Morphometric Variations in Cassava (Manihot esculenta Crantz) Whitefly, Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) from different Agro-Ecological Zones of Kerala, India

Article · May 2017

CITATIONS
0

READS
225

5 authors, including:

Harish e R
Indian Council of Agricultural Research
10 PUBLICATIONS **28** CITATIONS
SEE PROFILE

Mani Chellappan
Kerala Agricultural University
23 PUBLICATIONS **61** CITATIONS
SEE PROFILE

Ranjith M T
Kerala Agricultural University Thrissur
12 PUBLICATIONS **50** CITATIONS
SEE PROFILE

Ajinkya Ambavane
Kerala Agricultural University
17 PUBLICATIONS **66** CITATIONS
SEE PROFILE

Journal of Root Crops, 2016, Vol. 42 No. 2, pp. 90-102
Indian Society for Root Crops
ISSN 0378-2409, ISSN 2454-9053 (online)

Morphometric Variations in Cassava (*Manihot esculenta* Crantz) Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) from different Agro-Ecological Zones of Kerala, India

E.R. Harish[1], Mani Chellappan[2], T. Makesh Kumar[1], M.T. Ranjith[2] and A.R. Ambavane[2]

[1]Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram 695 017, Kerala, Kerala, India

[2]College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur 680 656, Kerala, India

Corresponding author: E.R. Harish; e-mail: harisher4@gmail.com

Received: 14 November 2016; Accepted: 22 December 2016

Abstract

The silverleaf whitefly, *Bemisia tabaci,* is the main limiting factor in the production of one of the most important tuber crops in the world, cassava. The cassava mosaic virus transmitted by the insect causes cassava mosaic disease and it accounts for about 40% reduction in the tuber yield. The present study evaluates the presence of different genetic groups, using morphometric variations among whitefly populations collected from all agro-ecological zones of Kerala, India. Studying fourteen different pupal characters and nine different adult characters could not establish significant variations in most of the characters studied, between these agro-ecological zones. But, the principal component analysis revealed the presence of more variations in populations collected from Sulthan Bathery compared to others. Further molecular studies using the populations could provide a clear cut idea about the presence of different genetic groups/ biotypes in these regions.

Key words: *Bemisia tabaci*, cassava, genetic groups, agro-ecological zones, morphometry, principal component analysis.

Introduction

Silverleaf whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most destructive invasive insect pests reported, infesting more than 900 species of plants and spreading more than 100 plant viral diseases (Global Invasive Species Database, 2016). This polyphagous insect is believed to be spread throughout the world as a result of transportation of plant products that were infested with whiteflies. Once established, *B. tabaci* quickly spreads and through its feeding habits and the transmission of diseases, it causes destruction to crops around the world. According to Misra and Lambda (1929) the first report of *B. tabaci* in India was from cotton fields of Punjab in 1905.

Tuber crops, which include cassava, is the third most important food crops after cereals and grain legumes. In India, cassava was introduced through Kerala by the Portuguese during the eighteenth century. The tubers of cassava saved many lives during famine conditions especially in Kerala. Now the crop has become an important industrial crop for production of sago, starch and eri silk rearing especially in Tamil Nadu, Kerala, Andhra Pradesh, Maharashtra and Assam states of India.

While considering the main limiting factor in cassava production, Cassava Mosaic Disease (CMD) stands out. The first report of cassava mosaic disease (CMD) was from East Africa in 1894 (Legg and Fauquet, 2004). Yield loss due to CMD ranges from 17% to 42% (Palaniswami

et al. 1996). Cassava mosaic disease (CMD) was first observed in Kerala during 1956 (Palaniswami *et al*. 1996) and different symptoms include leaf chlorotic mottle, distortion of leaves, crinkling and stunting of cassava plant parts.

B. tabaci is the vector responsible for transmission of *Cassava Mosaic Virus* in cassava, which causes CMD. De Barro et al. (2011) suggested that *B. tabaci* is not a single group of insects, but is a cryptic species complex consisting of 24 morphologically indistinguishable species and with 11 well-defined high-level groups. Polyphagous nature of *B. tabaci* makes it as a complex species and variation exists among the population in different cassava growing areas. Palaniswami et al. (2001) first reported the biology of cassava whitefly (*B. tabaci)* in India.

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. Accordingly, the present study focuses on differences in morphology of adult and pupal stages of *B. tabaci*, from different agro-ecological zones of Kerala, India.

Materials and Methods

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

Insect proof whitefly rearing cages were fabricated with dimensions of -5.5' X 2.5'- cylindrical iron frame fitted with black stiff net and white cotton cloth (with a mesh size of 50). Tapioca setts were planted (H-226) in grow bags of 60cmx30cmx30cm. The collected whiteflies were reared and multiplied in rearing cages up to six generations on cassava plants. The rearing conditions were 27-33ºC temperature and 65±5% relative humidity.

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 80ºC 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 specimens were examined under a binocular microscope. The body contents were expelled by making an incision and pumped the liquefied body contents out, using two fine spatulas. The excess KOH was pipetted off. The specimens were soaked in 70% ethanol for two minutes. The liquid was pipetted off. Finally, the

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

No	Zone	Place	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	118 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

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 include 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 are 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.

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.

Results and Discussion

Morphometric variations in *B. tabaci* pupa

For the study of pupa, 'puparium' (pupal case of late fourth nymphal stage with red eyes) was used (Fig.1).

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 (Table 2). For male pupal characters, variations in pupal length and width were found to be significantly different among populations (Table 3). Both in case of female and male pupa, whitefly populations from Sulthan Bathery 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 Sulthan Bathery also found to have the highest vasiform orifice length, operculum length, operculum width, lingula length, lingula width and distance between caudal setae among populations (except in case of operculum width for male pupa). In almost all other characters studied, there were variations in morphometry between populations collected from Sulthan Bathery and other 12 agro-ecological zones (Populations collected from SBY had lowest observations in other characters).

a. Principal component analysis (PCA) 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 Table 4 and 5. The principal component analysis divided these 14 traits into major principal components. The first principal component (PC1) accounts for maximum variability in the data with respect to succeeding components.

The scree plot of the principal components (PC) showed that the first five (female pupa) and four (male pupa) eigen values correspond to most of the variances in the dataset. The major principal components were extracted, the total cumulative variance of these principal components amounted to 86.3% and 80.6% 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%, PC2 for 17.5 per cent, PC3 for 12.3%, PC4 for 10.4% and PC5 for 8.8% (Table 4) of the total variation. For male pupa, the variables are grouped into four major principal components; in which PC1 accounted for 45.3%, PC2 for 15.1%, PC3 for 12.2% and PC4 for 8% (Table 5) of the total variation.

For female pupa, among the five principal components analysed, PC1 which made the largest contribution of 37.4% of the total variation, had shown highest positive contribution for pupal width, vasiform orifice length,

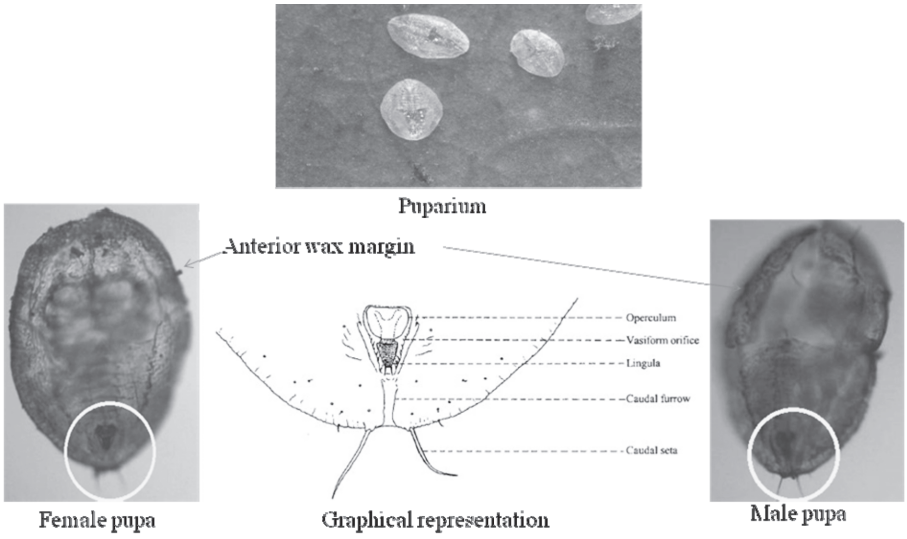

Fig.1. Female and male puparial characters of *B. tabaci*

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 4).

In case of male pupa, 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.

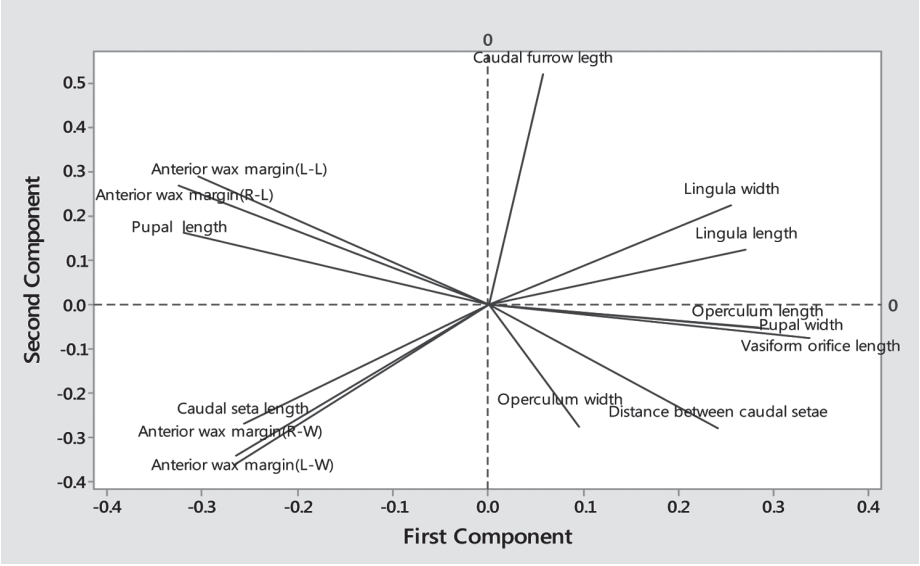

Fig. 2. Biplot/loading plot of the first two principal components showing relation among various *B. tabaci* female pupa characters

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 5).

The first two principal components contributing the major share of variances and were plotted to observe the relationships between measured whitefly traits/ variables (Fig. 2 and 3). In first and second principal components all the characters under consideration contributing diversity positively and up to second principal component 54.9% (female pupa) and 60.4% (male pupa) 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.

b. 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

Table 2. Characters of female pupa*

Place	Pupa length	Pupall 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]	Vasifor m orifice length	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

based on plot that had relatively large loading on both PC1 and PC2 axes (Fig. 2 and 3).

In case of biplot for female pupa (Fig. 2), there were near zero (angle 0-25º) 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 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 180º (150-160º) (r=cos180= -1) between their vectors.

In case of biplot for male pupa (Fig. 3), 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.

Table 3. Characters of male pupa*

Place	Pupa length	Pupall 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]	Vasifor m 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

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% of the total variation.

c. 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. 4 and Fig. 5. 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. 4)

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,

Table 4. PCA/ Eigen analysis of the correlation matrix (*B. 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 5. PCA/ Eigen analysis of the correlation matrix (*B. tabaci* male pupa)

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
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

VKA, TPA (quarter II). Quarter III, included only two entries PKD, SBY and quarter IV had only one entry SKM (Fig. 5).

Score plot analysis of female and male pupa did not provide any clear cut idea about the presence of different biotypes. But it was found that in case of both female and male pupa, SBY populations found to be present in a separate group.

Morphometric variations in adult *B. tabaci*

Different female and male adult characters were studied like antennal length, body length, body width, forewing length, forewing width, hind tarsal length and hind tarsal width. For adult male, additionally aedeagus length and clasper length were studied. Whitefly antenna found to have seven segments (Fig. 7). Hind tarsa (Fig. 7) and

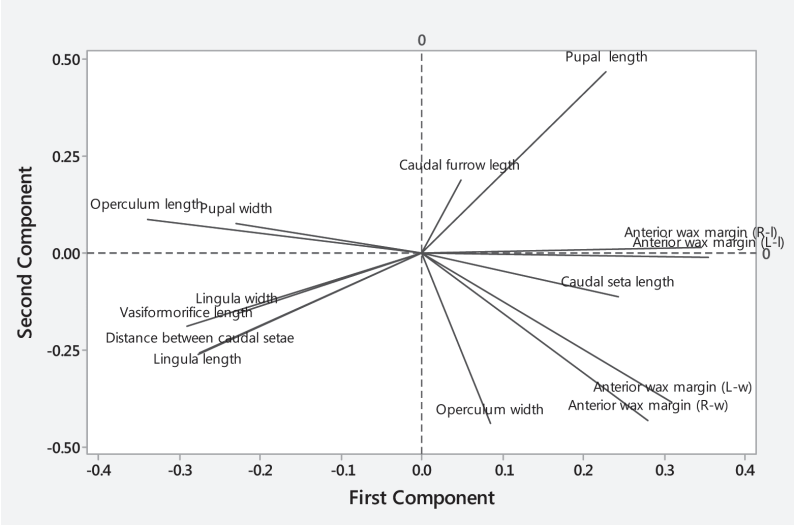

Fig. 3. Biplot/loading plot of the first two principal components showing relation among various *B. tabaci* male pupa characters

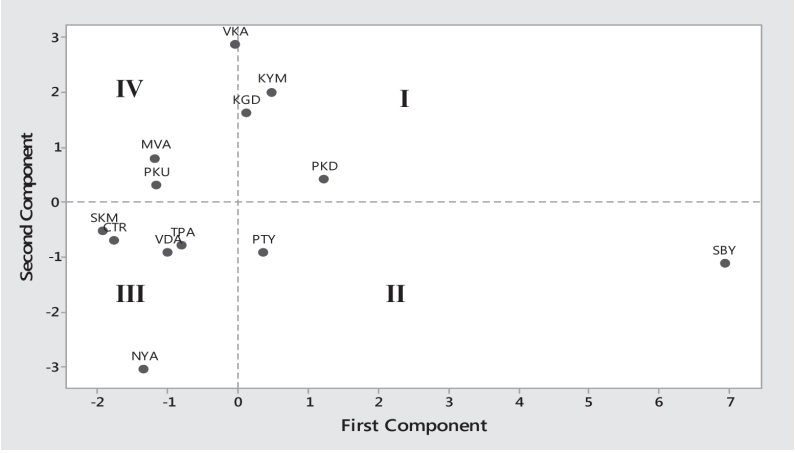

Fig. 4. Score plot of first two components for *B. tabaci* female pupa collected from various locations

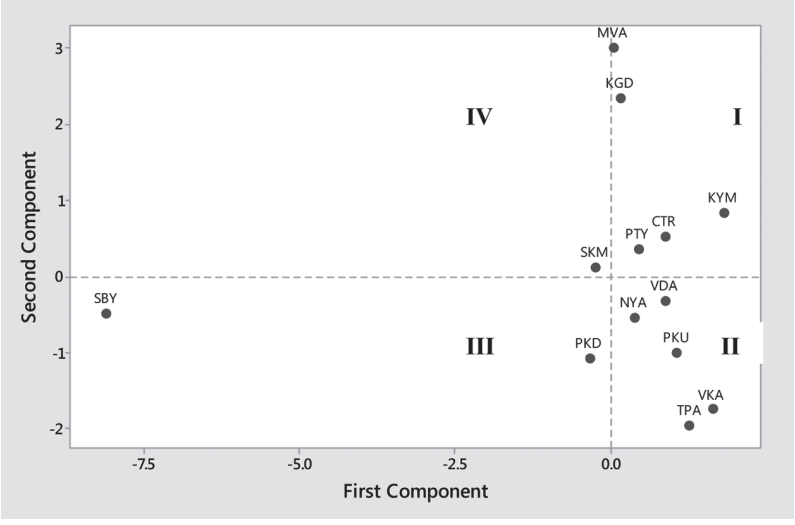

Fig. 5. Score plot of first two components for *B. tabaci* male pupa collected from various locations

characters of male *Bemisia* (Aedeagus and claspers) (Fig. 8) are very important in comparing whitefly.

Among 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 6 and 7).

In case of both adult female and adult male, whitefly populations from Sulthan 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.

a. Principal component analysis (PCA) for the estimated 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 Table 8 and 9.

The score plot of the principal components (PC) showed that the first

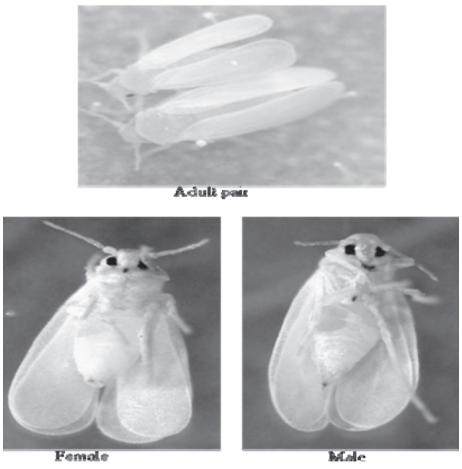

Fig. 6. Female and male *B. tabaci*

Table 6. Adult *B. tabaci* female characters*

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
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)

Table 7. Adult *B. tabaci* male characters*

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)

one (adult female) and two (adult male) eigen values correspond to most of the variances in the dataset. The major principal components were extracted, the total cumulative variance of these principal components amounted to 77.8% and 81.3% 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

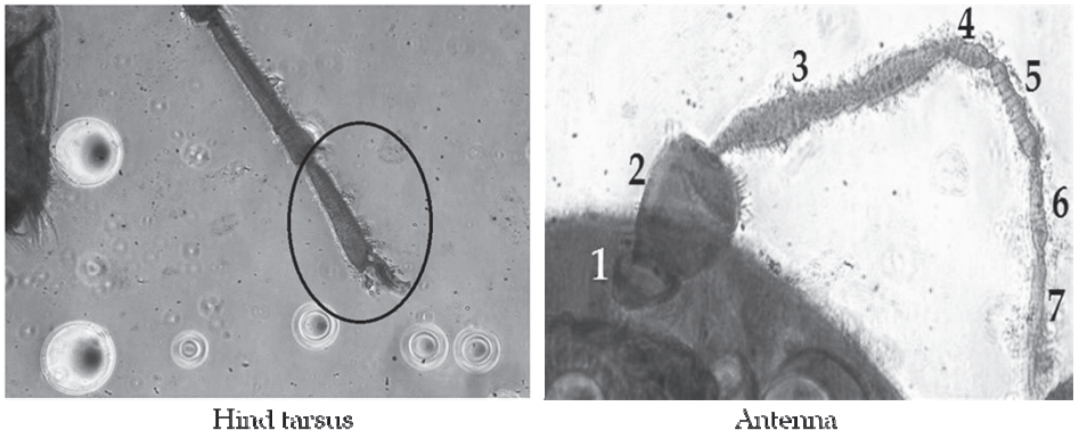

Fig.7. Hind tarsus and antenna of *B. tabaci*

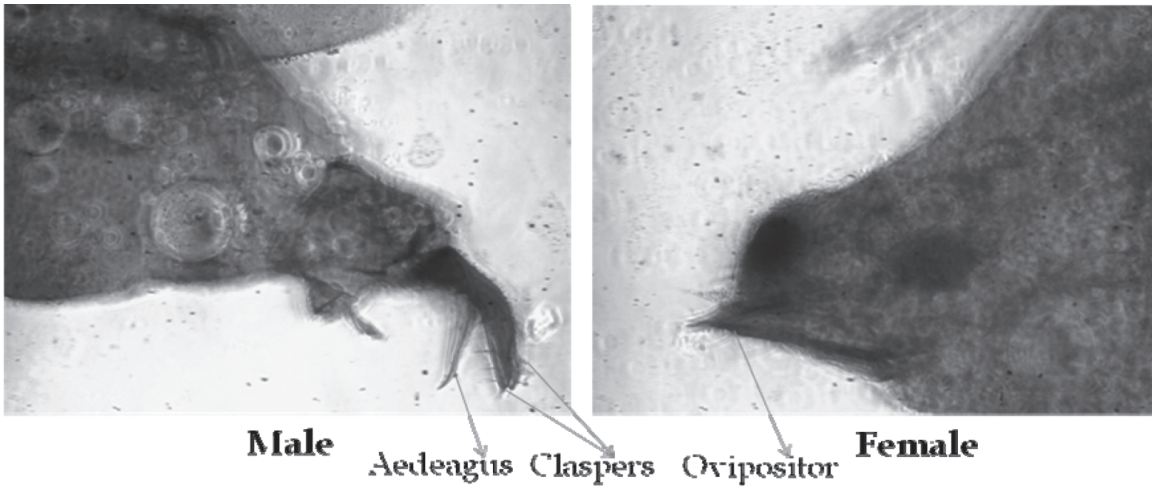

Fig.8. Genitalia of *B. tabaci*

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 variables into a single major group in case of adult female; which accounted for 77.8% (Table 8) of the total variation. For adult male, the variables are grouped into two major principal components; in which PC1 accounted for 60.8% and PC2 accounted for 20.5% (Table 9) of the total variation.

For adult female, the only principal component with a contribution of 77.8% 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 8).

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 (Fig. 9 and 10). The first two principal components contributing 87.6% of variation in adult female and for adult male, the first two principal components contributing 81.3% diversity. So other principal components were not considered as major contributing principal components but they were already

Table 8. PCA/ Eigen analysis of the correlation matrix (Adult female *B. 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

Table 9. PCA/ Eigen analysis of the correlation matrix (Adult male *B. 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

considered and measured in first two principal components.

b. 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. 9 and 10).

In case of biplot for adult female (Fig. 9), there were near zero (angle 0-25º) 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 180º (150-160º) (r=cos180= -1) between their vectors.

In case of biplot for adult male (Fig. 10), strong positive correlations observed between forewing width, body length, forewing length and hind tarsal width; aedeagus length and clasper length.

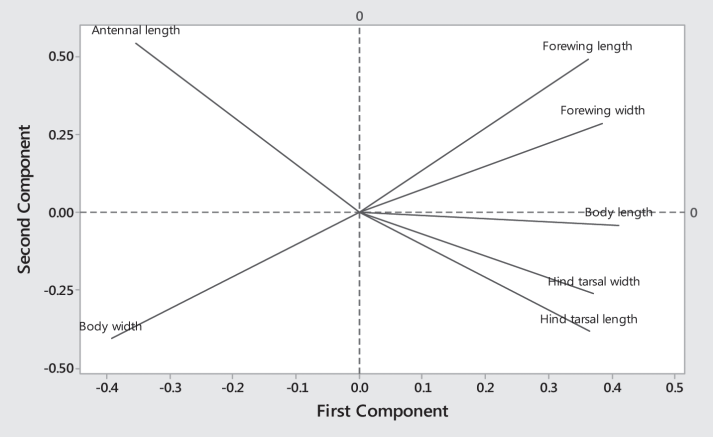

Fig. 9. Biplot/loading plot of the first two principal components showing relations among various *B. tabaci* adult female characters

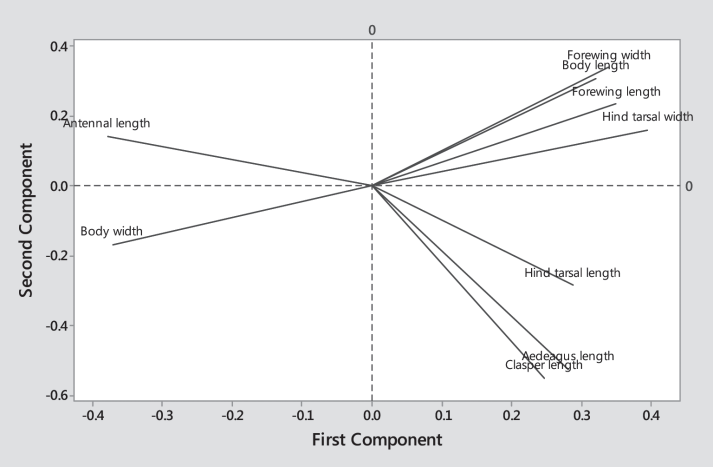

Fig. 10. Biplot/loading plot of the first two principal components showing relations among various *B. tabaci* adult male characters

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% of the total variation.

c. 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. 11 and Fig. 12. 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. 11).

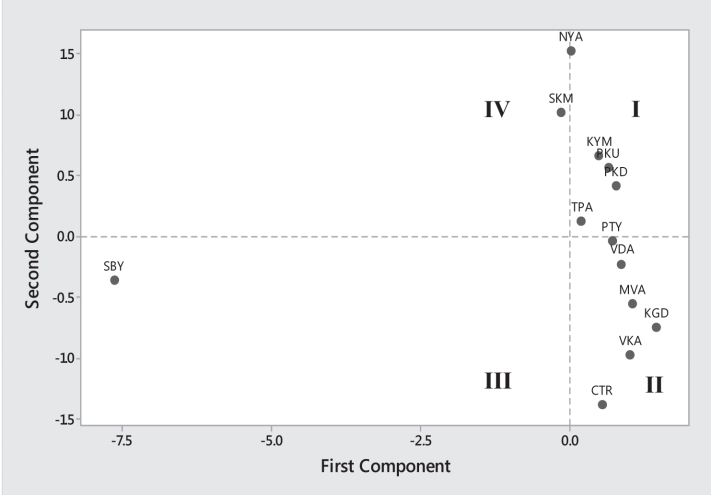

Fig. 11. Score plot of first two components for adult female *B. tabaci* collected from various locations

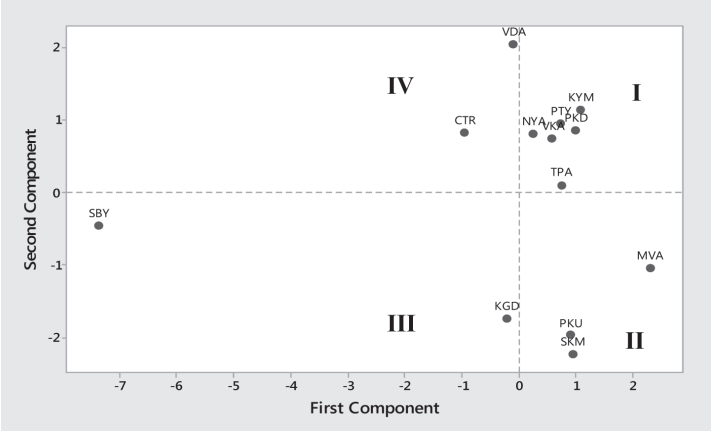

Fig. 12. Score plot of first two components for adult male *B. tabaci* collected from various locations

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. 12).

Score plot analysis of adult female and adult male did not provide any clear cut idea about the presence of different biotypes. But it was found that in case of both adult female and male, SBY populations found to be present in a separate group.

Conclusion

The present study to evaluate the presence of different genetic groups, using morphometric variations among whitefly populations collected from all agro-ecological zones of Kerala, India, using 14 different pupal characters and nine different adult characters provide the following conclusions:

i. Both in case of female and male pupa, whitefly populations from Sulthan Bathery (SBY) found to have lowest pupal length and highest pupal width. The populations collected from Sulthan Bathery also found to have the highest vasiform orifice length, operculum length, operculum width, lingula length, lingula width and distance between caudal setae between populations (except in case of operculum width for male pupa).

ii. In case of both adult female and adult male, whitefly populations from Sulthan Bathery found to have the highest antennal length and highest body width. For other morphometric characters studied, SBY populations had shown the lowest observations when compared with other 12 zones.

iii. Score plot analysis did not provide any clear cut idea about the presence of different

biotypes. But it was found that both in case of pupa and adult, SBY populations found to be present in a separate group.

Acknowledgement

We acknowledge AINPAO, Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur, Kerala and the Director, ICAR- Central Tuber Crops Research Institute (ICAR-CTCRI), Sreekaryam, Thiruvananthapuram, Kerala, for providing facilities and financial support for the study.

References

Chaubey, R. J., N. C. Naveen, and V. V. Ramamurthy. 2010. An illustrated taxonomic diagnosis of *Bemisia tabaci* (Gennadius) with additional notes on its life stages. *Ind. J. Entomol*., **72**: 321–325.

DeBarro, P. J., S. S. Liu., L. M. Boykin and A. B. Dinsdale. 2011. *Bemisia tabaci*: a statement of species status. *Ann. Rev. Entomol.,* **56**: 1-19.

Gill, R. J., and J. K. Brown. 2010. Systematics of *Bemisia* and *Bemisia* relatives: can molecular techniques solve the *Bemisia tabaci* complex conundrum-a taxonomist's viewpoint, pp 5–29. In: P. A. Stansly and S. E. Narahjo (eds.), *Bemisia*: Bionomics and Management of a Global Pest. Springer, Dordrecht, Germany.

Global Invasive Species Database (2016) Species profile: *Bemisia tabaci*. Downloaded from http://www.iucngisd.org/gisd/species.php?sc=106 on 20-12-2016.

Kalaisekar, A., V. V. Ramamurthy, J. V. Patil, A. Dhandapani, and N. S. Azad Thakur. 2012. Multivariate morphometrics of elytral colour polymorphism in seven-spotted ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *Curr. Sci*., **102**: 1418–1425.

Legg, J. and Fauquet, C. 2004. Cassava mosaic geminiviruses in Africa. *Plant Mol. Biol.*, **56**: 585-599.

Li, J., Q. Tang, R. Bai, X. Li, J. Jiang, Q. Zhai, and F. Yan. 2013. Comparative morphology and morphometrics of six biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from China. *J. Integ. Agri*., **12**: 846–852.

Malumphy, C. 2004: Diagnostic protocols for regulated pests *Bemisia tabaci*. OEPP/EPPO Bull., **34**: 281-288.

Malumphy, C., K. Walsh, M. B. Suarez, D. W. Collins, and N. Boonham. 2009. Morphological and molecular identification of all developmental stages of four whitefly species (Hemiptera: Aleyrodidae) commonly intercepted in quarantine. *Zootaxa,* **2118**: 1–29.

Misra, C. S. and S. K. Lambda. 1929. The cotton whitefly (*Bemisia gossypiperda* sp.). Bull. Agric. Res. Inst., Pusa, **196**: 1-7.

Palaniswami, M. S., Antony, B., Vijayan, L. and Henneberry, T. J. 2001. Sweet potato whitefly ecobiology, host interaction and its natural enemies. *Entomon.,* **26**: 256-262.

Palaniswami, M. S., Nair, R. R., Pillai, K. S. and Thankappan, M. 1996. Whiteflies on cassava and its role as vector of cassava mosaic disease in India. *J. Root Crops,* **22**: 1-8.

Tabachnick, B. G., and L. S. Fidell. 2007. Using multivariate statistics, 2nd ed. Allyn and Bacon, Boston, MA, USA.

Yan, F. M. 2001. Application of non- morphological characters in taxonomy of whiteflies (Homoptera: Aleyrodidae). *Entomotaxonomia,* **23**: 107–113.