Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera:Aleyrodidae) colonizing sweet potato and cassava in South Sudan

Beatrice C. Misaka¹, Everlyne N. Wosula², Philip W. Marchelo-d’Ragga¹, Trine Hvorsleb-Eide³,* and James P. Legg²

¹ Department of Agricultural Science, School of Natural Resources and Environmental Sciences, University of Juba, P. O. Box 82 Juba, South Sudan.
² International Institute of Tropical Agriculture, Dar es Salaam, Tanzania
³ Department of Plant Sciences, Norwegian University of Life Sciences (NMBU), P.O. Box 5003, 1432 Ås, Norway.
* Correspondence: trine.hvosleb-eide@nmbu.no; Telephone No. +4793433775

Abstract: *Bemisia tabaci* (Gennadius) is a polyphagous, highly destructive pest capable of vectoring viruses in most agricultural crops. Currently, information on the distribution and genetic diversity of *B. tabaci* in South Sudan is not available. The objectives of this study were to investigate the genetic variability of *B. tabaci* infesting sweet potato and cassava in South Sudan. Field surveys were conducted between August 2017 and July and August 2018 in 10 locations in Juba County, Central Equatoria State, South Sudan. Sequences of mitochondrial DNA cytochrome oxidase I (mtCOI) were used to determine the phylogenetic relationships between sampled *B. tabaci*. Six distinct genetic groups of *B. tabaci* were identified including three non-cassava haplotypes (Mediterranean (MED), Indian Ocean (IO) and Uganda) and three cassava haplotypes (Sub-Saharan Africa 1 sub-group 1 (SSA1-SG1), SSA1-SG3 and SSA2). MED predominated on sweet potato and SSA2 on cassava in all the sampled locations. The Uganda haplotype was also widespread, occurring in five of the sampled locations. This study provides important information on the diversity of *B. tabaci* species in South Sudan. A comprehensive assessment of the genetic diversity, geographical distribution, population dynamics and host range of *B. tabaci* species in South Sudan is vital for its effective management.

Keywords: *Bemisia tabaci*; genetic diversity; distribution; haplotype

1. Introduction

Cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batatas* (L.) Lam.) are key staple root crops that assure food security in sub-Saharan Africa. This is due to their high calorie content, low production inputs, adaptation to different soil types and resilience to climatic change compared to other major staple food crops [1-4]. Total production of cassava in Africa amounts to 177.8 million tonnes, while that of sweet potato is 27.7 million tonnes [5]. In South Sudan, cassava is the major food security crop after maize or sorghum in the Greenbelt and the Ironstone Plateau zones which include Western, Central and Eastern Equatoria (Greenbelt zone), Western Bahr el Ghazal State (Ironstone Plateau zone), and Lakes State [6]. In 2015, the estimated production area for cassava was 75,910 ha and total production was 1.1 million tonnes. These estimates, however, may not represent the actual production due to the ongoing civil unrest in the country [6]. Like cassava, sweet potato is widely grown by farmers in the cassava-producing areas of South Sudan. It is the third most widely grown crop in Eastern and Western Equatoria states after cassava and groundnut [7]. Data on cultivation area and production of sweet potato are not available. Due to the ongoing war, agricultural surveys...
are mostly done by non-governmental organizations, which focus most on major staple crops like maize, sorghum, cassava and groundnuts.

One of the major biotic factors that constrains the production of cassava and sweet potato in sub-Saharan Africa is virus diseases [8-13]. The most important virus diseases of cassava in sub-Saharan Africa are cassava mosaic disease (CMD) caused by cassava mosaic begomoviruses and cassava brown streak disease (CBSD) caused by cassava brown streak ipomoviruses [14-16]. The damage caused on cassava by CMD and CBSD can result in up to 82% yield losses [9,17]. CMD is prevalent wherever cassava is grown. In the 1990s, CMD was reported to be the most destructive virus disease in Western Equatoria Province in Southern Sudan before the independence of South Sudan from Sudan [18]. The cassava mosaic begomoviruses, *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), and *East African cassava mosaic virus-Uganda* (EACMV-UG) have been reported to be prevalent in South Sudan [7,19]. *Sweet potato chlorotic stunt virus* (SPCSV; genus *Crinivirus*) is the most important virus affecting sweet potato due to its ability to mediate severe synergistic disease with several other sweet potato-infecting viruses which results in major yield losses [12,20-22]. SPCSV is a component of sweet potato virus disease (SPVD), the most devastating virus disease of sweet potato worldwide, which is caused by co-infection of SPCSV and *Sweet potato feathery mottle virus* (SPFMV), an aphid-transmitted potyvirus [23-26]. Yield losses due to SPVD can amount to between 50% and 100% in East Africa [12,26,27]. The occurrence of SPCSV and SPFMV has been detected in South Sudan in a survey of sweet potato viruses conducted in 2015 in the sweet potato growing areas of Western Bahr el Ghazal, Eastern and Central Equatoria states [28].

*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a polyphagous highly destructive pest of food, fiber and ornamental crops globally [29,30]. It is known to damage host plants directly by feeding on phloem sap, and indirectly by excreting honeydew onto the surfaces of leaves, fruits and fiber, and transmission of plant viruses [29,31,32-34]. Honeydew secretion promotes the growth of sooty mold fungi (*Capnodium* spp.) on the surfaces of leaves and fruits and the contamination of cotton lint resulting in low quality grading [32,33]. *B. tabaci* causes its most severe crop damage through vectoring plant viruses. The insect is a vector of more than 350 plant virus species belonging to five genera including begomoviruses (family *Geminiviridae*), *Criniviruses* (family *Closteroviridae*), ipomoviruses (family *Potyviridae*), torradoviruses (family *Secoviridae*), and some carlaviruses (family *Betaflexiviridae*). Most of the viruses transmitted are begomoviruses [30,34,35-37]. *B. tabaci* is a vector of cassava mosaic begomoviruses (CMBs) and cassava brown streak ipomoviruses (CBSIs) that devastate cassava crops in East and Central Africa [38-40]. SPCSV (crinivirus) is transmitted by *B. tabaci*, *B. afer* sensu lato and *Trialeurodes abutilonea* (the banded-winged whitefly) [41-43]. Other viruses of sweet potato vectored by *B. tabaci* include *Sweet potato leaf curl virus* (SPLCV), *Ipomoea leaf curl virus* (ILCV) (begomovirus) and *Sweet potato mild mottle virus* (SPMMV) (ipomovirus) [43-47]. Outbreaks and spread of CMD and CBSD in cassava have been linked to the super-abundance of *B. tabaci* populations [9,10,48]. Rapid spread of SPCSV has also been associated with high *B. tabaci* populations, leading to high incidences of SPVD [49-51].

*Bemisia tabaci* is genetically complex with at least 34 cryptic species that are morphologically indistinguishable. These have been identified mostly through sequencing of a portion of the mitochondrial cytochrome oxidase I (mtCOI) gene [52-57]. In sub-Saharan Africa, *B. tabaci* is an important vector for plant viruses that infect cassava, sweet potato and other crops including tomato, cucurbits, eggplant, cotton and leguminous crops [48,58-61]. Two major groups of *B. tabaci* are known to occur in sub-Saharan Africa. One group colonizes sweet potato, vegetables and other crops but does not colonize cassava (non-cassava types). This group includes several putative species, including: Indian Ocean (IO), Mediterranean (MED), Middle East Asia Minor 1 (MEAM1) and Uganda [60,61]. The other cassava-colonizing group includes: Sub-Saharan Africa 1 to 5 (SSA1–5).

SSA1 has been separated into 5 sub-groups: SSA-subgroup1 (SSA1-SG1), SSA1-SG2, SSA1-SG3, SSA1-SG4 and SSA1-SG5 [10,58,62-65].

The mitochondrial DNA cytochrome oxidase I (mtCOI) marker has been the most widely used marker for phylogenetic studies of *B. tabaci*. It has a high degree of variability and has played an important role in characterizing genetic relationships between the *B. tabaci* cryptic species and...
haplotypes [53,56,66]. MtCOI has been used extensively in assessing the genetic variability, phylogeographic distribution, and identification of new invasive species of *B. tabaci* in Africa [10,60,62,67], and elsewhere [68-70]. Recent SNP-genotyping using NextRAD sequencing, however, revealed that mtCOI sequencing is not completely effective at distinguishing cassava-colonizing *B. tabaci* genotypes [71]. These were reclassified into six major groups designated: Sub-Saharan Africa East and Central Africa (SSA-ECA), Sub-Saharan Africa East and Southern Africa (SSA-ESA), Sub-Saharan Africa Central Africa (SSA-CA), Sub-Saharan Africa West Africa (SSA-WA), Sub-Saharan Africa 2 (SSA2) and Sub-Saharan Africa 4 (SSA4) [71,72]. Information on the occurrence and distribution of *B. tabaci* in sub-Saharan Africa is available, but there are no data for South Sudan. Assessing the nature of the problem posed by *B. tabaci* and the viruses it transmits in South Sudan and developing appropriate control strategies are currently impeded by the instability caused by the ongoing civil war. Data are urgently required on the genetic groups, haplotype diversity, geographical distribution and the phylogenetic relationships of *B. tabaci* in South Sudan. As a first step, this study sought to address this need by sampling and characterizing *B. tabaci* collected from cassava and sweet potato in Juba county, Central Equatoria State, South Sudan. We therefore aimed to provide the first description of the diversity of *B. tabaci* on sweet potato and cassava in South Sudan.

2. Materials and methods

2.1. Whitefly sampling

Adult whiteflies (*Bemisia tabaci*) were collected from sweet potato and cassava fields across 10 locations in Juba County (Central Equatoria State, South Sudan) between July and August 2018 (Table 1 and Figure 1). Whiteflies were also sampled from tomato and squash plants adjacent to sweet potato and cassava fields. *B. tabaci* adults collected from sweet potato plants in greenhouses at the University of Juba in August 2017 were also added to the field collections. In total 24 fields were sampled from the 10 locations. Whiteflies were aspirated alive and immediately preserved in 95% ethanol in vials, before being stored in the freezer at -20°C. Sweet potato and cassava leaves that contained *B. tabaci* nymphs were cut into small pieces, put into vials and also preserved in 95% ethanol before being stored in the freezer. *B. tabaci* were collected from several plants in each sampled field and at least 20 whiteflies were collected from each field.

2.2. DNA Extraction

DNA was extracted from single whiteflies, which were either adults or fourth instar nymphs. Insects were added to 3µl of lysis buffer in a 1.5ml Eppendorf tube then macerated. The lysis buffer contained 10mM Tris-HCL (pH 8.0, 50 mM KCL, 2.5 mM MgCl₂, 0.45% Tween-20, 0.01% Gelatine, and 60 µg/ml Proteinase). The mixture was then vortex shaken and spun down and immediately incubated on ice for 15 min. This was followed by incubation at 55°C in a water bath for 30 min and the lysate was stored at -20°C for downstream use. For PCR use, the lysate was diluted using sterile DPEC treated water in a ratio of 1:9.

| Host plant | Area/Payam  | Sampling site          | Latitude (°N) | Longitude (°E) | Elevation (masl) | Date        |
|------------|-------------|------------------------|---------------|---------------|-----------------|-------------|
| Cassava    | Rajaf West  | Lologo 2               | 04° 48.456'   | 31° 35.463'   | 468             | 31.07.2018  |
|            | Northern Bari| Lemon Gaba             | 04° 52.051'   | 31° 29.873'   | 487             | 03.08.2018  |
|            | Kondokoro   | Juba Na Bari-Jezira    | 04° 51.167'   | 31° 37.430'   | 457             | 04.08.2018  |

Table 1. Geographical information of sampling sites in Juba County, Central Equatoria State, South Sudan.
| Location            | Area Type            | Coordinates          | Area   | Date       |
|---------------------|----------------------|----------------------|--------|------------|
| Munuki Gudele 1 Block 7 | 04° 52.383' 33.025' | 478                  | 04.08.2018 |            |
| Northern Bari       | Gudele 2 Jopa        | 04° 52.852' 31.742'  | 472    | 04.08.2018 |
| Tomato              | Juba Na Bari-Jezira  | 04° 50.945' 37.392'  | 460    | 04.08.2018 |
| Munuki              | Gudele 1 Block 5     | 04° 52.849' 33.770'  | 466    | 27.07.2018 |
| Tomato              | Juba Na Bari-Jezira  | 04° 50.945' 37.392'  | 460    | 04.08.2018 |
| Squash              | Tokiman              | 04° 46.396' 36.334'  | 460    | 04.08.2018 |
| Sweet potato        | Beitery              | 04° 51.323' 32.009'  | 508    | 19.07.2018 |
| Munuki              | Mouna-Suk Hajer      | 04° 31.130' 34.483'  | 490    | 24.07.2018 |
| Lemon Gaba          | 04° 51.688' 30.104'  | 506                  | 25.07.2018 |
| Lemon Gaba          | 04° 31.965' 30.140'  | 490                  | 27.07.2018 |
| Munuki              | Gudele 1 Block 5     | 04° 52.849' 33.770'  | 466    | 27.07.2018 |
| Northern Bari       | Lemon Gaba           | 04° 51.850' 30.083'  | 498    | 28.07.2018 |
| Rajaf West          | Beitery              | 04° 51.669' 32.117'  | 488    | 28.07.2018 |
| Rajaf West          | Lologo 2             | 04° 48.443' 35.564'  | 473    | 30.08.2018 |
| Rajaf West          | Lologo 2             | 04° 46.447' 35.496'  | 476    | 31.07.2018 |
| Rajaf West          | Lologo 2             | 04° 48.344' 35.302'  | 474    | 01.08.2018 |
| Munuki              | Gudele 1 Block 5     | 04° 52.662' 33.690'  | 469    | 02.08.2018 |
| Northern Bari       | Lemon Gaba           | 04° 52.051' 29.873'  | 487    | 03.08.2018 |
| Kondokoro           | Juba Na Bari-Jezira  | 04° 50.945' 37.392'  | 460    | 04.08.2018 |
| Northern Bari       | Gudele 2 Jopa        | 04° 52.766' 31.757'  | 470    | 04.08.2018 |
| Juba University of Juba (Green house) | 04° 50.327' 35.225' | 494    | 10.08.2017 |
2.3. Mitochondrial COI (mtCOI) PCR amplification and sequencing

DNA extracted from 228 individual whiteflies from all the sampled locations was used for PCR amplification. Two sets of primers were used for amplification of a partial fragment of mtCOI, primer MM1: (5´-CTGAYATRGCKTTTCCCTCG-3´-F, 5´-TTACTGCAYWTTCTGCCAC-3´-R) (IITA lab) and primer set: 2195-Bt-F (5´-TGRTTTTTTGTTCGTCATCAGAAGT-3´) and C012-Bt-sh2-R (5´-TTTACTGCACCTTCTGCC-3´) [73]. These primers amplified ~1300bp and ~867bp respectively portions of the mtCOI gene. The PCR reaction contained 1X QuickLoad Master Mix (New England Biolabs, UK), 1mM MgCl2, 0.24μM of each primer, 2 μl DNA, and sterile distilled water to achieve the desired reaction volume of 25 μl. PCR was carried out at 95°C for 5min initial denaturation of template DNA, followed by 35 cycles at 94°C for 40s, 56°C for 30sec for annealing, and 72°C for 90sec for extension, with a final extension at 72°C for 10min. PCR products were run on a 1% agarose gel in 1 x TAE buffer stained with GelRed™ (Biotium, Fremont, CA, USA). DNA bands were visualized using a Gel Doc™ XR+ Gel Documentation System and only samples with intact bands were selected for sequencing. PCR products were sent to Macrogen Inc. (Maryland, USA) for purification and direct sequencing. DNA sequences were manually edited using Ridom Trace Edit v1.1.0 software. Sequences were assembled into contigs using CLC Main Workbench 7.0.2 (QIAGEN, Aarhus, Denmark). Multiple alignment of edited sequences was performed using Clustal W in MEGA version 7.0.26 [74] and sequences were trimmed to 744 nt. Construction of a maximum-likelihood phylogenetic tree was performed using MEGA with 1000 bootstrap replicates. Sequences were blasted using GenBank’s (NCBI) Blastn and selected reference sequences with 99% to 100% identity to our mtCOI sequences were included in the phylogenetic tree for comparison with previously published haplotypes. The extent of nt sequence variation within the identified B. tabaci groups was examined. Estimates were obtained for number of haplotypes, polymorphic sites (S), average number of nucleotide differences (k), nucleotide diversity (Pi), haplotype diversity (Hd), Theta per sequence and Theta per site and significance values using the mismatch distribution procedure of Dna-SP.
To determine whether sampled whitefly populations were stable or expanding, Tajima’s D and Fu’s Fs were calculated using Dna-SP 6.12.03.

3. Results and Discussion

*B. tabaci* whiteflies samples were collected from sweet potato (*Ipomeoa batata* L.), cassava (*Manihot esculenta* Crantz), tomato (*Solanum lycopersicum* L.) and squash (*Cucurbita pepo* L. ‘Zucchini’) from 10 locations in Juba County, Central Equatoria State, South Sudan. The locations included Tokiman (TOK), Lologo 2 (LO2), Beitery (BET), Juba Na Bari-Jezira (JNB-JZ), Mouna Suk Hajer (MO-SH), Gudele 1 Block 5 (GU1-B5), Gudele 1 Block 7 (GU1-B7), Gudele 2 Jopa (GU2-JP), Lemon Gaba (LEG), and University of Juba (UOJ). *B. tabaci* colonizing sweet potato were collected from 8 locations except Tokiman and Gudele 1 Block 7. *B. tabaci* on cassava were sampled from 5 locations: Lologo 2, Juba Na Bari-Jezira, Gudele 1 Block 7, Gudele 2 Jopa, and Lemon Gaba. On tomato, *B. tabaci* were collected from three locations including Juba Na Bari-Jezira, Gudele 1 Block 5 and Gudele 1 Block 7, whereas whiteflies on squash were collected from Tokiman (Table 2). *B. tabaci* fourth instar nymphs were also collected from sweet potato in Lologo 2 and from cassava in Gudele 1 Block 7. Most of the whiteflies sampled were from sweet potato and cassava, the main targeted crops of this study. As a result, the whiteflies collected from tomato and squash were from fields adjacent to either sweet potato or cassava plantings.

Table 2. Number of *B. tabaci* sequences obtained from sampled locations and host plants in Juba County, Central Equatoria State, South Sudan.

| Location                  | Host Plant | Sweet potato (Sp) | Cassava (Ca) | Tomato (To) | Squash (Sq) | Total |
|---------------------------|------------|-------------------|--------------|-------------|-------------|-------|
| Tokiman (TOK)             |            | -                 | -            | -           | 4           | 4     |
| Lologo 2 (LO2)            |            | 20                | 3            | -           | -           | 23    |
| Beitery (BET)             |            | 9                 | -            | -           | -           | 9     |
| Juba Na Bari-Jezira (JNB-JZ) |            | 3                 | 6            | 2           | -           | 11    |
| Mouna-Suk Hajer (MO-SH)   |            | 5                 | -            | -           | -           | 5     |
| Gudele 1 Block 5 (GU1-B5) |            | 6                 | -            | 11          | -           | 17    |
| Gudele 1 Block 7 (GU1-B7) |            | -                 | 41           | 2           | -           | 43    |
| Gudele 2 Jopa (GU2-JP)    |            | 4                 | 1            | -           | -           | 5     |
| Lemon Gaba (LEG)          |            | 25                | 6            | -           | -           | 31    |
| University of Juba (UOJ)  |            | 14                | -            | -           | -           | 14    |
| **Total**                 |            | 86                | 57           | 15          | 4           | 162   |

In total 183 whitefly samples were sequenced, out of which 162 produced high quality mtCOI sequences. There was a high level of diversity among *B. tabaci* populations collected from the sampled crop plants. The sequences obtained from sweet potato, tomato and squash grouped into three phylogenetically distinct groups, which included (MED), Indian Ocean (IO) and Uganda. The sequences from cassava were grouped into three distinct groups, SSA1-SG1, SSA1-SG3, and SSA2 (Figure 2). These groups were identified based on the topology of the phylogenetic tree and the clustering of the sequences obtained from this study relative to the reference sequences retrieved from GenBank. The predominant haplotype MED had a total of 90 whiteflies which accounted for 55.5% of all the whiteflies collected from the four host plants. Of these, 72 whiteflies (44.4%) were found on sweet potato (Table 3). The second most abundant haplotype was SSA2 with 43 whiteflies (26.5%), all of them found on cassava. SSA1-SG1 was a second haplotype found only on cassava for which there were 13 whiteflies (8%). The other haplotypes were Uganda which was present on sweet potato and had a total of 11 whiteflies (6.8%), Indian Ocean with 4 whiteflies (2.5% and SSA1-SG3 which was the least frequent haplotype with only 1 whitefly (0.6%) found on sweet potato (Table 3). A total of 45 selected sequences representing haplogroups found in this study have been submitted to GenBank under the following accession names (MN318379 - MN318423).
The clustering of the whiteflies SSA2, SSA1-SG1 and SSA1-SG3 into a distinct major clade separate from *B. tabaci* whiteflies that do not colonize cassava is consistent with what has been reported in other studies of *B. tabaci* from various cassava-growing countries in Africa [60,61,71]. The grouping of MED and Indian Ocean haplotypes is also consistent with what has been reported in previous studies [60,61]. Uganda, which was depicted by a clearly defined monophyletic grouping in our mtCOI sequence analysis has previously been identified as a genetically distinct haplotype occurring in East Africa [76,58].
Figure 2. Maximum likelihood phylogenetic tree constructed for mtCOI sequences obtained from *Bemisia tabaci* collected from 10 locations in Juba County, central Equatoria State, South Sudan between July and August 2018. Reference sequences from GenBank (▲) are included for comparison.
Table 3. Bemisia tabaci haplotype groups (numbers and percentages) on four host plants from Juba County, Central Equatoria State, South Sudan.

| B. tabaci haplotypes | Sweet potato | Cassava | Tomato | Squash | Total |
|----------------------|--------------|---------|--------|--------|-------|
| MED                  | 72 (44.4%)   | 1 (0.6%)| 13 (8.0%)| 4 (2.5%)| 90 (55.5%)|
| Indian Ocean         | 3 (1.9%)     | -       | 1 (0.6%)| -      | 4 (2.5%)|
| Uganda               | 10 (6.2%)    | 1 (0.6%)| -      | 11 (6.8%)|
| SSA1-SG1             | -            | 13 (8.0%)| -      | -      | 13 (8.0%)|
| SSA1-SG3             | 1 (0.6%)     | -       | -      | 10.6%)| -      |
| SSA2                 | -            | 43 (26.5%)| -      | -      | 43 (26.5%)|
| Total                | 86           | 57      | 15     | 4      | 162     |

We found that B. tabaci MED was predominant on sweet potato, tomato and squash in all sampled locations. MED is a globally important B. tabaci haplotype group which is thought to have originated from Africa. Consequently, there are numerous other reports of its prevalence on a wide range of crop and weed hosts [60,64,59]. B. tabaci MED has been reported to be extremely polyphagous and invasive [54], causing damage to both field and greenhouse crops [77]. It has also developed resistance to various insecticides under intensive production systems [78-80]. The presence of B. tabaci MED in all locations and on all sampled crop plants in our study in South Sudan suggests that this haplotype is an important pest of sweet potato and other crops in Juba County and likely also in other parts of South Sudan. Moreover, since SPCSV transmitted by B. tabaci is one of the most important viruses affecting sweet potato in this region of East Africa [12], it is likely that this is the main vector of this virus in South Sudan. Future investigations, however, should determine the relative abilities to transmit SPCSV of each of the three B. tabaci haplotypes occurring on sweet potato.

Since no similar studies have been conducted anywhere else in sub-Saharan Africa, this represents an important gap in the existing understanding of the relationship between B. tabaci haplotype groups and the viruses that they vector.

MED haplotype analyses revealed six haplotypes amongst the samples collected from South Sudan (Table 4). Two of these are previously described African MED haplotypes, whilst the other four are new unique haplotypes falling within the MED group. Haplotype diversity (0.51), nucleotide diversity (0.012) and a positive significant Tajima’s D (2.07283: P < 0.05) suggest that the population is undergoing balancing selection and has not undergone rapid recent expansion. Sixty-two of the 90 MED sequences (Haplotype 1) represent an important African MED haplotype for which there are a further 17 sequences in GenBank from Cameroon, Uganda and Nigeria. The samples in this haplotype were predominantly from sweet potato, although there were also individuals from tomato, squash and cassava, indicating that it could be sharing host plants. Haplotype 2 had 8 sequences from sweet potato which were identical to 13 sequences from GenBank originating from Sudan, Cameroon, Uganda and Burkina Faso. Haplotype 3 had 8 sequences from sweet potato and squash. These were most closely matched (99.7%) with a GenBank sequence from Uganda KX570768. Haplotypes 4 and 5 occurred on sweet potato and had 8 and 3 sequences respectively. These were most closely related (99.7%) to a sequence from China (MH908653). Haplotype 6, recorded from sweet potato, had 1 sequence sharing 99.9% homology with MH908653 from China. Currently GenBank hosts 944 MED sequences that comprise 168 haplotypes. 673 (71%) of the sequences cluster in three major haplotypes that are spread worldwide. Of the 168 haplotypes, 137 (81%) have only one sequence in GenBank, although it is possible that some of these may erroneously be considered as unique haplotypes due to the frequent occurrence of sequencing errors in mtCOI data submitted to this database.

Table 4. Population genetic analysis of Bemisia tabaci groups from Juba county, Central Equatoria region, South Sudan.

| Parameter               | All  | MED  | SSA2 | SSA1-SG1 | Uganda | Indian Ocean | SSA1-SG3 |
|-------------------------|------|------|------|----------|--------|--------------|---------|
| Sample size             | 162  | 90   | 43   | 13       | 11     | 4            | 1       |
| Number of haplotypes    | 13   | 6    | 2    | 1        | 1      | 2            | 1       |
haplotype in areas affected by the severe CMD epidemic which spread through Uganda in the 1990s. Cassava mosaic virus (EACMV), and East African cassava mosaic virus - Uganda (EACMV-UG) were detected in Western Equatoria states, African cassava mosaic virus (ACMV), East African in a baseline survey conducted on cassava in three times as many SSA2 individuals recorded compared to those of SSA1-SG1. Our data suggest that South Sudan could be an exception to this pattern, since there were more than West Africa to Kenya in the East), but in all cases it appears to be less frequent than SSA1 haplotypes. SSA1 haplotype co-occurs with others throughout its geographic range (stretching from Sierra Leone in Sudan. A recent continent-wide assessment of cassava-colonizing whiteflies from Uganda and Kenya between 2004 and 2010. Recent studies have noted the occurrence of SSA2 reported to be absent in more recent whitefly collections from cassava in Uganda and western Kenya [65,84], and replaced by SSA1-SG1 [10], although low frequencies of this haplotype were reported from Uganda and Kenya between 2004 and 2010. Recent studies have noted the occurrence of SSA2 on cassava in western Kenya and weedy hosts in Uganda [72,73,85]. The detection of SSA1-SG1 and SSA2 fourth instar nymphs of B. tabaci confirms that both haplotypes colonize cassava in South Sudan. A recent continent-wide assessment of cassava-colonizing B. tabaci in sub-Saharan Africa noted that SSA2 was the most widely distributed of the haplotypes recorded [72]. Significantly, this haplotype co-occurs with others throughout its geographic range (stretching from Sierra Leone in West Africa to Kenya in the East), but in all cases it appears to be less frequent than SSA1 haplotypes. Our data suggest that South Sudan could be an exception to this pattern, since there were more than three times as many SSA2 individuals recorded compared to those of SSA1-SG1.

In the 1990s, CMD was reported to be highly destructive in the Western Equatoria Province of pre-independence southern Sudan [18]. Furthermore, in a baseline survey conducted on cassava in 2005 in Eastern and Western Equatoria states, African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), and East African cassava mosaic virus-Uganda (EACMV-UG) were found to be the viruses affecting cassava [7]. SSA2 was shown to be the most abundant B. tabaci haplotype in areas affected by the severe CMD epidemic which spread through Uganda in the 1990s.
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It is quite likely that there may have been a similar association between virus and vector in southern Sudan during this period. However, whilst SSA1-SG1 subsequently displaced SSA2 as the predominant *B. tabaci* haplotype on cassava in Uganda, this change may not have happened further north in southern Sudan, with the result that SSA2 is currently the main cassava-colonizing *B. tabaci* haplotype in present day South Sudan. The reasons behind these contrasting patterns of population change in Uganda and South Sudan are not currently apparent but would be a useful topic for future study.

In this study, a single individual of non-cassava *B. tabaci* haplotype MED was collected from cassava. These rare occurrences have been reported elsewhere [61, 67,84]. However, previous studies have demonstrated that non-cassava *B. tabaci* whiteflies are unable to reproduce on and colonize cassava [86] partly since they are unable to feed effectively on cassava plant hosts [87]. In each of these instances, it has been concluded that whiteflies of non-cassava *B. tabaci* haplotypes occurring on cassava are present as visitors and not colonizing the crop.

4. Conclusions

This study presents the first report on the genetic diversity of *B. tabaci* whitefly populations collected from South Sudan. Six *B. tabaci* haplotype groups, including three non-cassava groups (MED, Indian Ocean and Uganda) and three cassava groups (SSA1-SG1, SSA1-SG3 and SSA2) were identified. MED and SSA2 were the most prevalent and most widely distributed amongst the sampled locations. The Uganda haplotype is also widespread and was identified from five of the locations. The discovery of six *B. tabaci* haplotype groups from the relatively small portion of South Sudan that was sampled does suggest that, like Uganda, this part of East Africa has a high level of whitefly diversity. This provides a strong indication that this part of Africa may have been a source for MED whiteflies that have had devastating global impacts as an invasive pest [88]. It is also significant that the MED species group of *B. tabaci* includes some of the most insecticide-resistant populations of whiteflies. Therefore, although *Bemisia* whiteflies may not be present on sweet potato and other host plants at high abundance levels, any future management efforts will need to apply extreme caution in the application of chemical insecticides in order to preclude the development of whitefly resistance. Whitefly populations observed in South Sudan were associated with transmission of viruses causing damaging disease in cassava and sweet potato. Improving understanding of the dynamic interactions between vector and virus will be important for each of these crop-virus-vector pathosystems. An essential first step in this task will be conducting a comprehensive assessment of the genetic diversity, geographical distribution, population dynamics and host range of *B. tabaci* species in South Sudan. This new knowledge will then provide the basis for the development of effective whitefly management strategies.

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