MITOCHONDRIAL DNA VARIATION OF *BEMISIA TABACI* (GENNADIUS) (HEMIPTERA: ALEYRODIDAE) INFESTING CASSAVA IN KENYA

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Abstract
Bemisia tabaci is a widely distributed crop pest affecting the yield of a broad range of agricultural, fiber, vegetable and ornamental crops. It is an extremely polyphagous pest that causes direct damage and can act as a vector of viral plant diseases. Populations of *Bemisia tabaci* that are morphologically indistinguishable and with different biological traits have been known to exist; they show differences in rates of development, host range, insecticide resistance and virus transmission efficiencies. The objective of this study was to investigate the biotype identity of *B. tabaci* infesting cassava in Kenya using the mitochondrial cytochrome oxidase I (mtCOI) sequence as a molecular marker. The mitochondrion cytochrome oxidase one gene fragments were phyologenetically analyzed using neighbor joining method. Results revealed two distinct haplotypes of cassava associated *B. tabaci* in Kenya. The first haplotype consisted *B. tabaci* collected from the Eastern and Coast provinces of Kenya in a sub-cluster with 68 boot strap value. They closely resembled *B. tabaci* genotypes from the southern parts of Africa (Mozambique, South Africa, Swaziland and Zambia) and *B. tabaci* isolate from West Africa (Ghana). The second haplotype comprised of *B. tabaci* collected from cassava growing regions in Nyanza and Western provinces of Kenya in a sub-cluster with 94 boot strap value. They shared close sequence homology with cassava *B. tabaci* from Uganda and Tanzania. The findings of this study form part of important documentation on *B. tabaci* biotype status in Kenya for pest management purposes and for the prevention of invasive biotypes which may be more dangerous.

Key words: Mitochondrial, DNA, *Bemisia tabaci,* cassava, Kenya

1.0 Introduction

Bemisia tabaci (Gennadius) (Hemiptera Aleyrodidae) is one of the most devastating tropical and sub-tropical agricultural pest (Byrne and Bellows, 1991; Brown *et al.,* 1995; Oliveira *et al.,* 2001) affecting the yields of a broad range of agricultural crops (Cahill *et al.,* 1996). It has been associated with more than 100 viral diseases which include Begomoviruses, Criniviruses, Carlaviruses and pomoviruses (Brown 1994, 2000; Maruthi *et al*., 2004a).

In cassava*, B. tabaci* pests cause direct damage to crops as a result of feeding on the phloem contents, and indirectly from excretion of honeydew onto the surfaces of leaves which results into the growth of sooty mold fungi, further interfering with photosynthesis (Byrne and Bellows, 1991). In addition, *it* is the vector of two most economically important cassava viral-pathogens, namely begomoviruses such as the African cassava mosaic virus (Harrison *et al*., 1986) which causes cassava mosaic diseases (CMD) and *Cassava brown streak virus* which causes cassava brown streak disease (CBSD) (Monger *et al.,* 2001). The serious damage caused by this whitefly pest in many countries has led researchers to study its biological and ecological characteristics in order to devise effective control strategies (Brown *et al*., 2000; De Barro *et al*., 2000, 2003).

Research outcome now reveals that morphologically indistinguishable populations exhibiting different biological traits exist within the *B. tabaci* species complex (Bird, 1957; Costa and Russell, 1975; Bedford *et al.,* 1994). The distinct biological traits include host-plant range and adaptability (Costa and Russell, 1975; Burban *et al*., 1992), inducement of plant physiological disorders (Costa and Brown, 1991; Perring *et al*., 1991) and plant virus transmission efficiency (Bird and Maramorosch, 1978; Bedford *et al*., 1994; Maruthi *et al*., 2002). Bird (1957) referred to the morphologically indistinguishable *B. tabaci* populations, as biotypes or host-races.

Recent studies of genetic variability involving certain African *B. tabaci* populations have revealed at least several indigenous *B. tabaci* haplotypes (Brown 2000, Kirk *et al*., 2000; Maruthi *et al.,* 2002; Abdullahi *et al*., 2003, 2004; Berry *et al*., 2004; Sseruwagi *et al.,* 2005b). *B. tabaci* diversity studies in Africa and indeed Kenya are important and specially geared towards monitoring the dynamics of cassava-associated *B. tabaci* populations and potentially to reveal new genetically distinct biotypes on cassava in Africa. This study was therefore initiated to study the genetic variability of cassava-associated *B. tabaci* from the four cassava growing provinces in Kenya using the mitochondrion cytochrome oxidase 1 (mtCOI) gene as a molecular marker.

2.0 Materials and Methods

2.1 Collection of Whiteflies

A *B. tabaci* collection survey was conducted in the year 2009/2010 in four major cassava growing provinces of Kenya; Western, Eastern, Nyanza and Coast provinces. Depending on availability of whiteflies, collections were made in at least one district per province. *B. tabaci* adults were collected from cassava in farmers' cassava fields or research centres (Table1). One sample was collected from egg plants that were growing adjacent to cassava in Coast province. Whiteflies were collected using an aspirator and transferred into well labelled falcon tubes containing 10mls of absolute ethanol. All the samples were stored at -20ºc until they were analyzed to establish their diversity. The collection sites are shown in table1.

2.2 Extraction of whitefly DNA

Ten whiteflies were selected randomly from each sample for DNA extraction. Total nucleic acids were extracted from individual adult whiteflies according to Frohlich *et al*. (1999) with some modifications. Individual whiteflies were placed on a section of parafilm and ground in 50 µL of ice-cold lysis buffer (10 mM Tris-HCl, pH 8.0, 1.0 mM EDTA pH 8.0, 0.1 M NaCl) using the tip of a 1ml blue tip. The lysis products were transferred from the parafilm into a microfuge tube using a 100µl pipette and then centrifuged at 10 000rpm for ten minutes to pellet the debris. The aqueous supernatant was used as the source of DNA for polymerase chain reaction (PCR) amplification

2.3 Polymerase Chain Reaction (PCR) Analysis

Polymerase chain reaction (PCR) was conducted to amplify a fragment (~ 800 bp) of the mtCOI gene using primers MT10/C1-J-2195 (5´-TTGATTTTTTGGTCATCCAGAAGT-3´) and MT12/L2-N-3014 (5´-CCAATGCACTAATCTGCCATATTA-3´) (Simon *et al*., 1994) per Frohlich *et al*. (1999). For each sample, a 25 µl reaction mixture was made up containing 3.0 µl DNA template, 2.5 µl 10x reaction buffer, 1.5 µl of 25 mM $MgCl_2$, 1.0 µl of 10mM dinucleotide

triphosphates (dNTPs), 1.0 µl each of primers MT10 and MT12 at 20 pm concentration and 0.25 µl Taq polymerase (Invitrogen). PCR conditions consisted of an initial denaturation at 95°C for 2 min (initial denaturation), followed by 1 min at 95°C (denaturation), 1 min at 52°C (primer annealing) and 1min at 72°C (amplification) for 30 cycles. A final extension of 10 min at 72°C was included and the reaction was held at 10 °C in a Gene Amp PCR System 9700 thermal cycler (Applied Bio-Systems). The PCR products were analysed using 1% agarose gel in 1.0x TBE buffer stained with ethidium bromide. Amplified DNA was viewed under UV trans-illumination.

2.4 Amplicon Purification, Sequencing and Phylogenetic Analysis

The amplified DNA of the expected size was purified using a QIAquick PCR Purification Kit (Qiagen Inc, USA), following the manufacturer's instructions. Purified DNA was bi-directionally sequenced at the Segolip Laboratory (ILRI) Kenya using the PCR primers MT10 and MT12 as sequencing primers in an automated sequencer (ABI 3700). Whitefly mtCOI sequences generated using the reverse primer were used for analysis in this study. They were blasted in the NCBI gene bank in order to confirm that they were the expected sequences. Three closely related neighbours in blast were selected for each query sequence. Sequences were manually edited using Chromas version 2.33, Technelysium Pty. Ltd) and aligned using the Clustal W (weighted) algorithm option in the MEGA 4.0 computer programme (Tamura *et al*., 2007). They were compared with *B. tabaci* reference mtCOI sequences available in the NCBI GenBank databases (Table 2). Some of the closely related neighbours in BLAST were selected for use as reference sequences in phylogenetic analysis. More reference whitefly mtCOI sequences were obtained from previous studies carried out around the world and were retrieved from the Genebank using their respective accession numbers (Table 2).

Phylogenetic analysis was done using the Neighbor-Joining method (Saitou and Nei, 1987). The genetic distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004). All positions containing gaps and missing data were eliminated from the dataset by (Complete deletion option). Phylogenetic analyses were conducted in MEGA 4 (Tamura *et al.*, 2007).

3.0 Results

3.1 PCR Amplification of mtCOI DNA

A total of 18 samples obtained from each of the four major cassava-producing regions of Kenya (Table1), were used to study the genetic diversity and distribution of cassava-associated *B. tabaci* genotypes in Kenya. A PCR fragment of the *mtCOI* gene (~800 bp) was obtained for each sample examined using the primer pair MT10/C1-J-2195 and MT12/L2-N-3014 (Figure 1).

3.2 Phylogenetic Analysis of Whitefly mtCOI Sequences

The edited sequences were subjected to pairwise and multiple alignments and a Phylogenetic tree that was predicted using neighbour joining method available in phylogenetic analysis with heterogenous pattern among lineages corresponding with gamma distribution. A nucleotide maximum composite likelihood model is shown (Figure 2).

Based on the phylogenetic analyses of the mtCOI sequences, members of the *B. tabaci* complex under this study grouped into three main clusters (Figure 2). All three major clusters were supported by high bootstrap (BS) value of 100, with several other sub-groups having BS scores of >60. The first main cluster comprised of genotypes from the Far East Old World (China, Pakistan, and Thailand). The second, main cluster comprised three members namely *B. tabaci* from the West Africa region, including Ivory Coast and Reunion Island. The third member of this cluster was *B. tabaci* isolate C7-KE from egg plant. It was highly similar with the *B. tabaci* isolate from Reunion Island in a strongly supported cluster with a BS value of 100.

The cassava-associated *B. tabaci* genotypes from sub-Saharan Africa and those under this study grouped into two distinct sub-clusters. *B. tabaci* in the first sub-cluster with a BS value of 94 were from the western region of Kenya and they shared close sequence homology with the *B. tabaci* from Uganda (UgTcNn45, Nkosi 2.2, 5MasaK, clone 30 Kych, clone 19MbaB), Tanzania (clone BuTZ) and Kenya (isolate 5) (Figure 2). The second sub-cluster with a BS value of 68 comprised isolates from the coastal and eastern cassava producing regions in Kenya. They closely resembled *B. tabaci* genotypes from the southern parts of Africa including Mozambique (Moz1), Malawi (clone 21

Malawi), South Africa (Lucia 6 and Lucia 9), Swaziland (SwMap 14), Zambia (Zamb2) and Ghana (isolate IMIDA) ((Figure 2).

4.0 Discussion and Conclusions

This study established the genetic variability of *B. tabaci* populations infesting cassava in Kenya using the *mtCOI* gene as the molecular marker. The mtCOI analysis separated *B. tabaci* infesting cassava in Kenya into two distinct haplotypes. The first haplotype comprised *B. tabaci* from the Kenyan coastal and eastern regions. Their closest relatives were found to have an origin in Mozambique (Moz1), Malawi (clone 21 Malaw), South Africa (Lucia 6 and Lucia 9), Swaziland (SwMap 14), Zambia (Zamb2) and Ghana (isolate IMIDA). This finding is similar to the study findings of Legg *et al*. (2002) which revealed two haplotypes (Ug 1 and Ug 2) of *B. tabaci* associated with cassava in Uganda. The closest relatives of Ug 1 haplotype were from Zambia, South Africa and Swaziland. In contrast, the closest relative identified for the second haplotype comprising *B. tabaci* from the western region of Kenya had an origin in Uganda (UgTcNn45, Nkosi 2.2, 5MasaK, clone 30 Kych, clone 19MbaB), Tanzania (clone BuTZ) and Kenya (isolate 5). This supports the findings of Abdullahi *et al*. (2003) in which one sample collected from cassava in Busia Kenya (kenya-cassava 60) closely related a Uganda-cassava 50 in a sub-cluster consisting other B. tabaci samples from Democratic Republic of Congo.

One sample of *Bemisia tabaci* collected from egg plant in Coast province was included in the analysis and was found to share close genetic similarity (99%) to the non cassava *B. tabaci* genotypes from Reunion Island and Ivory Coast. It was clearly separated from all African cassava populations, a result which is consistent with biological evidence for the host restriction of cassava and noncassava *B. tabaci* populations (Burban *et al*., 1992; Legg, 1996).

In this study, the mtCOI sequence was highly informative as a molecular marker in that it was useful for distinguishing the two distinct haplotypes of B. tabaci associated with cassava (the coastal-eastern haplotype and the western haplotype) in Kenya. Bemisia tabaci infesting cassava in the eastern coastal Kenya may be geographically isolated from the ones in the western region of the country due to the long distance between the two regions. This further makes it difficult for farmer to farmer exchange of planting materials thus limiting the transfer of the insect pest between the two regions. However, there was close genetic similarity between the western region populations in this study and the Uganda genotypes which may be attributed to the close proximity of this region to the cassava producing regions in Uganda that facilitates the exchange of cassava planting materials between the two regions.

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