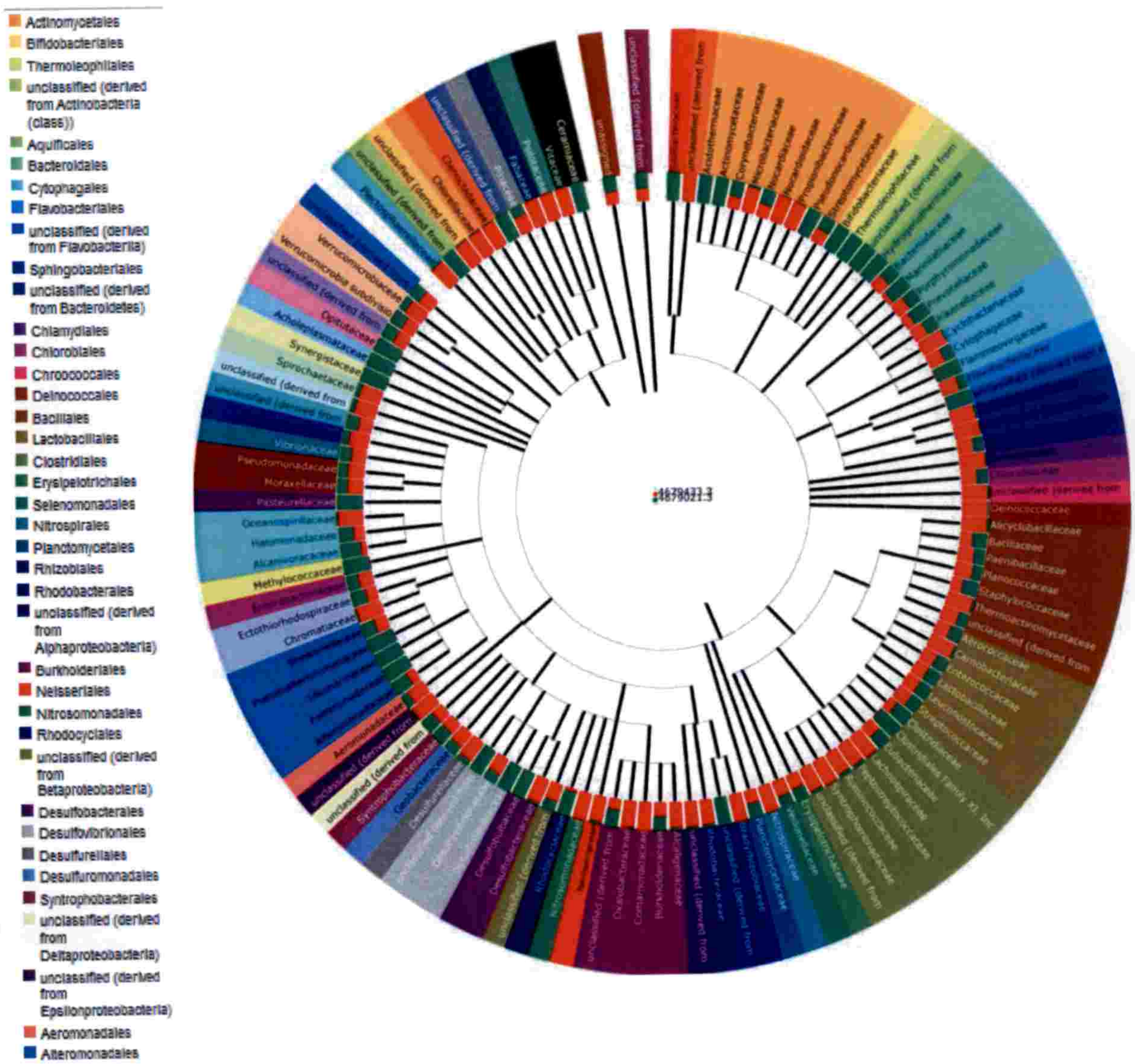

Fig. 22. Phylogenetic tree of bacteria at family level constructed in MG-RAST with illumine sequencing data set (Tree is present with orders (coloured slices) and families belong to each classes are given inside colored slices. The RDP database was used as annotation source, and a minimum identity cutoff (90 per cent) was applied).

Altogether 236 and 225 bacterial genera were present for P and H populations respectively. For P populations, *Bacillus* (35.57 %) was the most dominant group followed by *Arsenophonus* (24.69 %), *Vibrio* (5.83 %), *Riemerella* (4.53 %), *Lysinibacillus* (4.20 %), *Flavobacterium* (2.87 %), *Janthinobacterium* (2.94 %), *Sphingobacterium* (2.79 %), *Bacteroides* (1.90 %), *Enterococcus* (1.83 %) and for H populations, the order of abundance was *Bacillus* (82.27 %), *Alcanivorax* (8.19 %), *Staphylococcus* (5.58 %), *Pantoea* (1.13 %), *Lysinibacillus* (0.76 %), *Bacteroides* (0.44 %), *Alistipes* (0.22 %), *Photorhabdus* (0.21 %), *Terribacillus* (0.16 %) and *Enterococcus* (0.10 %). When the total reads were analyzed at species level, a total of 409 species were identified in sample P and total of 355 species were identified in sample H (Fig. 21). Analysis at species level showed similarity to the sequences deposited in the RDP database. Secondary endosymbiont of *Bemisia tabaci* [un-specified] (70.38 %), *Arsenophonus* endosymbiont of *Trialeurodes vaporariorum* (7.19 %), *Bacillus cereus* (4.07 %), *Bacillus megaterium* (3.78 %), *Bacillus flexus* (1.70 %), *Riemerella anatipestifer* (1.32 %), *Vibrio harveyi* (1.30 %), *Lysinibacillus sphaericus* (1.22 %), *Janthinobacterium sp.* J3 (0.86 %) and *Bacillus pumilus* (0.69 %) were the major 10 species identified for P populations. For H populations, the major species identified were *Bacillus thuringiensis* (72.62 %), *Alcanivorax sp. EPR 6* (7.58 %), *SBR proteobacterium* (6.97 %), *Staphylococcus pasteuri* (3.31 %), *Bacillus amyloliquefaciens* (1.75 %), *Staphylococcus sciuri* (1.63 %), *Bacillus megaterium* (1.10 %), *Pantoea dispersa* (1.02 %), *Lysinibacillus sphaericus* (0.70 %) and *Bacillus pumilus* (0.34 %).

4. 4. Study of *Bemisia tabaci – Cassava mosaic virus* interactions in cassava

Various types of interactions were studied between *B. tabaci* and *Cassava mosaic virus* (*Begomovirus*) - causing cassava mosaic disease. The different types of interactions studied included studies on dispersal, feeding, fecundity, adult longevity and life cycle. Three replications were used per cassava genotype.

4.4.1. Study on dispersal and settling pattern of *Bemisia tabaci* on different cassava genotypes

Average of one week's observations using ten male and ten female insects were recorded for the study (Table 19a and 19b). In three factorial CRD, presence of virus in the insect was denoted by factor A (virulence and non-virulence), sex of the insect by factor B, and different cassava genotypes by factor C.

Table 19a. Dispersal and settling pattern* of *Bemisia tabaci* on different cassava genotypes

(C) Cassava genotypes	(A)Virulence		Non-virulence	
	(B) Female	Male	Female	Male
R-CMR-9	10.05	10.50	15.21	16.02
R-CMR-128	12.09	11.53	16.25	16.10
IR-CMR-1	6.09	7.73	11.66	12.02
IR-CMR-102	6.10	6.25	10.37	9.62
S-H-226	4.28	5.82	12.20	12.30
S-H-165	4.99	5.26	12.60	12.33
PS-H-226	8.45	9.56	6.80	8.30
PS-H-165	7.79	8.65	5.37	6.55

NB: R-Resistant, IR-Infected but recovering, S-Susceptible, PS-Purposefully virus infected susceptible. * Unit- cm/s

19b. Critical difference and standard error for different factors and their interaction

Factors	C.D.	SE(d)	SE(m)
Factor (A)	0.248	0.126	0.089
Factor (B)	0.248	0.126	0.089
Interaction A X B	NIA	0.178	0.126
Factor(C)	0.496	0.252	0.178
Interaction A X C	0.702	0.356	0.252
Interaction B X C	0.702	0.356	0.252
Interaction A X B X C	NIA	0.504	0.356

NB: NIA-No interaction

In case of virulent female, fastest movement was observed in resistant cassava genotype CMR-128 (12.09 cm/s) and it was followed by resistant genotype CMR-9 (10.05 cm/s), purposefully virus infected susceptible genotype H-226 (8.45 cm/s),

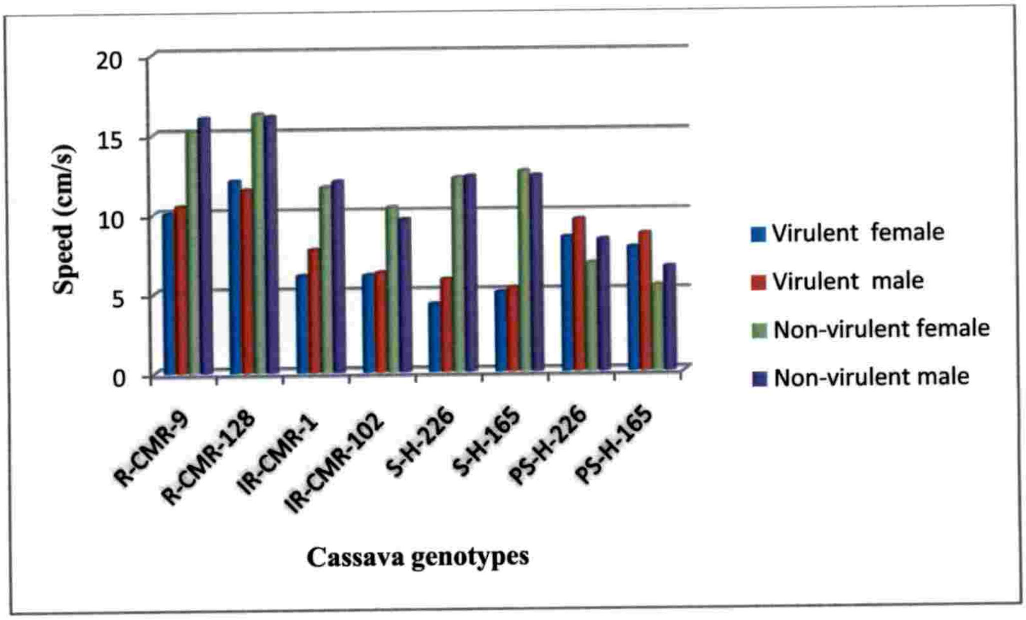

Fig. 23. Dispersal and settling pattern of *Bemisia tabaci* on different cassava genotypes (NB: R-Resistant, IR-Infected but recovering, S-Susceptible, PS-Purposefully virus infected susceptible. Average of one week observations using ten insects (Unit- cm/s).

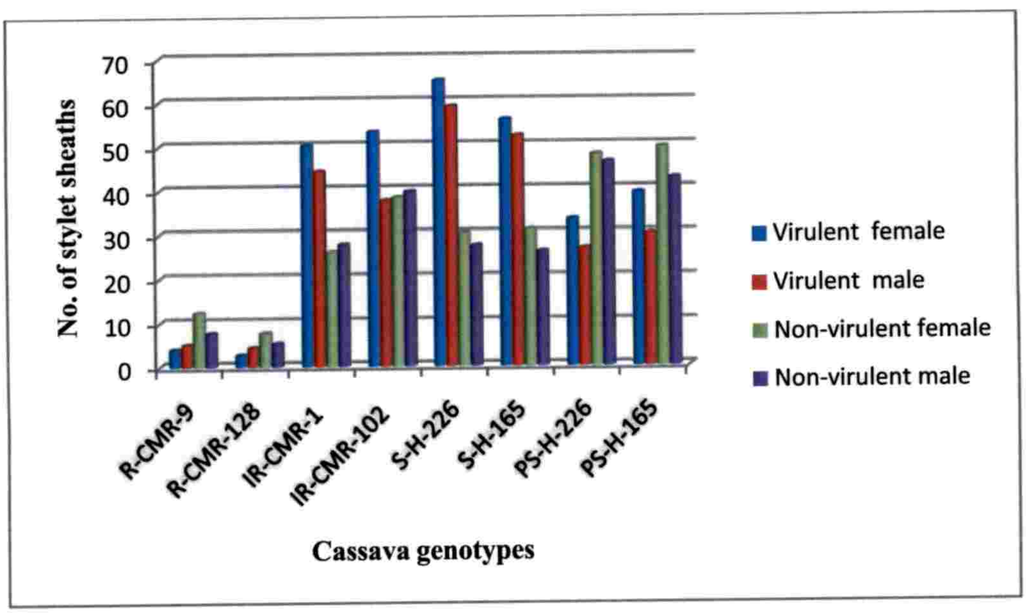

Fig. 24. Feeding pattern of *Bemisia tabaci* on different cassava genotypes (NB: R-Resistant, IR-Infected but recovering, S-Susceptible, PS-Purposefully virus infected susceptible. Average of ten insects. Unit - Number of stylet sheaths per plant).

purposefully virus infected susceptible genotype H-165 (7.79 cm/s), infected but recovering genotype CMR-102 (6.10 cm/s), infected but recovering genotype CMR-1 (6.09 cm/s), susceptible genotype H-165 (4.99 cm/s) and susceptible genotype H-226 (4.28 cm/s). In case of virulent male, decreasing order of speed of movement in different genotypes was, resistant cassava genotype CMR-128 (11.53 cm/s) > resistant cassava genotype CMR-9 (10.50 cm/s) > purposefully virus infected susceptible genotype H-226 (9.56 cm/s) > purposefully virus infected susceptible genotype H-165 (8.65 cm/s) > infected but recovering genotype CMR-1 (7.73 cm/s) > infected but recovering genotype CMR-102 (6.25 cm/s) > susceptible genotype H-226 (5.82 cm/s) > susceptible genotype H-165 (5.26 cm/s).

In case of non-virulent female, fastest movement was observed in resistant cassava genotype CMR-128 (16.25 cm/s) and it was followed by resistant genotype CMR-9 (15.21 cm/s), susceptible genotype H-165 (12.60 cm/s), susceptible genotype H-226 (12.20 cm/s), infected but recovering genotype CMR-1 (11.66 cm/s), infected but recovering genotype CMR-102 (10.37 cm/s), purposefully virus infected susceptible genotype H-226 (6.80 cm/s) and purposefully virus infected susceptible genotype H-165 (5.37cm/s). In case of non-virulent male, the maximum speed of movement was observed for resistant cassava genotype CMR-128 (16.10 cm/s) and it was followed by resistant cassava genotype CMR-9 (16.02 cm/s), susceptible genotype H-165 (12.33 cm/s), susceptible genotype H-226 (12.30 cm/s), infected but recovering genotype CMR-1 (12.02 cm/s), infected but recovering genotype CMR-102 (9.62 cm/s), purposefully virus infected susceptible genotype H-226 (8.30 cm/s) and purposefully virus infected susceptible genotype H-165 (6.55 cm/s).

In statistical analysis, using three factorial CRD, treatments in factor A (virulence and non-virulence), factor B (sex of the insect) and factor C (different cassava genotypes) were found to be significantly different with C.D. values 0.248, 0.248 and 0.496 respectively. Significant interaction effect was observed between factors A (virulence and non-virulence) and C (different cassava genotypes) (C.D. value 0.702); and factors B (sex of the insect) and C (different cassava genotypes) (C.D. value 0.702). There were no significant interactions observed between factors A and B; and A, B and C.

4.4.2. Feeding pattern of *Bemisia tabaci* on different cassava genotypes

Average of observations using ten insects, after counting the number of stylet sheaths (Fig. 25) on cassava leaves; one week after the release of male and female whiteflies were taken in the studies. Staining technique suggested by Backus *et al.* (1988) was used for studying the number of stylet sheaths present in leaves (Table 20a and 20b).

Table 20a. Feeding pattern* of *Bemisia tabaci* on different cassava genotypes

(C) Cassava genotypes	(A)Virulence		Non-virulence	
	(B) Female	Male	Female	Male
R-CMR-9	4.00	5.00	12.33	7.67
R-CMR-128	2.67	4.33	7.67	5.33
IR-CMR-1	50.33	44.33	26.00	27.67
IR-CMR-102	53.33	37.67	38.33	39.67
S-H-226	65.00	59.00	30.33	27.33
S-H-165	56.00	52.33	31.00	26.00
PS-H-226	33.33	26.67	48.00	46.33
PS-H-165	39.33	30.00	49.67	42.67

NB: R-Resistant, IR-Infected but recovering, S-Susceptible, PS-Purposefully virus infected susceptible. * Unit- No. of stylet sheaths.

20b. Critical difference and standard error for different factors and their interaction

Factors	C.D.	SE(d)	SE(m)
Factor (A)	2.280	1.141	0.807
Factor (B)	2.280	1.141	0.807
Interaction A X B	NIA	1.614	1.141
Factor(C)	4.561	2.283	1.614
Interaction A X C	6.450	3.228	2.283
Interaction B X C	NIA	3.228	2.283
Interaction A X B X C	NIA	4.565	3.228

NB: NIA-No interaction

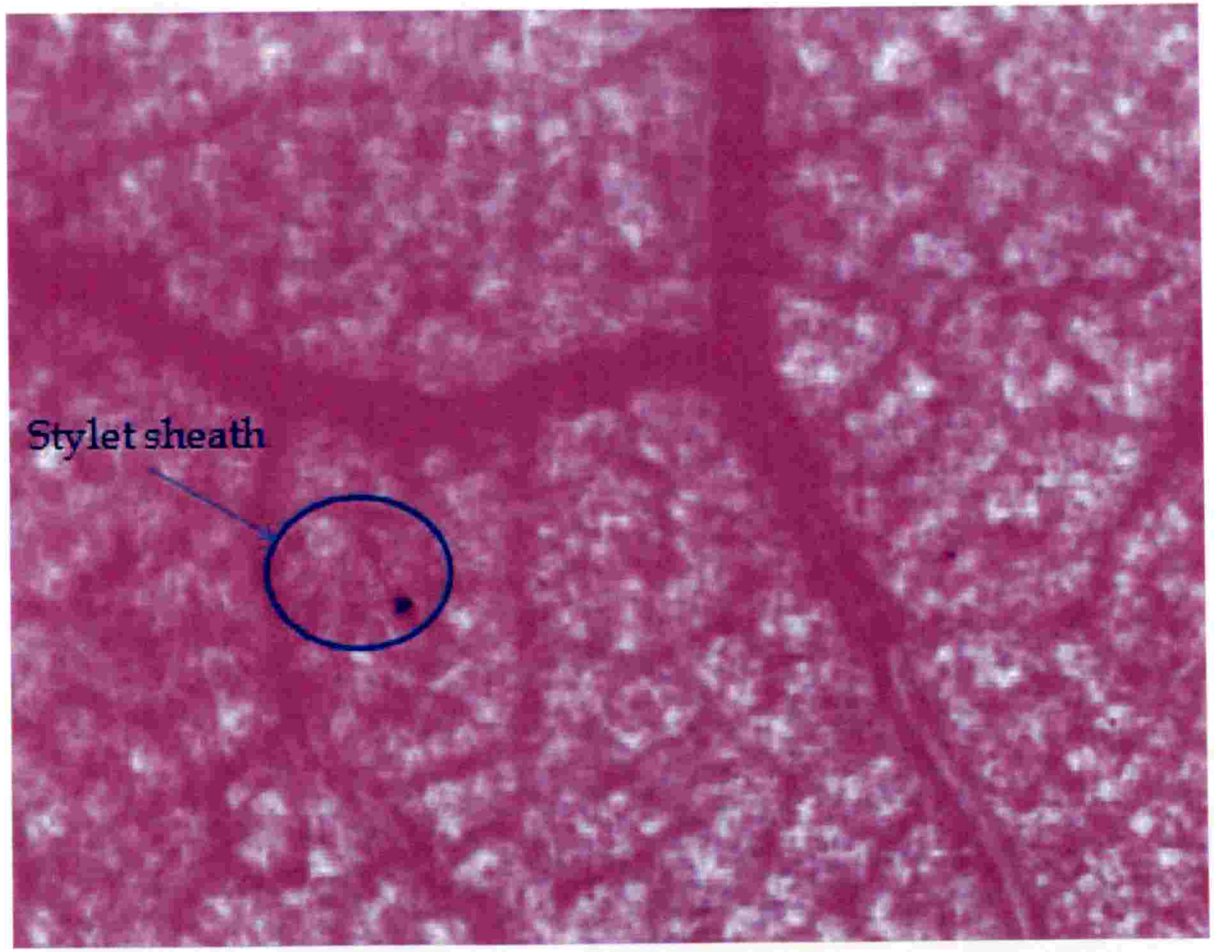

Fig. 25. Stylet sheath of *Bemisia tabaci* in cassava leaves

For virulent female, fastest movement was observed in susceptible genotype H-226 (65 cm/s) and it was followed by susceptible genotype H-165 (56 cm/s), infected but recovering genotype CMR-102 (53.33 cm/s), infected but recovering genotype CMR-1 (50.33 cm/s), purposefully virus infected susceptible genotype H-165 (39.33 cm/s), purposefully virus infected susceptible genotype H-226 (33.33 cm/s), resistant genotype CMR-9 (4 cm/s) and resistant cassava genotype CMR-128 (2.67 cm/s). In case of virulent male, speed of movement was highest in susceptible genotype H-226 (59 cm/s) and it was followed by susceptible genotype H- 165 (52.33 cm/s), infected but recovering genotype CMR-1 (44.33 cm/s), infected but recovering genotype CMR-102 (37.67 cm/s), purposefully virus infected susceptible genotype H-165 (30 cm/s), purposefully virus infected susceptible genotype H-226 (26.67 cm/s), resistant genotype CMR-9 (5 cm/s) and resistant cassava genotype CMR-128 (4.33 cm/s).

In case of non-virulent female, fastest movement was observed in purposefully virus infected susceptible genotype H-165 (49.67 cm/s) and it was followed by purposefully virus infected susceptible genotype H-226 (48 cm/s), infected but recovering genotype CMR-102 (38.33 cm/s), susceptible genotype H-165 (31 cm/s), susceptible genotype H-226 (30.33 cm/s), infected but recovering genotype CMR-1 (26 cm/s), resistant genotype CMR-9 (12.33 cm/s) and resistant cassava genotype CMR-128 (7.67 cm/s). In case of non-virulent male, the maximum speed of movement was observed for purposefully virus infected susceptible genotype H-226 (46.33 cm/s) and it was followed by purposefully virus infected susceptible genotype H-165 (42.67 cm/s), infected but recovering genotype CMR-102 (39.67 cm/s), infected but recovering genotype CMR-1 (27.67 cm/s), susceptible genotype H-226 (27.33 cm/s), susceptible genotype H-165 (26 cm/s), resistant genotype CMR-9 (7.67 cm/s) and resistant cassava genotype CMR-128 (5.33 cm/s).

In three factorial CRD, treatments in factor A (virulence and non-virulence), factor B (sex of the insect) and factor C (different cassava genotypes) were found to be significantly different with C.D. values 2.280, 2.280 and 4.561 respectively. Significant interaction effect was observed between factors A (virulence and non-virulence) and C (different cassava genotypes) (C.D. value 6.450). There were no significant interactions observed between factors A and B; B and C; and A, B and C.

4.4.3. Influence of cassava genotype variation on fecundity of *Bemisia tabaci*

In this study, the number of eggs laid for three generations by virulent and non-virulent female, reared on different cassava genotypes (one pair/ plant) was observed (Table 21a and 21b).

Table 21a. Influence of cassava genotype on fecundity* of *Bemisia tabaci*

(B) Cassava genotypes	(A)Virulence	Non-virulence
R-CMR-9	9.67	15.00
R-CMR-128	8.67	9.67
IR-CMR-1	41.33	44.33
IR-CMR-102	47.67	61.00
S-H-226	68.00	87.67
S-H-165	80.33	87.00
PS-H-226	22.33	23.33
PS-H-165	20.00	27.67

NB: R-Resistant, IR-Infected but recovering, S-Susceptible, PS-Purposefully virus infected susceptible. *Unit- No. of eggs laid.

21b. Critical difference and standard error for different factors and their interaction

Factors	C.D.	SE(d)	SE(m)
Factor (A)	6.01	2.94	2.08
Factor (B)	12.03	5.88	4.16
Interaction A X B	NIA	8.31	5.88

NB: NIA-No interaction

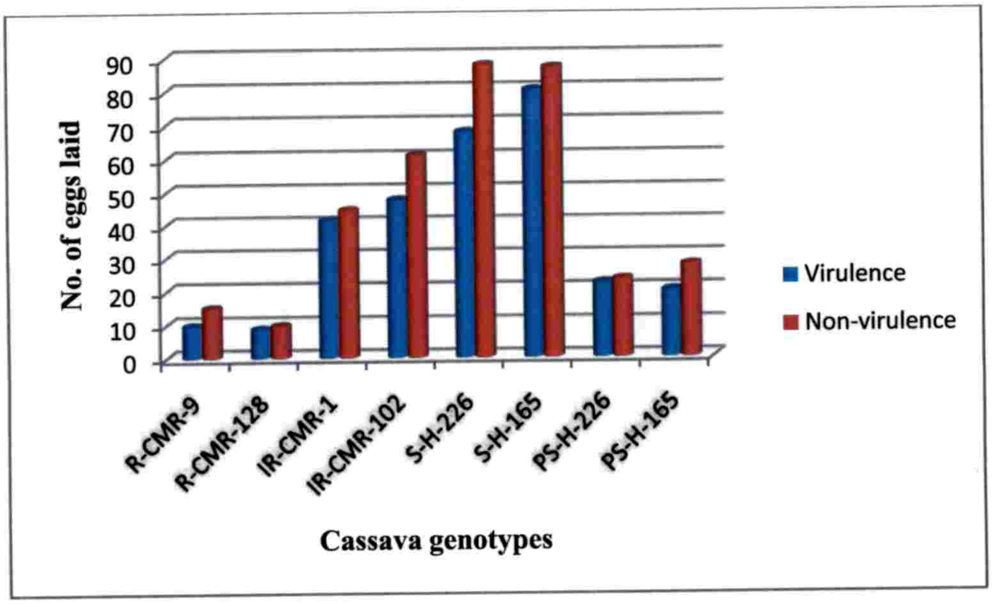

Fig. 26. Influence of cassava genotype on fecundity of *Bemisia tabaci* (NB: R-Resistant, IR-Infected but recovering, S-Susceptible, PS-Purposefully virus infected susceptible, Average ten insects. Eggs laid for 3 generations (one pair/ plant).

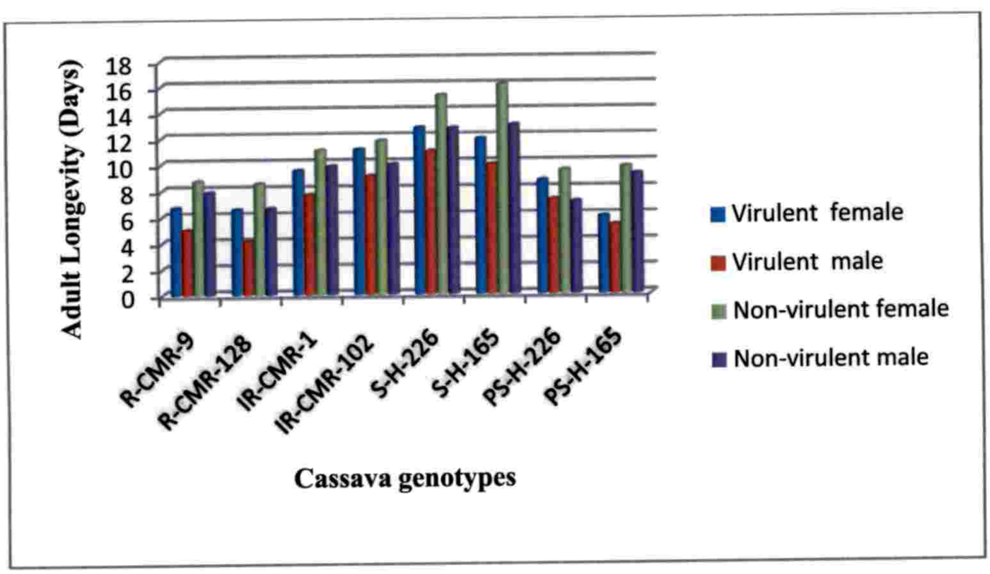

Fig. 27. Influence of cassava genotype on adult longevity of *Bemisia tabaci* (R-Resistant, IR-Infected but recovering, S-Susceptible, PS-Purposefully virus infected susceptible, average of ten insects (twenty pupae/ plant).

The highest number of eggs laid, in case of virulent ones, was in susceptible cassava genotype H-165 (80.33 cm/s) and it was followed by susceptible genotype H-226 (68 cm/s), infected but recovering genotype CMR-102 (47.67 cm/s), infected but recovering genotype CMR-1 (41.33 cm/s), purposefully virus infected susceptible genotype H-226 (22.33 cm/s), purposefully virus infected susceptible genotype H-165 (20 cm/s), resistant genotype CMR-9 (9.67 cm/s) and resistant cassava genotype CMR-128 (8.67 cm/s). In case of, non-virulent ones, maximum number of eggs was laid by susceptible cassava genotype H-226 (87.67 cm/s) and it was followed by susceptible genotype H-165 (87 cm/s), infected but recovering genotype CMR-102 (61 cm/s), infected but recovering genotype CMR-1 (44.33 cm/s), purposefully virus infected susceptible genotype H-165 (27.67 cm/s), purposefully virus infected susceptible genotype H-226 (23.33 cm/s), resistant genotype CMR-9 (15 cm/s) and resistant cassava genotype CMR-128 (9.67 cm/s).

In two factorial CRD, treatments in factor A (virulence and non-virulence) and factor B (cassava genotypes) were found to be significantly different with C.D. values 6.01 and 12.03 respectively. There were no significant interactions observed between factors A and B.

4.4.4. Influence of cassava genotype on adult longevity of *Bemisia tabaci*

The study was conducted by releasing 10 male and female pupae per plant and observed for three generations (Table 22a and 22b).

Table 22a. Influence of cassava genotype variation on adult longevity* of *Bemisia tabaci*

(C) Cassava genotypes	(A)Virulence		Non-virulence	
	(B) Female	Male	Female	Male
R-CMR-9	6.70	5.00	8.70	7.87
R-CMR-128	6.57	4.10	8.53	6.63
IR-CMR-1	9.47	7.67	11.00	9.80
IR-CMR-102	11.07	9.03	11.73	9.93
S-H-226	12.73	10.93	15.23	12.70
S-H-165	11.87	9.90	16.07	12.93
PS-H-226	8.70	7.23	9.43	7.07
PS-H-165	5.93	5.27	9.67	9.13

NB: R-Resistant, IR-Infected but recovering, S-Susceptible, PS-Purposefully virus infected susceptible. Unit- Days.

22b. Critical difference and standard error for different factors and their interaction

Factors	C.D.	SE(d)	SE(m)
Factor (A)	0.825	0.413	0.292
Factor (B)	0.825	0.413	0.292
Interaction A X B	NIA	0.584	0.413
Factor(C)	1.651	0.826	0.584
Interaction A X C	NIA	1.168	0.826
Interaction B X C	NIA	1.168	0.826
Interaction A X B X C	NIA	1.652	1.168

NB: NIA-No interaction

For virulent female, fastest movement was observed in susceptible genotype H-226 (12.73 cm/s) and it was followed by susceptible genotype H-165 (11.87 cm/s), infected but recovering genotype CMR-102 (11.07 cm/s), infected but recovering genotype CMR-1 (9.47 cm/s), purposefully virus infected susceptible genotype H-226 (8.70 cm/s), resistant genotype CMR-9 (6.70 cm/s) and resistant cassava genotype CMR-128 (6.57 cm/s) and purposefully virus infected susceptible genotype H-165 (5.93 cm/s). In case of virulent male, speed of movement was highest in susceptible genotype H-226 (10.93 cm/s) and it was followed by susceptible genotype H-165 (59.90 cm/s), infected but recovering genotype CMR-102 (9.03 cm/s), infected but recovering genotype CMR-1 (7.67 cm/s), purposefully virus infected susceptible genotype H-226 (7.23 cm/s), purposefully virus infected susceptible genotype H-165 (5.27 cm/s), resistant genotype CMR-9 (5 cm/s) and resistant cassava genotype CMR-128 (4.10 cm/s).

In case of non-virulent female, fastest movement was observed in susceptible genotype H-165 (16.07 cm/s) and it was followed by susceptible genotype H-226 (15.23 cm/s), infected but recovering genotype CMR-102 (11.73 cm/s), infected but recovering genotype CMR-1 (11 cm/s), purposefully virus infected susceptible genotype H-165 (9.67 cm/s), purposefully virus infected susceptible genotype H-226 (9.43 cm/s), resistant genotype CMR-9 (8.70 cm/s) and resistant cassava genotype CMR-128 (8.53 cm/s). In case of non-virulent male, speed of movement was highest in susceptible genotype H-165 (12.93 cm/s) and it was followed by susceptible genotype H-226 (12.70 cm/s), infected but recovering genotype CMR-102 (9.93 cm/s), infected but recovering genotype CMR-1 (9.80 cm/s), purposefully virus infected susceptible genotype H-165 (9.13 cm/s), resistant genotype CMR-9 (7.87 cm/s), purposefully virus infected susceptible genotype H-226 (7.07 cm/s), and resistant cassava genotype CMR-128 (6.63 cm/s).

In three factorial CRD, treatments in factor A (virulence and non-virulence), factor B (sex of the insect) and factor C (different cassava genotypes) were found to be significantly different with C.D. values 0.825, 0.825 and 1.651 respectively. Significant interaction effect was observed between factors A (virulence and non-virulence) and C (different cassava genotypes) (C.D. value 6.450). There were no significant interactions observed between factors A, B and C.

4.4.5. Influence of cassava genotype on life cycle of *Bemisia tabaci*

In this study, average life cycle of male and female insects was taken for three generations (Table 23a and 23b). In case of virulent female, speed of movement was maximum in resistant cassava genotype CMR-128 (30.77 cm/s) and it was followed

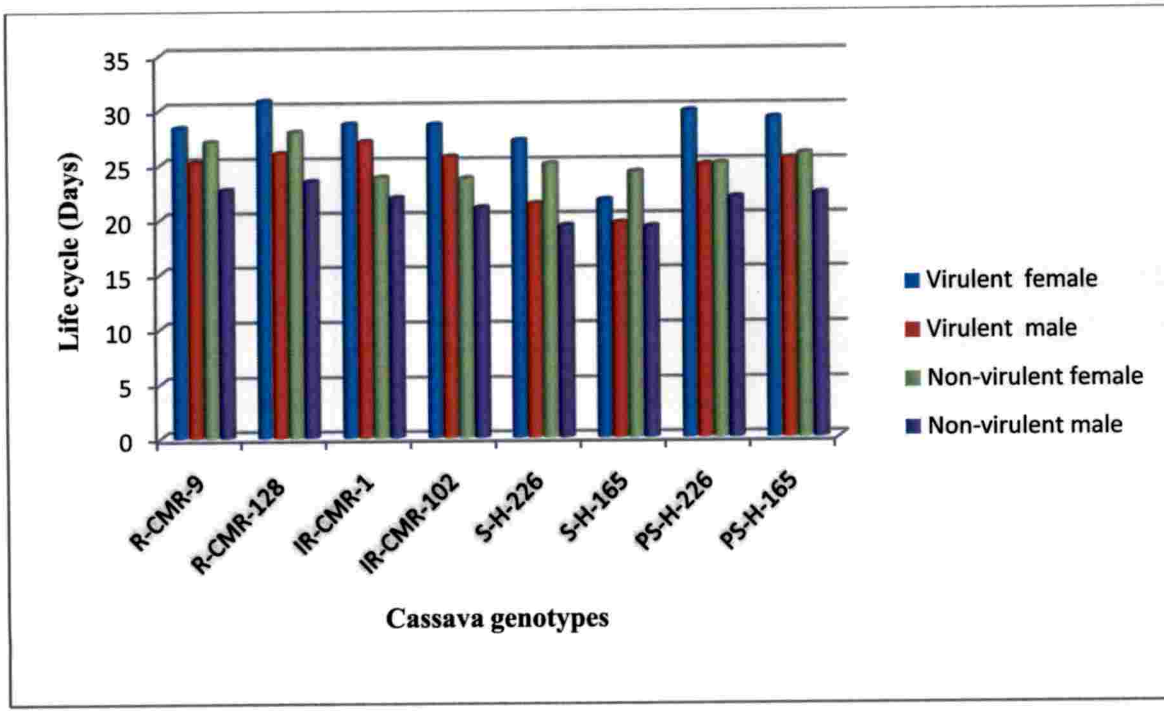

Fig. 28. Influence of cassava genotype on life cycle of *Bemisia tabaci* (R-Resistant, IR-infected but recovering, S-Susceptible, PS-Purposefully virus infected susceptible. Average of one adult pair studied for three generations).

by purposefully virus infected susceptible genotype H-226 (29.77 cm/s), purposefully virus infected susceptible genotype H-165 (29.13 cm/s), infected but recovering genotype CMR-1 (28.63 cm/s), infected but recovering genotype CMR-102 (28.57 cm/s), resistant genotype CMR-9 (28.33 cm/s), susceptible genotype H-226 (27.10 cm/s) and susceptible genotype H-165 (21.67 cm/s).

Table 23a. Influence of cassava genotype on life cycle* of *Bemisia tabaci*

(C) Cassava genotypes	(A)Virulence		Non-virulence	
	(B) Female	Male	Female	Male
R-CMR-9	28.33	25.20	27.00	22.67
R-CMR-128	30.77	26.00	27.90	23.43
IR-CMR-1	28.63	27.90	23.77	21.90
IR-CMR-102	28.57	25.67	23.67	21.00
S-H-226	27.10	21.33	25.00	19.33
S-H-165	21.67	19.57	24.23	19.23
PS-H-226	29.77	24.90	25.00	21.90
PS-H-165	29.13	25.43	25.87	22.23

NB: R-Resistant, IR-Infected but recovering, S-Susceptible, PS-Purposefully virus infected susceptible. Unit- Days.

23b. Critical difference and standard error for different factors and their interaction

Factors	C.D.	SE(d)	SE(m)
Factor (A)	1.072	0.537	0.379
Factor (B)	1.072	0.537	0.379
Interaction A X B	NIA	0.759	0.537
Factor(C)	2.144	1.073	0.759
Interaction A X C	NIA	1.518	1.073
Interaction B X C	NIA	1.518	1.073
Interaction A X B X C	NIA	2.146	1.518

NB: NIA-No interaction

For virulent male, speed of movement was highest in infected but recovering genotype CMR-1 (27.90 cm/s) and it was followed by resistant cassava genotype CMR-128 (26 cm/s), infected but recovering genotype CMR-102 (25.67 cm/s), purposefully virus infected susceptible genotype H-165 (25.43 cm/s), resistant genotype CMR-9 (25.20 cm/s), purposefully virus infected susceptible genotype H-226 (24.90 cm/s), susceptible genotype H-226 (21.33 cm/s) and susceptible genotype H-165 (19.57 cm/s).

In case of non-virulent female, fastest movement was observed in resistant cassava genotype CMR-128 (27.90 cm/s) and it was followed by resistant genotype CMR-9 (27 cm/s), purposefully virus infected susceptible genotype H-165 (25.87 cm/s), susceptible genotype H-226 (25 cm/s) = purposefully virus infected susceptible genotype H-226 (25 cm/s), susceptible genotype H-165 (24.23 cm/s), infected but recovering genotype CMR-1 (23.77 cm/s) and infected but recovering genotype CMR-102 (23.67 cm/s). For non-virulent male, speed of movement was highest in resistant cassava genotype CMR-128 (23.43 cm/s) and it was followed by resistant genotype CMR-9 (22.67 cm/s), purposefully virus infected susceptible genotype H-165 (22.23 cm/s), infected but recovering genotype CMR-1 (21.90 cm/s) = purposefully virus infected susceptible genotype H-226 (21.90 cm/s), infected but recovering genotype CMR-102 (21 cm/s), susceptible genotype H-226 (19.33 cm/s) and susceptible genotype H-165 (19.23 cm/s).

In three factorial CRD, treatments in factor A (virulence and non-virulence), factor B (sex of the insect) and factor C (different cassava genotypes) were found to be significantly different with C.D. values 1.072, 1.072 and 2.144 respectively. There were no significant interactions observed between factors A, B and C.

Discussion

5. DISCUSSION

The discussions on the results obtained from the study entitled "Characterization of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), for genetic variability, endosymbionts and vector-virus interactions in cassava" are summarized in this chapter to elucidate the various observations and findings.

5.1. Morphological characterisation of cassava whitefly, *Bemisia tabaci* from different agro-ecological zones of Kerala

Comparative morphometrics of puparia and adults of *B. tabaci* could be used to distinguish genetic variations (Li *et al.*, 2013). *B. tabaci* adults and pupae collected from 13 agro-ecological zones were subjected to morphometric studies, based on the methods described by Malumphy *et al.* (2009) and Chaubey *et al.* (2010). Total 21 characters of female and 23 characters of male whiteflies, were compared from places in 13 different agro-ecological zones of Kerala, *viz.*, Neyyattinkara, Sreekaryam, Vellanikkara, Vadakara, Sultan Bathery, Kasargod, Thodupuzha, Palluruthy, Pulikeezhu, Muvattupuzha, Kayamkulam, Palakkad and Chittur. According to Gill and Brown (2010), in terms of taxonomy or systematics, morphology is considered the foremost basis for species separation, and it is convenient for identification as well (Yan, 2001). The14 puparial and 9 adult measurements were evaluated in principal component analysis (PCA) to estimate the morphological variations (Johnson and Wichern, 2007).

5.1.1. Morphometric variations in *Bemisia tabaci* pupa

Total 14 characters of female and male pupae, were studied (Table 3 and 4). They were: pupal length, pupal width, length and width of right and left anterior wax margin, vasiform orifice length, operculum length, operculum width, lingula length, lingula width, caudal furrow length, caudal seta length and distance between caudal

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setae. Morphometric measurements for pupa and adults were similar to the reports of Li *et al.* (2013) and Chaubey *et al.* (2015a).

Among different pupal characters studied for female pupa (Table 3), only variations in pupal length, pupal width and length of right and left anterior wax margins were found to be significantly different among the populations collected from different agro-ecological zones of Kerala. For male pupal characters (Table 4), variations in pupal length and width were found to be significantly different among populations. Both in case of female and male pupa, whitefly populations from Sultan Bathery (SBY) were found to have the lowest mean pupal length (0.668mm and 0.582 mm respectively) and the highest pupal width (0.539mm and 0.422mm respectively). The populations collected from Sultan Bathery were also found to have the highest vasiform orifice length, operculum length, operculum width, lingula length, lingula width and distance between caudal setae among the different whitefly populations (except in case of operculum width for male pupa).

In multivariate analysis, 14 pupal characters were considered. First five and four principal components of female and male pupa respectively; obtained through PCA were selected using eigen values >1 (correspond to most of the variances in the dataset) and scree plot (Table 5, 6 and Fig. 2, 3). In case of biplot for female pupae (Fig. 4), the characters with strong positive correlations were operculum length, pupal width and vasiform orifice length; lingula length and lingula width; operculum width and distance between caudal setae; length of right and left anterior wax margin and pupal length; width of right and left anterior wax margin and caudal seta length. There were negative correlations between length of left anterior wax margin and distance between caudal setae; caudal seta length and lingula width.

In case of biplot for male pupa (Fig. 5), strong positive correlations were observed between length of right and left anterior wax margins and caudal seta length; pupal width and operculum length; lingula length, lingula width, vasiform orifice length and distance between caudal setae; width of right and left anterior wax margins and caudal furrow length and pupal length. Negative correlations were observed between pupal width and caudal seta length; length of right and left anterior wax margins and operculum length. In the score plot analysis of female pupa, SBY and PTY populations formed a separate group (quarter II) and for male pupa, only populations from SKM found in quarter IV (Fig. 6 and 7).

Similar studies were conducted by Jayasekera *et al.* (2010) and Chaubey *et al.* (2015a). The study conducted by Thomas and Gaur (2014), for the morphometric variations of different genetic group also shown that pupa of Asia I genetic group is comparatively broader, with smaller length and provided similar morphometric data for most of the characters. It is also in agreement with the study conducted by Chaubey *et al.* (2015a). Similar studies were conducted by Rosell *et al.* (1997) and they used the characters of 4th instars (pupae) for separating *Bemisia* spp. The results are also in agreement with the findings of Thomas *et al.* (2014).

5.1.2. Morphometric variations in adult *Bemisia tabaci*

For male adult insects, nine characters were compared and the characters were antennal length, body length, body width, forewing length, forewing width, hind tarsal length, hind tarsal width, aedeagus length and clasper length (Table 8). Seven characters except aedeagus length and clasper length were compared for adult female insects (Table 7).

In the study for, characters of adult female cassava whitefly, antennal length, body length, body width, forewing length and forewing width were found to be significantly different between the populations whereas, no significant difference was observed for hind tarsal length and width (Table 7). In adult male, antennal length, body length, body width, forewing length and forewing width were found to be significantly different between the populations; whereas, no significant difference was observed for hind tarsal length, hind tarsal width, aedeagus length and clasper length (Table 8). Both for, adult female and adult male, whitefly population from Sultan Bathery were found to have the highest antennal length (0.376 mm and 0.339 mm respectively) and highest mean body width (0.288 mm and 0.253 mm respectively). For other morphometric characters studied, SBY populations had shown the lowest observations when compared with other 12 zones. The results are in agreement with the peculiarities of Asia I genetic group of adult *B. tabaci*, as described by Thomas and Gaur (2014) and Chaubey *et al.* (2015a); with lower body length and higher body width and antennal length.

In multivariate analysis, a total of seven characters were considered for adult female and 9 characters were considered for adult male. For adult whitefly, the scree plot of the principal components (PC) showed that the first one (adult female) and two (adult male) eigen values contributed most to the variances in the dataset (eigen values >1) (Table 9, 10 and Fig. 8, 9).

In case of biplot for adult female (Fig. 10), the characters with strong positive correlations were forewing length and forewing width; hind tarsal length and hind tarsal width. There were negative correlations between body width and forewing length as well as antennal length and hind tarsal length. In case of biplot for adult male (Fig. 11), strong positive correlations were observed between forewing width, body length, forewing length and hind tarsal width; aedeagus length and clasper length. Negative correlation was observed between body width and hind tarsal width. In the score plot analysis of adult female, SBY populations formed a separate group (quarter III). While CTR and VDA populations (quarter IV) as well as SBY and KGD populations (quarter III) formed as separate groups in case of adult male (Fig. 12 and 13).

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In almost all other characters studied, there were variations in morphometry between populations collected from Sultan Bathery and other 12 agro-ecological zones. Subsequent molecular analysis showed that, the populations present in Sultan Bathery was Asia I genetic group and all others belong to Asia II- 5 genetic group (Table 16). (PCA provides information on both size and shape, and can detect genetic differences between populations (Klecka, 1975) and it had been used extensively for the study of variations within and between species (Shaw and Carlson, 1969; Blackman and Eastop, 1984). The morphometric results with the help of PCA indicate that there might be some morphological characters which can correlate with the genetic variation.

5.2. Molecular characterisation of cassava whitefly, *Bemisia tabaci*

So far only a single biotype/ genetic group of *B. tabaci* has been reported as a pest of cassava in India. It is known as cassava-strain (Lisha *et al.*, 2003) or Asia II-5 genetic group (Ellango *et al.*, 2015). Also, it was known that genetic variations can happen in insects based on geographical variations (Yu *et al.*, 2012). In the present study, genomic DNA of *B. tabaci* adults, occurring on cassava plants collected from 13-agro ecological zones of Kerala, were isolated using De Barro and Driver (1997) method. UV absorbance -A260/280 value (quality) in the range of 1.78 to 1.92 and quantity (ng/μl) in the range of 11.43 to 29.26 were obtained (Table 11).

5.2.1. Analysis using Inter Simple Sequence Repeats (ISSR) primers

According to Godwin *et al.* (1997), ISSR primers are very useful in the taxonomic studies of the closely related species. In the study, ten ISSR primers were screened out of 35, and used for the amplification of ISSR region in genomic DNA of *B.tabaci*, with thermal cycler and correspondingly programmed with primer specific annealing temperature (Table 13) to study the population genetic structure in *B.*

tabaci from different agro-ecological zones of Kerala. The primers were F1, I4, UBC 846, UBC 855, F2, UBC 827, ISSR 8, UBC 808, UBC 811and ISSR 3 (Table 13).

Dong *et al.* (2008) used six ISSR primers in his study on comparative analysis of population genetic structure in *B. tabaci.* According to Costa *et al.* (2016), ISSR primers provide highly discriminating information with better reproducibility, and are relatively abundant. Among these 10 primers only F1 and F2 were earlier tried for genetic variability studies in *B. tabaci* (Dong *et al.*, 2008).

In the whitefly genomic DNA samples, primer F1 formed 17 bands with four unique/polymorphic bands and primer I4 formed 23 bands with four unique bands. Six unique bands and a total of 19 bands are formed by the primer UBC 846. UBC 855 formed 21 bands with three unique bands and primer F2 formed 14 bands with six unique bands. Three unique bands and 22 total bands are formed by UBC 827. ISSR 8 formed 13 bands with no unique bands and UBC 808 formed 20 bands with four unique bands. Three unique bands are formed by UBC 811 with a total of 26 bands. Primer ISSR 3 formed 11 bands with four unique bands. The size of amplified fragments had ranged from 200 bp to 2900 bp. (Plates 22a-31).

Table 24. Amplification pattern of selected ISSR primers

Sl. No.	Primer	No. of bands	Polymorphic bands	Per cent polymorphism
1	F1	17	4	23.53
2	I4	23	4	17.39
3	UBC 846	19	6	31.58
4	UBC 855	21	3	14.28
5	F2	14	6	42.86
6	UBC 827	22	3	13.64
7	ISSR 8	13	0	0
8	UBC 808	20	4	20.00

9	UBC 811	26	3	11.54
10	ISSR 3	11	4	36.36
	Total	**186**	**37**	**19.89**

It was found that, out of 10 ISSR primers used, nine could result in the polymorphic pattern with overall polymorphism of 19.89 per cent (Table 24). The best polymorphism was observed for the primer F2 (42.86 per cent) (which is already used by Dong *et al.*, (2008), for their study), followed by ISSR 3 (36.36 per cent), and are comparatively better to use for the genetic variability study of whitefly. Whereas, ISSR 8 cannot be recommended for this type of study, as there was no polymorphism when the primer was used. Compared to the study conducted by Hameed *et al.* (2012) using RAPD primers, the per cent polymorphism (77 per cent to 19.89 per cent) is lesser; and the reason could be, the study was conducted in comparatively lesser areas, with the whiteflies collected only from a single host (cassava); even though ISSR proven to be better polymorphic (Costa *et al.*, 2016).

In general, dice coefficient values were utilized to construct dendrogram by un-weighted pair-group method analysis, UPGMA (Sneath and Sokal, 1973) based on presence or absence of ISSR bands (Table 14 and Fig. 14). Dendrogram analysis revealed the occurrence of two major clusters and an out group. The out group is formed by Sultan Bathery (SBY) sample, had shown the least similarity with all other samples (similarity coefficients in between 0.51-0.60). The biggest cluster formed had samples from Palakkad (PKD), Chittur (CTR), Kasargod (KGD), Thodupuzha (TPA), Vellanikkara (VKA), Vadakara (VDA), Neyyattinkara (NYA), Kayamkulam (KYM) and Sreekaryam (SKM) in the order in which the samples were closely related based on ISSR analysis. The second cluster had samples from Palluruthy (PTY), Muvattupuzha (MVA) and Pulikeezhu (PKU). The samples from Muvattupuzha (MVA) and Pulikeezhu (PKU) were very closely related. Similar, UPGMA dendrogram based comparisons were conducted for the genetic variability of *B. tabaci* by Lima *et al.* (2002), Hameed *et al.* (2012) and Yu *et al.* (2012).

The present study is in confirmation with the studies conducted by Perring *et al.* (1993), Dong *et al.* (2008) and Yu *et al.* (2012), using ISSR primers for analysing the genetic variability of *B. tabaci*. RAPD primers were also used (Lima *et al.*, 2002; Bao-Li *et al.*, 2003; Perumal *et al.*, 2009) in the genetic variability studies of *B. tabaci*. Abdullahi, *et al.* (2003), collected *B. tabaci* populations, from cassava and other plants in major cassava-cultivation areas of Sub-saharan Africa and from elsewhere around the world, and studied their biotype status and genetic variation using RAPD. They reported that populations of *B. tabaci* on cassava were distinct from non-cassava populations. Qiu *et al.* (2009) identified three major *B. tabaci* biotypes in China based on morphological and DNA polymorphisms.

5.2.1. Analysis using mitochondrial cytochrome oxidase 1 primer and DNA barcoding

DNA barcode can assign the unknown specimen at different developmental stages either as a complement to morphological analysis or as the primary diagnostic indicator in cases where the requisite morphological keys are unavailable (Hebert *et al.*, 2003). Analysis using *mtCO1* (mitochondrial cytochrome oxidase 1), uses a portion (typically between 600- 900 bp) of the mitochondrial gene cytochrome c oxidase subunit 1 (CO1). In the present study, *mtCO1* gene of *B. tabaci* was used to reveal genetic variability in the insect from different agro-ecological zones of Kerala.

The *mtCO1*sequences generated from whitefly consisted of 765 bp to 795 bp and homology of sequences with other reported sequences were analysed. Analysis of this mtDNA provides insight to understand the natural genetic diversity and population structures in organisms (Avise, 1994). Many researchers (Frohlich *et al.*, 1999; Legg *et al.*, 2002; Berry *et al.*, 2004 and Hsieh *et al.*, 2006) used the primers for the genetic variability studies in *B. tabaci*. The sequence showed significant homology to *B. tabaci* mitochondrial Cytochrome Oxidase CO1 gene already deposited in the public domain database using 'blast n' search tool.

All the 13 sequences were aligned using online software 'Clustal 2.1' and the respective values for their similarity were obtained by pair wise arrangement. The calculated values ranged from 83.50 to 100 (Table 15). Among all, the sample from Sultan Bathery (SBY) shows least similarity (83.50 - 85.63) in the matrix; while the others were in the range of 96.64 per cent to 100 per cent similarity. Dendrogram deduced from matrix of pair wise comparisons in *mtCO1* sequences of *B. tabaci* from the 13 agro-ecological zones of Kerala (MEGA-6, Neighbour joining method), showed that populations from Sultan Bathery was an out group (Fig. 15).

The gene sequences of all 13 whiteflies from all these zones were submitted to BOLD and corresponding barcode for *B. tabaci* was generated (Plate 31). This method had a potential for facilitating the identification of invasive insect pests (Floyd *et al.*, 2010). DNA barcoding also supplemented the morphological methods for identifying the invasive armyworm, *Spodoptera* species in Florida (Nagoshi *et al.*, 2011).

The 3.5% pair wise genetic divergence identified by Dinsdale *et al.* (2010) as being the boundary separating different species/ genetic groups. The similarities of the sequences were compared with the reference sequences of *B. tabaci* available in the NCBI data base (Ellango *et al.*, 2015). It was observed that all the samples except Sultan Bathery had 97 to 100 per cent similarity to Asia II-5 genetic group of *B. tabaci* (Table 16).

However, the sample from Sultan Bathery was found to belong to Asia I genetic group with 98 per cent similarity to the reference sequences. Similar works are done for the identification of the genetic groups from different parts of the world.

Chowda-Reddy, *et al.* (2012) could identify genetic groups, Asia I, Asia II-5, Asia II-7and Asia II-8 from different parts of India with the help of same primers used in the present study. Thomas *et al.* (2014) confirmed the presence of three putative species *viz.*, Asia 1, Asia II-1, and Asia II-7 on cotton cultivars in India, using sequence analyses using mitochondrial cyctochrome oxidase I. Roopa, *et al.* (2015) investigated the prevalence of a new genetic group, MEAM-K, of the whitefly *B. tabaci* in Karnataka, India, as evident from *mtCO1* sequences. Ellango *et al.* (2015), surveyed *B. tabaci* populations across India and the blast analysis of sequenced 850 bp of the mitochondrial CO1 (*mtCO1*) gene, with sequences from the *mtCO1* dataset showed the presence of one invasive group, MEAM1, and eight other groups of *B. tabaci* in India. i.e., Asia I, Asia I-India, Asia II-1, Asia II-5, Asia II-7, Asia II-8, Asia II-11 and China-3. Mitochondrial DNA variation of *B. tabaci* populations, infesting cassava in Africa was studied by Atuncha *et al.* (2011) and Mugerwa, *et al.* (2012). The results revealed two distinct haplotypes of cassava associated *B. tabaci*.

Significantly, so far only a single biotype/ genetic group of *B. tabaci* has been reported as a pest in cassava in India and is known as cassava-strain (Lisha *et al.*, 2003) or Asia II- 5 genetic group (Ellango *et al.*, 2015). Genetic variants (different genetic group) in cassava whitefly could be a warning sign and could lead to greater difficulty in tackling the pest and virus it carries. As the genetic group (Asia I) is first time reported as a pest in cassava, the result was confirmed by collecting settled nymhs, like 'red eyed nymphs' from the plants.

The reason for the Asia I biotype, to infest cassava plants is unknown and need to be investigated further. Presence of eggplants nearby could be one reason. Lisha *et al.* (2003) reported eggplant and cassava are common hosts for *B. tabaci*. Carabali *et al.*, (2005) opined that gradual adaptation to plants that were not previously hosts can happen and demonstrated the gradual adaptation of B biotype of *B. tabaci* to *Manihot* sp. through immediate hosts phylogenetically close to it.

Also many population of whiteflies that achieve high population densities may utilize certain plants as host, that otherwise would not have been utilized under lower population densities (Byrne and Bellows, 1991). Also, Yu *et al.* (2012) reported that genetic variations can happen in insects, based on geographical variations and a more detailed study on this aspect from more locations is required to know the exact reason for the presence of genetic variants in cassava whitefly.

5.3. Characterization of endosymbionts in *Bemisia tabaci*

This polyphagous agricultural pest harbour diverse bacterial communities in its gut and are reported to perform many diverse functions in whiteflies including its polyphagous nature and general fitness of the host.

In the present study, bacterial communities associated with cassava whitefly collected from different agro ecological zones of Kerala, India were compared. First, metagenomic DNA of *B. tabaci* were isolated by SDS based metagenomic DNA extraction procedure described by Zhou *et al.* (1996) and used the Illumina Next Generation Sequencing platform to reveal total bacterial community present. Analysis of hypervarible V3 region of 16S rRNA fragment resulted in 1,321,906 and 690,661 high quality paired end sequences with mean length of 150 base pairs. Highly diverse bacterial communities were present in the sample containing approximately 3,513 operational taxonomic units (OTUs). According to Fauquet *et al.* (2008), *P. aleyrodidarum* is the only primary endosymiont in whitefly and many secondary endosymbiotic genera are present in them like, *Rickettsia, Wolbachia, Arsenophonus, Cardinium, Fritschea* and *Hamiltonella,* based on 16S rRNA sequencing. Study conducted by Chiel *et al.* (2007) and Gueguen *et al.* (2010), for identifying bacterial community of *B. tabaci* also involved amplification of 16S rDNA of specific bacteria using bacteria specific primers.

Downstream analysis using QIIME (Caporaso *et al.*, 2010) and MG-RAST

(Meyer *et al.*, 2008) programmes showed a marked difference in the abundance of bacteria in the populations (Table 17 and 18). Altogether, 16 bacterial phyla were detected both in P and H samples. Among the phyla for P populations of *B. tabaci*, *Proteobacteria* was the most dominant which consisted of 87.57 per cent of total bacterial community and for H populations; *Firmicutes* was the most dominant with 82.67 per cent. It was followed by *Firmicutes* (9.29 %) for P populations and *Proteobacteria* (13.40 %) for H populations. *Bacteroidetes* was found 2.91 % in P populations and 0.84 % in H populations. Su *et al.* (2016), identified 27 different phyla of bacterial community associated with *B. tabaci* from different crops, in which *Proteobacteria* (94-98 %) was the most dominant, followed by *Bacteroidetes* (0.5-4.5 %) and *Firmicutes* (0.2-2 %), which is in agreement with the findings of the present study.

Oesi-Poku *et al.* (2012) and Jones *et al.* (2013) found that *Proteobacteria* is typically the predominant bacterial taxon in the gut of mosquitoes. In *H. armigera* larva, the major bacteria phyla detected were *Protecobacteria* followed by *Firmicutes* and *Actinobacteria* in the midgut (Priya *et al.*, 2012), gut and reproductive organs of both male and female fruit fly *Bactrocera minax*, gut of ground beetles (Jonathan *et al.*, 2007), and desert locust, *Schistocerca gregaria* (Dillon *et al.*, 2010). However, *Bacteriodetes* and *Firmicutes* were dominant in gut of termites (Xiang *et al.*, 2012) and bees (Mohr and Tebbe, 2006). According to Douglas (2015), *Actinobacteria* is the most dominant phylum of bacteria in whitefly, followed by *Bacteroidetes*, *Firmicutes*, and *Proteobacteria.* This variation can be attributed to the variation in hosts. According to Chandler *et al.* (2011), host diet has a great effect on the bacterial microbiome composition. Reads belongs to *Chlorobi, Actinobacteria, Planctomycetes, Verrucomicrobia, Spirochaetes, Tenericutes* and *Acidobacteria* were found to be the other phyla with only a few reads (<1%) for both P and for H populations.

According Thao and Baumann (2004a) and Himler *et al.* (2011), bacterial endosymbionts are essential for survival, spread and evolution of *B. tabaci*. The bacteria identified were found to perform a variety of roles in whiteflies; but all are not known. Their presence and functions in other insects can provide an idea about the possible roles (Table 25 and 26) in whitefly. *Proteobacteria* associated with insects aids in carbohydrate degradation (Delalibera *et al.*, 2005), synthesis of B vitamins and essential amino acids (EAA) (Bennet *et al.*, 2014) and pesticide detoxification (Werren, 2012). Some members of *Firmicutes* were beneficial to the insects in digestion of cellulose and hemicelluloses (Brown *et al.*, 2012). Higher termites harbour *Bacteroidetes*, in their hindgut, to degrade lignocellulose, with the host enzymes acting on the amorphous regions of cellulose and the symbiotic enzymes targeting the crystalline regions (Brune, 2014). These bacteria are also reported to induce cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella* (Hunter *et al.*, 2003). *Actinobacteria* exhibited diverse physiological and metabolic properties in insects like, production of extracellular enzymes and formation of wide variety of secondary metabolites (Schrempf, 2001). In termites, *Actinobacteri*a assisted in nutrient acquisition from polysaccharides including cellulose (Pasti and Belli, 1985; Watanebe *et al.*, 2003) and hemicellulose (Schafer *et al.*, 1996).

Chlorobi is a salivary associated unique bacterial community in *Anopheles culicifacies* (Sharma *et al.*, 2014) and their role in whitefly is unknown. According to Köhler *et al.* (2008) and Bignell (2010), the extreme alkalinity in some compartments of termite guts, supports the growth of specialized alkaline-tolerant symbiotic bacteria from *Planctomycetes*. It is found that, beetles and termites, which feed on wood or detritus, have higher populations of *Verrucomicrobia* in their gut.

(Colman *et al.*, 2012) and *Spirochaetae* are present in the hindgut of the wood-feeding termites (*Nasutitermes* spp.) (Köhler *et al.*, 2012). According to Sabree and Moran (2014), *Tenericutes* are present in termites and cockroaches. It is observed that, *Acidobacteria* can utilize plant polymers, including xylan and cellulose (Eichorst *et al.*, 2011) and it is also speculated that *Acidobacteria* can participate in the degradation of these polymers in the larval gut. *Acidobacteria* were identified from the larvae of the cerambycid *Leptura rubra* feeding on rotten softwood (Grünwald *et al.*, 2010).

Using 16S rDNA clone library sequences, Singh *et al.* (2012) identified more than 300 bacterial genera from whiteflies, including secondary endosymbionts like, *Cardinium*, *Wolbachia*, *Rickettsia* and *Arsenophonus*, *Bacillus*, *Enterobacter*, *Paracoccus* and *Acinetobacter.* They could also observe that, secondary endosymbionts were not uniformly distributed in different locations. In the present study, altogether 236 and 225 bacterial genera were present for P and H populations respectively. For P populations, *Bacillus* was the most dominant group followed by *Arsenophonus*, *Vibrio*, *Riemerella*, *Lysinibacillus*, *Flavobacterium*, *Janthinobacterium*, *Sphingobacterium*, *Bacteroides*, *Enterococcus* and for H populations, the order of abundance was *Bacillus*, *Alcanivorax*, *Staphylococcus*, *Pantoea*, *Lysinibacillus*, *Bacteroides*, *Alistipes*, *Photobacterium*, *Terribacillus* and *Enterococcus*. At species level, a total of 409 species were identified in sample P and total of 355 species were identified in sample H (Fig. 21). Secondary endosymbiont of *Bemisia tabaci* [un-specified], *Arsenophonu*s, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus flexus*, *Riemerella anatipestifer*, *Vibrio harveyi*, *Lysinibacillus sphaericus*, *Janthinobacterium sp.* J3 and *Bacillus pumilus* were the major 10 species identified for P populations. For H populations, the major species identified were *Bacillus thuringiensis*, *Alcanivorax sp. EPR 6*, *SBR proteobacterium*, *Staphylococcus pasteuri*, *Bacillus amyloliquefaciens*, *Staphylococcus sciuri*, *Bacillus megaterium, Pantoea dispersa*, *Lysinibacillus sphaericus* and *Bacillus pumilus*.

Table 25. List of endosymbiotic bacterial phyla in P and H populations of *Bemisia tabaci*

Sl. No.	Phylum	Host	Role/Function	Reference
1	*Proteobacteria*	*Bemisia tabaci* *Helicoverpa armigera* *Bactrocera minax* *Schistocerca gregaria* Mosquitoes Ground beetles	Carbohydrate degradation	Delalibera *et al.*, 2005
			Synthesis of B vitamins and essential amino acids	Bennet *et al.*, 2014
			Pesticide detoxification	Werren, 2012
2	*Firmicutes*	*B. tabaci* *H. armigera* *B. minax* *S. gregaria* Termites Ground beetles Bees	Digestion of cellulose and hemicellulose	Brown *et al.*, 2012
3	*Bacteroidetes*	*B. tabaci* *Encarsia pergandiella* Termites Bees	Digestion of cellulose and lignocellulose	Brune, 2014
			Cytoplasmic incompatibility in *E. pergandiella*	Hunter *et al.*, 2003
4	*Actinobacteria*	*B. tabaci* *H. armigera* *B. minax* *S. gregaria* Termites Ground beetles	Production of extracellular enzymes and formation of wide variety of secondary metabolites	Schrempf, 2001
			Digestion of polysaccharides including cellulose and hemicelluloses in termites	Pasti and Belli, 1985; Watanebe *et al.*, 2003; Schafer *et al.*, 1996
5	*Chlorobi*	*B. tabaci* *Anopheles culicifacies*	Salivary associated and role is unknown	Sharma *et al.*, 2014
6	*Planctomycetes*	*B. tabaci* Termites	Alkaline-tolerant and role is unknown	Köhler *et al.*, 2008; Bignell, 2010
7	*Verrucomicrobia*	*B. tabaci* Beetles Termites	Possible role in digestion of wood and detritus	Colman *et al.*, 2012
8	*Spirochaetes*	*B. tabaci* *Nasutitermes* spp.	Digestion of cellulose and hemicellulose	Köhler *et al.*, 2012
9	*Tenericutes*	*B. tabaci* Termites Cockroaches	Digestion of complex food particles	Sabree and Moran, 2014
10	*Acidobacteria*	*B. tabaci* *Leptura rubra*	Degradation of xylan and cellulose in larval gut	Grünwald *et al.*, 2010

Table 26. List ofendosymbiotic bacterial genera/species in P and H populations of *Bemisia tabaci*

S. No.	Phylum	Host	Role/Function/Use	Reference
1	*Bacillus*	*B. tabaci*	Production of amylase	Amund and Ogunsina, 1987; Oyewole and Odunfa, 1992
			Production of long-chain sugars	Davidson *et al.*, 1994
	a. *Bacillus thuringiensis*	*B. tabaci* *H. armigera*	Biocontrol agent	Raymond *et al.*, 2010; Walters *et al.*, 1995; El-Assal *et al.*, 2013
	b. *Bacillus cereus*	*B. tabaci* *H. armigera*	Biocontrol agent	Song *et al.*, 2014; El-Assal *et al.*, 2013
	c. *Bacillus pumilus*	*B. tabaci*	Kill 2nd nymphal instar of *B. tabaci*	Ateyyat *et al.*, 2010
	d. *Bacillus megaterium*	*B. tabaci* *Aphis pomi*	Production of medium-length sugars from sucrose	Davidson *et al.*, 1994
			Management of *Aphis pomi*	Aksoy and Ozman-Sullivan, 2008
	e. *Bacillus flexus*	*B. tabaci* *Phlebotomus papatasi*	Oviposition induction	Mukhopadhyay *et al.*, 2012
	f. *Bacillus amyloliquefaciens*	*B. tabaci*	Mosquito control programmes	Geetha *et al.*, 2014
2	*Arsenophonus*	*B. tabaci*	Virus transmission	Rana *et al.*, 2012
			Inhibition of reproduction	Gherna *et al.*, 1991; Duron *et al.*, 2008
			Reduction in fitness	Raina *et al.*, 2015
3	*Vibrio* a. *Vibrio harveyi*	*B. tabaci*	Production of luciferase	Schmidt *et al.*, 1989
4	*Riemerella*	*B. tabaci* *Nylanderia fulva*	Unknown	McDonald, 2012
5	*Alcanivorax*	*B. tabaci* Moss bugs	Nutrition	Santos-Garcia *et al.*, 2014
			Marine oil-spill degradation	McGenity *et al.*, 2012
6	*Lysinibacillus* a. *Lysinibacillus sphaericus*	*B. tabaci* *Aedes aegypti*	Biocontrol agent	El-Assal *et al.*, 2013
			Biocontrol agent against insecticide-resistant *Aedes aegypti*	Rojas-Pinzón and Dussán, 2017

(Contd..)

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7	*Staphylococcus*	*B. tabaci*	Production of medium length sugars from sucrose	Indiragandhi *et al*., 2010
	a. Staphylococcus pasteuri b. *Staphylococcus sciuri*	*B. tabaci* *Nylanderia fulva*	Biocontrol agents	Ateyyat *et al*., 2010; McDonald, 2012
8	*Flavobacterium*	*B. tabaci* Scale insects	Unknown	Rosenblueth *et al*., 2012
9	*Pantoea* a. *Pantoea dispersa*	*B. tabaci* *Locusta migratoria* *Aedes albopictus*	Production of semiochemical Guaiacol and aggregation of insects	Dillon and Charnley, 2002; Davis *et al*., 2013; Moro *et al*., 2013
10	*Bacteroides*	*B. tabaci* Termites Cockroaches Hemipterans	Utilization of nitrogenous waste products	Potrikus and Breznak, 1981
11	*Janthinobacterium* *a. Janthinobacterium* sp. J3	*B. tabaci* *Batocera horsfieldi*	Chitin degradation	Gleave *et al*., 1995; Xiao *et al*., 2005; Zhang *et al*., 2011
12	*Sphingobacterium*	*B. tabaci* *Teleogryllus occipitalis* Cerambycid beetles	Xylanolytic activity	Long *et al*., 2016; Zhou *et al*., 2009
13	*Alistipes*	*B. tabaci* *Hirudo verbena* Mice	Unknown *Alistipes finegoldii* attenuates colitis in mice	Maltz *et al*., 2014; Dziarski *et al*., 2016
14	*Photorhabdus*	*B. tabaci*	*Photorhabdus luminescens* is a bioluminescent entomopathogen	Schmidt *et al*., 1989
15	*Terribacillus*	*B. tabaci*	Unknown	An *et al*., 2007
16	*Enterococcus*	*B. tabaci*	Produce cyanide oxygenase and utilize cyanide as a nitrogenous growth substance	Fernandez and Kunz, 2005
17	*SBR proteobacterium*	*B. tabaci*	Pathogen associated with the disease syndrome "basses richesses" of sugar beet	Bressan *et al*., 2008

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Many *Bacillus* members are present in *B. tabaci*, and they may contribute in nutrition. As, *Bacillus* strains have the ability to produce amylase enzyme (Amund and Ogunsina, 1987; Oyewole and Odunfa, 1992), these amylases may be involved in the initial breakdown of cassava starch into simple sugars. *Bacillus megaterium* isolates were found to produce medium-length sugars from sucrose (Davidson *et al.*, 1994). Also, *Bacillus spp.* associated with *B. tabaci* may produce long-chain sugars which contribute to the stickiness of the honeydew of the insect (Davidson *et al.*, 1994).

Interestingly, *Bemisia* also harbours various entomopathogens, like *Bacillus thuringiensis* (Raymond *et al.*, 2010; Walters *et al.*, 1995) and *Bacillus cereus* (Song *et al.*, 2014) and are found to be very effective in whitefly management (El-Assal *et al.*, 2013). *Bacillus pumilus* is very effective in reducing the, 2nd nymphal instar populations (Ateyyat *et al.*, 2010) of *B. tabaci*. The entomopathogen, *Bacillus megaterium* is proven as very effective in the management of *Aphis pomi* (Aksoy and Ozman-Sullivan, 2008). Mukhopadhyay *et al.* (2012) reported the role of *Bacillus flexus* in oviposition induction of sand fly (*Phlebotomus papatasi*). *Bacillus amyloliquefaciens* has strong mosquito larvicidal and pupicidal action, and are used in mosquito control programmes (Geetha *et al.*, 2014).

Arsenophonus is reported to be important in virus transmission of whitefly (Rana *et al.*, 2012) and are abundant in P populations. GroEL molecular *chaperones* from *Arsenophonus* sp. are found to get associated with coat proteins of *Cassava mosaic virus* and help them from disintegration in the insect haemolymph. Similar results are reported by Morin *et al.* (1999) and Gottlieb *et al.* (2010) in case of *TYLC* (*Tomato yellow leaf curl virus*) for *Buchnera* GroEL and *Hamiltonella* GroEL respectively. Since, cassava plants from where P populations of whitefly are collected had shown high degrees (Scale 2-5) (Ikotun and Hahn, 1991) of CMD (Cassava Mosaic Disease) incidence, the study results are in agreement with the findings of Rana *et al.* (2012). Study result clearly brings out the direct role of whitefly endosymbiont, *Arsenophonus* in virus

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transmission. It is also to be noted that, cassava mosaic disease intensity was negligible (Scale 1-2) in Sultan Bathery (H populations), where *Arsenophonus* population was absent compared to plains (P populations). *Arsenophonus* has also been suspected to affect the reproduction of its host (Gherna *et al.*, 1991; Duron *et al.*, 2008). Raina *et al.* (2015) observed that the elimination of *Arsenophonus* and decrease in the bacterial symbionts diversity by antibiotic treatment led to increase in the fitness of *B. tabaci*.

Luciferases from luminous bacteria *Vibrio harveyii* is reported by Schmidt *et al.* (1989). Whereas, McDonald (2012) reported the presence of *Riemerella*, in ant species, *Nylanderia fulva*. Santos-Garcia *et al.* (2014) reported, symbiotic association of *Alcanivorax* in moss bugs to fulfill their nutritional requirements, resulting from their unbalanced diet; while, their role in marine oil-spill degradation is reported by McGenity *et al.* (2012). Apart from effective in whitefly management, *Lysinibacillus sphaericus* (El-Assal *et al.*, 2013) can also be used as a biological control agent against, insecticide-resistant *Aedes aegypti* (Rojas-Pinzón and Dussán, 2017). *Staphylococcus* from *Bemisia* was reported for their potential to produce medium length sugars from sucrose and contribute to the stickiness of the honeydew secreted by the host insect (Indiragandhi *et al.*, 2010). McDonald (2012) reported the presence of *Staphylococcus pasteuri* and *Staphylococcus sciuri* from ant species *Nylanderia fulva*; whereas, Ateyyat *et al.* (2010) reported their potential as biocontrol agents.

Rosenblueth *et al.* (2012) reported evolutionary relationships of flavobacterial endosymbionts with their scale insect hosts. The endosymbiont *Pantoea*, observed in the study may perform semiochemical effects, as it is already reported for *Pantoea agglomerans* which is producing a chemical Guaiacol and helps in the aggregation of Desert locust, *Locusta migratoria* (Dillon and Charnley, 2002; Davis *et al.*, 2013). *Pantoea dispersa* is reported in wild mosquito *Aedes albopictus* (Moro *et al.*, 2013). Evidence for the microbial utilization of nitrogenous waste products by *Bacteroides* has been obtained for termites, cockroaches and hemipterans (Potrikus and Breznak, 1981).

Janthinobacterium strains reported to have the capacity to degrade chitin (Gleave *et al.*, 1995; Xiao *et al.*, 2005) and *Janthinobacterium* sp. J3 isolated from the gut contents of *Batocera horsfieldi* larvae (Zhang *et al.*, 2011).

Sphingobacterium griseoflavum sp. nov., isolated from the insect *Teleogryllus occipitalis* living in deserted cropland (Long *et al.*, 2016) and *Sphingobacterium* isolate exhibiting xylanolytic activity has been isolated from the gut of a cerambycid larva (Zhou *et al.*, 2009). *Alistipes finegoldii* and *Alistipes putredinis* are reported in the gut of medicinal leech (*Hirudo verbena)* (Maltz *et al.*, 2014) and *Alistipes finegoldii* attenuates colitis in mice (Dziarski *et al.*, 2016). *Photorhabdus luminescens* is a bioluminescent entomopathogen, which comes under the genus *Photorhabdus*, reported in the study (Schmidt *et al.*, 1989).

Yandigeri *et al.* (2016) reported the presence of *Terribacillus halophilus* in various insects; even though their role is unknown. Bacterium found in *Bemisia, Enterococcus* sp. reported to produce cyanide oxygenase and utilize cyanide as a nitrogenous growth substance (Fernandez and Kunz, 2005). According to, Bressan *et al.* (2008) *SBR proteobacterium* is a pathogen associated with the disease syndrome "basses richesses" of sugar beet in France, and are spread by planthoppers - *Cixius wagneri*, *Hyalesthes obsoletus*, and *Pentastiridius leporinus*.

Our study revealed the composition and diversity of bacterial community associated with *B. tabaci* based on Illumina next generation sequencing of 16S rDNA amplicons. The study was not extrapolated to know the correlation of endosymbiont bacterium and genetic variability in whitefly, as the study conducted by Singh *et al.* (2012), already ruled out any such possibility. Mining out of functional diversity of bacterial community present in the insect revealed their role in making *B. tabaci* a successful vector and polyphagous pest of global importance. Our analysis also showed the presence of specific endosymbionts like *Asenophonus* which was found present only in high CMD infested area. Insecticidal toxin producing opportunistic bacteria like *B. thuringiensis*, *Bacillus* *cereus etc.* are also reported in *B. tabaci*. Further studies are required to determine the functional roles, if any, of these endosymbionts in making *B. tabaci* a successful vector and invasive plant pest. The knowledge generated through more elaborated studies on endosymbionts, can very well be utilized, not only for planning alternative pest management strategies but also for enhancing efficiency of beneficial insects.

5. 4. Study of *Bemisia tabaci* – *Cassava mosaic virus* interactions in cassava

The relationships between plant viruses, their herbivore vectors and host plants can be beneficial, neutral, or antagonistic (Jiu *et al.*, 2007). The different types of interactions studied between *B. tabaci* and *Cassava mosaic virus* include, study on dispersal, study on feeding, study on fecundity, study on adult longevity and study on life cycle.

5.4.1. Study on dispersal and settling pattern of *Bemisia tabaci* on different cassava genotypes

Significant variation was observed between virulent and non-virulent whiteflies, between male and female whiteflies and also between cassava genotypes with varying degrees of response to virus infection. Interaction between virulence /non-virulence of whiteflies and cassava genotypes, sex of the insect and cassava genotypes were also found to be significantly different. However, there was no significant variation in interaction between presence of virus and sex of the insect, between presence of virus, sex of the insect and cassava genotypes (Table 19a, 19b and Fig. 23).

The speed of movement of *B. tabaci* was more; when non-virulent insects fed on resistant genotypes as compared to virulent ones feeding on susceptible genotypes. Maximum speed of movement (16.25 cm/s) observed was by non-virulent female on resistant genotype--CMR-128 and minimum (4.28 cm /s) was by virulent female on susceptible genotype--H-226. The speed of movement indicates the non-preference of a genotype for feeding. Resistant genotypes are not preferred by both virulent and non-virulent whiteflies, and the susceptible varieties like H-226 and H-165 were preferred by both virulent and non-virulent ones. Among them, virulent ones preferred them more and showed lesser speed of movement (settled for feeding). The results indicate that this behavioural change should promote pathogen spread since non infective vector preference for infected plants will promote acquisition, while infective vector preference for non infected hosts will promote transmission.

Similar results were explained by Narasimhan and Arjunan (974), as they reported that CMD resistant cassava had lower whitefly population compared to susceptible variety. Also, Bellotti and Arias (2001) reported that the nymphal mortality was highest on the CMD resistant cassava clones, and lowest on the susceptible clones. When feeding on resistant genotypes whiteflies had less of fecundity, longer development periods, reduced size and higher mortality than when feeding on susceptible ones. Mortality is highest during the nymphal stages. Ingwell *et al.* (2012) proposed plant viruses alter insect behaviour to enhance their spread. They reported the first evidence that acquisition of a plant virus directly alters host selection behaviour by its insect vector. In accordance with the present results, Ning *et al.* (2015) demonstrated that female whiteflies are more efficient in both feeding and virus transmission.

5.4.2. Feeding pattern of *Bemisia tabaci* on different cassava genotypes

Variations were significant between virulent and non-virulent whiteflies, between male and female whiteflies and also between cassava genotypes with varying degrees of response to virus infection. Interaction between virulence /non-virulence of whiteflies and cassava genotypes also found to be significantly different. Whereas, there was no significant variation in interaction between, presence of virus and sex of the insect, sex of the insect and cassava genotypes, between presence of virus, sex of the insect and cassava genotypes were found (Table 20a, 20b and Fig. 24).

Feeding was more by virulent ones in susceptible genotypes, compared to non-virulent ones in resistant genotypes. Maximum feeding was observed (65 stylet sheaths/plant) in the genotype-H-226 by virulent female, where as minimum (2.67 stylet sheaths/plant) was in the genotype-CMR-128 by virulent female. The results were in agreement with Nair and Daniel (1983). They observed that *B. tabaci* have preference for certain varieties of cassava and found high population count on H-226 and H-165 and also adult flies prefer more cassava mosaic disease affected plants for feeding than unaffected ones. Cassava infested with EACMV-UG (*East African cassava mosaic virus*-Uganda) served as better host for *B. tabaci* than did by healthy plants (Colvin *et al.*, 2006). Infection of susceptible cassava by EACMV-UG apparently altered the phloem sap content by increasing the concentrations of at least one amino acid and this was associated with an increased population growth rate of *B. tabaci* (Colvin *et al.*, 2004). Acquisition of plant viruses can increase the feeding and crowding in vector insects (Pusag *et al.*, 2012). According to Liu *et al.* (2013) TYLCV-infected plants were more attractive, probing them more quickly and having a greater number of feeding bouts. Viruliferous whiteflies fed more readily than uninfected whiteflies and spent more time salivating into sieve tube elements that provided TYLCV a direct fitness benefit for the virus. According to Moreno *et al.* (2013), a plant virus manipulates the behaviour of its whitefly vector to enhance its transmission efficiency and spread. Fang *et al.* (2013) also demonstrated similar results for TYLCV.

5.4.3. Influence of cassava genotype variation on fecundity of *Bemisia tabaci*

Significant variation was observed between virulent and non-virulent whiteflies and also between cassava genotypes with varying degrees of response to virus infection (Table 21a, 21b and Fig. 26). But, the interaction between virulence /non-virulence of whiteflies and cassava genotypes was not found to be significantly different. Highest fecundity was observed in the genotype-H-226 (87.67 eggs/ plant) by non-virulent ones whereas lowest was in the genotype-CMR-9 (8.67 eggs/ plant) by virulent ones. Bellotti and Arias (2001) observed

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that resistance in cassava genotypes severely dented oviposition in *B. tabaci*. Mann *et al.* (2008) demonstrated similar results when they studied the performance of *B. tabaci* on healthy and *Cotton leaf curl virus* infected cotton. Virus infection increased percent egg viability of *B. tabaci*. Whiteflies deposited significantly fewer eggs on virus infected plants compared to healthy plants.

5.4.4. Influence of cassava genotype variation on adult longevity of *Bemisia tabaci*

Significant variations were observed, in between virulent, non-virulent whiteflies; between male, female whiteflies and also between cassava genotypes with varying degrees of response to virus infection (Table 22a, 22b and Fig. 27). Whereas, there was no significant variation observed in interaction between, the factors studied. Non-virulent females lived up to 16.07 days in the genotype-H-165, while virulent male died after 4.1 days (lowest adult longevity) in the genotype-CMR-128. Similar results were obtained for Bellotti and Arias (2001). Mann *et al.* (2008) reported that male and female whiteflies had shorter longevity on CLCuV infected plants compared with healthy plants.

5.4.5. Influence of cassava genotype variation on life cycle of *Bemisia tabaci*

Significant variation was observed in between virulent and non-virulent whiteflies, between male and female whiteflies and also between cassava genotypes with varying degrees of response to virus infection (Table 23a, 23b and Fig. 28). Whereas, there was no significant variation was observed in interaction between, the factors studied. Life cycle of females ranged from 30.77 days (CMR-128) to 21.67 days (H-165), whereas life cycle of males ranged from 27.90 days (CMR-1) to 19.23 days (H-165) and also, virus infection increased their total life cycle. The results are in agreement with the findings of Bellotti and Arias (2001). According to Van Lenteren and Noldus (1990), shorter development time as well as greater total oviposition on a host reflects the suitability of the plant, and virus infection in whiteflies could have made them to take more time for completion of their life cycle.

Studying these interactions precisely will help to understand the behavioural and physiological variations in whiteflies and other insects and this information could be a valuable tool to formulate management tactics against different viruses.

Future line of work

- Detailed correlation study between genetic and morphometric characters of whitefly from more locations.
- Presence of *B. tabaci* biotypes/genetic groups on different plants.
- Functional diversity of microbiota present in whitefly.
- Biochemical analysis of whitefly feeding on plants.

Summary

6. SUMMARY

Studies on "Characterization of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) for genetic variability, endosymbionts and vector-virus interactions in cassava" were conducted at Department of Agricultural Entomology, College of Horticulture during 2014 to 2016 and the results of investigation are summarized below.

- Surveys were conducted in different agro-ecological zones of Kerala, where cassava is grown, *viz*., Neyyattinkara, Sreekaryam, Vellanikkara, Vadakara, Sultan Bathery, Kasargod, Thodupuzha, Palluruthy, Pulikeezhu, Muvattupuzha, Kayamkulam, Palakkad and Chittur and collected different life stages of whitefly. *B. tabaci* populations collected were subjected to both morphological and molecular level studies, for studying their genetic variation.
- In morphometric studies, pupal and adult stages of both female and male whiteflies were used. Total 14 characters of both adult female and male pupa were studied and for male and female adults 9 and 7 characters respectively were studied.
- The characters studied for pupae were, pupal length, pupal width, length and width of right and left anterior wax margin, vasiform orifice length, operculum length, operculum width, lingula length, lingula width, caudal furrow length, caudal seta length and distance between caudal setae. For male adult insects, nine characters *viz*., antennal length, body length, body width, forewing length, forewing width, hind tarsal length, hind tarsal width, aedeagus length and clasper length, were compared. Seven characters except aedeagus length and clasper length were compared for adult female insects.
- Among different pupal characters studied for female pupa, only variations in pupal length, pupal width and length of right and left anterior wax margin were found to be significantly different among the populations

collected from different agro-ecological zones of Kerala. For male pupal characters, variations in pupal length and width were found to be significantly different among populations.

- Both in case of female and male pupa, whitefly populations from Sultan Bathery (SBY) found to have lowest pupal length (0.668mm and 0.582 mm, respectively) and highest pupal width (0.539mm and 0.422mm respectively). The populations collected from Sultan Bathery also found to have the highest vasiform orifice length, operculum length, operculum width, lingula length, lingula width and distance between caudal setae among whitefly populations (except in case of operculum width for male pupa).
- In multivariate analysis of female pupal characters, it was found that the characters with strong positive correlations were operculum length, pupal width and vasiform orifice length; lingula length and lingula width; operculum width and distance between caudal setae; length of right and left anterior wax margin and pupal length; width of right and left anterior wax margin and caudal seta length. There were negative correlations between length of left anterior wax margin and distance between caudal setae; caudal seta length and lingula width.
- For multivariate analysis of male pupa, strong positive correlations were observed between length of right and left anterior wax margins and caudal seta length; pupal width and operculum length; lingula length, lingula width, vasiform orifice length and distance between caudal setae; width of right and left anterior wax margins and caudal furrow length and pupal length. Negative correlations were observed between pupal width and caudal seta length; length of right and left anterior wax margins and operculum length.
- In the score plot analysis of female pupa, SBY and PTY (Palluruthy) populations formed a separate group and for male pupa, only populations from SKM (Sreekaryam) found in a different quarter.

- In the study for, characters of adult female cassava whitefly, antennal length, body length, body width, forewing length and forewing width were found to be significantly different between the populations; whereas, no significant difference was observed for hind tarsal length and width.
- In adult male, antennal length, body length, body width, forewing length and forewing width were found to be significantly different between the populations; whereas, no significant difference was observed for hind tarsal length, hind tarsal width, aedeagus length and clasper length.
- Both for, adult female and adult male, whitefly populations from Sultan Bathery found to have the highest antennal length (0.376 mm and 0.339 mm respectively) and highest body width (0.288 mm and 0.253 mm respectively). For other morphometric characters studied, SBY populations had shown the lowest observations when compared with other 12 zones.
- In multivariate analysis of adult female, the characters with strong positive correlations are forewing length and forewing width; hind tarsal length and hind tarsal width. There were negative correlations between body width and forewing length; antennal length and hind tarsal length.
- For multivariate analysis of adult male, strong positive correlations observed between forewing width, body length, forewing length and hind tarsal width; aedeagus length and clasper length. Negative correlations were observed between body width and hind tarsal width.
- In the score plot analysis of adult female, SBY populations formed a separate group. CTR (Chittur), VDA (Vadakara) populations and SBY, KGD (Kasargod) populations found as separate groups for adult male.
- In molecular analysis using Inter Simple Sequence Repeats (ISSR) primers, ten ISSR primers were screened out of 35, and used for the amplification of ISSR region in genomic DNA of *B.tabaci*, with thermal cycler and correspondingly programmed with primer specific annealing temperature to study the population genetic structure in *B. tabaci* from different agro-ecological zones of Kerala.

- The primers screened were F1, I4, UBC 846, UBC 855, F2, UBC 827, ISSR 8, UBC 808, UBC 811and ISSR 3.
- In the whitefly genomic DNA samples, primer F1 formed 17 bands with four unique/polymorphic bands and I4 formed 23 bands with four unique bands.
- Six unique bands and a total of 19 bands are formed by the primer UBC 846. UBC 855 formed 21 bands with three unique bands and primer F2 formed 14 bands with six unique bands.
- Three unique bands and 22 total bands are formed by UBC 827. ISSR 8 formed 13 bands with no unique bands and UBC 808 formed 20 bands with four unique bands.
- Three unique bands are formed by UBC 811 with a total of 26 bands. Primer ISSR 3 formed 11 bands with four unique bands. The size of amplified fragments had ranged from 200 bp to 2900 bp.
- It was found that, out of 10 ISSR primers used, nine could result in the polymorphic pattern with overall polymorphism of 19.89 per cent.
- The best polymorphism was observed for the primer F2 (42.86 per cent), followed by ISSR 3 (36.36 per cent), and are comparatively better to use for the genetic variability study of whitefly. Whereas, ISSR 8 cannot be recommended for this type of study, as there was no polymorphism when the primer was used.
- Dice coefficient values were utilized to construct dendrogram by un-weighted pair-group method analysis, UPGMA based on presence or absence of ISSR bands.
- Dendrogram analysis revealed the occurrence of two major clusters and an out group. The out group is formed by Sultan Bathery (SBY) sample had shown the least similarity with all other samples (similarity coefficients in between 0.51-0.60).
- The biggest cluster formed is having samples from Palakkad (PKD), Chittur (CTR), Kasargod (KGD), Thodupuzha (TPA), Vellanikkara (VKA), Vadakara (VDA), Neyyattinkara (NYA), Kayamkulam (KYM)

and Sreekaryam (SKM); the order in which the samples are closely related based on ISSR analysis. The second cluster is having samples from Palluruthy (PTY), Muvattupuzha (MVA) and Pulikeezhu (PKU). The samples from Muvattupuzha (MVA) and Pulikeezhu (PKU) are very closely related.

- The *mtCO1*sequences generated from whitefly consisted of 765 bp to 795 bp and homology of sequences with other reported sequences were analysed.
- All the 13 sequences were aligned using online software 'Clustal 2.1' and the respective values for their similarity were obtained by pair wise arrangement. The calculated values ranged from 83.50 to 100.
- The sample from Sultan Bathery (SBY) shows least similarity (83.50 - 85.63) in the matrix; while the others were in the range of 96.64 per cent to 100 per cent similarity. Dendrogram deduced from matrix of pair wise comparisons in *mtCO1* sequences of *Bemisia tabaci* from 13-agro-ecological zones of Kerala (MEGA-6, Neighbour joining method), shown that populations from Sultan Bathery is an out group.
- The gene sequences of all 13 whiteflies from all these zones were submitted to BOLD and corresponding barcode for *B. tabaci* was generated.
- The sample from Sultan Bathery found to belong to Asia I genetic group with 98 per cent similarity to the reference sequences.
- Asia I genetic group of *B. tabaci* is first time reported as a pest in cassava, the result was confirmed by collecting settled nymhs, like 'red eyed nymphs' from the plants.
- For the characterization of endosymbionts in *B. tabaci*, bacterial communities associated with cassava whitefly collected from different agro ecological zones of Kerala, India were compared.
- Metagenomic DNA of *B. tabaci* was isolated by SDS based metagenomic DNA extraction procedure and used the Illumina Next Generation

Sequencing platform to reveal total bacterial community present. Analysis of hypervarible V3 region of 16S rRNA fragment resulted 1,321,906 and 690,661 high quality paired end sequences with mean length of 150 base pairs. Highly diverse bacterial communities were present in the sample containing approximately 3,513 operational taxonomic units (OTUs). Downstream analysis using QIIME and MG-RAST programmes showed a marked difference in the abundance of bacteria in the two populations (P and H) of whitefly.

- Altogether, 16 bacterial phyla were detected both in P and H samples. Among the phyla for P populations of *B. tabaci*, *Proteobacteria* was the most dominant which consisted of 87.57 per cent of total bacterial community and for H populations; *Firmicutes* was the most dominant with 82.67 per cent. It was followed by *Firmicutes* (9.29 %) for P populations and *Proteobacteria* (13.40 %) for H populations. *Bacteroidetes* was found 2.91 % in P populations and 0.84 % in H populations.
- In the present study, altogether 236 and 225 bacterial genera were present for P and H populations respectively. For P populations, *Bacillus* was the most dominant group followed by *Arsenophonus*, *Vibrio*, *Riemerella*, *Lysinibacillus*, *Flavobacterium*, *Janthinobacterium*, *Sphingobacterium*, *Bacteroides*, *Enterococcus* and for H populations, the order of abundance was *Bacillus*, *Alcanivorax*, *Staphylococcus*, *Pantoea*, *Lysinibacillus*, *Bacteroides*, *Alistipes*, *Photobacterium*, *Terribacillus* and *Enterococcus*.
- At species level, a total of 409 species were identified in sample P and total of 355 species were identified in sample H. Secondary endosymbiont of *Bemisia tabaci* [un-specified], *Arsenophonu*s, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus flexus*, *Riemerella anatipestifer*, *Vibrio harveyi*, *Lysinibacillus sphaericus*, *Janthinobacterium sp.* J3 and *Bacillus pumilus* were the major 10 species identified for P populations.
- For H populations, the major species identified were *Bacillus thuringiensis*, *Alcanivorax sp. EPR 6*, *SBR proteobacterium*, *Staphylococcus pasteuri*, *Bacillus amyloliquefaciens*, *Staphylococcus*

sciuri, *Bacillus megaterium*, *Pantoea dispersa*, *Lysinibacillus sphaericus* and *Bacillus pumilus*.

- Mining out of functional diversity of bacterial community present in the insect, revealed their role in making *B. tabaci* a successful vector and polyphagous pest of global importance. Analysis also showed the presence of specific endosymbionts like *Asenophonus* which was found present only in high CMD infested area. Insecticidal toxin producing opportunistic bacteria like *B. thuringiensis*, *Bacillus cereus etc.* are also reported in *B. tabaci*.
- The different types of interactions studied between *B. tabaci* and *Cassava mosaic virus* include, study on dispersal, study on feeding, study on fecundity, study on adult longevity and study on life cycle.
- The speed of movement of *B. tabaci* was more; when non-virulent insects fed on resistant genotypes compared to virulent ones, fed on susceptible genotypes.
- Maximum speed of movement (16.25 cm/s) observed was by non-virulent female on resistant genotype--CMR-128 and minimum (4.28 cm /s) was by virulent female on susceptible genotype--H-226. The speed of movement indicates the non-preference of a genotype for feeding. Resistant genotypes are not preferred by both virulent and non-virulent whiteflies, and the susceptible varieties like H-226 and H-165 are preferred by both virulent and non-virulent ones. Among them, virulent ones preferred them more and show less speed of movement (settled for feeding).
- The results are an indication that, this behavioural change should promote pathogen spread since non infective vector preference for infected plants will promotes acquisition, while infective vector preference for non infected hosts will promote transmission.
- Feeding was more by virulent ones in susceptible genotypes, compared to non-virulent ones in resistant genotypes. Maximum feeding was observed (65 stylet sheaths/plant) in the genotype-H-226 by virulent female, where

as minimum (2.67 stylet sheaths/plant) was in the genotype-CMR-128 by virulent female.

- Maximum fecundity was observed in the genotype-H-226 (87.67 eggs/ plant) by non-virulent ones whereas; minimum was in the genotype-CMR-9 (8.67 eggs/ plant) by virulent ones.
- Virus infection increased percent egg viability of *B. tabaci*. Whiteflies deposited significantly fewer eggs on virus infected plants compared to healthy plants.
- Non-virulent females lived up to 16.07 days in the genotype-H-165, while virulent male died after 4.1 days (lowest adult longevity) in the genotype-CMR-128.
- Life cycle of females ranged from 30.77 days (CMR-128) to 21.67 days (H-165), whereas life cycle of males ranged from 27.90 days (CMR-1) to 19.23 days (H-165) and also, virus infection increased their total life cycle.

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CHARACTERIZATION OF *Bemisia tabaci* (Gennadius) (HEMIPTERA: ALEYRODIDAE), FOR GENETIC VARIABILITY, ENDOSYMBIONTS AND VECTOR-VIRUS INTERACTIONS IN CASSAVA

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ABSTRACT OF THE THESIS

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Characterization of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) for genetic variability, endosymbionts and vector-virus interactions in cassava

Abstract

Cassava is one of the important tuber crops cultivated all over the World. Cassava Mosaic Disease (CMD) is the most important limiting factor in its production. Silverleaf whitefly, *Bemisia tabaci* (Gennadius) is the vector responsible for the transmission of *Cassava mosaic virus* in cassava, which causes CMD. Genetic variation among the members of *B. tabaci,* makes them very difficult to manage. Endosymbionts present in the whitefly system could be a factor responsible for making them a successful sucking pest. There are various kinds of interactions existing between whitefly and the CMV. Studying these interactions precisely will help to understand the behavioural and physiological variations in whiteflies. In this background the present study, "Characterization of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), for genetic variability, endosymbionts and vector-virus interactions in cassava" was proposed and carried out at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, during Mrch 2014 to April 2016, with the objectives to analyse the genetic variability in cassava whitefly, characterization of its endosymbionts and elucidation of cassava whitefly - cassava mosaic virus interactions.

Various life stages of *B. tabaci* were collected from different cassava growing agro ecological zones of Kerala and reared in laboratory as well as in polyhouse at optimum conditions. Genetic variability study was conducted with 10 selected ISSR primers which had shown polymorphism in their banding pattern; with amplicon size ranged between 200bp to 2900bp. Phylogenetic analysis using NTsys software revealed the presence of two major clusters with Sultan Bathery population as out group. Similarity matrix had shown up to 49 per cent variation between the samples.

Polymerase chain reaction using mitochondrial *cytochrome oxidase1* primers, C1-J2195 and L2-N-3014 had given amplicon of 850bp. Nucleotide sequences had shown variation up to 16.5 per cent and dendrogram generated out of the sequences using MEGA-6 (Neighbor Joining Method) gave two clusters and one out group. Sequence similarity check using reference sequences from NCBI data base indicated the presence of two biotypes, AsiaI and AsiaII5 in cassava plants of Kerala.

Morphometric studies were conducted to assess the variations in different pupal and adult characters of thirteen whitefly populations. Significant variations were found in pupal length and pupal width of the biotypes. Pupal length varied between 0.746 mm to 0.668 mm and pupal width varied between 0.539 mm to 0.468 mm in female pupa. Out of 14 characters of pupa studied, variations in length and width were found to be significant. Among seven characters of adults studied, variations in wing, antennal length, body length and width were significant. AsiaI biotype was found to have lesser body length, but more width compared to AsiaII5. AsiaII5 was found to be an important biotype of *B. tabaci* infesting cassava in 12 out of the 13 locations surveyed.

Endosymbiont characterization from whitefly using Next Generation Sequencing (NGS) - Illumina platform revealed the variations in microbiota. At phylum level, *Proteobacteria* was found at 87.57 per cent in whitefly populations collected from plains. The populations from high ranges contained *Firmicutes* at 82.67 per cent. *Arsenophonus*, an 'indirect helper' for virus spread by protecting viral coat protein from degradation in insect system with their GroEL chaperones were found at 24. 69 per cent in *B. tabaci* populations collected from plains.

Behavioural and life cycle variation study of *B. tabaci* using six cassava genotypes had shown that virus infection in *B. tabaci* altered the dispersal and settling. Speed of movement observed to be maximum at 16.25 cm/s in non-virulent female whiteflies on the genotype CMR-9. Life cycle of virulent and non-virulent whiteflies was found to vary between 19.57 days to 30.77 days.

A thorough understanding of genetic variations, endosymbiont diversity and behavioural response to virus could help the researchers in planning proper management strategies for *B. tabaci*. In future, information generated of such kinds could also help the researchers and policy makers to foresee and manage any possible outbreak of the pest and avoid any havoc caused by them.

Appendices

Appendix I

Abbreviations and units used

Abbreviations

rpm: rotations per minute

DNA: deoxyribo nucleic acid

RNA: ribo nucleic acid

UV: ultra violet

bp: base pairs

CD: critical difference

MG-RAST: Metagenomic Rapid Annotations using Subsystems Technology

MEAM: Middle East Asia Minor

MED: Mediterranean

Units

g : gram

mg : milligram

mm: millimetre

% : per cent

^{0}C : degree Celsius

min: minutes

sec: seconds

ng: nanogram

μl: microlitre

Appendix II

Reagents of CTAB buffer

a. CTAB buffer (2X)

-2 per cent CTAB (W/V)

-100 mM Tris-HCl [pH-8]

-10 mM EDTA (pH-8)

-1.5 M NaCl

-2 per cent 2-ß mercaptoethanol

b. Chloroform: isoamyl alcohol (24:1 v/v)

c. 3 M sodium acetate

d. 70 and 95 per cent ethanol

e. Sterile distilled water

Reagent a. was autoclaved and stored at room temperature

Appendix III

Reagents required for agarose gel electrophoresis

1. Agarose - 0.8 per cent (for genomic DNA)

   - one per cent (for PCR product)

2. 50X TAE buffer (pH8.0)
3. Tracking/loading dye (6X)
4. Ethidium bromide (stock 10 mg/ml; working concentration 0.5 μg/ml)