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ASSESSMENT OF CASSAVA VARIETAL RESPONSE TO BEGOMOVIRUSES CAUSING AFRICAN CASSAVA MOSAIC DISEASE IN SOUTHWESTERN DEMOCRATIC REPUBLIC OF CONGO

ÉVALUATION DE LA RÉPONSE VARIÉTALE DU MANIOC AUX BÉGOMOVIRUS RESPONSABLES DE LA MOSAÏQUE AFRICAINE DU MANIOC DANS LE SUD-OUEST DE LA RÉPUBLIQUE DÉMOCRATIQUE DU CONGO

N.B. MAMBAKA [2,5], A. MUMBUMBU [2], T.Z. BAKELANA [1,2,4], F. TIENDRÉBÉOGO [2], A.O. ENI [2], J.S. PITA [2], A.N. NGOMBO [3] and M.M. MUENGULA [1,3]

[1] National Institute for Agronomic Studies and Research, DRC
[2] Regional Centre of Excellence for Transboundary Plant Pathogens, Epidemiology of Viruses in Central and West Africa, Science and Innovation Hub, Félix Houphouët-Boigny University, Abidjan BP 34, Ivory Coast
[3] University of Kinshasa, Faculty of agricultural and environmental sciences, Kinshasa, DRC
[4] University President Joseph Kasa Vubu, Faculty of agricultural and environmental sciences, Boma, DRC
[5] Kongo University, Faculty of agricultural and environmental sciences, Mbanza-Ngungu, DRC
Corresponding author: tony.bakelana@wave-center.org; bakelanatony@hotmail.com

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ABSTRACT

Cassava mosaic disease (CMD) remains one of the most serious constraints to cassava (*Manihot esculenta* Crantz) production in sub-Saharan Africa (SSA). In the Democratic Republic of Congo (DRC), the widespread use of infected planting materials and the diversity of Begomoviruses, increase the vulnerability of cassava varieties to the disease. This study aimed at evaluating the reaction of major cassava varieties to Begomovirus infections and to identify factors influencing their susceptibility under natural infection conditions in southwestern DRC. A field survey was conducted during March and April 2022, in 94 cassava fields located in the provinces of Kongo Central and Kinshasa. CMD incidence and severity; and the abundance of the whitefly vector (*Bemisia tabaci*) were assessed in the field; while molecular analyses were carried out to identify the Begomovirus species associated with infections. The results revealed an overall CMD incidence of 70.9%, dominated by single infections of African cassava mosaic virus (ACMV) (>65%); followed by mixed infections involving ACMV EACMV. The ACMV-UG variant was detected for the first time in Kongo Central province. Varietal responses differed considerably, with Ilona, Kilenda, Mahungu and Ngamaza showing relatively high tolerance; whereas Le Vieux and Vudungu were highly susceptible. Whitefly abundance, field age and

geographical location significantly influenced the risk of infection. These findings highlight the importance of varietal selection and integrated disease management for reducing CMD impact and improving cassava productivity in western DRC.

Key Words: ACMV-UG, *Bemisia tabaci*, *Manihot esculenta*

RESUME

La mosaïque africaine du manioc (Cassava Mosaic Disease, CMD) demeure l'une des principales contraintes à la production du manioc en Afrique subsaharienne. En République Démocratique du Congo (RDC), l'utilisation généralisée de matériel végétal infecté et la diversité des Begomovirus augmentent la vulnérabilité des variétés cultivées. Cette étude avait pour objectif d'évaluer la réponse des principales variétés de manioc face aux infections par les Begomovirus et d'identifier les facteurs influençant leur sensibilité dans des conditions d'infection naturelle élevée dans le sud-ouest de la RDC. Des prospections ont été réalisées entre mars et avril 2022 dans 94 champs de manioc situés dans les provinces du Kongo Central et de Kinshasa. L'incidence de la CMD, la sévérité des symptômes et l'abondance du vecteur *(Bemisia tabaci*) ont été évaluées au champ, tandis que des analyses moléculaires ont permis d'identifier les espèces de Begomovirus associées aux infections. Les résultats ont montré une incidence moyenne de la CMD de 70,9 %, dominée par les infections simples d'ACMV(>65 %), suivies d'infections mixtes impliquant ACMV et EACMV. La variante ACMV-UG a été détectée pour la première fois dans la province du Kongo Central. Les variétés Ilona, Kilenda, Mahungu et Ngamaza ont montré une tolérance relativement élevée, tandis que Le Vieux et Vudungu se sont révélées très sensibles. L'abondance des mouches blanches, l'âge des champs et la localisation géographique influencent significativement le risque d'infection. Ces résultats soulignent l'importance de la sélection variétale et de la gestion intégrée pour réduire l'impact de la CMD et améliorer la productivité du manioc dans l'ouest de la RDC

Mots Clés: ACMV-UG, *Bemisia tabaci*, *Manihot esculenta*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is among the main food crops in sub-Saharan Africa (SSA), providing food security for more than 200 million people. The crop is valued for adaptability to marginal environments, tolerance to drought and ability to produce acceptable yields under low-input farming systems (Nweke *et al*., 2002; FAO, 2020). In the DRC, it plays a central role in production systems and is the primary source of calories for households. However, national level productivity remains low, largely due to cassava mosaic disease (CMD), considered to be the most devastating viral disease affecting this crop in Africa (Patil and Fauquet, 2009; Legg *et al*., 2014).

CMD is caused by Begomoviruses (family *Geminiviridae*), primarily African *Cassava Mosaic Virus* (ACMV) and East *African Cassava Mosaic Virus* (EACMV); as well as several recombinant viruses such as East *African Cassava Mosaic Cameroon Virus* (EACMCV) (Fondong, 2017; Amoakon *et al*., 2023; Dye *et al*., 2023). These viruses are persistently transmitted by the whitefly, *Bemisia tabaci* (Casinga *et al.,* 2022; Likiti, 2024) and through vegetative propagation of infected cuttings. The emergence of more virulent ACMV–EACMV recombinants has led to severe epidemics in Central and East Africa, causing yield losses exceeding 80% in some areas (Fondong, 2017).

The regional West African Virus Epidemiology for Food Security (WAVE)

programme has strengthened surveillance and characterisation capacities for cassava viruses in West and Central Africa (Monique *et al.,* 2021; Mouketou et *al.,* 2022; Combala *et al.,* 2025). In the DRC, work conducted within this framework has highlighted a high diversity of CMD strains and a predominance of ACMV–EACMV co-infections, often associated with severe symptomatic expression (Pita *et al*., 2001; Soro *et al*., 2021; Likiti *et al* ., 2024).

Sustainable management of cassava disease, relies primarily on the use of resistant or tolerant varieties (Legg *et al.,* 2014). However, the effectiveness of these varieties is threatened by the rapid diversification of Begomoviruses *and* the spread of infected materials, through a largely informal seed system. In *southwestern* DRC, particularly in Kongo Central and Kinshasa, the response of local varieties to different viral strains remains poorly understood, even though this area represents a major cassava production and distribution region. The objective of this study to evaluate the response of the main cassava varieties to different forms of CMD *Begomovirus infection in southwestern DRC*.

MATERIALS AND METHODS

Study area and sampling device. The study was conducted during March and April 2022, in nine territories of Kongo Central and in the commune of Maluku, Kinshasa Province (Fig. 1). The survey was carried out within the framework of the WAVE Regional Programme (Epidemiology of Central and West African Virus Diseases for Food Security), following a harmonised protocol.

The survey targeted cassava fields of 3-6 months old; located along major accessible roads. On average, one field was selected every 10 Km, depending on accessibility and

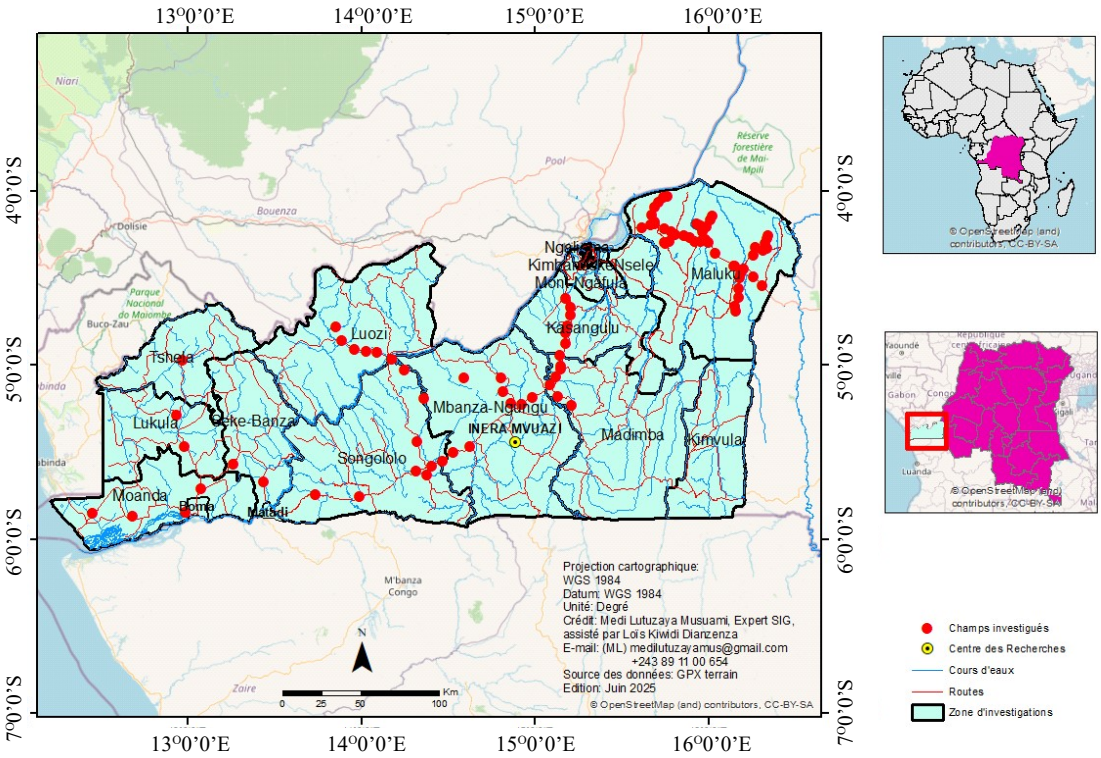

Figure 1. Map of the study area showing the surveyed localities in the provinces of Kongo Central and Kinshasa.

availability. In total, 94 fields were sampled (45 in Kongo Central and 49 in Kinshasa). Geographic coordinates (latitude, longitude, altitude) for every selected field, were recorded using a handheld GPS.

CMD visual assessment. In each field, 30 plants were randomly selected using an X-pattern diagonal selection procedure (15 per diagonal). Each plant was examined for the presence of typical CMD symptoms, according to the criteria described by Sseruwagi *et al.* (2004). Symptom severity was recorded on the International Iinstitute of Tropical Agriculture (IITA, 1990) standardised scale, ranging from 1 (absence) to 5 (severe stunting, curling, and reduced foliage). Incidence was calculated as the percentage of symptomatic plants per field.

Variety identification and infections types. The cultivated cassava varieties were identified using local names provided by the producers. Two main varietal groups were distinguished; namely, local and improved varieties. In fields with two or more varieties, only the dominant variety was considered for analysis. For each variety, the proportions of plants infected by primary transmission (*via* contaminated cuttings) and secondary transmission (*via* whiteflies) were estimated.

Abundance of whiteflies. Vector abundance was assessed in the same fields as disease symptoms. In each field, 30 plants were selected following a diagonal sampling approach. On each selected plant, the number of adult whiteflies was counted on the five uppermost apical leaves, following standardised field assessment procedures as described in previous studies (Kiwidi *et al*., 2026).

Collection of leaf samples. Between one and four leaf samples were collected per field, from both symptomatic and asymptomatic plants, according to the protocols of Sseruwagi *et al*. (2004) and WAVE (Combala *et al*., 2024). A total of 315 samples were obtained; dried and stored in herbarium presses until transfer to the laboratory.

Data management. Field data (symptoms, varieties, GPS coordinates, whitefly abundance, etc.) were entered *via* an iForm mobile application, on an Android smartphone. The data were synchronised to the iForm cloud and integrated into the WAVE Cube multidimensional database, in accordance with Soro *et al.* (2021).

DNA extraction. Total DNA was extracted from cassava leaves, using the CTAB method (Doyle and Doyle, 1987), adapted to the WAVE protocol. The DNA pellets were re-suspended in 100 µl of TE buffer and stored at -20 °C. The concentration was measured using Nanodrop One (Thermo Scientific) and adjusted to 50 ng μl^{-1} for PCR.

PCR and amplification conditions. Detection of CMD-associated viruses was performed by PCR, using Universal and Specific Primers for ACMV, ACMV-UG, EACMV and EACMCV (Table 1), according to the protocol of Delatte *et al*. (2005) and the WAVE (Combala *et al*., 2025) recommendations.

Each PCR reaction mixture (25 µl total volume) contained: 14.125 µl of nuclease-free water, 2.5 µl of PCR buffer (5×), 1 µl of MgCl, (25 mM), 0.25 µl of dNTPs (20 mM), 1 µl of each primer (10 mM), 0.125 µl of Taq polymerase (5 U/ ml), and 5 µl of template DNA (sample extract). The DNA amplification programme comprised of initial denaturation at 94 °C (4 min), followed by 35 cycles (94 °C/1 min, 55 °C/1 min, 72 °C/1 min), and then a final extension at 72 °C (10 min). The PCR products were visualised by electrophoresis on 1.2% agarose gel stained with Safeview™ Classic and migration with a 1 kbp marker.

Statistical analysis. All statistical analyses were performed using R version 4.4.1 (R Core Team, 2024). Normality of residuals and

TABLE 1. Primers used for the detection of Begomovirus in cassava leaf samples

Virus	Primers	primer sequences (5' - 3')	Target region	Expected size p	References
ACMV	JSP 001	ATGTCGAAGCGACCAGGAGAT	ACMV DNA-A(CP)	783	Pita *et al* ., 2001
	JSP 002	TGTTATTAATTGCCAATACT			
	ACMVBF	TCGGGAGTGATACATGCGAAGGC	ACMV DNA-B(BV1/BC1)	628	Matic *et al* ., 2012
	ACMVBR	GGCTACACCAGCTACCTGAAGCT			
	WAVE-508F	AAGGCCCATGTAAGGTCCAG	ACMV AV1/AC3	800	Combala *et al* ., 2024
	WAVE-1307R	GAAGGAGCTGGGGATTCACA			
EACMV	JSP 001	ATGTCGAAGCGACCAGGAGAT	EACMV DNA-A(CP)	780	Pita *et al* ., 2001
	JSP 003	CCTTTATTAATTTGTCACTGC			
	CMBRepF	CRT CAA TGA CGT TGT ACC A	EACMV DNA-A(AC1)	650	Alabi *et al* ., 2008
	EACMVRepR	GGT TTG CAG AGAACT ACA TC			
EACMCMV	WAVE-EA 1875F	TGTACCAGGCGTCGTTTGAA	EACMCMV AC1	800	Combala *et al* ., 2024
	WAVE-EA2674	TGTCCCCCGATCCAAAACG			
Seven CMVs	ACMV21for	GCAGTGATGAGTTCCCCGGTGCG	DNA-A(AC3-AC2-AC1) of ACMV, EACMV, EACMCMV, EACMKV, EACMMV, EACMZV and SACMV	552	Matic *et al* ., 2012
	ACMV21rev	ATTCCGCTGCGCGGCCATGGAGACC			

homogeneity of variances were were assessed using the Shapiro–Wilk and Bartlett tests (STATS package). As the data did not meet the assumptions of normality and homoscedasticity, even after applying logarithmic and square root transformations, non-parametric methods were used for further analyses.. Comparisons between varieties for cassava mosaic disease incidence, symptom severity, and whitefly abundance were performed using the Kruskal-Wallis test; followed by post-hoc comparisons corrected using the Benjamini-Hochberg (BH) method to control the false discovery error rate. The concordance between symptomatic expression and molecular detection (PCR) of Begomovirus was assessed using the Chi-square test of independence.

Binary logistic regression (glm, family = "binomial") was then used to identify the main factors explaining the probability of simple or mixed infection by the Begomoviruses responsible for the mosaic virus. This was done by considering symptom severity, field age, fly density, varietal resistance, and province of origin. Furthermore, the phenotypic structure of the varieties was studied using Gower distance (daisy, *cluster*) and visualised as a hierarchical dendrogram; constructed according to Ward 's method (hclust, stats) to explore the relationships between varieties and their resistance to CMD (Kaufman and Rousseeuw, 1990; Maechler *et al*., 2023). Graphical and exploratory analyses were performed using the ggplot2 and dplyr packages.

RESULTS

CMD intensity and whitefly abundance. Table 2 presents data for the CMD intensity and prevalence along with the abundance of whiteflies, the types of infection and the resistance status of the different varieties across all study sites. There were marked variability among cassava varieties in the incidence and severity of cassava mosaic disease, as well as in the abundance of *Bemisia tabaci* ($P < 0.05$ for all parameters). The mean incidence (70.95%, $P < 0.001$) indicates high prevalence of the disease in the sampled areas. The Ilona, Kilenda, Mahungu and Ngamaza varieties stood out with very low severity (score 1.00), confirming the high resistance (HR) endowed in them. On the other hand, varieties Le Vieux and Vudungu had high scores (4 to 5), indicating high susceptibility.

According to the classification adopted, the HR group includes varieties with an average severity score of 1; the R group those with a score between 1.01 and 2; and the S group, those with a score between 2.01 and 3 (Monique *et al.,* 2021). However, the observed differences in severity ($P = 0.017$) did not retain their significance after the Benjamini-Hochberg correction; suggesting inter-varietal variability, though real, remains moderate.

The mean abundance of whiteflies was estimated for each variety, showing considerable variation (Table 2). For example, Projet, Kimbungu, and Vudungu had the highest numbers of whiteflies per plant, whereas Kingawa, Le Vieux, and Samu showed low infestations. Identifying varieties in the field allowed us to link whitefly abundance with varietal susceptibility and resistance to CMD.

The predominance of primary infections indicates that contamination occurred mainly through planting materials (infected cuttings), rather than through secondary transmission *via* whiteflies. The majority of the varieties evaluated were susceptible to CMD, while only a few (4) exhibited high resistance (Ilona, Kilenda, Mahungu and Ngamaza). This situation highlights the vulnerability of local plant materials, and underscores the need to intensify the dissemination of improved varieties and strengthen participatory breeding programmes.

Molecular based distribution of viral infections. Molecular analyses has confirmed the presence of a diversity of simple and mixed viral infections among the cassava varieties in southwestern DRC (Table 3). Four viruses

TABLE 2. Incidence, severity, abundance of whiteflies, type of infection and resistance status of cassava varieties studied.

Cultivars	Number of fields	Incidence (%)	Severity	Abundant whitefly infestation	Type of infection (%)		RS
					Primary	Secondary	
Antiotta	1	60.00 ±14.14 d	3.25 ±1.50	11.97 ± 11.97 defgh	100	0	S
Dali	1	80.00 ±10.00 fg	3.33 ±0.57	3.00 ±3.00 abcdefghij	100	0	S
Dikando	5	100 ±0.00 i	3.46 ±1.40	0.67 ± 1.00a	100	0	S
Ilona	1	0.00 ±0.00a	1.00 ±0.00	2.00 ±2.00abcdefghi	0	0	HR
Kamanabua	1	100 ± 0.00i	3.00 ±0.00	6.00 ±6.00abcdefghij	100	0	S
Kigerard	18	100 ±0.00 i	3.17 ±1.51	3.86 ± 5.08abc	100	0	S
Kilenda	2	0.00 ± 0.00a	1.00 ±0.00	2.00 ±2.82abcdefghi	0	0	HR
Kimbungu	2	76.25 ± 8.53efg	3.18 ±1.25	22.93 ±11.86 days	100	0	S
Kimpembe	1	100 ±0.00 i	4.00 ±0.00	9.16 ±5.63 cdefgh	100	0	S
Kindombe	2	60.00 ± 5.00de	3.00 ±1.73	5.72 ± 5.75bcde	100	0	S
Kingawa	4	100 ± 0.00i	3.07 ±1.54	0.36 ±0.60ab	100	0	S
Kinkenge	1	100 ± 0.00i	2.50 ±1.73	4.63 ±1.35abcde	100	0	S
Kinzenga	1	100 ±0.00 i	3.75 ±1.54	14.40 ± 7.36fghi	100	0	S
Kivieki	1	100 ±0.00 i	2.33 ±1.73	2.00 ±1.00abcdefghi	100	0	S
Landrace	15	77.00 ± 5.77fg	3.21 ±1.89	9.59 ± 6.35ef	100	0	S
The old man	1	100 ±0.00 i	5.00 ±2.30	0.06 ±0.36ab	100	0	S
Lidame	1	100 ±0.00 i	2.66 ±1.48	1.00 ± 1.00abcdefghi	100	0	S
Lindalala	2	66.67 ±00.00def	3.66 ±0.00	6.30 ± 6.01bcde	100	0	S
Lueki	2	100 ± 0.00i	3.00 ±1.54	12.66 ± 12.05fgh	100	0	S
Mabindi	1	71.43 ± 0.00defg	3.66 ±2.30	1.76 ±1.97abc	100	0	S
Mahungu	2	20.00 ±10.00	1.00 ±0.00	0.50 ±0.70abcdefghj	100	0	HR
Malibuata	3	100 ± 0.00i	3.33 ±0.57	1.33 ±0.57abcdefghi	100	0	S
Maneli	2	100 ±0.00 i	3.00 ±1.00	6.50 ±2.12abcdefghij	100	0	S
Mapopo	1	40.00 ± 10.00c	3.25 ±1.50	16.83 ±13.63ghij	100	0	S
Matembele	1	100 ± 0.00i	4.16 ±0.40	2.00 ±2.00abcdefghi	100	0	S
Mayombe	1	0.00 ± 0.00a	5.00 ±0.00	1.70 ± 1.67abc	0	0	S

TABLE 2. Contd.

Cultivars	Number of fields	Incidence (%)	Severity	Abundant whitefly infestation	Type of infection (%)		RS
					Primary	Secondary	
Mpelonlongi	1	67.50 ±9.57def	3.00±1.41	17.50 ±12.00 hij	100	0	S
Muasi muindu	1	100 ±0.00 i	3.00±0.00	1.00 ±1.00abcdefghi	100	0	S
Ngamaza	1	0.00 ±0.00a	1.00±0.00	1.00 ±1.00abcdefghi	0	0	HR
Nicha	2	100 ± 0.00i	2.83±1.32	3.00 ±3.00abcdefghij	100	0	S
Nsumbakani	1	83.33 ± 0.00 gh	3.25±1.50	2.70 ± 2.07abcd	100	0	S
Obama	1	40.00±10.00c	4.33±1.15	1.20 ±1.89ab	0	0	S
Project	1	100 ±0.00i	3.25±1.50	37.86 ± 11.76k	100	0	S
Pululu	1	100 ±0.00 i	2.83 ±0.40	5.00 ±5.00abcdefghij	100	0	S
Rav	5	33.33 ±0.00bc	3.00±1.41	2.36 ± 2.65abc	100	0	S
Sadisa	1	85.71 ± 0.00gh	2.33 ±0.57	2.00 ±2.00abcdefghi	100	0	S
Emergency Medical Services	1	100 ±0.00i	2.50±1.29	0.23 ± 0.56abc	100	0	S
Six months	1	0.00 ± 0.00a	4.00 ±0.00	15.96 ± 8.39fghij	0	0	S
Vudungu	1	100 ± 0.00i	4.00 ±0.00	20.63 ±12.50 ij	100	0	S
Wingi	1	0.00 ±0.00a	3.33 ±1.15	1.53 ±3.20abc	0	0	S
Zizila	2	100 ±0.00 i	2.00 ±1.09	0.00 ± 0.000abcdefg	100	0	R
Total	94						
Overall average		70.95	3.12	6.86			
Resume (%)		51.06	44.34	164.05			
p-value (5%)		0.00000***	0.01715*	0.00000***			

RS = Resistance Status, HR = Highly Resistant, R = Resistant, S = Sensitive.

were specifically ideintified; namely *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Cameroon virus* (EACMCMV), and, in some samples, the *ACMV-UG* strain. The results showed a clear predominance of simple ACMV infections, detected in more than 65% of the positive varieties (Table 3); confirming the major role of this virus in the local epidemiology of cassava mosaic disease. Simple EACMV infections remained marginal (< 5%); while detection of *ACMV-UG* in some samples constitutes the first identification of this variant in southwest DRC.

Co-infections of ACMV + EACMV, ACMV + EACMCMV, and ACMV + EACMV + EACMCMV represented approximately 10, 0.5 and 3% of cases, respectively. The *Kamanabua* variety was 100% infected by the ACMV + ACMV-UG combination, indicating high compatibility for co-infection with closely related strains. Approximately 10% of samples were classified as "virus not detected" and 4% as "virus detected but not identified"; this suggests the possible presence of uncharacterised variants.

On the varietal level, Kimpembe, Samu, Lidame, Projet, Mpelonlongi, *Kindombe* and *Dali* exhibited exclusively single ACMV infections (Table 3), suggesting a specific susceptibility to this virus. Other cultivars, such as *Kinkenge , Lueki,* and *Rav,* showed a significant proportion of co-infections, reflecting increased exposure to multiple circulating viral strains. Conversely, some local varieties like *Kingawa, Kivieki, Mabindi, Projet*, and *Kinzenga* had a high proportion of healthy plants (>25%);, indicating either potential resistance or low infection pressure in the sampling areas.

There was fair agreement between field virus symptom observations and PCR detection results (Table 4). Out of the 263 samples analysed, 74.1% tested positive for at least one virus by PCR. Among symptomatic plants, 79.5% were indeed infected by CMD; while 20.5% tested negative to PCR. Conversely, 29.4% of asymptomatic plants had a negative PCR result, but 70.6% of them nevertheless carried a virus. This suggested the likely presence of latent infections, delayed symptom expression, or low viral loads not visible to the naked eye. The χ^2 test (P = 0.1809) indicated that this discrepancy was not statistically significant, but the proportion of asymptomatic infections showed the limitations of visual diagnosis, justifying the need for molecular screening for more reliable monitoring of CMD.

Risk factors associated with viral infection. Table 5 presents the results of binary logistic regression models, evaluating the main factors associated with the probability of simple and mixed infection of cassava plants by *Begomovirus* responsible for cassava mosaic disease.

Results show that, for the simple infection model, the probability of infection was significantly influenced by the age of the field, the prevalence of *Bemisia tabaci,* and the Province of origin (P< 0.05). The overall model was highly significant (Ä Deviance = 39.84; P < 0.001), demonstrating its explanatory robustness. The risk of infection increased sharply with field age (Estimate = 2.46; P < 0.001) and whitefly population density (Estimate = 0.10; P = 0.002); highlighting the cumulative impact of vector pressure and duration of exposure. Furthermore, plants from Kongo Central had a higher probability of infection than those from Kinshasa (Estimate = 0.52; P = 0.03). This was likely related to agroecological differences or cultivation practices. The severity of symptoms was marginally significant (P = 0.05), which suggests that field observations remained broadly consistent with molecular diagnoses; although confirmation by PCR remains necessary for a reliable assessment.

In contrast, the complementary model focusing on co-infections revealed a significant effect only for plant age (P = 0.015). This non-

TABLE 3. Distribution of simple and mixed viral infections according to cassava varieties

Cultivars	Simple infections			Co-infections				Unidentified virus	Virus not detected	Healthy
	ACMV	EACMV	ACMV-UG	ACMV+ EACMV	ACMV+ EACMCMV	ACMV+ ACMV-UG	ACMV+ EACMV+ EACMCMV			
Antiotta	50	0	Not tested	25	0	-	25	0	0	0
Dali	66.66	0	Not tested	0	0	-	0	0	0	0
Dikando	0	0	Not tested	0	0	-	0	26.66	53.33	20
Ilona	0	0	0	0	Not tested	0	-	Not tested	-	-
Kamanabua	0	0	0	0	Not tested	100	-	Not tested	-	-
Kigerard	51.78	10.71	Not tested	16.07	0	-	0	5.35	10.71	7.14
Kilenda	33.33	Not tested	0	-	Not tested	0	-	Not tested	0	-
Kimbungu	36.36	9.09	Not tested	27.27	0	-	0	0	27.27	0
Kimpembe	100	0	Not tested	0	0	-	0	0	0	0
Kindombe	66.66	0	Not tested	0	0	-	0	0	0	0
Kingawa	7.14	0	Not tested	0	0	-	0	0	50	28.57
Kinkenge	0	0	Not tested	50	0	-	50	0	0	0
Kinzenga	0	0	Not tested	0	0	-	0	75	0	25
Kivieki	33.33	0	Not tested	0	0	-	0	0	0	33.33
Landrace	50	1.92	Not tested	7.69	0	-	1.92	16.25	6.25	6.25
The old man	66.66	0	Not tested	0	0	-	0	33.33	0	0
Lidame	66.66	0	Not tested	0	0	-	0	0	0	0
Lindalala	33.33	50	Not tested	0	0	-	0	16.66	0	0
Lueki	66.66	0	Not tested	16.66	16.66	-	0	0	0	0
Mabindi	0	33.33	Not tested	33.33	0	-	0	0	0	33.33
Mahungu	Not tested	Not tested	0	-	-	-	-	Not tested	-	-
Malibuata	33.33	0	Not tested	0	0	-	0	0	0	0
Maneli	0	Not tested	0	-	-	0	-	Not tested	-	-
Mapopo	50	0	Not tested		0	-	0	0	50	0
Matembele	16.66	Not tested	0	-	-	0	-	Not tested	-	-
Mayombe	0	100	Not tested	0	0	-	0	0	0	0
Mpelonlongi	100	0	Not tested	0	0	-	0	0	0	0

TABLE 3. Contd.

Cultivars	Simple infections			Co-infections				Unidentified virus	Virus not detected	Healthy
	ACMV	EACMV	ACMV-UG	ACMV+ EACMV	ACMV+ EACMCMV	ACMV+ ACMV-UG	ACMV+ EACMV+ EACMCMV			
Muasi muindu	Not tested	Not tested	0	-	-	-	-	Not tested	-	-
Ngamaza	Not tested	Not tested	0	-	—	-	-	Not tested	-	-
Nicha	16.66	0	0	0	-	0	-	Not tested	-	-
Nsumbakani	75	0	Not tested	25	0	-	0	0	0	0
Obama	0	0	Not tested	0	0	-	0	0	100	
Project	75	0	Not tested	0	0	-	0	0	0	25
Pululu	22.22	0	66.66	0	-	0	-	Not tested	-	-
Rav	40	5	Not tested	35	10	-	10	0	0	0
Sadisa	0	0	0	0	0	-	0	0	0	0
Emergency Medical Services	100	0	Not tested	0	0	-	0	0	0	0
Six months	66.66	0	Not tested	33.33	0	-	0	0	0	0
Vudungu	66.66	0	Not tested	33.33	0	-	0	0	0	0
Wingi	33.33	0	Not tested	0	0	-	0	0	66.66	0
Zizila	0	0	16.66	0	-	0	-	Not tested	-	-

TABLE 4. Concordance between symptom expression and PCR detection

Expression of symptoms	PCR negative	Simple infection (ACMV; EACMV; EACMCMV)	Co-infections	Unidentified virus	Total
Symptoms absent	20 (29.4%)	26 (38.2%)	22 (32.3%)	0	68
Symptoms	40 (20.5%)	95 (48.7%)	29 (14.8)	31 (15.8%)	195
Total samples	60	121	51	31	263

TABLE 5. Results of binary logistic regressions on simple and mixed infections of cassava plants

Explanatory variables	Estimate (β)	Standard error	Z-value	P-value	Meaning	Model concerned
(Intercept)	-1.418	0.857	-1.66	0.098	ns	Simple infection
Severity of symptoms	0.583	0.298	1.96	0.050	*	Simple infection
Age of the field	2.458	0.616	3.99	<0.001	***	Simple infection
Abundance of whiteflies	0.103	0.033	3.13	0.002	**	Simple infection
Province	0.519	0.240	2.16	0.031	*	Simple infection
(Intercept)	-0.992	1.139	-0.87	0.384	ns	Co-infection
Severity	0.089	0.380	0.24	0.814	ns	Co-infection
Varietal resistance	0.426	0.978	0.44	0.663	ns	Co-infection
Age of plants	0.992	0.409	2.43	0.015	*	Co-infection
Whitefly density	-0.018	0.034	-0.53	0.597	ns	Co-infection
Province	0.204	0.353	0.58	0.564	ns	Co-infection

ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

linear relationship suggests that older or intermediate-aged plants were approximately 2.7 times more likely to be co-infected than younger plants, due to a progressive accumulation of viral load and prolonged exposure to vectors . The other variables (severity, varietal resistance, whitefly density, and geographic location) did not show a significant effect; suggesting that co-infection was more closely linked to temporal and epidemiological factors than to varietal or environmental parameters.

Phenotypic structure and relationship with CMD resistance. The dendrogram based on Gower distance (Fig. 2) reveals a marked phenotypic structure among the studied cassava varieties, illustrating a wide morphological variability linked to the observed leaf and petiole characteristics. This diversity testifies to the richness of local plant materials and the influence of farmers' selection and exchange practices; where some varieties bearing different names could correspond to similar genotypes. The distribution of varieties into several distinct groups, thus reflects the coexistence of real diversity and possible varietal redundancy within the farmers' germplasm.

However, this structure only partially corresponds to the classification based on CMD resistance. Highly resistant varieties do

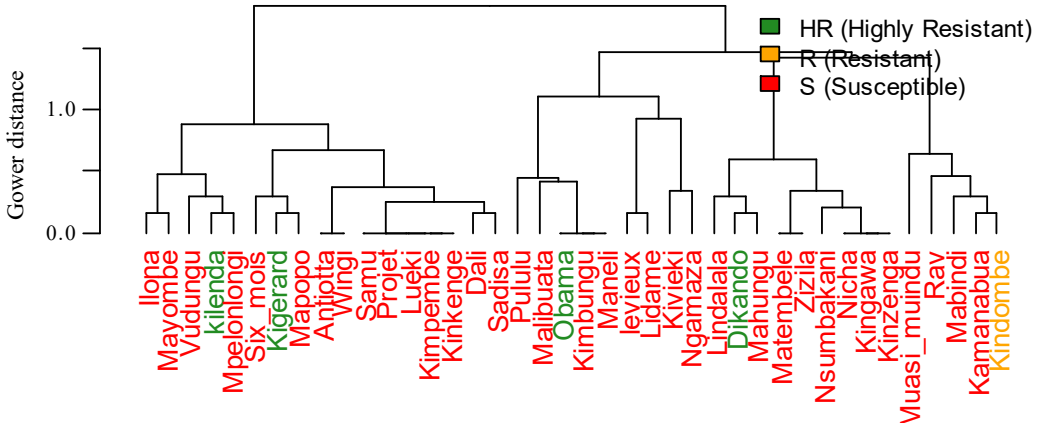

Figure 2. Dendrogram of cassava varieties based on phenotypic characteristics.

not form homogeneous clusters; while susceptible varieties are widely dispersed (Fig. 2), suggesting that resistance is not strictly linked to visible morphological traits. These results indicate that tolerance to CMD likely arises from underlying genetic or physiological mechanisms, and that an approach combining phenotypic and molecular analyses would be necessary to better characterise genetic diversity and identify potential morphological markers associated with resistance.

DISCUSSION

The high incidence of cassava mosaic disease (~70.95%) confirms the persistent high epidemic pressure in the producing areas of southwestern DRC, consistent with observations in Central and East Africa where cassava mosaic disease remains a major constraint on cassava production (Legg and Fauquet, 2004; Owor *et al.,* 2004). This high prevalence often results in yield losses of up to 80%, depending on the variety and environmental conditions, and also poses a serious threat to cassava productivity (Thresh *et al.,* 1994; Kouakou *et al.,* 2024). The varieties Ilona, Kilenda, Mahungu, and Ngamaza exhibited very low severity, validating their high resistance status, while the varieties Le Vieux and Vudungu showed high susceptibility. However, the lack of statistical significance after Benjamini-Hochberg correction, suggests that intervarietal variability, although real, remains moderate, probably due to environmental and microclimatic influences on symptom expression, as already highlighted by Legg *et al*. (2014).

The average abundance of *Bemisia tabaci* (6.86 individuals/plant) and the variability observed between varieties confirm that vector pressure is not uniform. This result supports the observations of Sseruwagi *et al*. (2004), according to which whitefly density depends not only on ecological conditions, but also on the host variety. The predominance of primary infections indicates that disease spread originates mainly from infected cuttings, which is consistent with the results obtained by Legg *et al*. (2015) and Fondong (2017), who showed that contaminated plant material is the main source of initial dissemination before secondary transmission by vectors.

Molecular analysis revealed the dominance of uncomplicated ACMV infections (>65%), confirming its leading role in the epidemiology of the CMD complex in Central Africa (Vernier *et al.,* 2018; Pita *et al.,* 2001). Uncomplicated EACMV infections were rare, but the detection of ACMV-UG in the study area represents a novel observation for Kongo Central and suggests the introduction or local evolution of derived variants. Co-infections of ACMV + EACMV (~10%) and ACMV + EACMV + EACMCMV (~3%) reflect the increasing

complexity of viral populations, a likely consequence of recombination and the exchange of plant material between areas (Legg *et al.,* 2014; Fondong, 2017). The significant proportion of "virus not detected" or "unidentified" samples could indicate the circulation of as-yet-uncharacterised strains, a phenomenon already documented by Ndunguru *et al.* (2015) in Tanzania.

The partial concordance between symptoms and PCR detection (79.5% of symptomatic individuals were actually infected, but 70.6% of asymptomatic individuals tested positive for PCR) illustrates the limitations of visual diagnosis, particularly in the face of latent infections or those with low viral load. Such discrepancies, not statistically significant in this study, were also reported by Sseruwagi *et al.* (2004), highlighting the need to integrate molecular testing for the reliable detection of mixed or latent infections.

Risk factor analysis using logistic regression shows that the probability of single infection is influenced by field age, *Bemisia tabaci* density, and province of origin. These results confirm previous observations that older plants, with longer exposure to vectors, have an increased risk of infection (Legg and Fauquet, 2004). Whitefly density is positively correlated with viral prevalence, validating their key role in transmission dynamics (Thresh *et al.,* 1994; Legg *et al.,* 2015). The higher risk observed in Kongo Central could be attributed to agroecological differences or cultural practices that promote vector survival. In contrast, co-infections are primarily associated with plant age, suggesting a cumulative effect of exposure over time, without a marked influence from varietal or environmental characteristics. This also demonstrates that the longer the plant is exposed, the greater the chance it has of being contaminated by many viruses at the same time (Fargette *et al*., 2006).

Analysis of phenotypic structure reveals significant morphological diversity among varieties, but without a clear correlation to CMD resistance. This lack of direct correlation between morphological traits and viral tolerance confirms that resistance depends more on internal genetic or physiological mechanisms than on visible foliar or petiole traits (Pita *et al.,* 2001; Legg *et al.,* 2014). Nevertheless, the phenotypic similarity of some resistant varieties suggests the possibility of using certain traits as indirect indicators, a finding that requires further molecular analysis.

From a practical standpoint, these results highlight several major implications. First, the selection and dissemination of resistant varieties (Ilona, Kilenda, Mahungu, Ngamaza) must be strengthened in seed multiplication programs. Second, the high proportion of primary infections justifies the implementation of sanitation and plant material certification programs. Third, the control of *Bemisia tabaci* remains a crucial pillar of integrated management, particularly through the use of repellent plants, biological controls, or less attractive varieties. Finally, epidemiological surveillance should systematically incorporate molecular detection to monitor the emergence of new viral strains.

CONCLUSION

This study reveals a high prevalence of cassava mosaic disease in western DRC, dominated by single ACMV infections, but also including ACMV + EACMV, ACMV + EACMCMV, and ACMV + EACMV + EACMCMV co-infections, as well as the new detection of the ACMV-UG strain in the region. Varieties Ilona, Kilenda, Mahungu, and Ngamaza confirmed their high resistance; while Le Vieux and Vudungu proved susceptible. *Bemisia tabaci density*, field age, and location significantly influenced the probability of infection, reflecting a strong interaction between varietal and agroecological factors. The lack of correlation between morphological traits and resistance suggests a genetic basis for the latter. These results support the need to strengthen breeding, sanitation, and enhanced virological surveillance programs to secure

cassava production in western DRC. But also the strengthening of the selection and dissemination of resistant varieties, integrated vector management, awareness-raising of producers and finally analysing the real economic impact of co-infections on yields.

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