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Diversity of whitefly *Bemisia tabaci* genetic groups and its implications on managementof this invasive pest

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ABSTRACT: Whitefly, *Bemisia tabaci* is a global invasive pest. The intra-species diversity in the whitefly, *B. tabaci*, presents a case study of several genetic groups observed to occur across the globe.As per the current understanding, it is considered as a species complex comprising of 44 genetic groups. Phylogeographic analysis using mitochondrially encoded Cytochrome C Oxidase-I gene as a molecular marker has widely been used as a tool for identification of *B .tabaci* genetic groups across the world. The association of endosymbionts with the biological attributes among the *B.tabaci* species complex is also illustrated. Although, two whitefly genetic groups, MEAM1 and MED have wide spread occurrence across the globe, Asian genetic groups like Asia I Asia II 1 and Asia II-7 are predominant in India and other Asian countries. Studies on heat shock tolerance, host utilization, salivary effector proteins and salivary polyphenoloxidase and peroxides, virus transmission and profile of various detoxification enzymes may throw more light on the biological attributes of dominant Asian genetic groups.The utility of other genetic markers such as nuclear genes and possibly microsatellite markers may also be explored to further support the hypothesis of invasiveness of certain whitefly genetic groups.

Key words: invasive whitefly, *Bemisia tabaci,* genetic diversity, management, India

Introduction

Whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), is listed among the top hundred invasive alien Species of the world by the International Union for the Conservation of Nature and Natural Resources

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(IUCN; http://www.issg.org/database/species/search.asp?st=100ss). As per the current understanding, *B.tabaci* has been regarded as a species complex comprising of 44 morphologically identical but genetically diverse subspecies (Kanakala& Ghanim, 2019). It is present in all continents except Antarctica. It has a host range of over 1000 species of agricultural and horticultural crops (http://www.issg.org/database). *B. tabaci* causes direct damage to the crops as a sap sucking pest and indirect damage as vector of more than 200 viruses belonging to seven different categories: geminiviruses, luteoviruses, closteroviruses, nepoviruses, carlaviruses, rod-shaped DNA viruses, and potyviruses (Zaidi *et al.*, 2017). Control of this pest is very difficult because of its wide host range, global invasiveness and ability to evolve resistance to insecticides. Whiteflies have evolved resistance against more than 40 active constituents of pesticides (Naveen *et al.,* 2017). Insecticide resistance, virus vector interactions, host plant plasticity are some of the biological traits which act as driving force for the spread of this invasive pest. This paper gives an overview of genetic diversity in *B.tabaci* with special reference to management of the pest.

B. tabaci taxonomic and biotype status

Whitefly, *B. tabaci*, was initially described as *Aleurodes tabaci* (Gennadius), as a pest of tobacco crop from Greece in 1889. Taxonomic descriptions and revisions over the years rename this pest under 22 different names, owing to the morphological plasticity of nymphal stages of *B. tabaci*. However, all the subsequent redescriptions later on had been clubbed as synonymous with *B. tabaci.* The biotype concept came into existence in *B. tabaci,* consequent of the invasion of a *B. tabaci* population in southern United States during 1980s. Biochemical studies based on esterase profile and host plant range revealed that the invasive species was different from the indigenous populations. The invasive population was designated as 'B biotype', while the native populations was referred to as the 'A biotype'. Later on, the characterization of allozymes and techniques like RAPD-PCR (randomly amplified polymorphic DNApolymerase chain reaction) confirmed significant differences between A and B biotypes. A series of subsequent biological and molecular investigationshave led to the identification of 11 biotypes (A, B, B2, D, E, G, H, K, J, L, and M) of *B. tabaci*. Of these 11 biotypes tested, only B and B2 could cause specific physiological changes in host plants. Earlier, it was held that B biotype could be distinguished from other biotypes by its capacity to induce squash silver leafing, but, this capacity has since been observed for the B2, M5, and J groups in subsequent studies. There were other traits such as the dispersal capacity and insecticide resistance but clear lack of quantified measures, the biotypes could not be separated across the whole of *B. tabaci*. The A and B biotypes could not be conclusively separated based on initial examinations on morphological characters of fourth instar which are the principal species distinguishing features in whitefly taxonomy. But based on certain additional characters such as absence of the fourth anterior margin setal pair, width of the thoracic tracheal folds, and width of wax extrusions from the folds B and A biotype could be separated. Mating studies revealed that they were incompatible. This has led to re-description of 'B' biotype as *B. argentifolii* Bellows and Perring, 1994 (Bellows *et al.* 1994). However the separate species status for *B. argentifolii* could not sustain long due to insufficient biological data and for lack of clear picture on phylogenetic structure of *B. tabaci.*

Phylogeographic analysis of *B. tabaci*

The degree of genetic relatedness has to be relied upon to separate the groups across the whole of *B. tabaci* species complex. Although, the esterase banding pattern, allozymes and RAPDs could provide some insights into genetic variability within *B. tabaci*, the deployment of molecular markers such as mitochondrial cytochrome oxidase 1 (*mtCO1*) and nuclear markers such as Internal transcribed spacer-1 (*ITS1*) uncovered the phylogenetic structure of *B. tabaci* groups. The global relationships of *B. tabaci* could be clearly resolved by comparisons of a large number of individual sequences of *B. tabaci* populations through Bayesian analyses. Frolich *et al.*, (1999) was the first to do phylogeographic analysis of ten*B. tabaci* collections across the Middle East, New World, North Africa and India, by using molecular markers based on mitochondrial CO1 (*mtCO1*) gene and it has become the preferred marker of choice for phylogeographic analysis of *B. tabaci* populations in the years to come. Boykin *et al.*, (2007) generated a dataset by retrieving *mtCO1sequences* of *B. tabaci* populations from GenBank. Subsequent analysis by Dinsdale *et al.*, (2010) clearly defined the quantifiable limits to subdivide the *B. tabaci* groups; two clear sub divisions were suggested one at one at 11% and the other at 3.5% for assigning the genetic group and sub groups of *B. tabaci*. A consensus data set was curated with which the *B. tabaci* genetic groups could easily be identified by using Bayesian analysis. A number of newer genetic groups have been added since the original descriptions based on subsequent studies (De Barro *et al.* 2011; Firdaus *et al.* 2013; Ellango *et al.* 2015; Hu *et al.* 2018) and as per a recent study (Kanakala and Ghanim, 2019), *B. tabaci* is a species complex composed of at least 44 morphologically indistinguishable species.

Global genetic diversity of *B .tabaci*

Kanakala and Ghanim, 2019 explored the global genetic diversity of *B. tabaci* species complex by analysis of available 4,253 mitochondrial cytochrome oxidase I (mtCOI) sequences, retrieved from Gen Bank. Using Bayesian phylogenetic analysis, it described the global distribution of *B. tabaci* species complex. On the basis of 4.0 % pair wise divergence in *mtCOI* sequences within *B. tabaci* species, 44 distinct genetic groups have been identified. Asian continent contains the highest number of *B. tabaci* species, with a total of 28 native and invasive species distributed across 13 countries. China represents the highest distribution of 16 *B. tabaci* species followed by India with 11 species of the *B. tabaci* species complex (India Asia I, Asia I-India, Asia II 1, Asia II 5, Asia II 7, Asia II 8, Asia II 11, Asia II 13, MEAM K, China 3, MEAM1).

Invasive groups of *B. tabaci* species complex

The diversity of *B. tabaci* genotypes in many geographical regions has seen dramatic changes in the recent past with the establishment of invasive *B. tabaci* genetic groups, MEAM1 and MED commonly referred to as B and Q biotypes (De Barro *et al.*, 2011). A driving factor in the establishment of *B. tabaci* B and Q biotypes in more than 40 countries across the globe has partly been driven by their insecticide resistance traits. The invasiveness of these genetic groups of B. *tabaci* has been attributed to virus transmission capability (Brown, 2007), insecticide resistance (Horowitz and Ishaaya, 2014) and other biological attributes which have enabled them to displace the native/indigenous genetic groups in several countries across the globe. In recent times the MEAM 1 and then MED those have spread

globally with MEAM1 reported from 42 countries while MED from 44 countries, showing that these species are themost highly diverse and distributed on a worldwide scale (Kanakala and Ghanim, 2019). However, indigenous genetic groups like Asia I and Asia II-1 have prevailed over invasive biotypes like B in the Indian subcontinent region owing to its improved virus transmission ability and resistance to insecticides (Naveen *et al.,* 2017).

Compared to the two invading species, MEAM1 and MED, currently our knowledge on indigenous members of *B. tabaci* complex in Asia is very limited. Samplings of *B. tabaci* populations in Asian countries in the recent past have shown that Asia II group extends from Asia Minor through the Indian Subcontinent into China. Analysis of the genetic diversity of Asia I Asia II 1 in the Indian subcontinent revealed that the average number of pairwise nucleotide differences **(k)** and nucleotide diversity **(π)** were relatively higher in Asia 1 (n= 77) and Asia II 7 (n =14) than in Asia II 1 (n= 551), the most common species in the region. Haplotypes network analysis revealed 29 haplotypes among the 551 sequences of Asia II 1 from Pakistan and India. One haplotype was dominant (63%), occurring in all populations from both countries and in all cotton-growing areas of Pakistan. Three other haplotypes with a relatively high frequency (7%) and two with a low frequency (1%) were also found in both the countries. There were seven Asia II 1 haplotypes unique to India and 16 unique to Pakistan (Ashfaq *et al.*, 2014). A detailed survey by Ellango *et al.*, (2015) established the predominance of Asia II-1 in Northern India and Asia I in southern India.

MEAM 1 has proved to be particularly adept at displacing other members of the species complex. But, it has not been so in Punjab, Pakistanand regional abundance of Asia II one might be sufficient to prevent invasion by MEAM1 (Ashfaq *et al.*, 2014). Similarly, a variant of B biotype (MEAM K) was reported from India (Roopa *et al.*, 2015).However it is found to be non invasive and it has so far been confined only to the Kolar region of Karnataka state in India. Considering the widespread distribution of *B. tabaci* Asia II-1 in Northern India and documentation of strong insecticide resistance *B. tabaci* populations from the regions adjoining the Pakistan, Asia II-1 has become the most diverse *B.tabaci* genetic group in vast stretches of land area in India.

The well resolved phylogeny of *B. tabaci s*pecies could be analyzed using molecular dating. The molecular clock analyses of *mtCO I* data elucidated that the genus *Bemisia* separated from the other members of the subfamily around 86 million years (mya). The majority of the *B. tabaci* species complex, diversified between 6030 mya. The diversity of Asian species in the *B. tabaci* species complex started around ~36 mya. While, divergence of Asia II occurred around 28 mya, Asia 1 diversification could be dated to 11 mya (Boykin *et al.,* 2013). The highly invasive species MED and MEAM1 diverged from the Indian Ocean species around 12 mya. Shannon entropy (http://www.hiv.lanl.gov), a measure of variation in DNA and protein sequence alignments calculated for *B. tabaci* species complex revealed that all the species other than MED and MEAM 1 were found to have higher entropy scores indicating that these two are the "breakout" species in the complex. It was speculated by Boykin *et al.,*(2013)that based on molecular divergence data, the two predominant members of *B. tabaci* in Asian region *viz.*, Asia II 1 and Asia I may turn out to be the breakout species of Asian members of *B. tabaci* species complex as like MED and MEAM 1. However, further analysis like Shannon entropy of Asian

members of *B. tabaci* in relation to Asia I and Asia II 1 may confirm their breakout status.

Biological traits of *B.tabaci* influenced by endosymbionts

The intracellular symbionts, display strict host association referred as 'obligate' are essential for the host and are maternally transmitted. Whitefly, *B.tabaci* harbours an obligate symbiont, *Porteira aleyrodidarum* and a number of facultative or secondary symbionts like *Arsenophonus*, *Cardinium*, *Hamiltonella*, *Wolbachia*, and *Ricketsia*. These secondary endosymbionts, although non-essential offer certain adaptive capabilities such as high temperature tolerance, host plat plasiticity and resistance to parasitoids. Several earlier studies have shown that insecticide susceptibility levels of insect populations vary with the infection frequencies of secondary symbionts (Kontsedalov *et al.*, 2008; Ghanim and Kontsedalov, 2009). Increased susceptibility of B biotype populations had been correlated with the presence of *Rickettsia (*Kontsedalov *et al.*, 2008) and it was speculated that *Rickettsia* might have possessed some fitness for its whitefly host making it vulnerable to the environmental stress.Elaborate studies are needed to assess temporal shifts in the infection frequencies of endosymbionts and their role in fitness adaptive traits of Asian genetic groups of whitefly, *B. tabaci*.

Conclusions and way forward

With the large diversity of *B. tabaci* and parasitoid diversity present in the Indian subcontinent (Evans, 2008) supports the hypothesis that this region represents an important Old Worldcenter of diversification and evolution of *B. tabaci* (http://www.issg.org/database). Diversity in the cropping systems in different agro climate zones of India provides an ideal platform for proliferation of *B. tabaci* throughout the year. Short life cycle and host plant plasticity enable the whiteflies being subjected to continuous selection pressure due to insecticides which are the mainstay of plant protection paradigms. There is a need for regular monitoring, spatial and temporal distribution of *B. tabaci* genetic groups for sustained management of *B. tabaci* in the Indian subcontinent.

Further studies on heat shock tolerance, host utilization, salivary effector proteins and salivary polyphenoloxidase and peroxides, virus transmission, profile of various detoxification enzymes may throw light on the biological traits of dominant Asian genetic groups Asia I and Asia II 1 which would provide insights into the evolution perspectives of the invasiveness of these genetic groups. The utility of other genetic markers such as nuclear genes and possibly microsatellite markers may well be explored to further support the hypothesis of invasiveness.

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