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MORPHOMETRICS OF *BEMISIA TABACI* (GENNADIUS) (HEMIPTERA: ALEYRODIDAE) SPECIES COMPLEX FROM COTTON

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ABSTRACT
The whitefly *Bemisia tabaci* (Gennadius) is now recognized as a species complex and many of its genetic groups are important agricultural pests. *B. tabaci* specimens collected from three cotton cultivars were subjected to genetic characterization and morphometric studies. The cotton cultivars selected were namely HS1300, P86 and LRA5166. 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. Thus, this study adds to our knowledge of the host-associated morphological variations and genetic diversity of *B. tabaci* populations.

INTRODUCTION

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is currently recognized as a species complex with 28 genetic groups or morphologically indistinguishable species. Some groups within this species complex are important pests of a range of agricultural, horticultural, and ornamental crops. While the genetic complexity and economic importance of *B. tabaci* has been recognized, its species status has been the subject of debate for decades despite the considerable effort in investigating its taxonomy and systematics. Both immature and adults cause direct damage through feeding, production of copious amount of honey dew, and indirectly as a vector of the plant viruses, primarily *Begomoviruses* (Geminiviridae) (Kalaria *et al*., 2013). Until the findings of De Barro *et al*. (2011) these genetically different genetic groups of *B. tabaci* species complex were named as biotypes and a number of these had been reported for a long time. De Barro *et al*. (2011) concluded its nature as a species complex with 24 morphologically indistinguishable species on the basis of genetic distance and 4 more species were added by Hu *et al*. (2011) from China. Gill and Brown (2010) examined the species status and the morphological variations in puparia within *B. tabaci* and their relatives. Different genetic groups were found co-occuring in the same cotton field (Thomas *et al.,* 2014). This concluded that it is a complex of cryptic species which have evolved over time in isolation probably without the need to change morphologically, but these studies advocated for more thorough morphological studies.

Simultaneously, Tay *et al*. (2012) revealed that the original *B. tabaci* belongs to Mediterranean genetic group in their revision of the species complex through matching the *Aleurodes tabaci* collected in 1889, and on the basis of morphological and molecular data. The examination of type specimen revealed the morphological variation in cement gland of females, and lateral view of aedegues of male. These suggest the need for an intense study of the taxonomic characters of populations belonging to its genetic groups. This also implies the need for deciphering the genetic groups in more details, since as of now the groups are determined solely on the basis of DNA sequences. Hence, this study analyzes the intraspecific variations in the morphometrics of populations of *B. tabaci* from different cotton cultivars categorized on the basis of genetic differences. The results obtained are presented herein.

MATERIALS AND METHODS

Sample collection

B. tabaci specimens were collected from the cotton cultivars cultivated in the research farm of the Indian Agricultural Research Institute (IARI), New Delhi, India. The puparia (late fourth nymphal stage with red eyes) were collected along with leaf and kept in the petri plates with wet sponge for adult emergence were processed following the procedure of Philips and Jesudsan (2013). The newly emerged adults were preserved in 70% ethyl alcohol for molecular identification and simultaneously the puparial cases left in the petri plates were collected for morphometrics.

Morphometrics

The samples collected from the cotton cultivars were analysed

for the determination of genetic groups. A standard protocol was used for DNA isolation and PCR amplification using mtCO1 markers. PCR product was sequenced directly and the obtained sequence were aligned and compared with global data set (Boykin *et al*. 2007) for the determination of the genetic group. The samples studied were found to fall under three genetic groups namely Asia 1, Asia II-1 and Asia II-7. A sample of about 30 puparia collected from each of these genetic groups and was examined under Wild M8 stereozoom microscope. These were mounted on microslides following Jesudasan and David (1991) by initially soaking them in 10% KOH for 12- 24 hr followed by rinsing in ethyl alcohol. Specimens were then cleared in distilled water using fine entomological needle and fine brush and then dehydrated in 70% and 90% ethyl alcohol. Dehydrated specimens were kept in clove oil for clearing for 20 min and then mounted with DPX mountant.

These slide mounted specimens were examined for total of 62 characters helpful in defining *B. tabaci* (Takahashi, 1954; Jesudasan and David, 1991; Martin *et al*., 2000) under Leica DM 1000 phase contrast compound microscope at 10x-100x. The data obtained were subjected to single factor ANOVA for screening the statistically significant ones. Multivariate morphometric analyses through Principal Component Analysis (PCA; SAS procedure PRINCOMP, SAS version 9.1.3), was carried out on these significant ones to find out the characters that are distinctive in defining the genetic groups.

RESULTS AND DISCUSSION

Of the 62 characters converted to measurements or observations evaluated 48 characters showed significant variations when subjected to single factor ANOVA in terms of F value at the probability level ≤0.01 (Table 1). The results reveal that length of puparia, caudal furrow and operculum were more in Asia II-1 genetic group while breadth, length of antennae, and lingula were more in Asia I group. Length of vasiform orifice was more in Asia II 7 genetic group. The details of the highly significant characters are shown in Table 2. These 48 significant characters were considered for multivariate analysis and the first nine principal components obtained through PCA were selected using eigen values >1 and scree plot.

The scattergraph for the Prin 1 and 2 are shown in Fig. 1, which indicate that the *B. tabaci* genetic groups from the cultivar HS1300 (Asia II 1) fall in the quarter 4. The scattergraph of Prin 2 and Prin 3 shown in Fig. 2 reveal that the populations from cultivars P86 (Asia 1 group), HS1300 (Asia II 1 group) and LRA5166 (Asia II 7) are scattered in the quarters 1, 2, 3 and 4. Prin 1 plotted against Prin 2 revealed the separation of populations from the cultivar HS1300 (Asia II 1 group) from LRA5166 (Asia II 7 group) and P86 (Asia 1 group) (Fig. 1). Similar trend could be seen when the Prin 2 was plotted against Prin 3.

The taxonomic status of *B. tabaci* species complex has been problematic due to the continuous morphological differences which were considered by the taxonomists to represent intraspecific variations (Rossell, *et al.,* 1997). However genetic differentiation and behavioral differences among the populations had lead to a proliferation of alternative genetic groups or putative species (De Barro, *et al*., 2011). The approach herein investigating the morphological variations considering the three most commonly occurring genetic groups on cotton along with morphometrics has enabled separation of the Asia 1 group form the other two. Previous studies by Calvert *et al*. (2001) showed that morphological characters used to separate A and B biotypes were not useful to separate other biotypes. Recently Li *et al*. (2013) had reported that some characters could differentiate six biotypes or the genetic groups. Though length and breadth of puparia can vary depending on the nature of host plant leaf, the characters on antenna, vasiform orifice and lingula were found significantly varied between the genetic groups. Thus, the morphometric distinctions that have emanated in the present study could be applied to more genetic groups from different host plants and geographical regions to check their stability and reliability. The results indicate that there might be some morphological characters which can correlate with the genetic variation and these genetically and morphologically different groups might in future be identified as distinct species status.

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