Asian Journal of Plant Pathology

ISSN 1819-1541

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Plant Pathology 6 (1): 1-12, 2012
ISSN 1819-1541 / DOI: 10.3923/ajppaj.2012.1.12

Occurrence and Distribution of Cassava Mosaic Begomovirus Related to Agro-ecosystems in the Sud-kivu Province, Democratic Republic of Congo

[1,2]E. Bisimwa, [2]J. Walangululu and [1]C. Bragard

[1]Earth and Life Institute, Applied Microbiology, Phytopathology, Universite catholique de Louvain, Croix du Sud 2 boite L 07.05.03., 1348 Louvain-la-Neuve/Belgium

[2]Faculte des Sciences Agronomiques, Universite Catholique de Bukavu, BP 285 Bukavu, Democratic Republic of Congo

Corresponding Author: E. Bisimwa, Earth and Life Institute, Applied Microbiology, Phytopathology, Universite catholique de Louvain, Croix du Sud 2 boite L 07.05.03., 1348 Louvain-la-Neuve/Belgium Tel: +3210474023 Fax: +3210478697

ABSTRACT

Cassava mosaic disease epidemiology and molecular characterization of the related viruses monitored during this study in the Sud-Kivu region, Democratic Republic of Congo. Collected epidemiologic data showed a negative correlation with the altitude (elevation) on the disease incidence and its vector population. Disease incidence was 9.5 to 37.8% associated to less than 3 insects per leaf when the elevation is higher than 1500 m while 65 to 100% and more than 10 insects per leaf were recorded where elevation was less than 1000 m. No impact of rainfall has been recorded. Associated molecular cassava begomovirus diagnostic focused on AC2 and AC4 genes revealed only two species occurring in this area, African Cassava Mosaic Virus (ACMV) and East African cassava mosaic virus-Uganda (EACMV-UG) with more EACMV-UG and mixed infections in low elevation area while ACMV were predominant in high elevation sites. Phylogeny analysis revealed a dual coexistence of indigenous and Ugandan spread among ACMV isolates while all EACMV isolates were restricted to Ugandan spread (95 to 100% of amino acids identity). No recombination was revealed in this study, isolates diversity was restricted to nucleotide substitution.

Key words: Cassava mosaic disease (CMD), virus species diversity, great lakes region, altitude, *Bemisia tabaci*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz, Family Euphorbiaceae) is a major food crop in Sub-Saharan Africa and nourishes more than 60% of the Democratic Republic of Congo (DRC) population (FAOSTAT, 2009). Its production is constrained by several pests and diseases among which Cassava Mosaic Disease (CMD) transmitted by whiteflies, *Bemisia tabaci*, is the most important (Legg *et al*., 2006).

CMD is well known since the 1930s (Kufferath and Ghesquiere, 1932) but its impact on yield harvest was relatively stable. Before the 1990s, only two virus species were known as CMD causal agents. Actually, seven begomovirus species widely distributed in Sub-Saharan Africa are

recognized: the African Cassava Mosaic Virus (ACMV) (Hong *et al*., 1993; Swanson and Harrison, 1994), the East African Cassava Mosaic Virus (EACMV) and EACMV-like strains (Deng *et al*., 1997; Zhou *et al*., 1997; Legg and Ogwal, 1998; Legg, 1999; Legg *et al*., 2001, 2004, 2006), the East African Cassava Mosaic Cameroon Virus (EACMCV) (Fondong *et al*., 2000; Ndunguru *et al*., 2005), the East African Cassava Mosaic Malawi Virus (EACMMV) (Zhou *et al*., 1998), the East African Cassava Mosaic Zanzibar Virus (EACMZV) (Maruthi *et al*., 2004), the South African Cassava Mosaic Virus (SACMV) (Berrie *et al*., 2001; Ranomenjanahary *et al*., 2002; Mabasa and Rey, 2007), the Indian cassava mosaic virus (Adjata *et al*., 2008, 2009). Recently, the South East African Cassava Mosaic Virus (SEACMV) was diagnosed in Madagascar and is proposed as a new species by Harimalala *et al*. (2011).

Following the spread of the epidemic CMD severe, Legg and Fauquet, (2004) have proposed a geographic distribution map on which ACMV occurred in 20 countries, EACMV in 10, EACMV-UG in 11, EACMCV in 5, SACMV in 2, EACMZV in 2 and EACMMV in 1. Since this period the only recorded changes are the ICMV presence in Togo (Adjata *et al*., 2008, 2009), SACMV in Madagascar and the proposed new species SEACMV in Madagascar (Harimalala *et al*. 2011).

The begomovirus species diversity is depending on several phenomenon among which recombination (Zhou *et al*., 1997), pseudo-recombination (Fondong *et al*., 2000; Pita *et al*., 2001a) and nucleotides substitution (Duffy and Holmes, 2009) are involved in new species and strains emergence. Despite the DRC second position of African producers, cassava mosaic begomovirus occurrence is less documented and reported. Neuenschwander *et al*. (2002) study in western DRC, Monde *et al*. (2010) in Yangambi region, Janssens (2001) and Muyolo (1987) represent the easily available information on cassava in DRC.

Regarding the rapid spread of CMD severe and virus diversity throughout Eastern and Central Africa, this study was planned to provide a preliminary diagnostics and distribution of the Cassava Mosaic Begomoviruses (CMBs) based on field data collection and molecular diagnosis. AC2, involved as Post Transcriptional Gene Silencing (PTGS) suppressor and AC4, an activator of the virus pathogenicity (Vanitharani *et al*., 2004; Vanitharani *et al*., 2005) were considered in molecular analysis related to their probable role in plant-virus interaction in expressing the disease symptoms. Additionally, AC2, AC4 and the coat protein are the most diversified zones on DNA of begomovirus genome. Based on the AC2 and AC4 sequences analysis, virus diversity and related causes were assessed.

MATERIALS AND METHODS

Field data and leaf samples collecting: Surveys were conducted in the South-Kivu province, Eastern D.R. of Congo in different cassava growing villages. From Bukavu town, the administrative centre of the province, five directions (Southern, Western, Northern, North-Western and the costal lake-Kivu). Twenty two villages and a total of 150 fields were covered during the survey (data not shown).

Within the selected fields, all the plants on the diagonals were systematically observed independently of the field square. For each plant examined, CMD incidence, CMD severity, the whitefly number (counted from the five top youngest leaves) and the type of infection (whitefly infection or cutting-borne infection) were recorded as proposed by Hahn *et al*. (1980) and adapted by Okao-Okuja *et al*. (2004) and Sseruwagi *et al*. (2004).

Leaf samples were systematically collected in the survey area on diseased plants. Samples were kept in alcohol prior to DNA extraction, Polymerase Chain Reaction (PCR) diagnosis and sequencing realised in the plant pathology laboratory at the Universite catholique de Louvain in Belgium.

Total DNA extraction, PCR amplification and sequencing: Total DNA was extracted from collected leaf samples using the protocol described by Dellaporta (Dellaporta *et al*., 1983) and the FastDNA^{R} Kit with FastPrep^{R} instruments (Qbiogene, inc., CA).

DNA-A AC2 and AC4 regions specific primers described by Monde *et al*. (2010) were used.

The mix PCR was prepared in a final volume of 50 μL using H_2O depc 26.25 μL, $MgCl_2$ 25 mM 5 μL, GoTaq® 5x flexi buffer 10 μL, Dntp 100 mM 1.5 μL, each upstream and downstream primer 1 μL, GoTaq®DNA polymerase (Promega) 5 units μL^{-1} 0.25 μL, extract DNA samples 5 μL. The PCR was cycling by a thermocycler ICycler Biorad® version 4.006 at 94°C for 2 min for denaturizing, followed of 38 cycles of amplification at 94°C for 30 sec for denaturizing, hybridization at 58°C for 30 sec; 72°C for 1 min for elongation. The final elongation has been done at 72°C for 7 min. The revelation was made in 1.2% agarose gel under UV after electrophoresis at 120 V in ethidium bromide. Direct sequencing using Biosequencer Genetic analyser 3100 was made with PCR product diluted tenfold in distilled water. Each amplicon was sequenced many times forward and reverse. Partial sequences produced were translated in amino acids by a molecular toolkit (www.vivo.colostate.edu) and submitted to EMBL EBI server. NCBI accessions numbers were attributed with accessions FN433646 to FN433697. Concatenated amino acids of AC2 and AC4 from the NCBI Genbank and those collected in DR Congo were used to carry out phylogenetic trees using MEGA4.0 software (Tamura *et al*., 2007). The evolutionary history was determined using the Neighbour-Joining method with the complete deletion option and 1000 replicates for bootstrap values. The expressed mutations in amino acids sequences are shown in front of respective phylogenetic trees by alignment (Fig. 1 and 2).

Concatenated amino acids sequences of AC2 and AC4 of the following NCBI accessions were used: Cameroon [CM] [AF366902][1]; Nigeria [NG] [AJ427910][1]; Uganda [UG] [AF126800, AF126802][1]; [AJ717533, AJ717534, AJ618958, AM502326, AM502327, AM502329, AM502334][2]; Ivory Coast [IC] [AF259894][1] and Tanzania [TZ] [AY795988][2].

- [(1)]: ACMV and [(2)]: EACMV

Molecular diversity of the analysed sequences were reported in the geographic map of Sud-Kivu (Fig. 3) established in basis of general map of soil occupation of DR Congo provided by UCL-Geomatics, Louvain la Neuve, Belgium, 2007 where only elavation is highlightened.

RESULTS AND DISCUSSIONS

CMD incidence, severity, infection type and whitefly population: CMD incidence, its severity, whitefly population and virus diagnostics are presented in Table 1. The field data collected showed a clear geographic and ecological distribution of CMD incidence, its severity and the whitefly population in the surveyed area. CMD mean incidence values were moderate to high, ranging from 9.48 to 100% with the highest values recorded in the low altitude of Uvira (high temperature values, grassland and intensive agriculture environment) and Bunyakiri (high

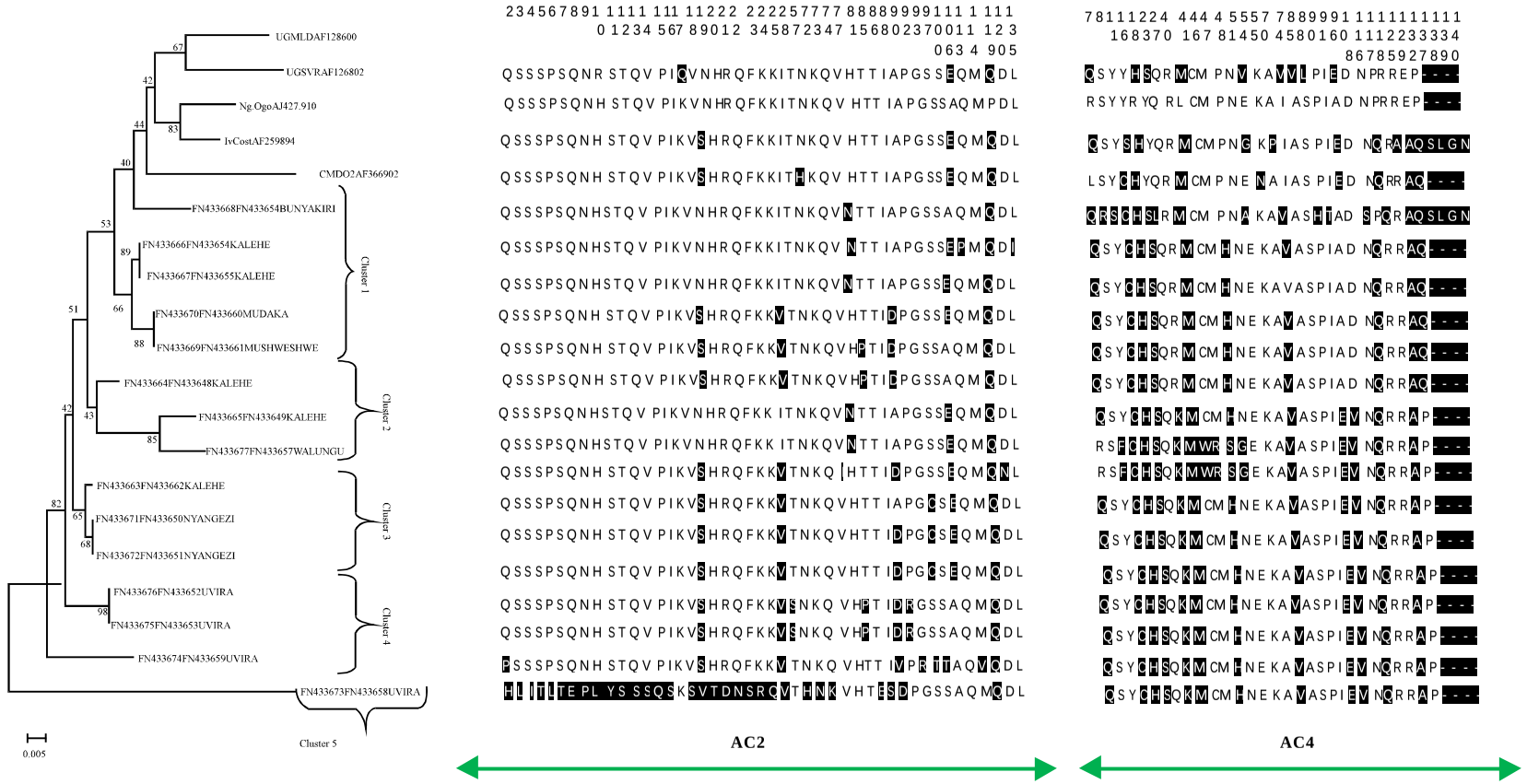

Fig. 1: Phylogenetic and molecular analysis based on amino acids in concatenated sequences of AC2 and AC4 for ACMV inferred from the Neighbour-joining method (Saitou and Nei, 1987) using MEGA software version 4.0 with complete deletion option. Bootstrap values (1000 replicates) are shown as percentages in the branches (Tamura *et al*., 2007). The phylogenetic tree was constructed with AC2 and AC4 sequences from Cameroon [CM] AF366902; Ivory Coast [IC] AF259894; Nigeria [Ng] AJ427910; Uganda [UG] AF126800, AF126802 and Democratic Republic of Congo FN433646, FN433647, FN433648, FN433649, FN433650, FN433651, FN433652, FN433653, FN433654, FN433655, FN433656, FN433657, FN433658, FN433659, FN433660, FN433661, FN433662, FN433663, FN433664, FN433665, FN433666, FN433667, FN433668, FN433669, FN433670, FN433671, FN433672, FN433673, FN433674, FN433675, FN433676, FN433677

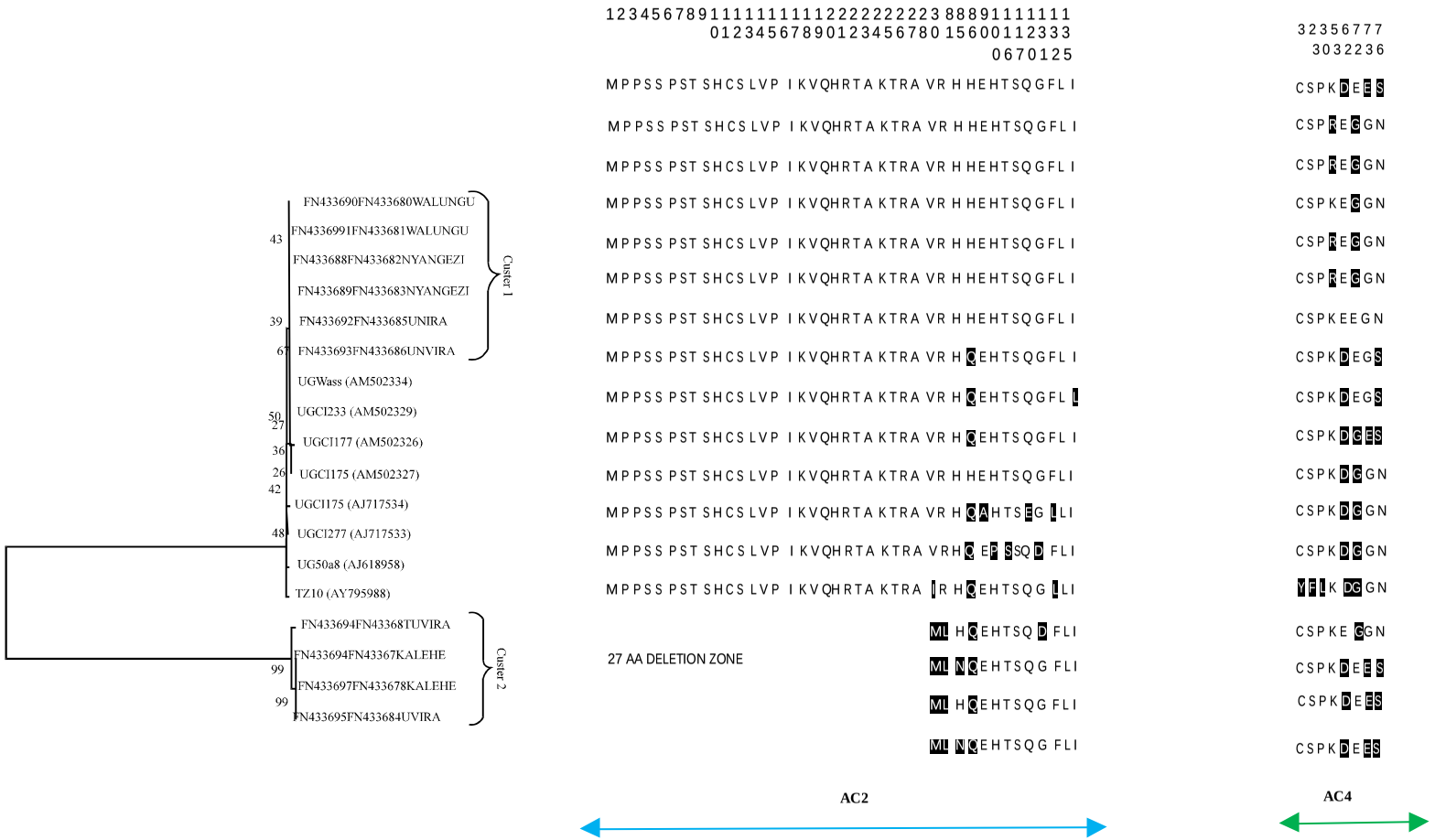

Fig. 2: Phylogenetic and molecular analysis based on amino acids in concatenated sequences of AC2 and AC4 for EACMV-UG inferred from the Neighbour-joining method (Saitou and Nei, 1987) using MEGA software version 4.0 with complete deletion option. Bootstrap values (1000 replicates) are shown as percentages in the branches (Tamura *et al*., 2007). The phylogenetic tree was constructed with AC2 and AC4 sequences from Uganda [Ug]AM502327, AM502329, AM502326, AM502334, AJ717534, AJ717533, AJ618958; Tanzania [Tz]AY795988 and Democratic Republic of Congo FN 433678, FN433679, FN433680, FN433681, FN433682, FN433683, FN433684, FN433685, FN433686, FN433687, FN433688, FN433689, FN433690, FN433691, FN433692, FN433693, FN433694, FN433695, FN433696, FN433697

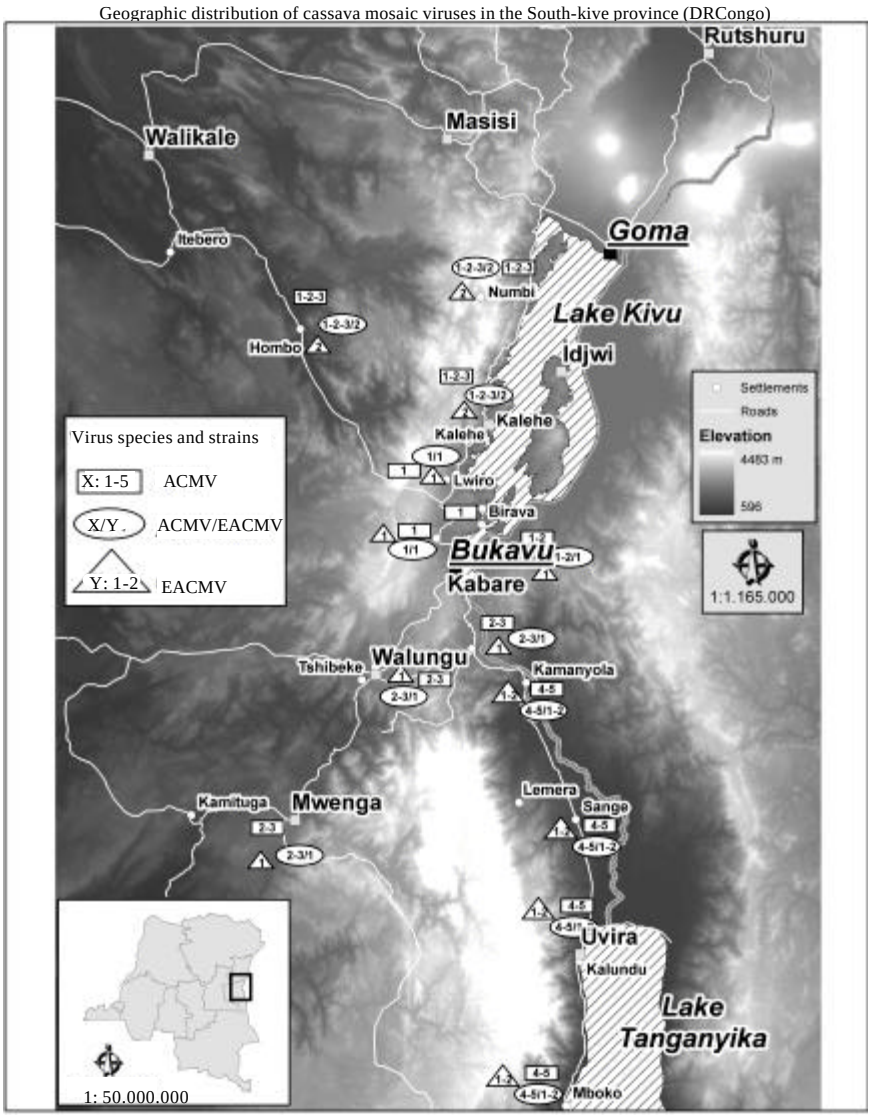

Fig. 3: Cassava mosaic Begomovirus distribution on the Sud-Kivu map. The location of sampling were made in the basis of the general map of DR Congo. Five different isolates of ACMV (1 to 5) and two isolates of EACMV-UG (1 and 2) were identified by molecular analysis based on AC2 and AC4 genes. Fractional numbers (X/Y) represent the dual infections by both ACMV and EACMV-UG

temperature values, secondary forest) where more fields were recorded with 85 to 100% of diseases plants than anywhere else (Table 1). In those villages more than 50% of infected plants were recorded with high severity score 3-5 and cassava asymptomatic plants in the fields were rare. The high incidence and severity values were positively correlated to whitefly population (7-17.9 insects

Table 1: CMD incidence, severity and whitefly population and CMD diagnostic in the South-kivu province. Number with different succeeding alphabets are significantly different at p = 0.05 level, mean incidence was estimated as a percentage of CMD diseased plants in a field and in a village. Mean severity was estimated by using a 1-5 scale proposed by Sseruwagi *et al.* (2004) where score 1 is attributed to a cassava plant CMD symptom free and 5 to a cassava plant with CMD severe symptoms. Whitefly population was estimated as a mean whitefly number per leaf on the 5 top cassava leaves

		Locations			Mean	Mean	Whitefly	ACMV Accesion reference		EACMV accessions reference	
Axis	Villages	Latitude	longitude	Altitude (m)	incidence	severity	population	AC4	AC2	AC2	AC4
North	Kalehe Ihusi	2°05'07" S	28°54'10" E	1602	93.5±6.73^{ab}	3.7±0.4^{bc}	10.4±2.22^{cd}	FN433648	FN433664	FN433697	FN433678
North	Kalehe Kasheke	2°05'48" S	28°54' 16"E	1616	54.9±24.56^{ef}	2.7±0.2^{f}	10.4±2.3^{cd}	FN433662	FN433663		
North	Kalehe Cibanda	2°05'50" S	28°54' 22"E	1618	39.5±19.66^{fgh}	3.2±0.4^{cde}	11.3±1.49^{bc}				
North	Kalehe Muhongoza	2°5'53" S	28°54'29" E	1607	76±16.00^{cd}	3.5±0.2^{bc}	7.2±1.3^{d}	FN433654	FN433666		
North	Kalehe Buloho	2°05'54"	28°54'37" E	1590	80.6±17.18^{cd}	3.9±0.22^{b}	7.56±0.88^{cd}	FN433649	FN433665	FN433696	FN433679
North	Kalehe Bushushu	2°05'56" S	28°54'50" E	1574	32.1±24^{gh}	3±0.8^{de}	8.4±1.69^{cd}	FN433655	FN433667		
North-West	Bunyakiri Irunga	2°04'33" S	28°34'24"E	1171	100±0^{a}	3.9±0.2^{b}	8.5±4.9^{cd}				
North-West	Bunyakiri Bulambika	2°05'37" S	28°35'05" E	1132	81.3±10.65^{bc}	4.5±0.32^{a}	7.8±1.7^{cd}	FN433654	FN433668	FN433696	FN433679
North-West	Bunyakiri Kando	2°7'40" S	28°35'53" E	1180	65±10de	3.3±0.4^{cde}	8.7±0.57^{cd}				
North	Katana	2°13'28" S	28°49'52" E	1618	25.2±16^{ghi}	3±0.5^{de}	1±1^{e}	FN433647			
North	Lwiro	2°15'06" S	28°49'34" E	1638	9.5±4 i	2.7±0.7^{f}	2±1.2^{e}				
North	Kavumu	2°16' S	28°48' E	1760	22.61±20^{hi}	2.5±0.3^{f}	2±1.4^{e}	FN433646		FN433696	FN433679
North	Mudaka Kalambo	2°21'50" S	28°52'59" E	1473	52.2±11^{ef}	2.8±0.3^{ef}	1±1^{e}	FN433660	FN433670		
Costal Lake Kivu	Luhihi	2°17 S	28°52' E	1828	37.8±24^{fgh}	2.9±0.5^{ef}	1±0.6^{e}				
Costal Lake Kivu	Mushweshwe	2°18'27" S	28°52' E	1525	77.7±22^{cd}	2.6±0.16^{f}	2±1.16^{e}	FN433661	FN433669		
Costal Lake Kivu	Birava	2°20'52" S	28°53'21" E	1528	60.5±24de	2.7±0.2^{f}	2±1.6^{e}				
South-West	Walungu	2°42' 29"S	28°39' E	1560	41.7±10^{fg}	2.9±0.7^{ef}	3±1.9^{e}	FN433656	FN433677	FN433690	FN433680
								FN433657	FN433677	FN433691	FN433681
South	Nyangezi	2°39' S	28° 52' E	1450	67.2±11.83^{de}	3.2±0.3^{cde}	3±0.8^{e}	FN433650	FN433671	FN433688	FN433682
								FN433651	FN433672	FN433689	FN433683
South	Uvira Kamanyola	2°47' S	29° 00 E	1000	73.5±9.27^{cd}	2.7±0.3^{f}	16±2.4^{a}	FN433658	FN433673	FN433695	FN433684
South	Uvira Luvungi	2°50' S	29°03' E	978	70.2±17.86^{de}	3±0.4^{de}	15.3±2.98^{ab}	FN433659	FN433674	FN433692	FN433685
South	Uvira Sange	3°05' S	29°06' E	950	88.8±10.55^{abc}	3.4±0.40^{bcde}	15.4±3.18^{a}	FN433652	FN433676	FN433693	FN433686
South	Uvira Kiliba	3°14' S	29°10' E	870	89.5±9.47^{abc}	3.5±0.40^{bcd}	17.9±3.09^{a}	FN433653	FN433675	FN433694	FN433687
					5%: 18.36	5%: 0.5		5%: 4			

Mean values with same letters are not signifficantly LSD different LSD at p = 0.05

per leaf) depending on the local environment temperature. Xie *et al.* (2011) have mentioned that whitefly fecundity and development were significantly increased at 24°C comparing to 15, 18 and 21°C.

However, great values of CMD incidence, severity and whitefly population were recorded at Kalehe, despite its location in altitude, moderate temperature and intensive agriculture environment. The lowest values were observed in the high altitude (Lwiro, Kavumu, Katana, Luhihi) where 9.5 to 37.8% of infected plants were recorded. The lowest value was observed in Lwiro (9.5%) where most of the cassava plants on the fields were asymptomatic, CMD severity score moderate (2-3) and whitefly population low (2 insects per leaf).

The high incidence and CMD severity score observed in the low altitude area (Uvira, Kalehe and Bunyakiri) was recorded in a positive correlation with the EACMV presence, alone or in mixed infections, in cassava fields with 57.2; 64.2 and 63.7% EACMV-positive samples respectively. These zones can be considered with the high epidemic index 3 (Legg *et al.*, 2006). All sampled fields were predominantly grown with local and susceptible genotypes, among which Nambiyombiyo, M'Bailo and Cizinduka are most cultivated in altitude zones whereas Nakarasi and Naunde are most cultivated in Uvira zones.

The CMD severity score all around the covered area was ranged from 2.3 at Kavumu to 5 at Bunyakiri and Uvira with a mean score of 3.5 for the three most affected zones (Uvira, Kalehe and Bunyakiri). There was no genotypes variability on the incidence and severity, homogenous results have been recorded for all the local genotypes observed.

The whitefly number counted on upper cassava leaves varied from the different villages with low mean value of 1-3 insects per leaf in the high altitude (Katana, Luhihi, Lwiro, Kavumu, Nyangezi, Walungu and Mudaka) to 17.9 insects per leaf, the highest value observed in low altitude in Uvira-Kiliba. The positive correlation of white fly population, CMD incidence and severity and EACMV presence reaffirms the hypothesis EACMV to be easily transmitted by whitefly than ACMV (Colvin *et al.*, 2004). The type of infection was homogenously distributed in each ecological zone, roughly 75 to 80% for cutting-borne infection and 20 to 25% for whitefly infection, except in Kamanyola where whitefly infection is 32.6 versus 67.4% for cutting-borne infection. These results suggest an ecological impact, mainly the temperature, on CMD vector development and its impact on the disease transmission and spread.

PCR diagnostic showed that only two species are occurring in the region, ACMV and EACMV species. They were diagnosed alone or in mixed infections in all cassava fields in the survey area except in the villages all along and near the lake Kivu (Luhihi, Mushweshwe and Birava) where only ACMV has been found (87%). EACMV-UG was found alone or in mixed infections with ACMV. Great rates of mixed infections were frequently associated to severe CMD symptoms observed at Bunyakiri, Kalehe and Uvira as it has been observed in Uganda, Kenya, Tanzania and Rwanda, suggesting a synergistic interaction (Harrison *et al.*, 1997; Legg, 1999; Pita *et al.*, 2001b; Fondong *et al.*, 2000).

The blast of EACMV sequences showed high similarity to EACMV-UG (more than 98% of nucleotide and amino acids homology) (Fig. 2). This study is a report on EACMV-UG spread to Sud-Kivu province where it hasn't been reported yet. The high frequency of EACMV-UG isolates in the north and south means the spread was realized from two different movements, at the north probably from Uganda when at the south, the spread is probably from Burundi, where it has been reported earlier (Bigirimana *et al.*, 2004).

The concatenated AC2 and AC4 amino acids sequences alignment with Ugandan isolates showed that AC2 region of both ACMV and EACMV-UG is less diversified with less than 6% of amino acids substitution. The same observation was made for AC4 of EACMV-UG with less than 4% of amino acids substitution while the same region of ACMV was the most diversified with 6 to 11% of amino acids substitutions (Fig. 1 and 2). The AC4 of ACMV variability percentage is closed to what have been mentioned by Ndunguru *et al.* (2005) and Duffy and Holmes, (2009) for cassava begomovirus diversity and evolution and confirms ACMV occurring in the region for a long time, while the EACMV-UG less diversity is related to its recent introduction in the region.

The phylogenetic trees of different isolates established based on concatenated amino acids of AC2 and AC4 (Fig. 1 and 2) showed a single ACMV group of truncated AC4 gene presenting a premature codon stop and segregated in five different clusters while all EACMV-UG isolates were segregated in two different clusters.

The three first groups of ACMV are widely distributed in the region and linked to moderate CMD symptoms, while the groups 4 and 5 are restricted to Uvira zone and linked to severe CMD symptoms. The cluster 5 contains one isolate which is completely different from the other isolates and contains a specific fragment length of twenty five amino acids (22.9% of amino acids substitution) at the beginning of the AC2 region. The blast of the differential fragment didn't reveal any recombination from a known begomovirus, nucleotides and amino acids substitution was the only one way of the virus diversity and evolution identified in this study.

The EACMV-UG isolates are closed to Uganda isolates and segregated in two clusters. The first cluster contains isolates widely distributed in the surveyed area which have homogenous AC2 region and weak AC4 amino acids substitution (3.8%) comparing to Ugandan isolates. The second cluster contains isolates restricted to kalehe and Uvira zones which present a deletion of twenty seven amino acids on the beginning of AC2 region, while AC4 region contains a weak amino acids substitution (3.8%). This particular isolates, both ACMV and EACMV-UG, restricted to Kalehe and Uvira is one of the probable causes of severe CMD symptoms observed in this area beside a great rate of ACMV/EACMV dual infections and the intensive cultivation of local and susceptible cassava genotypes.

The spread to Great Lake region (DRC, Rwanda and Burundi) of EACMV-UG from East African countries was expected to introduce the cassava begomovirus diversity reported by Ndunguru *et al.* (2005) in its origin region, in contrast no cassava begomoviruses species diversity has been reported by this study similar to the Rwandan and Burundian cases where both ACMV and EACMV-Ug only were reported (Bigirimana *et al.*, 2004; Legg *et al.*, 2001). In the East-Northern DRC, only those two species were reported by Monde *et al.* (2010) study. In relation to these observations, it can be assume that the Ugandan CMD spread to Great Lake countries was realized before recombination processes. However, recombinations result from the coexistence of several begomovirus in the same environment and no additional favourable conditions are reported to influence this phenomenon. In this way, frequent begomovirus recombinations reported in East Africa can easily occur in the Great lake region and there is no reason to not fear such threat in the Great Lake region where the two species coexistence is reported since ten years. Curiously, the first phenomenon of begomovirus recombination in Uganda was reported related to severe CMD disease (Deng *et al.*, 1997; Zhou *et al.*, 1997) only four years after Hong *et al.* (1993) report on the coexistence of ACMV and EACMV in East Africa region. Thus, there is no evident explanation of this and more care is needed to permanently monitor the disease epidemiology and aetiology in this region.

ACKNOWLEDGMENTS

This study was achieved under a Belgian technical cooperation (BTC) scholarship associated to technical support of the Earth and Life Institute Applied Microbiology-Phytopathology of the University catholic of Louvain in Belgium and the Catholic University of Bukavu in DR Congo. Authors are grateful to UCL-ENGE-Geomatics for providing the map.

REFERENCES

Adjata, K.D., E. Muller, M. Aziadekey, Y.M.D. Gumedzoe and M. Peterschmitt, 2008. Incidence of cassava viral diseases and first identification of East African cassava mosaic virus and Indian cassava mosaic virus by PCR in Cassava (Manihot esculenta Crantz) fields in Togo. Am. J. Plant Physiol., 3: 73-80.

Adjata, K.D., E. Muller, M. Peterschmitt, O. Traore and Y.M.D. Gumedzoe, 2009. Molecular evidence for the association of a strain of uganda variant of east african cassava mosaic virus to symptom severity in cassava (Manihot esculenta Crantz) fields in togo. Am. J. Biochem. Biotechnol., 5: 196-201.

Berrie, L.C., E.P. Rybicki and M.E. Rey, 2001. Complete nucleotide sequence and host range of South African cassava mosaic virus: Further evidence for recombination amongst begomoviruses. J. Gen. Virol., 82: 53-58.

Bigirimana, S., P. Barubanze, R. Obonyo and J.P. Legg, 2004. First evidence for the spread of East African cassava mosaic virus: Uganda (EACMV-Ug) and the pandemic of severe cassava mosaic disease in Burundi. Plant Pathol., 53: 231-231.

Colvin, J., C.A. Omongo, M.N. Maruthi, G.W. Otim Nape and J.M. Thresh, 2004. Dual begomovirus infection and high Bemisia tabaci populations: Two factors driving spread of cassava mosaic disease pandemic. Plant Pathol., 53: 577-584.

Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA mini-preparation: Version II. Plant Mol. Biol. Rep., 1: 19-21.

Deng, D., G.W. Otim Nape, A. Sangare, S. Ogwal, R.N. Beachy and C.M. Fauquet, 1997. Presence of a new virus closely associated with cassava mosaic outbreak in Uganda. Afr. J. Root Tuber Crops, 2: 23-28.

Duffy, S. and E.C. Holmes, 2009. Validation of high rates of nucleotides substitution in Geminiviruses: Phylogenetic evidence from East African cassava mosaic viruses. J. Gen. Virol., 90: 1539-1547.

FAOSTAT, 2009. FAO Database. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fondong, V.N., J.S. Pita, M.E.C. Rey, A. de Kochko, R.N. Beachy and C.M. Fauquet, 2000. Evidence of synergism between African cassava mosaic virus and the new double recombinant Geminivirus infecting cassava in Cameroon. J. Gen. Virol., 81: 287-297.

Hahn, S.K, E.R. Terry and K. Leuschner, 1980. Breeding cassava for resistance to cassava mosaic disease. Euphytica, 29: 673-683.

Hong, Y.G, D.J. Robinson and B.D. Harrison, 1993. Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses in cassava. J. Gen. Virol., 74: 2437-2443.

Harrison, B.D., X. Zhou, G.W. Otim-Nape, Y. Liu and D.J. Robinson, 1997. Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. Ann. Applied Biol., 131: 437-448.

Harimalala, M.A., J. Villemot, M. Hoareau, I. Zinga and S. Ranomenjanahary *et al*., 2011. Phylogeny and phylogeography of begomoviruses associated with mosaic disease of cassava in Madagascar. Proceedings of the 13 th Meetings of Plant Virology (RVV 2011), January 16-20, 2011, Aussois, France.

Janssens, M., 2001. Cassava. In: Crop Production in Tropical Africa, Raemaekers, R.H. (Ed.). Directorate General for International Co-operation, Brussels, pp: 194-218.

Kufferath, H. and J. Ghesquiere, 1932. Cassava mosaic. Report Soc. Biol., 109: 1146-1148.

Legg, J.P. and S. Ogwal, 1998. Changes in the incidence of African cassava mosaic virus disease and the abundance of its whitefly vector along South-North transects in Uganda. J. Applied Entomol., 122: 169-178.

Legg, J.P., 1999. Emergence, spread and strategies for controlling the pandemic of cassava mosaic virus disease in East and Central Africa. Crop Prot., 18: 627-637.

Legg, J.P., G. Okao-Okuja, R. Mayala and J.B. Mahinyuza, 2001. Spread into Rwanda of the severe cassava mosaic virus disease pandemic and the associated Uganda variant of the East African Cassava Mosaic Virus (EACMV-Ug). Plant Pathol., 50: 796-796.

Legg, J.P., F. Ndjelassili and G. Okao-Okuja, 2004a. First report of cassava mosaic disease and cassava mosaic geminiviruses in Gabon. Plant Pathol., 53: 232-232.

Legg, J. and C. Fauquet, 2004b. Cassava mosaic geminiviruses in Africa. Plant Mol. Biol., 56: 585-599.

Legg, J.P., B. Owor, P. Sseruwagi and J. Ndunguru, 2006. Cassava mosaic virus disease in East and Central Africa: Epidemiology and management of a regional pandemic. Adv. Vir. Res., 67: 355-418.

Mabasa, K.G. and M.E.C. Rey, 2007. Epidemiology of cassava mosaic disease and its whitefly (Bemisia tabaci) vector in the lowveld region of South Africa. South Afr. J. Sci., 103: 3-3.

Maruthi, M.N., S. Seal, J. Colvin, R.W. Briddon and S.E. Bull, 2004. East African cassava mosaic Zanzibar virus: A recombinant begomovirus species with a mild phenotype. Arch. Virol., 149: 2365-2377.

Monde, G., J. Walangululu, S. Winter and C. Bragard, 2010. Dual infection by cassava begomoviruses in two leguminous species (Fabaceae) in Yangambi, North-Eastern Democartic Republic of Congo. Arch. Virol., 155: 1865-1869.

Muyolo, G., 1987. Current status of major diseases of cassava in Zaire and progress in their control. Seminar on Diseases and Pests of Major Food Crops in Central Africa. CTA, BADC, IRAZ, ISABU. Bujumbura, Burundi, pp: 197-206.

Ndunguru, J., J.P. Legg, T.A.S. Aveling, G. Thompson and C.M. Fauquet, 2005. Molecular biodiversity of cassava Begomovirus in Tanzania: Evolution of cassava geminiviruses in Africa and evidence for East Africa being a center of diversity of cassava geminiviruses. Virol. J., 2: 21-21.

Neuenschwander, P., J. Hughes, F. Ogbe, J.M. Ngatse and J.P. Legg, 2002. Occurrence of the Uganda variant of East African Cassava Mosaic Virus (EACMV-Ug) in Western Democratic Republic of Congo and the Congo Republic defines the westernmost extent of the CMD pandemic in East/Central Africa. Plant Pathol., 51: 385-385.

Okao-Okuja, G., J.P. Legg, L. Traore and A.M. Jorge, 2004. Viruses associated with cassava mosaic disease in Senegal and Guinea Conakry. J. Phytopathol., 152: 69-76.

Pita, J.S., V.N. Fondong, A. Sangare, G.W. Otim-Nape, S. Ogwal and C.M. Fauquet, 2001a. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. J. Gen. Virol., 82: 655-665.

Pita, J.S., V.N. Fondong, A. Sangare, R.N.N. Kokora and C.M. Fauquet, 2001b. Genomic and biological diversity of the African cassava geminiviruses. Euphytica, 120: 115-125.

Ranomenjanahary, S., R. Rabindran and D.J. Robinson, 2002. Occurrence of three distinct begomoviruses in cassava in Madagascar. Ann. Applied Biol., 140: 315-318.

Saitou, N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

Sseruwagi, P., W.S. Sserubombwe, J.P. Legg, J. Ndunguru and J.M. Thresh, 2004. Methods of surveying the incidence and severity of cassava mosaic disease and whitefly vector populations on cassava in Africa: A review. Virus Res., 100: 129-142.

Swanson, M.M. and B.D. Harrison, 1994. Properties, relationships and distribution of cassava mosaic geminiviruses. Trop. Sci., 34: 15-25.

Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol., 24: 1596-1599.

Vanitharani, R., P. Chellappan, J.S. Pita and C.M. Fauquet, 2004. Differential roles of AC2 and AC4 of cassava geminiviruses in mediating synergism and suppression of Posttranscriptional gene silencing. J. Virol., 78: 9487-9498.

Vanitharani, R., P. Chellappan and C.M. Fauquet, 2005. Geminiviruses and RNA silencing. Trends Plant Sci., 10: 144-151.

Xie, M., F.H. Wan, Y.H. Chen and G. Wu, 2011. Effects of temperature on the growth and reproduction characteristics of Bemisia tabaci B-biotype and Trialeurodes vaporariorum. J. Applied Entomol., 135: 252-257.

Zhou, X., Y. Liu, L. Calvert, C. Munoz, G.W. Otim-Nape, D.J. Robinson and B.D. Harrison, 1997. Evidence that DNA-A of a Geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. J. Gen. Virol., 78: 2101-2111.

Zhou, X., D.J. Robinson and B.D. Harrison, 1998. Types of variation in DNA-A among isolates of East African cassava mosaic virus from Kenya, Malawi and Tanzania. J. Gen. Virol., 79: 2835-2840.