MOLECULAR DIVERSITY OF WHITEFLY, *Bemisia tabaci* (HEMIPTERA ALEYRODIDAE) FROM SELECTED CASSAVA GROWING REGIONS OF KENYA USING MITOCHONDRIAL CYTOCHROME OXIDASE 1 GENE

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AGRICULTURE AND TECHNOLOGY

2018

Molecular diversity of whitefly, *Bemisia tabaci* (Hemiptera Aleyrodidae) from selected cassava growing regions of Kenya using mitochondrial cytochrome oxidase 1 gene

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A thesis submitted in partial fulfilment for the degree of Master of Science in Agricultural and Environmental Biotechnology in the Jomo Kenyatta University of Agriculture and Technology

2018

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

To my parents, my dear wife Abigael Kerubo and my children, Livia Moraa and Abraham Rosana for the love, care and immeasurable encouragement. Above all, I offer my sincere gratitude to the Almighty God for making this journey possible.

ACKNOWLEDGMENTS

I appreciate Dr. Steven Nyanjom Ger and, Prof. Elijah M. Ateka of JKUAT for the good supervision they accorded me. I also wish to thank the Bill and Melinda Gates Foundation through the Mikocheni Agricultural Research Institute for financially supporting this work within the framework of the Cassava Viruses Diagnostics Project. Special thanks go to Samuel Mwaura, Timothy Onyinge for their invaluable technical support in molecular analysis. I also wish to pass special thanks to Prof. Laura Boykin of the University of Western Australia for guidance in data analysis and by helping access resources provided by the Pawsey Supercomputing Centre with funding from the Australian Government and the Government of Western Australia.

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LIST OF ABBREVIATIONS AND ACRONYMNS

ACMV	African cassava mosaic virus
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
CBSD	Cassava brown streak disease
CBSVs	Cassava brown streak viruses
CMBs	Cassava mosaic begomoviruses
CMD	Cassava Mosaic Disease
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EACMKV	East African cassava mosaic virus (Kenya type1)
EACMV	East African cassava mosaic virus
EACMV-KE2	East African cassava mosaic virus (Kenya type 2)
EACMV-UG	East African cassava mosaic virus (Ugandan type)
EACMZV	East African cassava mosaic Zanzibar virus

EDTA	Ethylenediamine tetra-acetic acid
ITSI	Internal transcribed spacer I
mtCOI	Mitochondrial cytochrome oxidase I gene
PCR	Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
RNA	Ribonucleic Acid

ABSTRACT

Whiteflies, *Bemisia tabaci*, (Gennadius) are major insect pests that affect many crops such as cassava, tomato, beans, cotton, cucurbits, potato, sweet potato, various species of fruits and ornamental plants. *Bemisia tabaci* transmits viral diseases namely cassava mosaic and cassava brown streak diseases which are the main constraints to cassava production causing huge losses estimated in excess of US$ 100 Million annually among many small-scale farmers in East Africa. The current work aimed at determining the phylogenetic relationships and population genetic structure among whiteflies in major cassava growing areas of Kenya. Surveys were carried out between 2013 and 2015 in major cassava growing areas (Western, Nyanza, Eastern, and Coast regions). Adult whiteflies were collected from the five top most leaves of the cassava plants, and the samples from each field for DNA extraction and amplification of mitochondrial cytochrome oxidase I gene. The whitefly population is associated with the continued transmission and spread of the dual pandemic of cassava begomoviruses (CMD and CBSD). The amplified mtCOI gene sequences and phylogenetic trees were constructed using Bayesian methods to understand the genetic diversity across the study regions. The population genetic structure was analyzed by AMOVA that showed F_{ST} 0.2000. Phylogenetic analysis revealed two distinct *Bemisia tabaci* species present in Kenya, sub-Saharan Africa comprising five different clades (A-E) with percent sequence similarity ranging from 97.7 to 99.5%.Clades B, C, D and E are predominantly distributed in Western and Nyanza regions of Kenya whereas clade B is dominantly found along the Coast, Eastern and parts of Nyanza. *Bemisia tabaci* clade A clusters together with sub-Saharan Africa 2-(SSA2) recorded a percent sequence similarity of 99.5%. The sub-Saharan Africa 2 is species associated with CMD and is found in western Kenya region that borders Uganda. This work also reports the identification of SSA2 after a 15 years break in Kenya. More information is needed to determine if these species are differentially involved in the epidemiology of cassava viruses.

Keywords: *Bemisia tabaci*; genetic diversity; mt-COI; cassava; Kenya

CHAPTER ONE

INTRODUCTION

1.1 Background information

Cassava (*Manihot esculenta*; Crantz, Euphorbiaceae) is an important source of food to more than one fifth of the world's population across Africa, Asia, and South America (Cossa, 2011). From the time of its introduction, the crop has spread and gained prominence as a major food and staple crop for many communities in sub-Saharan Africa (Nweke, 1996). Cassava is ranked the third most important source of carbohydrates in Africa, and it is the second most important food crop after maize in western and coastal regions of Kenya (Mwangombe *et al*., 2013). The cassava crop has a wide range of uses; it is a food security-crop which is estimated to be consumed by approximately 500 million people in Africa (Sseruwagi, 2005; Were *et al*., 2007). Cassava is also a cash crop, livestock feed, and a raw material for industrial uses such as pharmaceuticals, starch, and alcohol production (Wyatt & Brown, 1996; IITA, 2003). Cassava roots are a rich source of carbohydrate while leaves are high in proteins, minerals, and vitamins. The roots of cassava save many lives during famine conditions especially in various parts of Kenya that experience drought, thereby playing a major role in food security and contributing to poverty reduction (Nweke, 1996). Despite these benefits, the crop is affected by two viral diseases namely; cassava mosaic disease (CMD) and cassava brown streak virus disease (CBSD). CMD has been a major biotic constraint to cassava production in Africa (Mugerwa *et al*., 2012). In Kenya, the disease is predominantly caused by Geminiviruses, namely African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) (Simon *et al*., 2006). Symptoms of CMD include leaf chlorotic mottle, distortion of leaves, stem twisting, crinkling and stunting of cassava plant parts (Were *et al*., 2007; Mugerwa *et al*., 2012). The pandemic affected cassava growing areas in East and Central African countries. It has caused severe losses in cassava yields estimated at 50% (IITA, 2003; Legg *et al*., 2006; Legg *et al*., 2013-14; Aduwa *et al*., 2016). CBSD, on the other hand, is caused by Cassava brown streak virus (CBSV) and Uganda cassava brown streak virus (UCBSV) (Legg *et al*., 2014; Ndunguru *et al*., 2015). Recent studies have revealed existence of several species of CBSD (Ndunguru *et al*., 2015). The disease is characterized by severe chlorosis and necrosis on infected leaves, giving them a yellowish, mottled appearance. Chlorosis may be associated with the veins, spanning from the mid vein, secondary and tertiary veins, or rather in blotches unconnected to veins (Ntawuruhunga *et al*., 2016). Brown streaks may appear on the stems of the cassava plant, but in some varieties, a dry brown-black necrotic rot of the cassava root exists, which may progress from a small lesion to the whole root. Finally, the roots may become constricted due to the tuber rot with overall plant stunting, thereby reducing production (Mohammed *et al*., 2012). The viruses causing CMD and CBSD are transmitted by the whiteflies (*Bemisia tabaci*) and through infected cuttings. Heavy infestation by *B. tabaci* on cassava leads to the presence of honey dew and sooty mould that affects the photosynthetic structures reducing cassava production (Were *et al*., 2007; Palaniswami *et al*., 2011). In the present study, the population genetic structure and the phylogenetic relationships of CMD transmitting *Bemisia tabaci* was investigated. The findings demonstrate that within the cassava growing areas in Kenya, different clades are distributed. This explains the continued spread of *Bemisia tabaci* associated diseases CMD and CBSD, leading to reduced yields of cassava. *Bemisia tabaci* is highly polyphagous affecting a wide range of host plant species (edible, ornamental, and fibre crops) in both tropical and subtropical regions (F. Diaz *et al*, 2015). The species complex of *Bemisia tabaci* also has high reproductive rate, high capacity for dispersion and resistance to several insecticides which complicates control mechanism (Perring, 2001). *Bemisia tabaci* has expanded all over the world continents along the tropics and subtropics (F. Diaz *et al*, 2015). The population of this species complex from these different locations varies in their responses to local climates, host plants, virus transmissions and disease resistance has led to rise into several clades and sub clades (Oliveira *et al*., 2001). The species complex of *Bemisia tabaci* vector are morphologically indistinguishable, cryptic species have been differentiated with genetic markers which includes allozymes, RAPD,AFLP, RFLP and microsatellites markers (De Barro., 2005). Recent studies reports that most B. tabaci groups have been defined by sequence variation in the mitochondrial COI gene and Nuclear ITSI gene (Frohlich *et al*., 1999; Boykin *et al*., 2007). Globally *Bemisia tabaci* species complex has emerged into 37 biotypes (Dinsdale *et al*., 2010; Boykin *et al*., 2014). In recent research in Kenya three species clades (SSAI, SSA2 and Reunion) have been identified that keep varying from time to time (Mugerwa *et al*., 2012). Adult *Bemisia tabaci* whiteflies are easily located on the underside surfaces of young apical leaves of young cassava plants (Sesruwagi, 2005). The species complex infest heathy young cassava plants during first three to five months after planting , symptoms of viral diseases, CMD and CBSD infections are observed (Ndunguru *et al*., 2014). Reproductive behavior studies has been reported that *Bemisia tabaci* reproduce parthenogenetically (Maruthi *et al*., 2001, 2004a). Females that are unmated produce males (haploid), while mated females produce both males and females(diploid)(Byrne and Bellows, 1991)

1.2 Statement of the problem

Over the past two decades, severe outbreaks of *B. tabaci* in many parts of East Africa have become more frequent leading to increased spread of CMD and CBSD infections and great losses to cassava farmers. A survey (Western, Nyanza, Eastern and Coastal regions) of the genetic diversity of CMD and CBSD associated begomoviruses across the major cassava growing areas of Kenya, have implicated the vector of the viruses to hinder production of cassava across the region. *B. tabaci* has high genetic variability due to over use of pesticides leading to resistance development (Arlindo *et al*., 2013) has persisted to transmission of cassava viral diseases. This research study determined the different *B. tabaci* distribution and biotypes using mtCOI gene and infer the phylogenetic relationships and the population genetic structure of species populations associated with the spread of CMD and CBSD in the cassava growing regions in Kenya.

1.3 Justification

Bemisia tabaci is a vector of CMD and CBSD. The diseases have continued to devastate cassava crops posing a threat to many lives, (Mugerwa *et al.,* 2012). The purpose of this study is to have insight on various *B. tabaci* species or subspecies that are contributing to transmission of begomoviruses in cassava. The findings would contribute to effective management of the pest through early detection and developing resistant crops. The transmission rates of the CMD and CBSD has been estimated to be between 13-22% within the sub-Saharan cassava growing regions (Maruthi *et al*., 2004). Development of resistant cultivars necessitates development a sound knowledge base of the vector, its biotypes, and their distribution. This requires rapid and accurate techniques for whitefly biotype detection and subsequent identification to facilitate studies of whitefly epidemiology and genetic diversity. This information would be important in the designing of more efficient crop protection strategies thereby sustaining cassava production in the region.

1.4 Hypothesis

1. There is no species diversity, distribution and evolutionary relationship of *Bemisia tabaci* population from selected sites of cassava growing in Kenya.

2. There is no phylogenetic relationship among the *Bemisia tabaci* population from the selected sites of cassava growing in Kenya.

1.5 Objectives

1.5.1 General objective

To determine the diversity, phylogenetic relationship, distribution and genetic structure of *B. tabaci* population in cassava growing areas of Kenya using mitochondrial cytochrome oxidase 1 gene.

1.5.2 Specific objectives

1 To identify *B. tabaci* clades circulating in major cassava growing regions in Kenya.

2. To establish distribution of *B. tabaci* clades in major cassava growing areas in Kenya.

3. To determine the population genetic structure of *B. tabaci* populations from major cassava growing areas in Kenya.

4. To infer phylogenetic relationship among *B. tabaci* populations from cassava growing areas of Kenya using molecular tools.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Bemisia tabaci* species complex

The *B. tabaci* species complex is globally distributed and the putative species are named based on their geographic locations; Mediterranean; Middle East-Asia Minor 1; Middle East-Asia Minor 2; Indian Ocean; Asia I; Australia/Indonesia; Australia; China; China 2; Asia II 1; Asia II 2; Asia II 3; Asia II 4; Asia II 5; Asia II 6; Asia II 7; Asia II 8; Italy; sub-Saharan Africa 1 (SSA1); sub-Saharan Africa 2 (SSA2); sub-Saharan Africa 3 (SSA3); sub-Saharan Africa 4 (SSA4); sub-Saharan Africa 5 (SSA5); New World; and Uganda (Mugerwa *et al*., 2012). Recent studies have reported new species (Asia II 9, Asia II 10, Asia III, and China 3, and Asia I-India and New World 2), for a total number of 34 morphologically (Boykin *et al*., 2012) indistinguishable species reported in the *B. tabaci* complex (Dinsdale *et al*., 2010, Lee *et al*., 2013, Boykin *et al*., 2014). The worldwide spread of emerging species, such as *B. tabaci* MEAM1, also known as *B. argentifolii*, and *Bemisia tabaci* MED, continue to cause severe crop losses which are expected to increase, resulting in higher pesticide use on many crops (tomato, beans, cassava, cotton, cucurbits, potato, sweet potato). In East Africa, there are two distinct cassava-associated *B. tabaci* putative species, sub-Saharan Africa 1 (SSA1) and sub Saharan Africa 2 (SSA2) (Legg *et al*., 2002). In Kenya, limited studies on *B. tabaci* have been carried out in Eastern, Coastal Kenya, Nyanza, and the Western regions of Kenya, and the putative *B. tabaci* species found to be widely spread among the affected crops remain elusive (Riis *et al*., 2000).

2.2 Taxonomy and nomenclature *Bemisia tabaci*

Bemisia tabaci (Gennadius) is also referred to as *Bemisia gossypiperda* (Misra & Lamba); *Bemisia longispina* (Priesner & Hosny, 1934) and *Bemisia nigeriensis* (Corbett, 1936). Its taxonomic position: Insecta: Hemiptera: Homoptera: Aleyrodidae. The insect is commonly known as tobacco whitefly, sweet potato whitefly, and cotton whitefly. The genus *Bemisia* contains 37 species and is thought to have originated from Asia (Mound & Halsey, 1978, Boykin *et al*., 2014), but recently molecular studies reveals that the insect originated from Africa (Campbell *et al*., 1996). Three distinct groups of *B. tabaci* have been identified by comparing their mitochondrial 16S ribosomal subunits. These are: (a) New World, (b) India/Sudan, (c) remaining Old World (Frohlich & Brown, 1994). First reports of a newly evolved biotype of *B. tabaci*, the B biotype, appeared in the mid-1980s (Brown *et al*., 1995b). Commonly referred to as the silverleaf whitefly or poinsettia strain, the B biotype has been shown to be highly polyphagous and almost twice as fecund as previously recorded strains and has been documented as being a separate species, also named *B. argentifolii* (Bellows *et al*., 1994). The B biotype is able to cause phytotoxic disorders in certain plant species, e.g. silverleaf in squashes (Bedford *et al*., 1992, 1994a). A distinctive nonspecific esterase banding pattern is also helpful in identification (Brown *et al*., 1995a), but not infallible (Byrne *et al*., 1995). The authors' described morphological characters are, however, highly debatable and are presently under investigation. As one example of the problems involved, one may note that the presence or absence of spines on the 'puparium' is now known to be determined by the smoothness or hairiness of the leaves of the host plant (Bedford *et al*., 1994a), yet the absence of a small anterior submarginal seta on the 4th larval instar/puparium stage has been described as one of the identifying morphological features of so-called *B. argentifolii*. No Old World populations of *B. tabaci* studied so far can be distinguished from so-called *B. argentifolii* by this or other morphological features, although these Old World populations do not induce phytotoxic disorders or exhibit B biotype esterase banding patterns. It may be noted, finally, that several other biotypes have been described (Brown *et al*., 1995b), which supports the idea of a species complex, rather than of a number of distinct species such as *B. argentifolii*.

2.3 Ecology of *B. tabaci*

B. tabaci has been recorded infecting a wide diversity of host plants in sub-Saharan Africa (Legg J.P., 2013). Host-associated morphological plasticity in nymphs led initially to the description of a large number of *Bemisia* species whose adults were indistinguishable, although these were subsequently synonymized under the single name, *Bemisia tabaci*. More recently, combining morphological and molecular analyses has highlighted the cryptic nature of the *Bemisia tabaci* species complex (Brown J. K, 2010). Molecular markers have been used to propose putative species delimitation boundaries within the *B. tabaci* complex, and it seems likely that future work combining bioassays with mating studies and molecular characterization will lead once again to the division of the complex into several distinct species, each with unique names (Dinsdale *et al*., 2010).

2.4. Host range

Bemisia tabaci is an extremely polyphagous species. It colonizes mainly annual, herbaceous plants including over 500 species from 74 families (Brown *et al*., 1995, Cossa, 2011). *B. tabaci* is known to have a host range that is highly variable. Examples of *B.tabaci* host plants include avocado, banana, cabbage, capsicum, cassava, cauliflower, citrus, coconut, cotton, eggplant, garlic, guava, legumes, mango, mustard, onion, peachy, pepper, radish, squash, soybean, tomato, and tobacco (Palaniswami *et al*., 2011). However, species of *B. tabaci* vary with respect to geography, fecundity, dispersal behaviour, insecticide susceptibility, natural enemy complex, invasive behaviour, plant virus transmission, and complement endosymbionts (Brown *et al*., 1995). In West Africa and Uganda, differences in host selection, has been documented among different *B. tabaci* species (Navas-Castillo *et al*., 2011).

2.5 Whiteflies as insect vectors

There are over 1500 whitefly species known worldwide in approximately 126 genera (Martin, 2004). *Bemisia tabaci* is a species complex that is globally distributed (Martin, 2004) and important because a number of the species that make up the complex are known to damage commercially important plant species either through direct feeding or through the transmission of more than 150 plant viruses primarily belonging to the genus

Begomovirus (family: Geminiviridae) (Navas-Castillo *et al*., 2011; Thompson., 2011; Smith *et al*., 2015). It is, therefore, important to control whiteflies with the aim of reducing virus transmission and agronomic losses. The male adult whitefly is about 0.8 mm while the female is approximately 1 mm in length. Both sexes have wings that are generally opaque and covered with a whitish powder or wax (Martin, 2004).

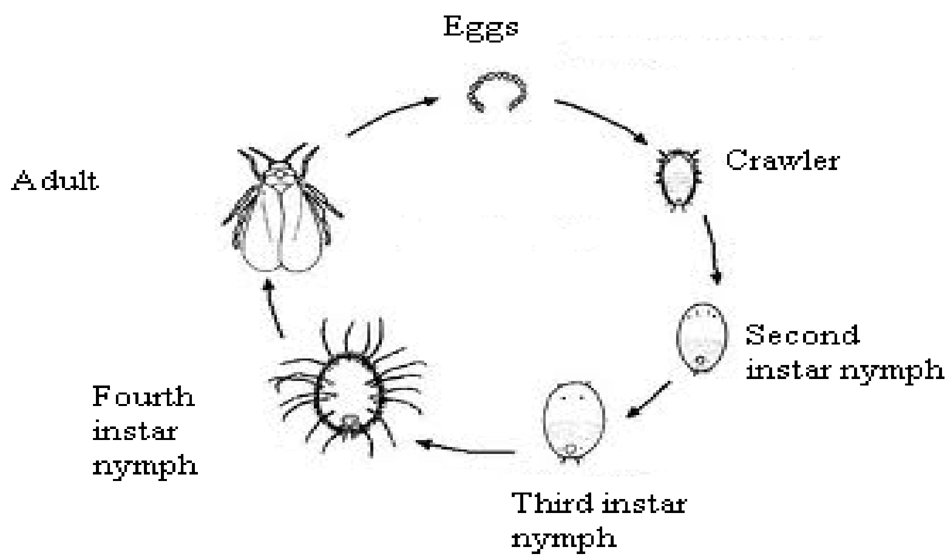


Figure 2.1 Life cycle of whitefly-*B. tabaci* (Suresh *et al*., 2013)

The whiteflies undergo incomplete metamorphosis (Figure 2.1). The females lay 50 to 400 eggs underneath the leaves. The whitish eggs range from 0.10 mm to 0.25 mm and change to a brown colour towards the time of hatching within five to seven days. Female whiteflies are diploid and emerge from fertilized eggs whereas male whiteflies are haploid and emerge from unfertilized egg (Martin, 2004).

After the egg stage, the whitefly hatchling develops through four instar stages. In the first instar, commonly called the crawler, the nymph is 0.3 mm in size and grows to be 0.6 mm until the fourth instar stage. During the first instar stage the body is greenish in colour and flat in body structure. The mobile whitefly nymph walks on plants to find a suitable area on the leaf with adequate nutrients and moults into four other instar or nymphal stages over the span of 40–50 days until it reaches adulthood. During moulting, the whitefly nymphs shed their silver skins, which are left on the leaves. At the feeding sites, the nymphs use parts of their mouth to pierce into the plant and suck the plant's cell sap. The next stage is the pupal stage when the eyes become a deep red colour; the body colour becomes yellow, while the body structure thickens. After development is completed, adult whiteflies have light yellow bodies and white wings (Figure 2.2, EPPO, 2006).The active adult *B. tabaci* are largely responsible for virus spread from plant to plant (Suresh *et al* 2013).

Figure 2.2 *Bemisia tabaci* SSA1 species on cassava (Photo: Laura M. Boykin)-The underside of the cassava leaf heavily infested with adult *B. tabaci*, nymphs and egg stack.

2.6 Population dynamics of *B. tabaci*

The pest that affects cassava crop has a wide range of biological characteristics such as multivoltism, broad host range, high reproductive rate, ability to migrate greater distances and virus vector has contributed to increased challenge to developing a sustainable integrated management mechanism (Gerling *et al*., 2001). Super abundance of *B. tabaci* population and being highly polyphagous have contributed to increased population growth rate leading to rise in the rapid expansion of CMD and CBSD in cassava crop within sub-Saharan countries (Legg, 2002; Legg, J.P. *et al*., 2013). The understanding of the host-virus-vector interaction and dynamics of the vector provides vital information useful in overall management of the vector and associated diseases. The knowledge would also be useful in managing the current threats to food security for millions of people within sub Saharan countries.

2.7 Economic significance of *B. tabaci*

B. tabaci has been known as a minor pest of cotton and other tropical or semi-tropical crops in the warmer parts of the world and, until recently, has been easily controlled by insecticides. In the southern states of the USA in 1991 it was estimated to have caused combined losses of 500 million USD to the winter vegetable crops (Perring *et al*., 1993) through feeding causing damage and plant virus transmission. *B. tabaci* is also a serious pest in glasshouses in North America and Europe. The feeding of adults and nymphs causes chlorotic spots to appear on the surface of the leaves. Depending on the level of infestation, these spots may coalesce until the whole of the leaf is yellow, apart from the area immediately around the veins. Such leaves are later shed. The honeydew produced by the feeding of the nymphs covers the surface of the leaves and can cause a reduction in photosynthetic potential when colonized by moulds. Honeydew can also disfigure flowers and, in the case of cotton, can cause problems in processing the lint. With heavy infestations, plant height, number of internodes and quality and quantity of yield can be affected (e.g. in cotton). The larvae of the B biotype of *B. tabaci* are unique in their ability to cause phytotoxic responses to many plant and crop species. These include a severe silvering of courgette leaves, white stems in pumpkin, white streaking in leafy brassica crops, uneven ripening of tomato fruits, reduced growth, yellowing and stem blanching in lettuce and kai choy (*Brassica campestris*) and yellow veining in carrots and Lonicera (Bedford *et al*., 1994a, 1994b). *B. tabaci* is the vector of over 60 plant viruses in the genera Geminivirus, Closterovirus, Nepovirus, Carlavirus, Potyvirus and a rod-shaped DNA virus (Markham *et al*., 1994). The *Bemisia tabaci* 5 geminiviruses are by far the most important agriculturally, causing yield losses to crops of between 20 and 100% (Brown & Bird, 1992). Geminiviruses cause a range of different symptoms which include yellow mosaics, yellow veining, leaf curling, stunting and vein thickening. Quite a while a million ha of cotton was being decimated in Pakistan by cotton leaf curl bigeminivirus (CoLCV) (Mansoor *et al*., 1993), and important African subsistence crops such as cassava are affected by geminiviruses such as cassava African mosaic bigeminivirus (ACMV). Tomato crops throughout the world are particularly susceptible to many different geminiviruses, and in most cases exhibit yellow leaf curl symptoms. Most of these epidemics in the Old World are attributed to tomato yellow leaf curl bigeminivirus (TYLCV) but may also be caused by other geminiviruses. TYLCV has also recently been recorded in the New World, but several others, exclusively American, tomato geminiviruses have now been described, e.g. tomato mottle bigeminivirus (EPPO/CABI, 1996). Tobacco leaf curl (TLCV), watermelon chlorotic stunt (WCSV), squash leaf curl (SLCV) and bean golden mosaic (BGMV) bigeminiviruses also cause heavy yield losses in their respective hosts. Dual infections have also been shown to occur. Several of these viruses are now quarantine pests in the EPPO region (e.g. bean golden mosaic, squash leaf curl, tomato mottle bigeminiviruses, and lettuce infectious yellows closterovirus, tomato yellow leaf curl bigeminivirus (EPPO/CABI, 1996).

The emergence of the B biotype of *B. tabaci*, with its ability to feed on many different host plants has given whitefly-transmitted viruses the potential to infect new plant species. This has already been shown to have occurred in the Americas. Europe has three known geminiviruses within this group. Two have been shown to be no longer transmissible by *B. tabaci*, tobacco leaf curl bigeminivirus and abutilon mosaic bigeminivirus, possibly through many years of vegetative propagation of their ornamental host plants (Bedford *et al*., 1994a). The other is the readily transmissible tomato yellow leaf curl bigeminivirus that is causing major crop losses within the tomato industries of Spain and Italy. The possibility exists that indigenous weed species may also be reservoirs for this and other yet to be identified geminiviruses worldwide. A newly identified *B. tabaci* transmitted closterovirus is now reported to be causing severe damage to cucumbers and melons in Spain (EPPO, 2006).

2.8 Control of *B.tabaci*

Most recently molecular study suggests that whitefly, *Bemisia tabaci* complex are the most globally destructive pest that originated in Africa. This pest is probably thought to have spread throughout the tropics, sub tropics and finally into the temperate zones and cold countries' greenhouses (Campbell *et al*., 1996). The pest *B. tabaci* are readily controlled by use of insecticides in the field and glass houses conditions; however problems with effective control in wide range of crops have been experienced by many farmers due to insecticide resistance and increased fecundity of the *B. tabaci* biotypes. Research indicates that no single control treatment can be used in a long term basis against the pest and the integration of a number of different control agents for effective level of control (Integration pest management) is recommended (EPPO, 2010). Regions where the pest problems are localized needs assessing individually and an appropriate IPM programme. Natural enemies of *B. tabaci* which are localized on a few crop hosts causes damage either directly or through virus transmission have been thought to control them. Information generated across ten sub Saharan African countries (Ghana, Benin, Nigeria, Cameroon, Uganda, Kenya, Sudan, Tanzania, Malawi and Madagascar) is that there are a few aphelinid parasitiods and a few predators attacking this pest. Gerling *et al*, 2001 has reported 117 predator species that attack *B. tabaci* exist globally and many of these are found in Africa (Legg J.P. *et al*., 2013). Biological control agents such as *Eretmocerus mundus* Mercet, *Encarsia sophia* Girault and Dodd (Otim, 2007), *Encarcia formosa* and *Verticillium lecanii* are moderately successful (Nedsdam, 1992; Otim, 2007). Predators are of coccinelids, lacewings, bugs, spiders and mites that are found adjacent to whitefly population on cassava plants and other associated plant hosts within East African countries (Riis *et al.,* 2000). Biological agents do not lower the infestation of *B. tabaci* to a level that stops virus transmission. Classical biological control is oftenly integrated with a reduced level of chemical spray to minimize the impact of pesticides within the environment (Dent, 2000)

2.9 Species molecular markers

Molecular markers have been used to study insect populations and their phylogenetic relationships. These markers have been used to examine protein-coding genes, major ribosomal RNA genes, and non-coding regions (Sseruwagi, 2005; Sseruwagi *et al*., 2006). The mitochondrial (mtCOI) DNA marker is the most commonly used, but other markers have also been used—for example, the ribosomal RNAs (Caterino *et al*., 2000) and a ribosomal nuclear marker of the internal transcribed spacer I (ITSI) region sequences (De Barro, 2005). These markers have the advantage of relative ease of isolation and amplification and are amenable to straightforward analyses. Mitochondrial cytochrome oxidase I (mtCOI) has the highest degree of variability for the *B. tabaci* species compared to the nuclear genes mentioned above, therefore becoming the most widely used marker for phylogenetic studies of *B. tabaci* globally (Sseruwagi *et al*., 2006; Boykin *et al*., 2007). The mitochondrial cytochrome oxidase I (mtCOI) marker (Frohlich *et al*., 1999) and ITSI region sequences (De Barro, 2005-6) have also been used to study the genetic variability and evolutionary relationships among *B. tabaci* from different geographical locations and host-plant species (Lee *et al*., 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Determination of phylogenetic relationship among B. tabaci samples from cassava growing areas of Kenya using molecular tools

3.1.1 *B. tabaci collection sites*

The study was conducted in major cassava growing areas of Kenya from 2013 to November

2015. The regions surveyed were Bungoma, Busia, Kakamega, Homabay, Migori, Kisumu, Siaya, Kilifi, Kwale, Taita Taveta, Nyamira, Kitui, and Machakos counties (Table 3.1). These geographical locations share a similar agro-ecology, where western region counties of Kenya are characterized by bimodal rainfall ranging from 950 to 1500 mm annually; temperature ranging from 18.4^{o} C to 25.4^{o} C altitude ranges of 900–1800 m, and savannah grassland. In the Eastern region, the altitude ranges from 1000 to 1800 m, with a rainfall potential of 500–760 mm (Orodho, 2006). The Coast region has rainfall ranging from 500 to 1000 mm annually, temperature ranges between 22.4 ^{o} C and 30.3 ^{o} C, altitude ranges of 900–1800 m, and savannah grassland. The Geo-cordinates (latitude and longitude) were recorded using a Geographical Positioning System (GPS) for each sampled field from all regions (Appendix 1).

Table 3.1 Collection areas of *Bemisia tabaci* from cassava from major cassava growing regions in Kenya

Region	Counties	Host plant	Number Fields
Western	Busia,	Cassava	12
	Bungoma	cassava	12
Nyanza	Siaya,	cassava	12
	Kisumu	cassava	12
	Homabay	cassava	12
	Migori	cassava	12
	Nyamira	Cassava	12
Coast	Taita/Taveta	cassava	12
	Kilifi	Cassava	12
	Kwale	Cassava	12
Estern	Kitui and Machakos	cassava	12
	Machakos	Cassava	12

3.1.2 Collection of B. tabaci population

In each of the regions, 12 fields were randomly selected approximately 10 km apart along by the roadside. In each field after consent from individual farmers, infected cassava plants were assessed for *B. tabaci* population along X-shaped transects (Sseruwagi, 2005; Table 3.1). In all the sites surveyed, 15 to 20 adult whiteflies were collected from the five top most cassava leaves from ventral surfaces using an aspirator.

The adult *B.tabaci* whiteflies were collected from 3 to 5 months old cassava plants during the months of June to September. Collection of the *B. tabaci* population was done when the weather is cool, early in the morning and during the evening The collected adult whiteflies were preserved in absolute ethanol (Thermo Fisher Scientific., UK) and stored at -20 ^{o}C. in sampling bottles until analysis in the laboratory (Sseruwagi, 2005, Mware *et al*., 2012).

3.1.3 DNA extraction

One adult female *Bemisia tabaci* whitefly preserved in ethanol was selected from the 12 sampling sites for DNA extraction. They were washed in distilled water and dried on filter paper for few seconds. The samples were then homogenized with a micro pestle in a 1.5 ml Eppendorf tube containing 50 µl of STE buffer (0.1M NaCl, 10mM Tris-HCl, pH 8.0, 1.0 mM EDTA). Proteinase K was then added to STE buffer and the lysate was incubated for 15 minutes at a temperature of 65 ^{o}C, and then further heat treated at 95 ^{o}C for 10 minutes. The lysis product was centrifuged briefly for 5 minutes at 10000 rpm at 4 ^{o}C and immediately placed on ice before PCR amplification.

3.1.4 PCR amplification of mtCOI DNA

A total of 94 samples were collected based on the description above. These collections were used to study the genetic diversity (by extracting DNA from individual whiteflies from each sample collection) and their distribution in the various cassava growing zones in Kenya. Polymerase chain reaction was conducted using two primers; MT10/C1-J-

2195 (5’-TTGATTTTTTGGTCATCCAGAAGT-3’) and MT12/L2-N-3014 (5’-TCCAATGCACTAAT-CTGCCATATTA-3’), to amplify mitochondria cytochrome oxidase I (mtCOI) DNA. All reactions contained 0.15 µl of 60ng/ µl of dNTPs , 0.5 µl of each primer10um/10pmole, 0.2 µl of Taq DNA polymerase, 5 µl of 5 x My Taq Reaction buffer, and 5.0 µl of DNA template (5 x Taq master enhancer) and topped up to final reaction volume of 25 µl with 13.80 µl nuclease free water. The contents were vortexed briefly and quickly spun. Initial denaturation of template DNA was conducted for 3 minutes at 94^{o} C followed by 30 cycles of denaturation at 94° C for 30 seconds, primer annealing at 52°C for 30 seconds, and extension at 72°C for 1 minute. The final extension of 10 minutes was run at 72° C and the reaction held at 4°C in an Applied Biosystems 2720 thermal cycler (Singapore) (Frohlich *et al*., 1999).

3.1.5 Gel electrophoresis and DNA sequencing

The PCR products were electrophoresed in 2 % agarose gel stained in ethidium bromide (Biotium, CA, USA) in 1 x_Tris acetate ethylenediaminetetraacetic acid (TAE) buffer and the resolved bands visualized under Benchtop 2 U V transilluminator, (Cambridge UK). The gel was photographed using an Electrophoresis Documentation and Analysis System 120 digital camera (Canon USA., Inc.). PCR products of the expected size (850 bp) were excised from the agarose gel and purified using Qiagen gel Purification kit (QIAGEN Inc, San Diego, CA, USA) as per the manufacturer’s procedure. Sequencing was outsourced at Bioscience Centre for Eastern and Central Africa, Nairobi, Kenya in collaboration with University of Wisconsin Biotechnology Centre, Madison, USA, and done bi-directionally using the amplification primers. DNA sequences produced in this study were identified using the BLASTn algorithm at GenBank (http://www.ncbi.nlm.nih.gov). The sequences of COI were submitted to GenBank using Bankit, a web-based data submission tool (Appendix 1).

3.1.6 Phylogenetic analysis of mtCOI sequences

B. tabaci mtCOI sequences 44 in number were edited manually using the Optimal Alignment method of DNAMAN (version 5.0; Lynnon BioSoft, Québec, Canada) program to produce a consensus sequence (~850 bp) for each individual adult whitefly. The edited consensus sequences were aligned using Clustal W (MEGA 6.06-ClustalW) (Thompson *et al.,* 1994). Upon using MrBayes version 3.2.1 (Ronquist *et al*., 2012) that employs Markov Chain Monte Carlo (MCMC) sampling to approximate the posterior probabilities of phylogenies (Peter, 1995); the posterior probabilities are shown above the branches (Appendix 2). MrBayes 3.2.1 was run in parallel on the Magnus supercomputer (located at Pawsey Supercomputer Centre, Perth, Western Australia) utilizing the BEAGLE library (Ayres *et al*., 2012

3.2 Determination of population genetic structure of *B. tabaci* populations from selected sites in Kenya

3.2.1 Analysis of molecular variance (AMOVA)

Polymerase chain reaction (PCR) of the mitochondrial cytochrome oxidase I (mtCOI) gene was used to assess the genetic structure of whiteflies (Gorsane *et al*., 2011). Molecular variance (AMOVA) was determined using ARLEQUIN software version 3.0 (Excoffier *et al*., 2005) to describe the distribution of genetic variability between defined groups, between populations inside each group and between all populations (Mugerwa *et al*., 2012; Dhia *et al*., 2013).

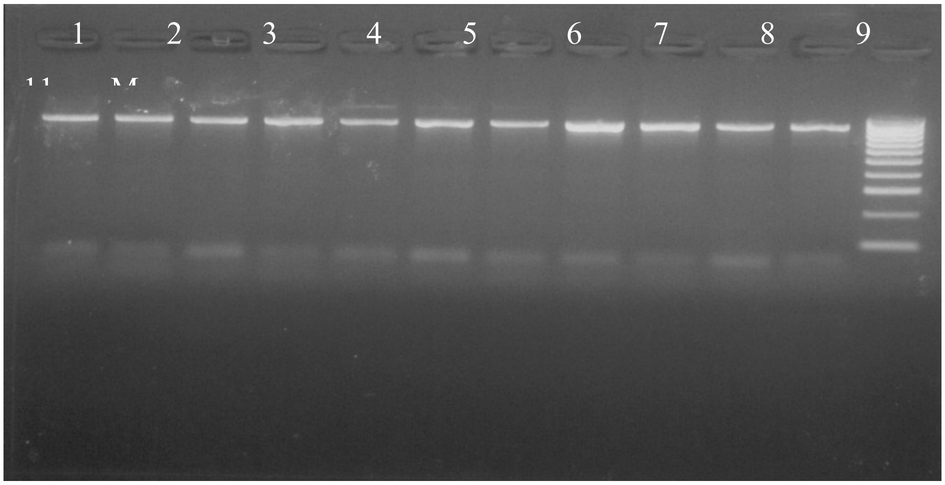
CHAPTER FOUR

RESULTS

4.1 Genetic variability among B. tabaci from cassava growing areas of Kenya

4.1.1 Distribution of Bemisia tabaci putative species on Cassava in Kenya

Amplification of the mtCO1 gene resulted in an 850 bp fragment (Figure 4.1) for each adult whitefly using primer pair MT10 and MT12. 23 out of 94 samples gave negative results during PCR amplification analysis. 71 samples amplified and purified for sequencing obtaining 44 samples which were manually edited to consensus sequences from both forward and reverse sequencing was obtained size approximately 850pb of *B. tabaci* individual whiteflies. Sequences with low quality and bases were not conclusively identified were excluded. The 44 mtCOI sequences from this study were combined with other mtCOI sequences from global whitefly samples found in www.whiteflybase.org. The final dataset composed of 659 sequences



-850bp

Figure 4.1: Agarose gel of PCR-amplified product (~850 bp) size. Lanes 1-12 are female individual whitefly (Bemisia tabaci). Lane M: DNA ladder of 2 kbp.

These data are also mapped onto Figure 4.3, and there is an interactive map found at http://beta.whiteflybase.org/datamap/.

Table 4.1: Geographic distribution and proportion (%) of two genetic groups of *Bemisia tabaci*

Location	No. of samples	SSA Clade A	SSA Clade B	SSA Clade C	SSA Clade D	SSA Clade E
Western	7 (15.9%)	1 (2.27%)	2 (4.55%)	2 (4.55%)	2 (4.55%)	-
Nyanza	16(36.36%)	-	12 (27.27%)	-	2 (4.55%)	2(4.55%)
Eastern	3 (6.81%)	-	3 (6.81%)	-	-	-
Coast	18(40.91%)	-	18 (40.91%)	-	-	-

The results of the current study reveal the distribution of two different *Bemisia tabaci* sub-Saharan Africa species in Kenya (Figure 4.1). The putative species (Boykin, 2014) found in my study are from the sub-Saharan Africa species SSA1 and SSA2, in which five distinct clades were identified and labelled (clades A–E) (Appendix 1 and Figure 4.2). The first clade (A) can be compared to the SSA2 putative species (Dinsdale *et al*., 2010) with sequence similarity of 99.5% which was also referred to as the "invader" (Legg *et al*., 2002). According to (Legg *et al*., 2002) SSA2 is frequently found in western Kenya and is an invasive vector in cassava growing areas which is associated with increased incidences of CMD in various geographical regions in East Africa (Maruthi *et al*., 2005).

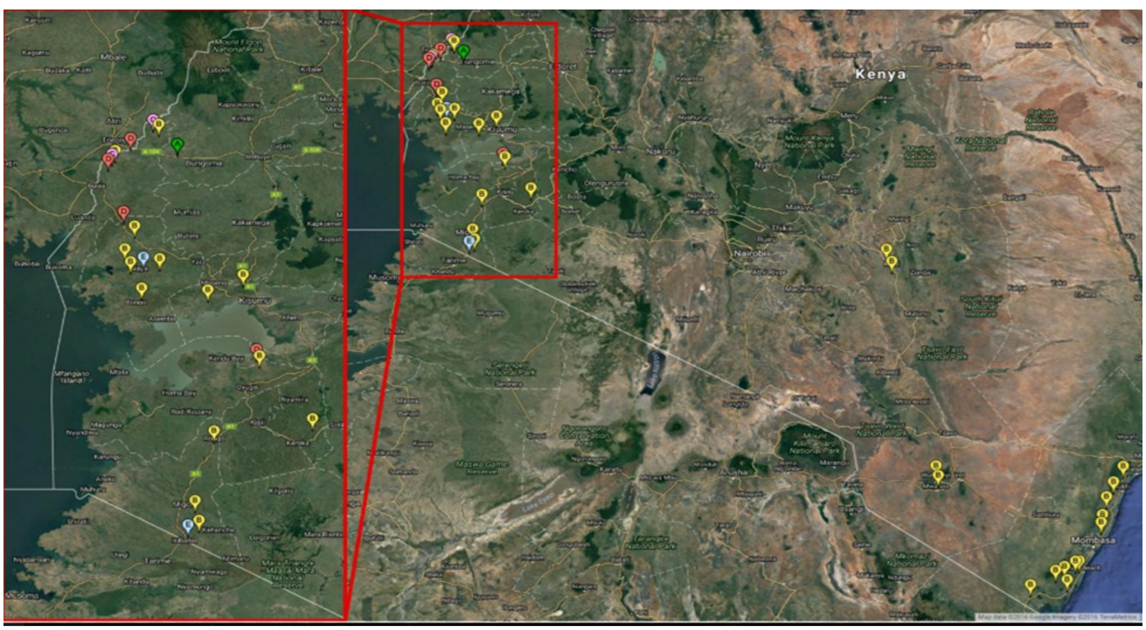


Figure 4.2: Map of Kenya showing distribution of cassava whiteflies included in this study. The red box highlights the collection sites near Lake Victoria, and the red

box to the left is a blow-up of that region. The different coloured circles with letters represent the five different clades of *Bemisia tabaci* species (A–E or SSA-A–E) as indicated in Appendix 1 in the column labelled "Genetic Group".(Manani *et al*., 2017)

The geographic distribution of the species found in Kenya is intriguing. Only SSA-B was found at the coast while around Lake Victoria there was four different genetic entities (SSA-A, B, C, D) identified (Figure 4.4 and Table 4.2). The second clade (B) clustered with other *B. tabaci* sequences that have sequence similarity (97%–98.8%) from throughout east and southern Africa (Esterhuizen *et al*., 2013) (Figure 4.4). Thirdly, clade C specimens were collected from one county, Busia, and two samples from the region form a unique clade to Kenya not found before in previous sampling efforts in the region. Fourthly, clade D, sampled from counties surrounding the Lake Victoria Basin and from Busia, is also unique to Kenya. Finally, the last clade (E) clustered with the sub-Saharan Africa (SSA1) based on analysis done including previously published sequences from southern Uganda (Legg *et al*., 2002).). MrBayes 3.2.1 was run with a GTR + I + G model of molecular evolution, utilizing four chains for 30 million generations and trees were sampled every 1000 generations. All runs reached a plateau in likelihood score, which was indicated by the standard deviation of split frequencies (0.0015), and the potential scale reduction factor (PSRF) was close to one, indicating that the MCMC inverged.

Distinct clades A–E blown up below with new sequences generated in this study highlighted in green.

(a) Clade A

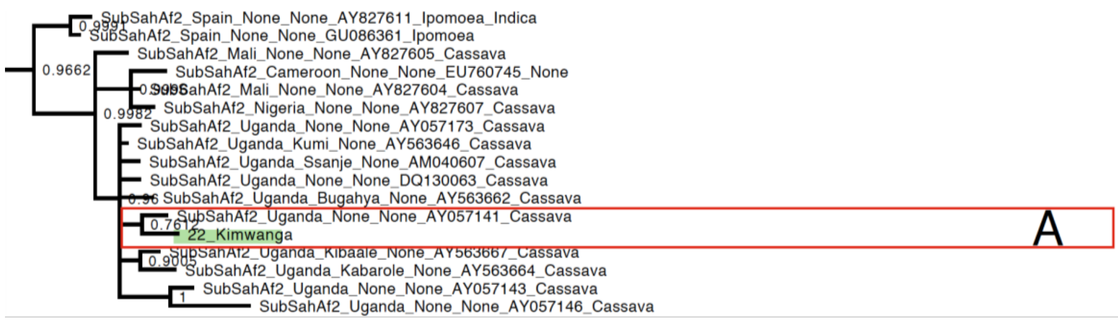


Figure 4.4a. MrBayes phylogenetic tree based on the mitochondrial cytochrome oxidase I gene sequences for B. tabaci collected in Kenya, representing clade A , highlighted green

(b) Clade B

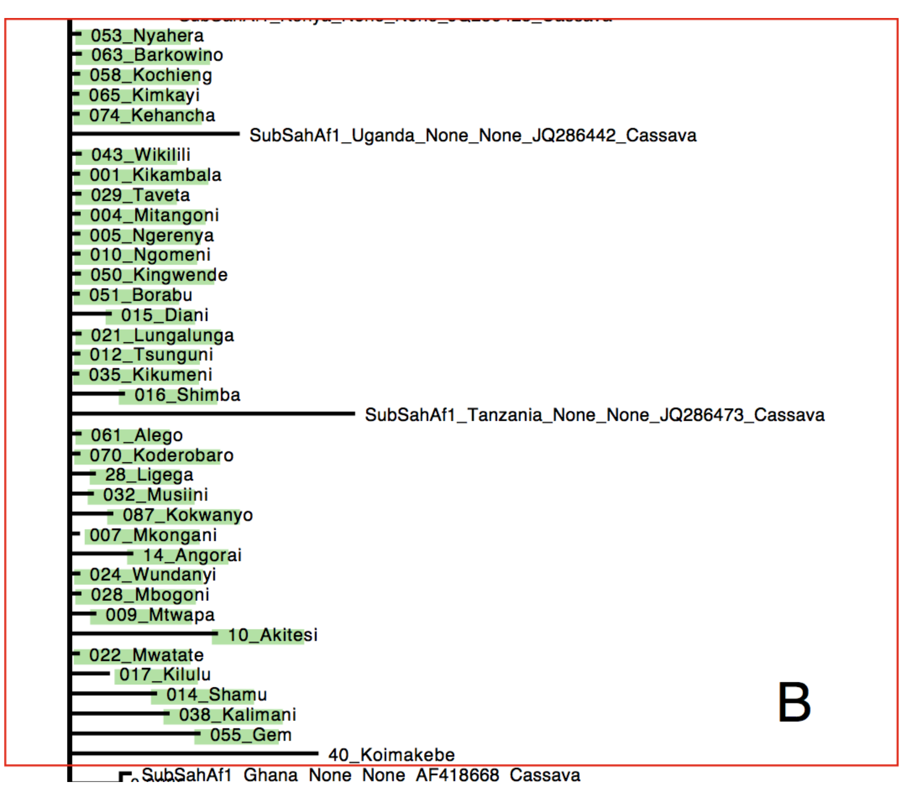


Figure 4.4b. MrBayes phylogenetic tree based on the mitochondrial cytochrome oxidase I gene sequences for *B. tabaci* collected in Kenya for Clades B

Figure 4.4c. MrBayes phylogenetic tree based on the mitochondrial cytochrome oxidase I gene sequences for *B. tabaci* collected in Kenya for Clades C-E

4.2 The population genetic structure of *B. tabaci* from the selected sites in Kenya

4.2.1 Analysis of molecular variance (AMOVA)

A hierarchical AMOVA (Excoffier *et al*., 2005) was conducted to assess the genetic differentiation of the *B. tabaci* populations in cassava growing areas in Kenya (Table

4.3). The four populations were grouped into the SSA species, with the five clades. Comparative results from this study revealed significant differences among groups/clades ($P < 0.001$, FCT = 0.0000), among populations within groups ($P < 0.001$, FSC = 0.2000), and within populations ($P = 0.05$, FST = 0.20000). The highest contribution to the total variance was the differences among groups (120%). A similar result was obtained with the Tajima and Nei distance method (data not shown).

Table 4.2: Hierarchical analysis of molecular variance and F-statistics of genetic differentiation of Bemisia tabaci populations from cassava growing areas in Kenya

Source of Variation	df	Sum of Squares	Variance components	% of Variation	F-statistics	P- Value
Among groups	3	0.0000	0.0000Va	0.00	0.0000	<0.001
Among populations within group	40	-0.0000	-0.08889Vb	-20	0.20000	<0.001
Within populations	220	117.333	0.53333Vc	120	0.20000	<0.001
Total	263	117.333	0.44444			*p<0.05

The *B. tabaci* complex was grouped according to species (groups), among population within groups and within populations. The population expansion may not be uniform due to the population dynamics of the pernicious vector. All comparisons between populations from different cassava growing regions were significantly different from zero to 0.17 (Appendix 3). The pairwise comparisons between regions indicate some aspect of genetic structure within the *B. tabaci* species complex collected in Kenya. Genetic variability among groups of *Bemisia tabaci collected* from the four regions growing cassava show no variation generally. Variation within SSA1 and SSA2 variation is very high bringing a huge difference percentage difference very high (Table 4.2).

Table 4.3: The spatial expansion testing for populations collected in Kenya for the period 2013-2015

Values	**Statistics**
Mismatch observed mean	1.067
Mismatch observed variance	1.067
Tau	2.321
Theta	0.001
M	1.986
Harpending's Raggedness index	0.7866
Sum of Squared deviation	0.15519673

CHAPTER FIVE

DISCUSSION

5.1 The population genetic structure of *B. tabaci* from selected sites in Kenya

In this study Amova analysis on the genetic structure of *B. tabaci* shows that genetic variability is low. The species complex using COI demonstrates that genetic differences are small thus evident that geneflow between genetic populations is scanty (De Barro, 1995). Analysis of molecular variance confirmed differences between whitefly samples collected from the coastal, eastern western and Nyanza. Cassava *B. tabaci* sequences (2013-15 collections) shows significant values that are comparable with previous analyses (Legg *et al*., 2002, 2013). On population genetics the mismatch analysis results reveals a significant change of SSA1-SSA2 population (Table 4.3). Wide genetic exploration of *B. tabaci* suggests that the populations are genetically heterogeneous. Genetic distances between populations and sub populations support the view that these populations are reproductively isolated (De Barro *et al*., 2007). The individuals within each population display considerable genetic heterogeneity which is revealed by the genetic distances (0.402).These results indicate that *B. tabaci* whiteflies within the cassava growing areas are not significantly differentiated. When compared to other studies of *B. tabaci* population globally our findings relatively high level of genetic variability (Dhia *et al*., 2013). Reproductive studies that were undertaken crossing between SSA1 and SSA2 confirmed the two putative species are reproductive compartible (Maruthi *et al*., 2004) indicating an aspect of genetic structure of gene flow within the groups. The presence of clade B along the lake region which predominate the coastal region is as result of expansion due to transport by human activities such as transportation of infested planting materials of cassava (Diaz *et al.,* 2015)

5.2 Phylogenetic relationships among *B. tabaci* from cassava growing areas of Kenya

Cassava is one the most important crop host for *B. tabaci* in Africa, and the insect occurs on cassava wherever it is grown, within the sub-Saharan region the insect is comprised of five putative species SSA1, SSA2, SSA3, SSA4, and SSA5 (Legg *et al*., 2002; Abdulahi *et al*.,2003). There is insufficient data to accurately represent the putative species distribution of each five species. Current information suggests SSA1 is the most widely distributed species in East, Southern and West Africa (Legg *et al*., 2002). SSA3 and SSA4 reported in Cameroon, Congo DRC and Uganda while SSA5 only in South Africa (Esterhuizen *et al*., 2013). Using the mtCOI gene as a molecular marker, this study determined the occurrence of two *B. tabaci* species. They form five distinct but closely related clades within the sub-Saharan Africa (SSA1 and SSA2) species (Table 4.1). SSA 1-B is the most abundant clade found in Kenya and is widely distributed along the coast of Kenya and around Lake Victoria basin area. This corresponds to where cassava is grown in Kenya (Simon *et al*., 2006). The nature of the agro-ecological zones where cassava is grown in Kenya is hot and humid (De Barro *et al*., 2000), which also supports many other crops that become alternative hosts of whiteflies, such as sweet potato and tomato, thereby increasing the spread of the vector and transmission of viral diseases in cassava. Whitefly collections from cassava fields (three to six months) from major cassava growing areas in Kenya have a close relationship with the virus diseases that are widely distributed in different agro-ecological zones (low, medium, and high altitude at less or greater than 1000 m above sea level (Ndunguru *et al*., 2015). The climate around Lake Victoria Basin is an equatorial one with temperatures modified by the relatively high elevations surrounding the basin, such as Mt. Elgon. Temperatures and rainfall are relatively lower than the typical equatorial conditions to be classified as a sub-humid climate (temperature range between 20^{o} C to over 35^{o} C). The rainfall ranges between 1000 mm and 1500 mm with no distinct dry season in the year, thereby creating a favourable atmosphere for SSA whiteflies to spread throughout the Lake Basin and riparian states. However, along the coast, the climatic conditions are quite different thereby highlighting the diverse habitat SSA-B can inhabit. CMD and CBSD are both cassava virus pandemics that cause 100% loss to cassava during production in most parts of East Africa (Maruthi *et al*., 2005). There is continued spread of CBSD in most areas of East Africa affected by CMD, probably because of large populations of *B. tabaci* during the early planting seasons when the rains are available (Legg *et al*., 2014). In terms of CBSD, the coast is reported to be the original epidemic zone of CBSD, while the Great Lakes regions are the most recent epidemic zone (Legg *et al*., 2011). One hypothesis is that the native range of SSA-B is along the coast in Kenya and appears to be displacing the local species of Nyanza and the Western regions by invading these new areas. The alternative hypothesis is that SSA-B originated in the Lake zone and has been moved to the coast *via* the movement of cuttings that could potentially be infested with whitefly nymphs. To test these hypotheses, a thorough population genetics study is needed to detect migration patterns of SSA-B. There are other members of the *B. tabaci* complex that are also widespread and invasive. For example, it has been reported that in many parts of the world there has been an explosive outbreak of *B. tabaci* species in the tropics and sub-tropic regions, where species *B. tabaci* MEAM1 and *B. tabaci* MED are extremely polyphagous (Brown *et al*., 1995). These species have the ability of exhibiting resistance to many insecticides, high fecundity, and the capability to displace their competitors (Brown, 2007). Through close monitoring of *B. tabaci* MEAM1, it has been observed to cause significant losses through its ability to rapidly expand its population, transmit Geminiviruses on the host crops, and overcome the effects of insecticides (Thompson, 2003), the same could be true for SSA-B. In addition, (Figure 4.3) shows that clades C and D of *B. tabaci* are unique and are spread around Lake Victoria Basin of Kenya and Uganda where the cassava crop is highly affected by the CMD and CBSD epidemics. Work done on the epidemiology of both CMD and CBSD in most parts of East Africa is associated with the presence of *B. tabaci* (Legg, 2010), however, most previous studies have failed to correctly characterize the species of *B. tabaci* found. Clade C can only be found in one locality in Busia County away from the Lake Victoria Basin region (Figure 4.1). The viral diseases are rapidly spread from plant to plant and between fields by the whitefly vectors, *B. tabaci*, and producing the phenomenon of a spreading severe disease "front" that advanced through the southern part of Uganda and to the neighbouring countries (Legg *et al.,* 2014). Phylogenetic analysis of the whiteflies in Kenya indicates the occurrence of five different clades of *B. tabaci* from the surveyed areas, which suggests close relationships within the cryptic species complex as well as the evolutionary history within the riparian states of East Africa (De Barro, 2012). In the past, clade A (SSA2) was a major genetic group prevalent in areas affected greatly by the CMD pandemic that most recently has been reported to be absent from cassava collections in Uganda (Sseruwagi *et al*.,2006, Mugerwa *et al*., 2012, Legg *et al*.,2013) and in western Kenya (Mugerwa *et al*., 2012, Ayres *et al*.,2012). This study reports the detection of SSA2 in western Kenya along the boundary of Kenya and Uganda. SSA2 is referred to as an invasive whitefly species ("invader/Ug2") that is associated with areas that are severely affected by CMD in Uganda (Otim-Nape *et al*., 1997, Jarvis *et al*., 2012). The putative species SSA2 most probably moved from Uganda to Kenya through exchange of infested cassava planting materials and/or environmental influences, such as the effect of strong winds. There are many viral species and many whitefly species circulating in East Africa. The causal viruses of CMD are: African cassava mosaic virus-Kenya (ACMV-K), and East African cassava mosaic virus (EACMV), East African cassava mosaic Zanzibar virus (EACMZV), Uganda variant strain of the EACMV (EACMV-Ug), and East African cassava mosaic Kenya Virus (EACMKV). Cassava brown streak disease is linked to two recognized species, Cassava brown streak virus and Uganda cassava brown streak virus. Many more viruses are being uncovered as more genomes are characterized (Ndunguru *et al*., 2015, Alicai *et al*., 2016). The challenge now is to match these many viruses of CMD and CBSD with the many species in the *Bemisia tabaci* species complex. We can no longer assume the vector is "*B. tabaci*" as there are many species present in the region where these devastating viruses are circulating. All studies in the future to include barcoding of the vector they are finding in their survey data and the transmission studies.

5.3 Conclusions and Recommendations

In sub-Saharan Africa, cassava remains primarily a subsistence crop to farmers, but little attention has been directed to a better understanding and significance on whitefly management strategies on cassava, and this has led to high populations and continued spread of the vector and transmission of the virus diseases in cassava crops (Legg *et al*., 2014). The notorious plant virus vector *B. tabaci* in Kenya are mainly two species (SSA1 and SSA2) grouped into five distinct clades within major cassava growing areas in Kenya. The species SSA2 thought to have come to extinct it is still present in western Kenya. The five clades have a less than 3.5% divergence in mtCOI (Dinsdale *et al*., 2010), but they may differ in terms of their biology, fecundity, virus transmission, and mating ability (Mugerwa *et al*., 2012), and, as such, further biological studies are needed. The genetic structure of the *B. tabaci* complex species within the cassava growing areas in Kenya remains low. In addition, the SSA2 putative species requires further investigation of its role in cassava virus disease epidemiology.

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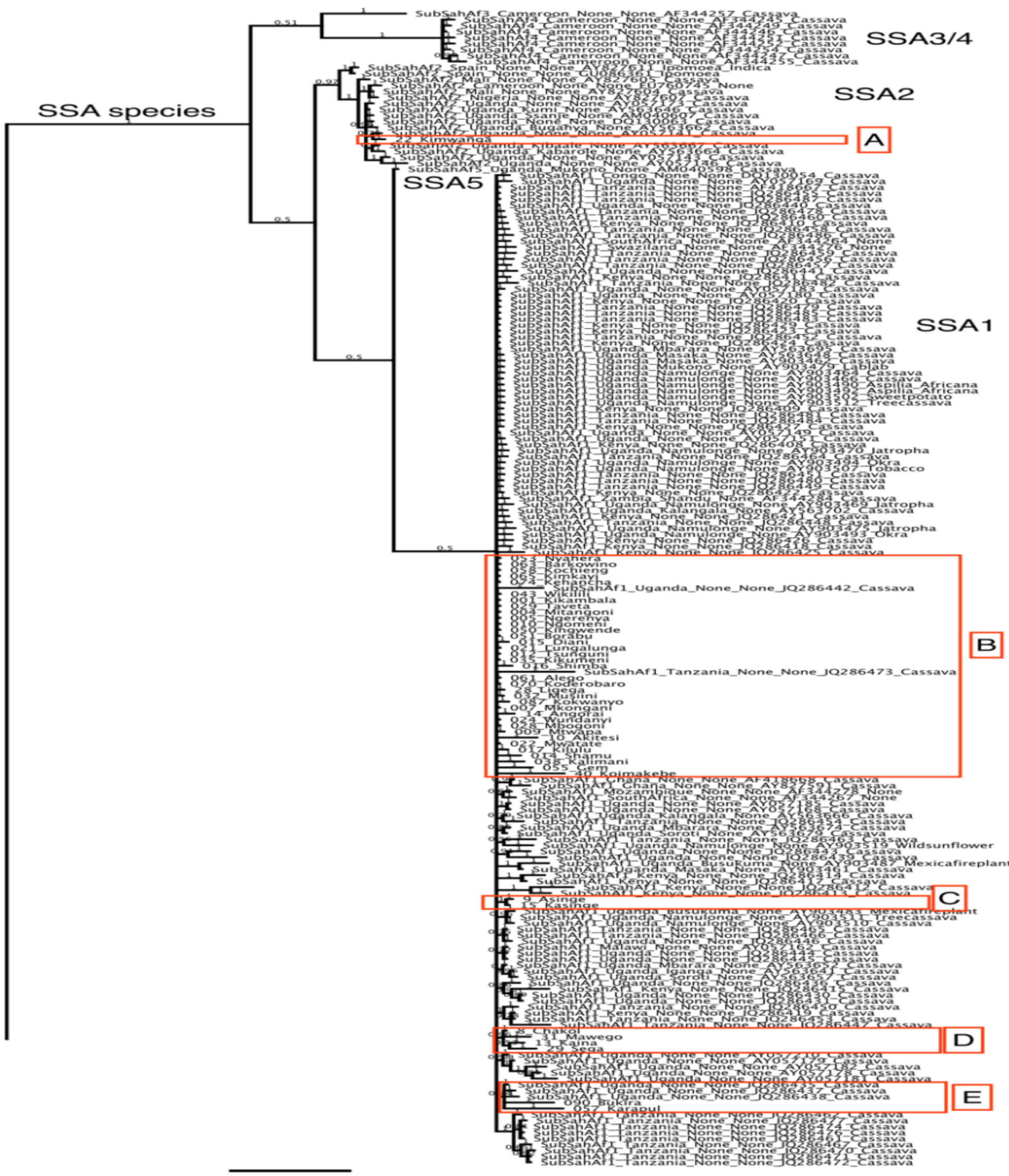
APPENDICES

Appendix 1 B. tabaci collected in Kenya and used in this study.

SampleNo.	County	Area	Field	Geographic Location	Altitude	Genetic	Accession No.
1	Siaya	Ligega	NF3	N00.20321, E034.27061	1330	SSA-B	KY523872
2	Siaya	Sega	NF4	N00.27314, E034.22346	1243	SSA-D	KY523885
3	Siaya	Gem	NF5	N00.03174, E034.25409	1957	SSA-B	KY523894
4	Siaya	Karapul	NF8	N00.05285, E034.30654	3132	SSA-E	KY523892
5	Kisumu	Nyahera	NF2	S00.03051, E034.71960	1459	SSA-B	KY523852
6	Siaya	Kochieng’	NF10	N00.09266, E034.23024	1256	SSA-B	KY523854
7	Siaya	Alego	NF14	N00.04531, E034.37395	1409	SSA-B	KY523870
8	Kisumu	Kitmikayi	NF22	S00.10358, E034.57415	1176	SSA-B	KY523855
9	Siaya	Barwino	NF16	S00.09508, E034.29937	1509	SSA-B	KY523853
10	Homabay	Koderobaro	NF33	S00.78241, E034.59921	1416	SSA-B	KY523871
11	Migori	Bukira west	NF34	S01.23.442, E034.4920	1236	SSA-E	KY523887
12	Homabay	Kokwanyo	NF27	S00.42202, E034.78882	1275	SSA-B	KY523875
13	Busia	Chakol	WF12	N00.52575, E034.16062	1152	SSA-D	KY523877
14	Busia	Asing’e	WF13	N00.54886, E034.17672	1152	SSA-C	KY523880
15	Busia	Aktesi	WF14	N00.56531, E034.18919	1153	SSA-B	KY523886
16	Busia	Kaina	WF17	N00.62388, E034.25398	1177	SSA-D	KY523876
17	Busia	Ang’orai	WF18	N00.69299, E034.37015	1230	SSA-B	KY523879
18	Busia	Kasing’e	WF19	N00.71344, E03434693	1293	SSA-C	KY523881
19	Bungoma	Kimwanga	WF26	N00.59644, E034.44701	1233	SSA-A	KY523874
20	T. Taveta	Wundanyi	CF30	S03.39443, E038.36485	1339	SSA-B	KY523882
21	Homabay	Mawego	NF31	S0038946, E034.77587	1424	SSA-D	KY523888
22	Migori	Kiomakebe	NF40	S01.21158, E034.53714	1562	SSA-B	KY523895
23	Migori	Kehancha	NF41	S01.11733, E034.52141	1418	SSA-B	KY523856
24	Nyamira	Borabu	NF44	S00.72273, E035.00735	1926	SSA-B	KY523864
25	Kilifi	Kikambala	CF1	S03.86207, E039.74467	86	SSA-B	KY523858
26	Kilifi	Mitongani	CF5	S03.69020, E039.77895	61	SSA-B	KY523860
27	Kilifi	Ngerenya	CF7	S03.54610, E039.83553	53	SSA-B	KY523861
28	Kilifi	Mkongani	CF9	S03.39686, E039.91546	20	SSA-B	KY523878
29	Kilifi	Mtwapa	CF12	S03.93400, E039.73534	38	SSA-B	KY523884
30	Kwale	Ngomeni	CF13	S04.12535, E039.63130	25	SSA-B	KY523862
31	Kwale	Tsunguni	CF15	S04.18433, E039.55974	15	SSA-B	KY523867

32	Kwale	Shamu	CF17	S04.30154, E039.54972	42	SSA-B	KY523891
33	Kwale	Diani	CF18	S04.30731, E039.51998	90	SSA-B	KY523865
34	Kwale	S. Hills	CF19	S04.35600, E039.42932	104	SSA-B	KY523869
35	Kwale	Kilulu	CF21	S04.39463, E039.35639	85	SSA-B	KY523890
36	Kwale	Kikoneni	CF22	S04.42855, E039.31791	70	SSA-B	KY523868
37	Kwale	Lungalunga	CF25	S04.53264, E039.14732	64	SSA-B	KY523866
38	Kwale	Kingwende	CF27	S04.48550, E039.45035	22	SSA-B	KY523863
39	T. Taveta	Mwatate	CF28	S03.48400, E038.38088	830	SSA-B	KY523889
40	T.Taveta	Mbogoni	CF36	S03.41414, E037.70263	729	SSA-B	KY523883
41	T. Taveta	Taveta	CF37	S03.46017, E037.69030	720	SSA-B	KY523859
42	Kitui	Kalimani	EF18	S01.31211, E037.95479	1232	SSA-B	KY523893
43	Kitui	Wakilili	EF25	S01.42728, E037.99684	1135	SSA-B	KY523857
44	Machakos	Musiini	EF4	S01.42061, E037.36724	1413	SSA-B	KY523873

Appendix 2: Entire MrBayes phylogenetic tree based on the mitochondrial cytochrome oxidase I gene sequences for B. tabaci collected in Kenya



Appendix 3 A pairwise distance comparison of the mitochondrial cytochrome oxidase I (mtCOI) nucleotide sequence, expressed as percent nucleotide divergence between adult Bemisia tabaci populations identified on cassava in Kenya as calculated using Clustal algorithm (Thompson et al. 1994)

001_Kikambala	001_	8_Ch	9_As	10_A	13_K	14_A	15_K	22_K	28_L	30_K	31_M	29_S	40_K	004_	050_	051_	053_	055_	057_	058_	061_	065_
8_Chakol	0.07																					
9_Asinge	0.08	0.07																				
10_Aktesi	0.07	0.07	0.06																			
13_Kaina	0.06	0.06	0.08	0.09																		
14_Angorai	0.08	0.06	0.05	0.08	0.07																	
15_Kasinge	0.08	0.06	0.04	0.07	0.07	0.04																
22_Kimwanga	0.14	0.16	0.14	0.15	0.16	0.14	0.14															
28_Ligega	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.14														
30_Kobuya	0.06	0.08	0.06	0.07	0.08	0.06	0.07	0.14	0.06													
31_Mawego	0.07	0.06	0.10	0.09	0.08	0.08	0.07	0.16	0.07	0.07												
29_Sega	0.08	0.07	0.07	0.08	0.08	0.07	0.07	0.13	0.06	0.07	0.09											
40_Kiomakebe	0.10	0.12	0.10	0.13	0.12	0.11	0.11	0.17	0.10	0.11	0.12	0.11										
004_Mitangoni	0.04	0.06	0.08	0.08	0.07	0.08	0.08	0.15	0.06	0.08	0.08	0.08	0.11									
050_Kingwende	0.03	0.07	0.07	0.07	0.08	0.09	0.08	0.14	0.06	0.07	0.08	0.08	0.10	0.04								
051_Borabu	0.03	0.07	0.08	0.08	0.07	0.08	0.08	0.14	0.06	0.07	0.08	0.08	0.10	0.04	0.03							
053_Nyahera	0.04	0.06	0.06	0.06	0.05	0.07	0.07	0.14	0.04	0.06	0.06	0.06	0.11	0.05	0.05	0.05						
055_Gem	0.06	0.08	0.08	0.08	0.07	0.09	0.09	0.16	0.07	0.08	0.09	0.08	0.14	0.07	0.07	0.07	0.05					
057_Karapul	0.06	0.07	0.08	0.09	0.07	0.09	0.09	0.16	0.06	0.08	0.09	0.09	0.13	0.07	0.07	0.07	0.06	0.08				
058_Kochieng	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.14	0.05	0.06	0.06	0.06	0.11	0.05	0.06	0.06	0.03	0.05	0.06			
061_Alego	0.04	0.06	0.06	0.06	0.05	0.07	0.07	0.15	0.05	0.06	0.07	0.07	0.12	0.05	0.05	0.05	0.03	0.05	0.04	0.04		
065_Kimkayi	0.04	0.05	0.06	0.06	0.05	0.07	0.06	0.13	0.04	0.05	0.07	0.05	0.10	0.05	0.04	0.04	0.04	0.05	0.05	0.03	0.03	
063_Barkowino	0.04	0.06	0.06	0.06	0.05	0.07	0.06	0.14	0.04	0.06	0.07	0.06	0.11	0.06	0.05	0.05	0.03	0.05	0.05	0.03	0.04	0.02
005_Ngerenya	0.04	0.06	0.08	0.08	0.07	0.08	0.08	0.15	0.06	0.08	0.08	0.08	0.11	0.00	0.04	0.04	0.05	0.07	0.07	0.05	0.05	0.05
007_Mkongani	0.04	0.08	0.08	0.08	0.07	0.08	0.08	0.15	0.07	0.08	0.09	0.08	0.11	0.04	0.05	0.04	0.05	0.07	0.08	0.06	0.05	0.05
009_Mtwapa_KALRO	0.03	0.07	0.08	0.07	0.08	0.09	0.08	0.14	0.06	0.08	0.08	0.08	0.11	0.04	0.03	0.04	0.05	0.06	0.07	0.05	0.05	0.04
010_Ngomeni	0.03	0.07	0.07	0.08	0.07	0.09	0.08	0.14	0.07	0.08	0.08	0.07	0.11	0.03	0.03	0.04	0.05	0.07	0.07	0.05	0.05	0.05
012_Tsunguni	0.03	0.08	0.07	0.08	0.07	0.09	0.07	0.13	0.06	0.07	0.08	0.07	0.10	0.04	0.04	0.04	0.05	0.06	0.07	0.05	0.05	0.04
014_Shamu	0.06	0.09	0.08	0.09	0.08	0.09	0.09	0.15	0.07	0.08	0.10	0.09	0.11	0.06	0.06	0.06	0.07	0.09	0.08	0.07	0.06	0.07
015_Diani	0.03	0.07	0.08	0.07	0.07	0.09	0.08	0.14	0.06	0.07	0.08	0.07	0.10	0.04	0.04	0.03	0.05	0.07	0.07	0.06	0.06	0.04
016_Shimba_hills	0.04	0.08	0.08	0.07	0.07	0.08	0.08	0.14	0.06	0.06	0.08	0.08	0.10	0.04	0.04	0.04	0.05	0.07	0.07	0.05	0.05	0.04
017_Kilulu	0.04	0.07	0.09	0.08	0.07	0.09	0.08	0.16	0.07	0.08	0.09	0.07	0.11	0.05	0.04	0.04	0.06	0.07	0.07	0.06	0.06	0.05
021_Lungalunga	0.02	0.07	0.07	0.07	0.06	0.08	0.07	0.13	0.05	0.06	0.07	0.07	0.10	0.04	0.03	0.03	0.05	0.06	0.06	0.05	0.04	0.03
022_Mwatate	0.04	0.07	0.08	0.08	0.08	0.09	0.08	0.14	0.07	0.08	0.08	0.07	0.11	0.04	0.04	0.04	0.07	0.08	0.07	0.06	0.06	0.06
070_Koderobaro	0.05	0.06	0.07	0.07	0.08	0.08	0.07	0.15	0.05	0.07	0.07	0.07	0.12	0.05	0.05	0.06	0.05	0.07	0.06	0.04	0.05	0.04
074_Kehancha	0.04	0.06	0.06	0.06	0.06	0.07	0.06	0.14	0.04	0.06	0.07	0.06	0.11	0.05	0.05	0.05	0.03	0.05	0.05	0.03	0.04	0.02
087_Kokwanya	0.05	0.06	0.05	0.06	0.06	0.06	0.06	0.13	0.04	0.04	0.07	0.06	0.11	0.06	0.06	0.06	0.04	0.06	0.06	0.04	0.04	0.03
090_Bukira_west	0.05	0.07	0.08	0.08	0.08	0.08	0.08	0.15	0.06	0.07	0.08	0.07	0.13	0.07	0.06	0.07	0.04	0.07	0.06	0.06	0.04	0.04
032_Musiini	0.04	0.07	0.07	0.07	0.07	0.08	0.08	0.13	0.06	0.07	0.07	0.07	0.11	0.04	0.04	0.04	0.05	0.06	0.07	0.04	0.06	0.05
035_Kikumeni	0.04	0.07	0.07	0.07	0.08	0.09	0.09	0.15	0.06	0.08	0.09	0.07	0.12	0.04	0.04	0.04	0.05	0.07	0.07	0.05	0.05	0.05
038_Kaliwani	0.05	0.09	0.10	0.09	0.10	0.09	0.09	0.15	0.08	0.09	0.09	0.09	0.12	0.07	0.06	0.06	0.07	0.10	0.09	0.08	0.08	0.07
041_Kitui_ATC	0.04	0.07	0.07	0.09	0.09	0.07	0.08	0.15	0.06	0.08	0.08	0.09	0.10	0.05	0.05	0.04	0.06	0.08	0.08	0.06	0.06	0.06
043_Wakilili	0.03	0.07	0.06	0.06	0.07	0.08	0.08	0.14	0.06	0.07	0.08	0.08	0.11	0.03	0.03	0.03	0.04	0.06	0.06	0.04	0.04	0.04
029_Taveta	0.03	0.07	0.07	0.07	0.08	0.09	0.08	0.14	0.07	0.07	0.08	0.08	0.10	0.04	0.02	0.03	0.06	0.07	0.06	0.06	0.05	0.05
028_Mbogoni	0.05	0.08	0.08	0.08	0.09	0.08	0.08	0.13	0.07	0.07	0.08	0.09	0.10	0.06	0.05	0.05	0.07	0.09	0.08	0.07	0.06	0.06
024_Wundanyi	0.05	0.08	0.08	0.08	0.09	0.08	0.08	0.13	0.07	0.07	0.08	0.09	0.10	0.06	0.05	0.05	0.07	0.09	0.08	0.07	0.06	0.06

001_Kikambala	063_	005_	007_	009_	010_	012_	014_	015_	016_	017_	021_	022_	070_	074_	087_	090_	032_	035_	038_	041_	043_	029_	028_
005_Ngerenya	0.06																						
007_Mkongani	0.06	0.04																					
009_Mtwapa_KALRO	0.04	0.04	0.04																				
010_Ngomeni	0.04	0.03	0.05	0.04																			
012_Tsunguni	0.04	0.04	0.04	0.04	0.03																		
014_Shamu	0.07	0.06	0.06	0.06	0.07	0.06																	
015_Diani	0.04	0.04	0.04	0.04	0.04	0.04	0.06																
016_Shimba_hills	0.04	0.04	0.05	0.04	0.04	0.03	0.06	0.04															
017_Kilulu	0.05	0.05	0.04	0.05	0.04	0.04	0.06	0.04	0.04														
021_Lungalunga	0.04	0.04	0.04	0.03	0.03	0.03	0.06	0.03	0.03	0.04													
022_Mwatate	0.06	0.04	0.05	0.04	0.05	0.04	0.06	0.05	0.05	0.05	0.04												
070_Koderobaro	0.04	0.05	0.07	0.06	0.05	0.06	0.08	0.06	0.06	0.06	0.06	0.06											
074_Kehancha	0.03	0.05	0.05	0.05	0.05	0.04	0.06	0.05	0.04	0.06	0.04	0.06	0.05										
087_Kokwanya	0.04	0.06	0.07	0.06	0.06	0.05	0.07	0.06	0.05	0.07	0.04	0.07	0.06	0.03									
090_Bukira_west	0.05	0.07	0.07	0.07	0.07	0.06	0.08	0.06	0.07	0.07	0.05	0.08	0.06	0.05	0.05								
032_Musiini	0.05	0.04	0.06	0.04	0.04	0.04	0.06	0.04	0.04	0.06	0.04	0.05	0.06	0.04	0.05	0.06							
035_Kikumeni	0.05	0.04	0.06	0.04	0.03	0.03	0.07	0.04	0.05	0.05	0.04	0.06	0.05	0.05	0.06	0.07	0.04						
038_Kaliwani	0.08	0.07	0.06	0.06	0.07	0.06	0.07	0.07	0.07	0.06	0.06	0.06	0.09	0.07	0.08	0.09	0.07	0.07					
041_Kitui_ATC	0.06	0.05	0.04	0.05	0.05	0.04	0.06	0.05	0.05	0.05	0.05	0.05	0.07	0.06	0.06	0.07	0.06	0.06	0.06				
043_Wakilili	0.05	0.03	0.04	0.03	0.03	0.03	0.06	0.04	0.03	0.04	0.03	0.04	0.06	0.04	0.04	0.06	0.03	0.03	0.06	0.05			
029_Taveta	0.04	0.04	0.05	0.04	0.03	0.03	0.06	0.04	0.04	0.04	0.03	0.04	0.05	0.05	0.06	0.06	0.04	0.04	0.07	0.05	0.03		
028_Mbogoni	0.07	0.06	0.06	0.06	0.06	0.05	0.07	0.05	0.05	0.06	0.04	0.06	0.08	0.06	0.06	0.07	0.06	0.07	0.05	0.05	0.05	0.05	
024_Wundanyi	0.07	0.06	0.06	0.06	0.06	0.05	0.07	0.05	0.05	0.06	0.04	0.06	0.08	0.06	0.06	0.07	0.06	0.07	0.05	0.05	0.05	0.05	0.00