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Contribution of cassava-maize-common beans inter-cropping system to the management of cassava mosaic disease and its vector

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ABSTRACT

Cassava mosaic disease (CMD) is reported as the most important constraint on cassava in Sub-Saharan Africa. The understanding of the disease epidemiology, the genetic diversity of the virus and its vector is a key factor for the disease management. In this study, cropping system associating cassava, common beans (*Phaseolus vulgaris*) and maize, the most frequent cassava cropping system in Eastern DR Congo, was investigated to understand the disease characteristics and existing interactions within this pathosystem: cassava mosaic begomoviruses (CMBs), cassava and non-cassava plants, *Bemisia tabaci* population and agro-ecosystems in which they are found. Different geographic locations mainly depending on the altitude levels showed a significant influence on CMD incidence and severity, and whitefly population. CMD and its vector pressure were high in low altitude, but significant decrease was observed in intercropped fields. Under high epidemic pressure, respective decreases of 19% and of 33.3% were recorded on disease incidence and whitefly population. Only two CMBs (ACMV and EACMV-UG) were detected both in cassava plants and in whiteflies collected on cassava and other plant species. In contrast, no CMBs were detected in non-cassava associated plants. *Bemisia tabaci* characterization based on *MtCOI* sequences analysis revealed the presence of a single haplotype close to Ug1, irrespective of the plant species on which the insects were collected. The low diversity of the CMBs and whitefly population combined with the impact of local intercropping system cassava, common beans and maize are discussed, underlining the interest of the other ways of CMD management besides crop resistance strategies.

KEYWORDS: cassava mosaic disease (CMD), cassava mosaic begomoviruses (CMBs), intercropping system, *Bemisia tabaci*, Democratic Republic of Congo (DRC), South-Kivu

INTRODUCTION

Cassava is one of the most cultivated tropical crops and nourishes more than 700 million of the world population. Africa produces more than fifty percent of the world's production [1]. Cassava cultivation is an economical opportunity for African farmers due to its high yield under various environmental conditions including drought, low fertility and acid soils [2, 3, 4]. It produces food for family subsistence and cash. Its cultivation is compromised by biotic and abiotic constraints among which cassava mosaic disease (CMD) caused by several cassava mosaic begomoviruses (CMBs) is the most important [2]. It causes 25 to 95% of yield loss depending on the infection period, virus types, varieties and ecological conditions [5, 6, 7, 2, 8, 9].

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CMD management includes several strategies. Three of them are mostly used. The first one consists of preventing infections, delaying the time of infection or minimizing the effects of infection once it has occurred. The second and significant is the use of phytosanitation (uprooting the diseased plants and selecting disease-free stems for new planting) combined with the third concerning the development and deployment of resistant varieties [2]. Thresh & Cooter [3] mentioned that cultural practices, vector control and mild-strain protection should also be included to complete CMD management strategies but underlined that insufficient information is available on their use in Africa. Intercropping and varietal mixtures in which resistant varieties are used to 'protect' susceptible local cultivars have been shown to provide some degree of protection for CMD-susceptible material in Uganda [10, 11]. Although CMD pandemic has risen and spread through the interaction of virus-vector and host plant, less attention has been given to the vector management [2] while CMD pandemic has spread with super-abundant whitefly populations in epidemic and post epidemic areas [12, 2]. Okorogri *et al*. [13] have studied the re-infection rate of cassava mosaic disease on free genotypes and showed that under high epidemic pressure, virus-free cassava plants from tissue culture were re-infected four weeks after planting. Seven weeks after planting, more than sixty percent of cassava plants were re-infected. CMD management strategies which include the vector management are promising.

In the tropics, cassava is usually grown alone or together with one or more other crops, including legumes and cereals. In DR Congo (South-Kivu province), cassava is commonly grown intercropped with common beans (*Phaseolus vulgaris*) and maize. This cropping system is also the most commonly used by local farmers in Rwanda [14].

The intercropping system is mentioned to be a sustainable crop production system in Africa. It is associated with the rational use of the land, increasing and diversifying harvest products, and an integrated soil fertility management approach [15]. It is also mentioned as an alternative approach for crops pests and diseases management in different pathosystems resulting in chemical use reduction [16, 17]. Its impact on bacterial blight severity and yield of common beans and maize was attested by Fininsa [18]. Zinsou *et al*. [19] reported a cassava bacterial severity reduction induced by the intercropping system while Pridham & Entz [20] have observed a suppression of weeds and diseases in intercropped wheat resulting in grain yield increase as also observed on rice by Wang Han [21].

The great diversity of the genotypes grown in the cropping systems is expected to influence the incidence and severity of pests and diseases. However, this possibility has received only limited attention especially in the case of CMD in Uganda and adjacent parts of eastern and central Africa [10]. There is limited evidence on the spread of CMD in cassava grown with other crop species (banana, sweet potato, cereals and legumes) that may have beneficial effects through improving overall land productivity and by decreasing whitefly vector populations, whitefly activity and virus spread [3]. There is evidence from Ivory Coast and Uganda studies that the spread of CMD is influenced by host plant population density. Disease incidence was high at the widest spacing adopted and alongside footpaths or around gaps in otherwise continuous stands of cassava [22, 23, 24]. Similar results were reported by Fondong *et al*. [25] in a field trial where cassava was grown alone and intercropped with maize and/or with cowpea in Cameroon. The intercropping system has reduced both CMD incidence and whitefly population for cassava planting until six months after plantation when maize and cowpea were harvested. Afterwards, whitefly population increased and CMD transmission expected but no significant yield loss could be induced. Separately, Ogbe *et al*. [26]; Alabi *et al*. [27] and Monde *et al*. [28] have diagnosed CMBs presence in legumes, weeds and other non cassava species and showed that the virus produced mosaic symptoms. Until now, none of the available studies have analyzed the interactions of cassava, non cassava plants, whiteflies and CMBs in relation with agro-ecosystems in which they evolved.

This study aims to provide information on (i) CMD epidemiologic characteristics (incidence, severity and infection type), (ii) whitefly population

and its characterization, and (iii) CMBs in whiteflies, cassava and non cassava associated species in farmers fields, where cassava is grown alone and inter-cropped with common beans (*Phaseolus vulgaris*) and maize in different agro-ecosystems in the South-Kivu province, Eastern DRC.

MATERIALS AND METHODS

This study is based on an epidemiological survey in the South-Kivu province, eastern Democratic republic of Congo (DRC) in 8 villages where cassava is grown both in monoculture and intercropped with maize and beans in farmer's fields. The selected villages are located in different agro-ecological conditions (forest, savanna semi-arid zones, low to high altitude, and costal lake zone with frequent strong wind). The selected villages were split up into three altitude agro-ecosystems. In the first, the tropical zone in low altitude (climate type Aw_{1-3}, altitude < to 1000 m, rainfall < 1300 mm/year, annual mean temperature > 24 °C), Luvungi, Sange and Kiliba villages were selected. The second, tropical zone mid-altitude (climate type Aw_3, altitude 1000-1400 m, rainfall > 1300 mm/year, annual mean temperature 20-24 °C), Kalehe, Katana, Kavumu and Mudaka villages were selected. The third, tropical zone in high altitude (climate type Cw, altitude > 1400 m, Rainfall > 1300 mm/year, annual mean temperature 12-20 °C), Walungu and Nyangezi villages were selected. In mid and high altitude, clay soils are predominant, while in low altitude, sandy soils with alluvial deposits are predominant.

In each village, three fields were monitored in each group (monoculture and intercropping) and CMD epidemiologic data (incidence, severity, whitefly population, cutting-borne infection and whitefly infection) were recorded for each observed plant. CMD incidence was determined as a percentage of diseased plants while CMD severity was recorded as per a 1-5 scale as proposed by Hahn *et al*. [29] and adapted by Sseruwagi *et al*. [30] where 1 is recorded for a healthy plant with no CMD symptoms and 5 for a severely diseased plant. Whitefly population was estimated as the mean whitefly number per leaf on the five top cassava leaves. Suspicion of cutting-borne infection was recorded for a cassava plant with CMD symptoms on all the plant leaves while whitefly infection was recorded when only upper new leaves were diseased [30].

Cassava, bean, sweet potato, cocoyam leaves and whitefly insects were collected from the studied area for later PCR CMBs diagnostic and whitefly characterization. Cassava and bean leaves were collected from intercropped fields while sweet potato and cocoyam leaves were collected from around these fields. Whiteflies were collected from cassava, beans and sweet potato plants.

Total DNA was extracted from the collected leaf samples using the protocol described by Dellaporta [31] and the FastDNA^R Kit with FastPrep^R instruments (Qbiogene inc., CA).

DNA-A AC2 and AC4 region specific primers for ACMV and EACMV diagnostic were used whereas MT10 and C1J2195 were used for *Bemisia tabaci MtCOI* region amplification [32, 28].

PCR amplicons were sequenced using a Bio Genetic Analyser 3100. Then sequences were compared with NCBI database of CMBs and *Bemisia tabaci MtCOI*, homology percentages were established using Clustalw EBI server for multiple sequence alignment. Phylogenetic tree analysis was carried out using MEGA 4.0 software [33].

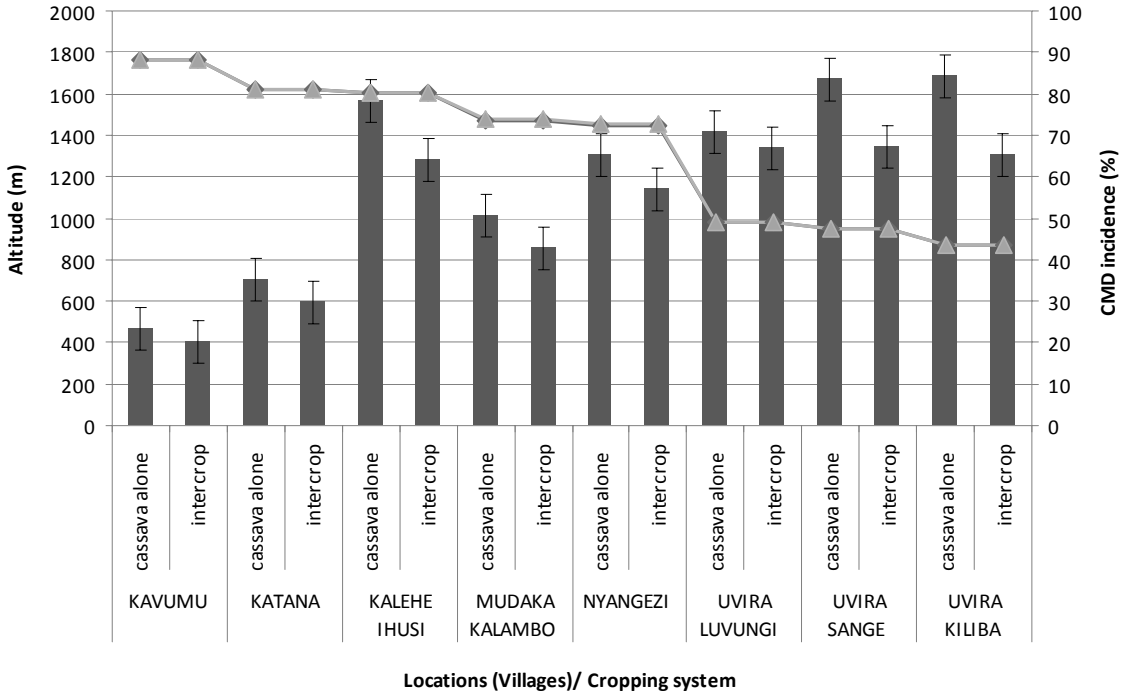
Collected field data were analyzed with general analysis of variance (ANOVA) and simple correlation using GenstatDiscovery edition 3 (www.vsni.co.uk).

RESULTS AND DISCUSSIONS

Intercropping impact on CMD and its vector population

The results on the intercropping impact on CMD and whitefly population are presented in Figure 1. a. b. c. These results showed a significant difference of CMD epidemiologic data with a clear influence of location site (altitude) and cropping system (monoculture and intercropping). Kalehe and Uvira locations have the highest values. In Uvira, we have recorded 72.9%, 3.31 and 15 respectively for CMD incidence, severity and whitefly number when in Kalehe 71.1%, 3.34

(a)


Altitude (m)
CMD incidence (%)
cassava alone
intercrop
KAVUMU
KATANA
KALEHE IHUSI
MUDAKA KALAMBO
NYANGEZI
UVIRA LUVUNGI
UVIRA SANGE
UVIRA KILIBA
Locations (Villages)/ Cropping system

(b)

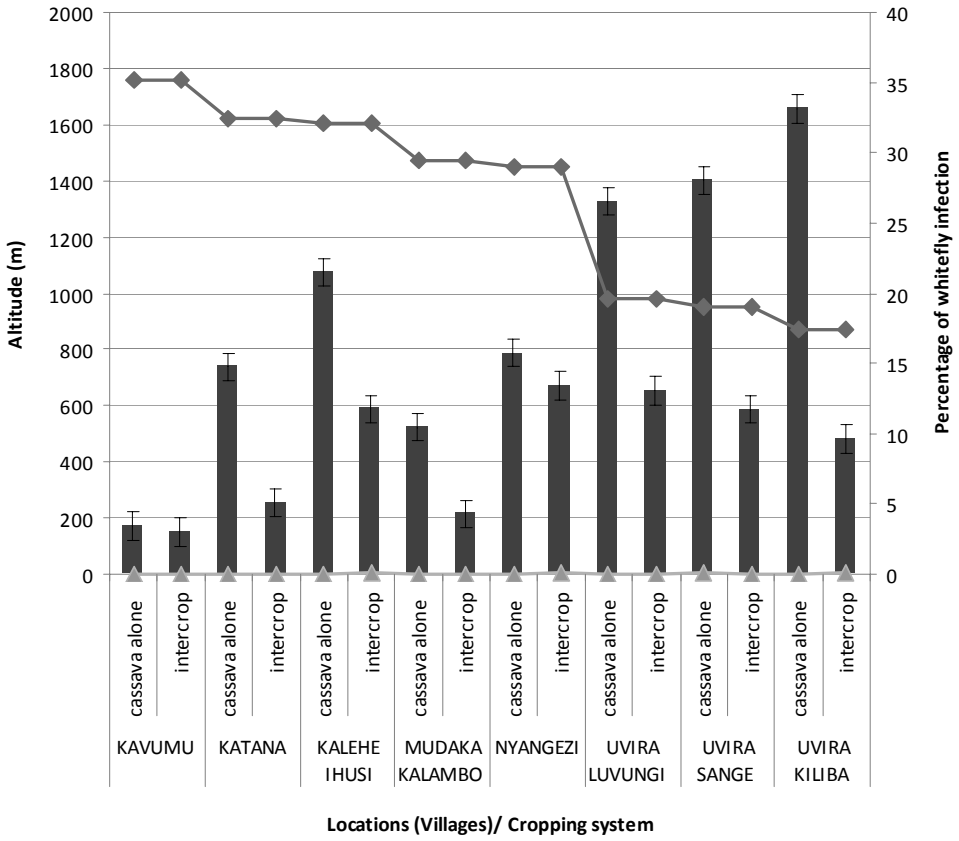
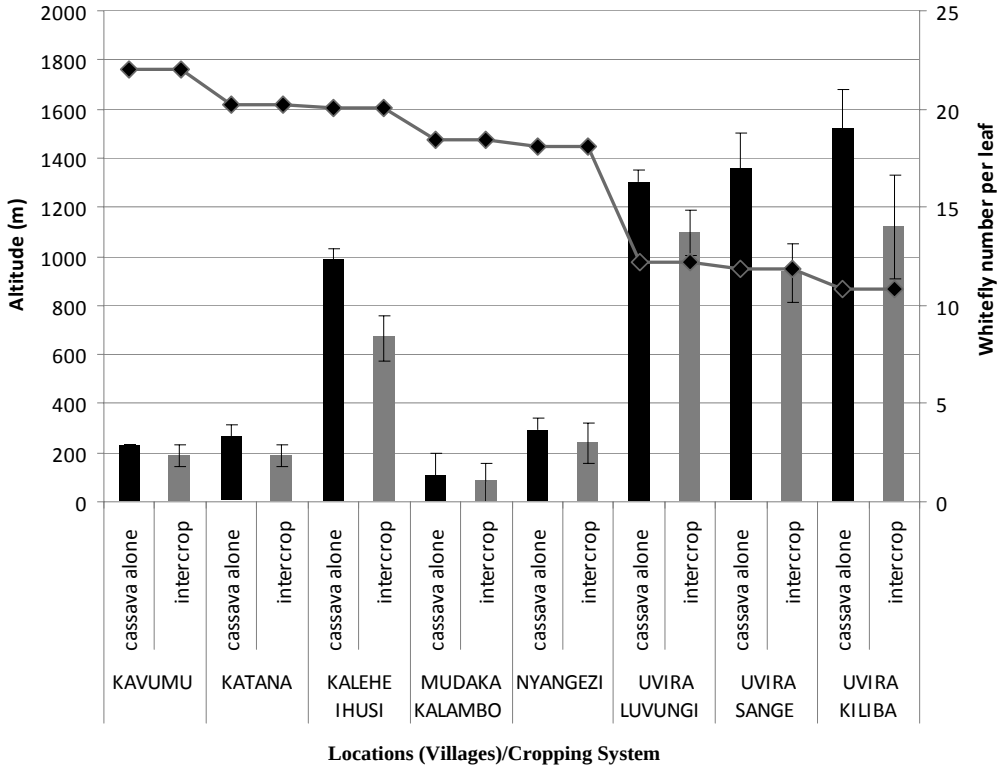

Altitude (m)
Percentage of whitefly infection
cassava alone
intercrop
KAVUMU
KATANA
KALEHE IHUSI
MUDAKA KALAMBO
NYANGEZI
UVIRA LUVUNGI
UVIRA SANGE
UVIRA KILIBA
Locations (Villages)/ Cropping system

Figure 1

Figure 1 continued..

(c)



(d)

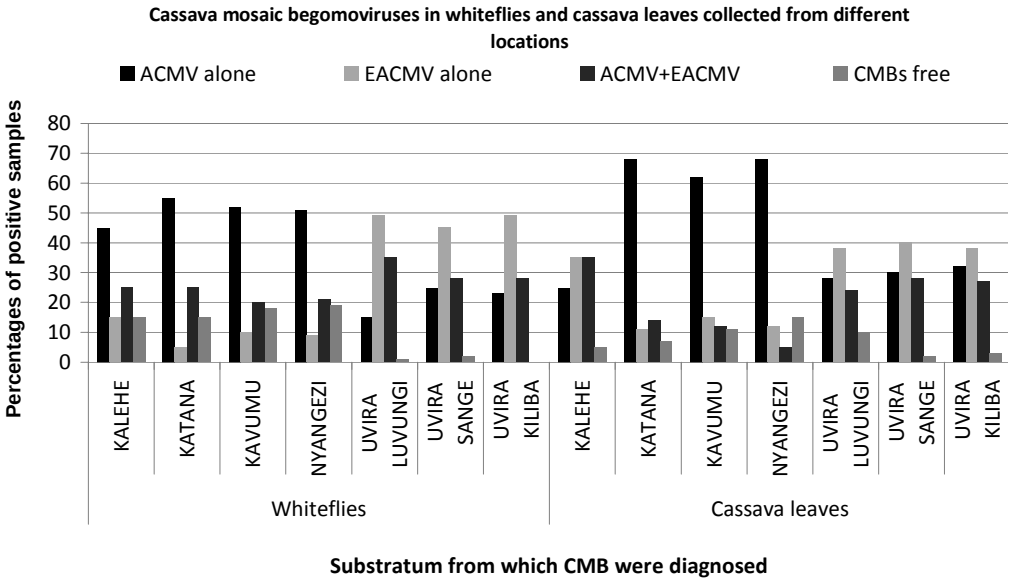


Figure 1. Cassava mosaic disease incidence (a), whitefly infection (b) and whitefly number per leaf (c) in cassava fields when cassava is grown alone and intercropped with maize and beans under different altitude agro-ecosystems in the South-Kivu province, and begomovirus diagnostic in whiteflies and cassava leaves collected from different locations (d). Altitude values are indicated by the curve while CMD incidence, whitefly infection and whitefly numbers values are mentioned with dark and clear histograms respectively for cassava alone and cassava intercropped with maize and beans.

and 10.33 were observed. Moderate values have been recorded at Mudaka and Nyangezi. The lowest values were recorded in the villages located at a high altitude, Katana and Kavumu. CMD recorded data were respectively 26.7%, 2.9 and 2.77 for incidence, severity and whitefly number on cassava leaves. The correlation analysis showed a strong negative relationship between temperature and the CMD incidence (r = -0.794) and the whitefly number per leaf (r = -0.94) while temperature relation with CMD severity was weak (r = -0.35). Curiously, this relationship was not observed for Kalehe, which is also located in high altitude but CMD incidence and severity scores were high. This situation was probably due to the intensive and predominant cultivation of "Nambiyombiyo", one of the local CMD-susceptible varieties cultivated in this area.

Comparison of cassava monoculture and intercropping showed a significant decrease of CMD recorded data when cassava is grown in mixture cropping system (Fig. 1). Intercropping CMD incidence values reduction are respectively 19, 16, 14, 12, 9, 6, 4 and 3 for Kiliba, Sange, Kalehe, Mudaka, Nyangezi, Katana, Luvungi and Kavumu villages. These results suggest that when CMD incidence values in monoculture are high, the intercropping CMD incidence reduction is also high; there is a light decrease in the incidence in intercropping system when CMD incidence in monoculture is low. Intercropping is more profitable under very high CMD pressure than under moderate or low CMD pressure. Similar results were observed for whitefly population and whitefly infection. In cassava monoculture fields whitefly population and related infection were more predominant than in intercropped fields where cutting borne infection were high. Regarding these results, planting clean material will offer less CMD infection rates during the cultivation.

In the same agro-ecosystem conditions, for example, in Kalehe and Uvira where CMD pressure is among the highest, the intercropping system can reduce 26.3% to 33.3% of whitefly population. Comparable results were recently reported by Night *et al.* [14] in Rwanda. Knowledge on the impact of the intercropping system on whitefly population is an important contribution to the disease management especially because virus-free plants can be re-infected by the vector only four weeks after planting [13]. Several factors are expected to produce such impact. Local environment (humidity, local temperature) may influence the insect growth, fecundity and reproduction, combined with the physical barrier provided by maize plants, which is probably involved in hampering the insect mobility. In this way (local conditions' influence), it's clear that maize plants' role is preponderant because of its rapid growth speed and final great height compared to cassava plants while beans plants are important to produce nitrogen amendment to reinforce cassava plant health and tolerance to CMD. Spitel & Van Huis [34]; Pridham & Entz [20]; Zinsou *et al.* [19] mentioned a direct influence of soil fertility and fertilization on plant cassava growth and its tolerance to disease. Ogbe *et al.* [35] and Ossom's [36] studies have mentioned a significant correlation between increasing levels of nitrogen and CMD severity symptom while Lusembo *et al.* [37] observed that intercropping cassava and *Centrosema pubescens* (leguminous species) have improved soil nitrogen and organic matter profitable for subsequent crops.

The environment modification provided by intercropping system can also provide favorable conditions to *B. tabaci* natural enemies [16] or interferes with its virus transmission capacity.

However, it's possible that the simultaneous presence of alternate *B. tabaci* food crops in the same environment can reduce insect population and its pressure on each crop regarding the preference of the insect to use each crop. In this way, there is food key distribution of whitefly population on different crops which considerably reduces the insect number and the impact on CMD transmission to cassava plants. This observation can preliminarily explain why Fondong *et al.* [25] have observed an increase of whitefly population on cassava plants when maize and bean ended their cycle. Tscharntke *et al.* [38] highlighted a pest population decrease when agro-ecosystem is diversified and discontinuous due to high insect mortality rate associated to low fecundity. O'Rourke [39] attributed such impact to environment change which induces pest natural enemies' emergence when the ecosystems increased in diversity.

In the same way as this study, Trenbath [16]; Gomez-Rodriguez & Zavaleta-Mejia [17]; Fininsa [18]; Zinsou *et al*. [19] and Pridham & Entz [20] have observed a positive effect of crop mixture on cassava bacterial blight control, beans common bacterial blight severity and fungal spores dissemination and development.

These significant results incite, in near future, to intensively discuss and experiment the importance of crop production systems in sub-Saharan Africa. Recent agroforestry approaches using a large number of intercropping tree species should be tested to provide a significant contribution to improve crops yield and pest management in farmer's conditions. According to Monde *et al*. [28] and Tilman *et al*. [40, 41, 42] observations, the mixture of cassava, other crops and agroforestry species will provide a discontinuous agro-ecosystem unfavorable for the epidemic development of the disease and its vector.

Nevertheless, in sub-Saharan Africa, farmers usually do sowing or planting of crops at random with no respect to crop spacing out or disposition. This makes it difficult to determine the optimal crop density and different crop species' disposition and distribution in the field, which may give significant and useful results. There is a need to investigate the optimal crop density and disposition in the intercropping systems to improve its efficiency for CMD and its vector management.

CMBs diagnostic in cassava, beans, sweet potato, cocoyam leaves and in whiteflies

For better understanding of the interactions between whitefly population, crop species and the virus spread, CMBs have been diagnosed in whiteflies and plant leaves.

Results of CMBs diagnostic in whiteflies (Figure 1d) demonstrated the presence of ACMV and EACMV in single and mixed infections. From all the collected insect samples 38% of whiteflies were infected by ACMV alone, 26% by EACMV alone, 26% by the mixture ACMV + EACMV while only 10% didn't show infection. The CMBs diagnostic in whiteflies from different locations showed that ACMV is predominant in insects collected from high altitude, while EACMV is predominant in those collected from low altitude. Such situation has been previously observed in cassava leaves collected earlier, there were more EACMV positive samples in Kalehe and Uvira than elsewhere, while more ACMV positive samples were collected from Katana, Kavumu, Mudaka and Nyangezi, in high altitude where whitefly populations are less abundant.

No difference has been observed for CMBs in insects collected from different plants (cassava, beans, sweet potato and cocoyam). All insects showed to be infected by both ACMV and EACMV independently of the plant from which they were collected. This observation is demonstrating the probable food adaptation of whitefly populations on different crop and weeds in tropical environment, since *Bemisia tabaci* can colonize non-cassava plant species [43]. This simultaneous presence of both ACMV and EACMV in mixed infections under high epidemic environment such as Uvira and Kalehe, might provide favorable conditions to generate new recombinations not reported yet in this area.

CMBs diagnostic in other plant leaves didn't show any virus' presence in beans, sweet potato and cocoyam leaves. Ogbe *et al*. [26], Alabi *et al*. [27] and Monde *et al*. [28] have diagnosed CMBs presence in leguminous (*Fabaceae*) species (*Senna occidentalis*, *Leucaena leucocephala*, *Glycine max*, *Centrosema pubescens* and *Pueraria javanica*) and other weeds species (*Combretum confertum* and *Manihot glaziovii*). These studies showed that the virus was able to develop and produce mosaic symptoms on these plant leaves. Nevertheless, it is far from being demonstrated that such a plant could play a role as a virus reservoir, since no back transmission has been experienced yet. In relation to this, it was thought that common beans (*Phaseolus vulgaris*), the most cultivated leguminous species intercropped with cassava and other herbaceous neighboring cassava fields should also be alternate hosts of CMBs. This study didn't show cassava viruses in common beans, sweet potato and cocoyam plants and maintained the hypothesis of a simple food relation with whiteflies. In conclusion, intercropping cassava-beans and maize can be considered as the most effective cropping system for CMD and its vector management.

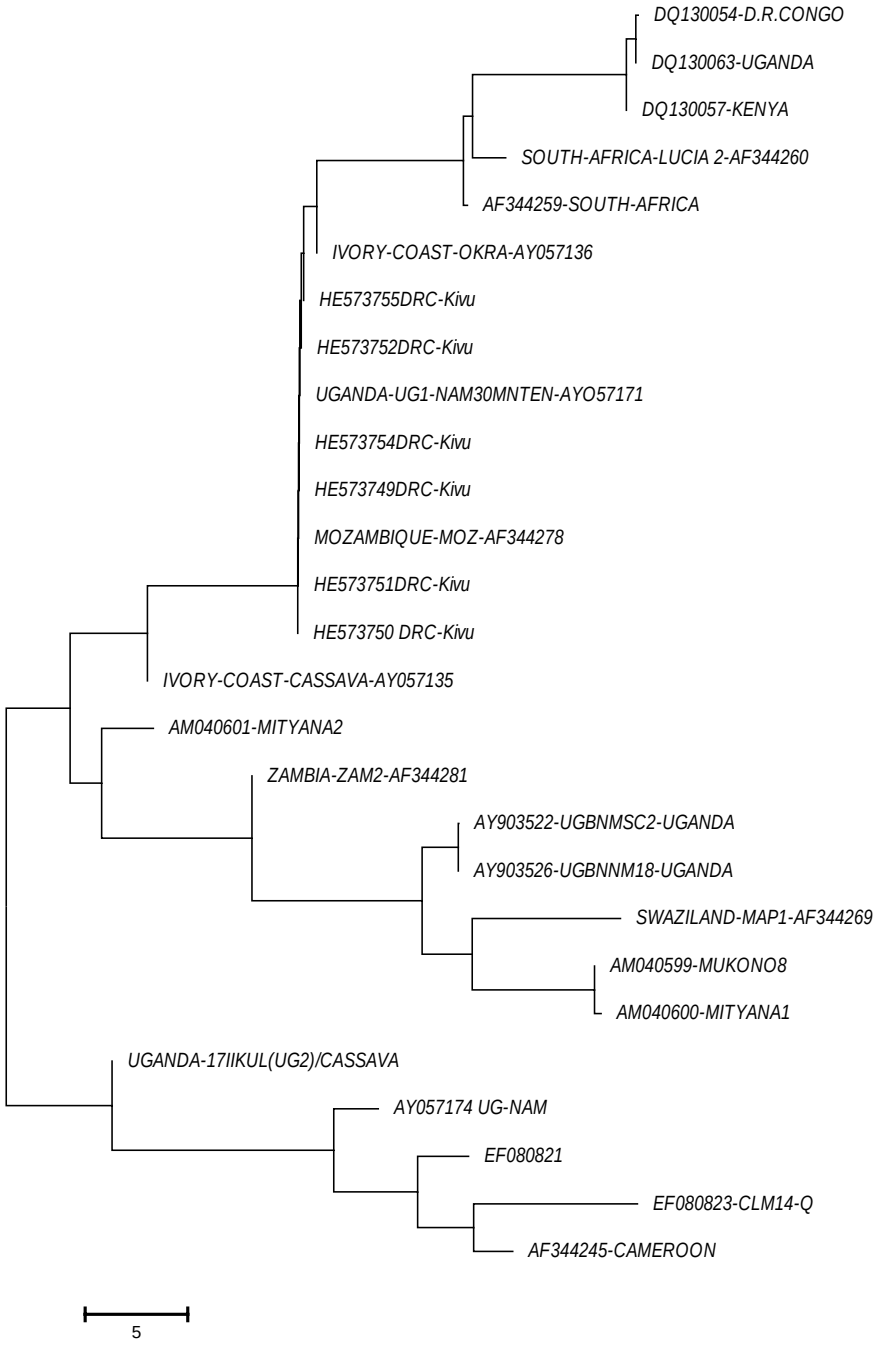


Figure 2. Phylogenetic analysis was conducted by MEGA4 software using nucleotide sequences of *Bemisia tabaci MtCOI* region of insects collected from the South-Kivu province (Eastern DR Congo). Accession numbers of other sequences were from NCBI Genbank database. The evolutionary history was inferred using the neighbor joining method with the complete deletion option. To construct the phylogenetic tree, sequences of the following origins were used: Uganda [UG]: AM040599, AM040600, AM040601, AY057158, AY057171, AY157174, AY903522, AY903526, DQ130063; Kenya [Ke]: DQ130057; Democratic Republic of Congo [DRC]: DQ130054; Cameroon [CM]: AF344245; Ivory-Coast [IC]: AY057135, AY057136; Zambia [ZAM]: AF344281; Mozambique [MOZ]: AF344278; Swaziland [SWAZ]: AF344269 and South Africa [SA]: AF344260, AF344259. *Bemisia tabaci Mt*COI sequences from our study area are represented by the following accessions: HE573749, HE573750, HE573751, HE573752 and HE573754.

The *Bemisia tabaci* diversity analysis based on the mitochondrial cytochrome oxydase I (*Mt*COI) sequences (~750 bp) suggest that there is only one haplotype (clade). All sequences have revealed 95-99% of nucleotide identity independent of the plant from which the insects were collected (cassava or beans). There is a significant *Bemisia tabaci* population homogeneity; nucleotide sequences showed only 11.4% sites of difference and showed a high nucleotide similarity to the common East African clade, known as Ug1 occurring in Uganda. High nucleotide identity rates have been observed compared to Kenyan (99-100%), Mozambican, Zambian, Swazi and South African collections (94-98%) whereas 81-86% of nucleotide identity were recorded for West African (Ivory Coast and Cameroon) and Ug2 collections (Figure 2).

In contrast with the Ugandan and neighbor East African countries, this study has demonstrated that South-Kivu agro-ecosystems have a low CMBs and whitefly diversity compared to Uganda and other East, South and west African countries where more species were recorded [44, 45, 43].

This situation is a typical case in which the complexity of the CMD pathosystem is minimal, offering an opportunity for the disease management under the local intercropped crop system which associates cassava, beans and maize. In this regard, cassava genotypes screening and evaluation to CMD should be undertaken at Kalehe and Uvira (Kiliba and Sange) where the disease and its vector pressure are high (high risk of infection), while seed multiplication must be done in altitude area where there is a great chance to produce virus free materials due to low pressure of the disease and its vector.

CONFLICT OF INTEREST STATEMENT

The authors are not aware of any affiliations, funding, memberships or financial holdings that might be perceived as a conflict of interest with regard to this publication.

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