Universal Journal of Agricultural Research 9(6): 266-276, 2021 http://www.hrpub.org
DOI: 10.13189/ujar.2021.090605

Evaluation of Ten Cassava Varieties for Resistance to Cassava Mosaic Disease in Burkina Faso

Monique Soro[1,2], Koussao Somé[3,4,*], Fidèle Tiendrébéogo[2,3], Justin S. Pita[1,5], Rahim Romba[6], Bouma James Néya[2,3], Daouda. Koné[1,7]

[1]Laboratoire de Biotechnologie, Agriculture et Valorisation des Ressources Biologiques, UFR Biosciences, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire
[2]Laboratoire de Virologie et de Biotechnologies Végétales, Institut de l'Environnement et de Recherches Agricoles (INERA), 01 BP 476 Ouagadougou 01, Burkina Faso
[3]Laboratoire Mixte International Patho-Bios, IRD-INERA, 01 BP 476, Ouagadougou 01, Burkina Faso
[4]Laboratoire de Génétique et de Biotechnologies Végétales, Institut de l'Environnement et de Recherches Agricoles (INERA), 01 BP 476 Ouagadougou 01, Burkina Faso
[5]Central and West African Virus Epidemiology (WAVE), Pôle Scientifique et d'Innovation de Bingerville, Université Félix Houphouët-Boigny (UFHB), Bingerville, Côte d'Ivoire
[6]Laboratoire d'Entomologie Fondamentale et Appliquée, Unité de Formation et de Recherche en Sciences de la Vie et de la Terre (UFR-SVT), Université Joseph Ki Zerbo, Ouagadougou, Burkina Faso
[7]Centre d'Excellence Africain sur le Changement Climatique, la Biodiversité et l'Agriculture Durable (WASCAL/CEA-CCBAD, Université Félix Houphouët-Boigny), PSI-Université Félix Houphouët-Boigny 22 BP 582 Abidjan 22, Côte d'Ivoire

Received September 16, 2021; Revised October 28, 2021; Accepted November 21, 2021

Cite This Paper in the following Citation Styles

(a): *[1] Monique Soro, Koussao Somé, Fidèle Tiendrébéogo, Justin S. Pita, Rahim Romba, Bouma James Néya, Daouda. Koné, "Evaluation of Ten Cassava Varieties for Resistance to Cassava Mosaic Disease in Burkina Faso," Universal Journal of Agricultural Research, Vol. 9, No. 6, pp. 266 - 276, 2021. DOI: 10.13189/ujar.2021.090605.*

(b): *Monique Soro, Koussao Somé, Fidèle Tiendrébéogo, Justin S. Pita, Rahim Romba, Bouma James Néya, Daouda. Koné(2021). Evaluation of Ten Cassava Varieties for Resistance to Cassava Mosaic Disease in Burkina Faso. Universal Journal of Agricultural Research, 9(6), 266 - 276. DOI: 10.13189/ujar.2021.090605.*

Abstract The whitefly-transmitted cassava mosaic disease (CMD) caused by cassava mosaic geminiviruses (CMGs) is the most important disease threatening the production of cassava (*Manihot esculenta* Crantz). Among the main measures for controlling CMD, the use of resistant varieties seems to be among the best methods. This study was conducted in 2017/2018 cassava growing season at three locations to evaluate the level of resistance to CMD of 7 elite cassava varieties widely used by farmers and 3 local cassava varieties cultivated in Burkina Faso. Both morphological and molecular markers were used to screen these varieties against CMGs infection. Morphological markers revealed 8 varieties as highly resistant (TMS 91/02312, TMS 92/0067, TMS 92/0325, TMS 92/0427, TMS 4(2)1425, TMS 94/0270, TMS 30572 and Boborola) whilst the two others (Nouhao and Santidougou) were resistant. The molecular markers linked to *CMD1* and *CMD2* genes were detected in all varieties. The molecular marker associated with *CMD3* gene was detected only in the 8 highly resistant varieties. However, whitefly number per plant and disease pressure were low during this study. It was, therefore, concluded that a better assessment of resistance of cassava varieties to CMD in Burkina Faso could be obtained by combining agro-inoculation and molecular screening.

Keywords Geminiviruses, Simple Sequence Repeats, Sequence Characterized Amplified Region, Resistance, Disease

1. Introduction

Cassava (*Manihot esculenta* Crantz, Family: *Euphorbiaceae*) is an important food crop in most of the tropical regions of Africa, Asia and Latin America [1]. It is native from the Northern Amazonian basin [2], [3] and

was probably introduced into West Africa (Gulf of Guinea) in the 16th century by Portuguese [4]. It was spread quickly thanks to its resilience, flexibility of harvest and diversity of uses [5], [6]. Cassava is the second most important root and tuber crop after the potato (*Solanum tuberosum* L.) with a global production of more than 303 million tons in 2019. In the same period, Africa contributed 192.1 million tons, more than half of the world supply [7]. About 33.2% of world's cassava is produced in West Africa, Nigeria being the top producer with 19.5% of the global production [7]. Cassava is a major staple for more than 700 million people in tropical and subtropical developing countries and enhances food security in these countries [8]–[10]. It is a valuable food security crop, particularly to smallholder farmers in Sub-Saharan African countries [11] and consequently a source of incomes for many processors and traders [9]. The high calorie yield per hectare (250 kcal/ha/day), drought tolerance, hardiness in stressful environments, flexibility of harvesting time are the major advantages of this crop compared to many other crops [5], [12], [13]. However, cassava production is negatively affected by several pests and diseases, among which, cassava mosaic disease (CMD) could be the major constraint.

In Africa, CMD is caused by nine distinct cassava mosaic geminiviruses (CMGs) species: *African cassava mosaic Burkina Faso virus* (ACMBFV) [14], *African cassava mosaic virus* (ACMV) [15], E*ast African cassava mosaic Cameroon virus* (EACMCMV) [16], *East African cassava mosaic Kenya virus* (EACMKV) [17], *East African cassava mosaic Malawi virus* (EACMMV) [18], *East African cassava mosaic virus* (EACMV) [19], *East African cassava mosaic Zanzibar virus* (EACMZV) [20], *Cassava mosaic Madagascar virus* (CMMGV) and *South African cassava mosaic virus* (SACMV) [21]. Among these nine species, the presence of ACMV, ACMBFV and the Uganda strain of EACMV (EACMV-UG) was reported in Burkina Faso [14], [22], [23]. Recently EACMCMV was also detected in the country [24]. CMD causes chlorosis on cassava leaves, which reduces photosynthetic activity, ultimately leading to stunted growth of plant and lowered yields ranging from 20 to 95% [4].

Among the main measures for controlling CMD, the use of resistant varieties is one of the best methods. It reduces both production losses that are caused by the disease and the inoculum source in crops, especially in varieties that suppress virus accumulation [25], [26]. Resistance to CMD was first obtained from a cross between cassava and its relative *Manihot glaziovii* Muller von Argau [27]. After three backcrosses into cassava to obtain suitable storage roots, several improved cultivated cassava genotypes of the Tropical Manihot Selection (TMS) series, with resistance to CMD were identified [28]. The CMD resistance gene "*CMD1*" from *M. glaziovii* is polygenic and recessive [25], [29]. A SSR marker, SSRY40 (linkage group D of cassava genetic map) [30], [31], was found to be associated with this resistance and explained 48% of the phenotypic variance of CMD resistance [31]. A second source of resistance "*CMD2*", which is located on Linkage group R of the molecular genetic map of cassava, monogenic with a dominant effect, was discovered in Nigerian landraces of the Tropical Manihot Esculenta (TME) series [25], [26]. Simple sequence repeat (SSRY28, NS158 and NS169) and sequence characterized amplified region (RME1) molecular markers were found to be associated with *CMD2* gene and explained 70% of the phenotypic variance [25], [26], [32]–[34]. Recently, a new CMD resistance gene, designated as "*CMD3*", was described in the elite cultivar TMS 97/2205 [34]. TMS 97/2205 was derived from crosses of TMS 30572 (*CMD1* resistant type) and TME 6 (*CMD2* resistant type) [35]. The SSR marker NS198 was found to be associated with *CMD3* gene and explained 11% of the phenotypic variance [34].

In Burkina Faso, cassava was introduced by farmers decades ago from Ghana and Côte d'Ivoire [36]. It has long been cultivated around vegetable gardens for domestic consumption. Formerly considered as a neglected crop, cassava has become a cash crop, since the formal introduction of improved varieties from the International Institute of Tropical Agriculture (IITA) in 2003 and constitutes a major national commercial priority. Cassava production has spread in Burkina Faso with the support from the " Programme de Development Agricole " and the government initiatives to increase internal supply [36]. Despite these efforts, cassava production remains relatively low compared to the expected demanded. Indeed, national demand was estimated at 124,917 tons in 2017 while the annual production was 22,104 tons [37]. To increase cassava productivity in Burkina Faso, the government, through the national research institute, has introduced some improved high-yielding varieties from IITA [35], [38], [39]. Since their introduction in Burkina Faso to date, no study on their resistance to CMD under farmer's conditions has yet been conducted. This paper presents the results of a study aiming to estimate the level of resistance to CMD under natural conditions of 7 elite cassava varieties widely grown by farmers and 3 local cassava varieties of Burkina Faso.

2. Materials and Methods

2.1. Plant Material

Thirteen distinct cassava varieties were used for the study. For ten of them their status regarding the infection to CMD was assessed (Table 1). The experiment was conducted in three locations. Prior to the experiment, the health status of the planting material was assessed. Each of the ten varieties was subjected to polymerase chain

reaction (PCR) using the following primers: JSP001 (5'-ATGTCGAAGCGACCAGGAGAT-3') and JSP002 (5'-TGTTTATTAATTGCCAATACT-3') for ACMV-like virus coat protein (CP) gene detection and JSP001 and JSP003 (5'-CCTTTATTAATTTGTCACTGC-3') for EACMV-like virus CP gene detection [19]. Only CMD-free planting materiel (those which were negative to the virus's detection using the primers mentioned above) was used for the evaluation of resistance to CMD. In each of the three experimental locations, CMD infected cuttings of the local variety from farmer's fields were used as inoculum to allow natural infection by the isolates of the virus naturally present. Details of the cassava varieties are provided in Table 1.

2.2. Experimental Sites and Field Layout

The experiment was conducted in three locations during the cassava growing season 2017/2018. The locations were Léo (11°4'6.24''N, 2°6'7.30''O and 322 m altitude) in the Southern part of the country, Gourpouo (11°2'56.10''N, 2°54'46.30''O and 257 m altitude) in the Southwestern part and Savili (12°5' 7.16''N, 2°2'16.77''O and 340.4 m altitude) in the Centre (Figure 1). These three locations are in Savannah part of Burkina Faso characterized by an annual rainfall ranged from 600 to 900 mm. In each location, the experiment was conducted in a randomized complete block design with three replications containing eleven varieties (the ten varieties from clean planting material and the local infested variety of each location). In each block of replication, the variety was planted in two rows of five plants for a total of ten plants per plot separated from the next variety by one row of the infected local variety to be the source of virus inoculum. Clean planting materials (for each variety) of relatively uniform size were selected for the trial. Planting was done on ridges with the spacing of 1 m between consecutive plant and 1 m between consecutive ridges for a planting density of 10,000 ha^{-1}. The blocks were 2 m apart.

No fertilizer or pesticide was applied during the experiment. Plots were hoe-weeded when necessary.

2.3. CMD Symptoms Severity, Disease Incidence and Whitefly Population Assessment

Each plant was assessed visually for the presence or absence of adult whiteflies and CMD symptoms (chlorotic mosaic of the leaves, leaf distortion, and stunted growth). The number of adult whiteflies as well as CMD symptom score was recorded at 1, 2, 5, and 10 months after planting. CMD symptom severity score was recorded using a scale of 1 (no symptoms) to 5 (very severe symptoms) [40]. For each variety, the CMD incidence was calculated as the percentage of infected plants in relation to the number of plants assessed [41] and CMD symptoms severity score as the mean rating of symptomatic and non-symptomatic plants. The number of adult whiteflies for each variety was determined by counting whiteflies on the top five fully expanded leaves of each plant. This was done to assess the relationship of whitefly population and the CMD symptom severity and the incidence of disease.

Table 1. The characteristics of cassava varieties used in the study

Variety	Type of variety	Role of variety in the study	CMD status before planting	Place of collection	Origin
TMS 30572	Improved	CMD resistance assessment	CMD free	INERA/Burkina	IITA/Nigeria
TMS 4(2)1425	Improved	CMD resistance assessment	CMD free	INERA/Burkina	IITA/Nigeria
TMS 91/02312	Improved	CMD resistance assessment	CMD free	INERA/Burkina	IITA/Nigeria
TMS 92/0067	Improved	CMD resistance assessment	CMD free	INERA/Burkina	IITA/Nigeria
TMS 92/0325	Improved	CMD resistance assessment	CMD free	INERA/Burkina	IITA/Nigeria
TMS 92/0427	Improved	CMD resistance assessment	CMD free	INERA/Burkina	IITA/Nigeria
TMS 94/0270	Improved	CMD resistance assessment	CMD free	INERA/Burkina	IITA/Nigeria
Boborola	Local	CMD resistance assessment	CMD free	Boborola	Burkina Faso
Nouhao	Local	CMD resistance assessment	CMD free	Nouhao	Burkina Faso
Santidougou	Local	CMD resistance assessment	CMD free	Santidougou	Burkina Faso
Local Léo	Local	Source of inoculum at Léo	CMD	Léo	Burkina Faso
Local Gourpouo	Local	Source of inoculum at Gourpouo	CMD	Gourpouo	Burkina Faso
Local Savili	Local	Source of inoculum at Savili	CMD	Savili	Burkina Faso

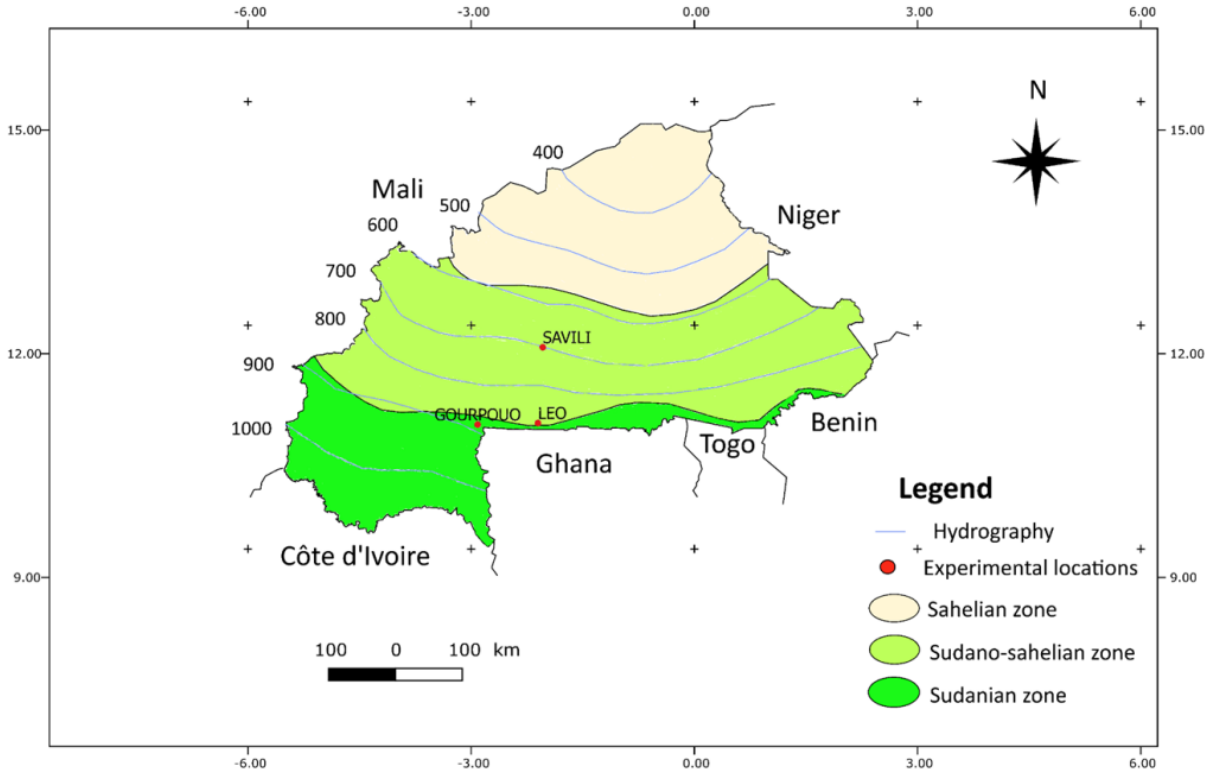

Figure 1. The map of Burkina Faso showing experimental locations

2.4. Resistance to CMD Classification

Varieties with a mean CMD symptom severity score of "1" were classified as highly resistant (HR), those with score "2" were resistant (R), while score "3" were classified as susceptible (S) and scores "4" and "5" classified as highly susceptible (HS) [29], [42].

2.5. Detection of the Presence of Genes Conferring Resistance to CMD

Total DNA was extracted from cassava leaves of each variety using the CTAB protocol according to Permingeat *et al* [43]. The concentration of DNA of each sample was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and adjusted to 200 ng/µL. The thirteen varieties were screened with four flanking markers (SSRY28, NS158, NS169 and RME-1) linked to the *CMD2* gene [25], [26], [29], [33], [34]. SSRY40 marker linked to *CMD1* gene was used for this resistance gene detection [31] and NS198 marker was used for *CMD3* gene detection [34]. TME 3 (source of the *CMD2* gene) and TMS 30572 (source of the *CMD1* gene) were included as a positive control for polymerase chain reaction (PCR). In the absence of TMS-97/2205, the source of the *CMD3* gene, all the varieties showing bands at a size of 196 bp were considered to have the *CMD3* gene [34]. The product sizes and other marker information's are provided in Table 2. The PCR was done in a final volume of 25 µl, containing 2.5 µl of 10x of BD reaction buffer, 1.5 µl or 2.5 µl of 25 mM of MgCl2, 0.5 µl of 10 mM of dNTPs, 0.5 µl of 10 µM of each primer, 0.1 µl of 5U/µl of FIREPol® DNA Polymerase (Solis BioDyne, Teaduspargi, Estonia) and 200 ng of DNA template of each sample. The DNA amplification was carried out in a SimpliAmp™ Thermal Cycler (Life Technologies Holdings Pte Ltd, Singapore). The PCR temperature profile was set at 94°C for 2 minutes for initial denaturation, followed by 35 cycles of amplification at 94ºC for 30 seconds, 50°C or 55°C for 1 minute and 72ºC for 1 minute. The final elongation step was performed at 72°C for 5 minutes. PCR amplified products were subjected to 2% (RME1) and 3 % (SSRY28, SSRY40, NS158, NS169 and NS198) agarose gels electrophoresis, stained with ethidium bromide. The electrophoresis was performed at 100V for one and a half hour and gels were visualized using a Compact Digimage System, UVDI series (MS major science, Saratoga, USA).

Table 2. Marker information's for *CMD1*, *CMD2* and C*MD3* genes detection

CMD Gene	markers	Type of Marker	Right primer	Left primer	Expected Product size (bp)	Ann temp (°C)	$MgCI_2$ (mM)
CMD1	SSRY40	SSR	TGCATCATGGTCCACTCACT	CATTCTTTTTCGGCATTCCAT	231	55	1.5
CMD2	SSRY28	SSR	GCTGCGTGCAAAACTAAAAT	TTGACATGAGTGATATTTTCTTGAG	180	55	1.5
	NS158	SSR	TGAAATAGTGATACATGCAAAAGGA	GTGCGAAATGGAAATCAATG	166	55	2.5
	NS169	SSR	GCCTTCTCAGCATATGGAGC	GCCTTCTCAGCATATGGAGC	319	55	2.5
	RME1	SCAR	AGAAGAGGGTAGGAGTTATGT	ATGTTAATGTAATGAAAGAGC	700	50	2.5
CMD3	NS198	SSR	TGGAAGCATGCATCAAATGT	TGCAGCATATCAGGCATTTC	196	55	2.5

2.6. Statistical Analysis

Data analysis was performed using R software version 3.6.1 (R Development Core Team, July 2019). The difference in the mean number of whiteflies and mean symptoms severity score of CMD between varieties were assessed using the Generalized Linear Model and Tukey's pairwise mean comparison test. A pairwise comparison of proportions was used based on a G-test with correction of BY [44] to compare the incidences of CMD between varieties. Pearson correlation coefficient was used to assess the relationship between whitefly abundance and symptom severity or incidence of the CMD. The map of Burkina Faso showing experimental locations was designed using QGIS software version 2.18.26 (Online available from https://qgis.org/downloads/).

3. Results

3.1. Whitefly Number per Plant

The whitefly abundance observed during the study was very low (1.5) with significant difference between varieties ($p < 0.001$) (Table 3). The highest mean number of whiteflies per plant was found on the variety Santidougou (2.4) and the lowest mean number on the variety Boborola (0.5). There was significant difference of whitefly mean number between cassava varieties in each experimental site (Table 4). At Léo, the highest mean number of whiteflies per plant was found on the variety Santidougou (4.5) and the lowest mean number on the variety Boborola (0.3). At Gourpouo, the highest mean number of whiteflies was found on TMS 30572 (2.8) and the lowest mean number on the variety TMS 92/0325 (0.6). While at Savili, the highest mean number of whiteflies was found on TMS 30572 (1.4) and the lowest on Boborola and Nouhao (0.3). For most of the varieties, the mean number of whiteflies per plant was higher at Léo than the other experimental locations (Figure 2). No relationship was found between whitefly abundance and severity of the CMD, nor between whitefly abundance and incidence of the disease ($R^2 = 0.31$, $p = 0.09$).

Table 3. Whitefly mean number, disease severity, disease incidence and resistance status of cassava varieties involved in the study.

Varieties	WN	SEV	INC	RS
TMS 30572	1.9abc	1.00c	0.0a	HR
TMS 4(2)1425	0.9ef	1.00c	0.0a	HR
TMS 91/02312	1.5cd	1.00c	0.0a	HR
TMS 92/0067	1.1de	1.00c	0.0a	HR
TMS 92/0325	1.2de	1.00c	0.3a	HR
TMS 92/0427	2.1ab	1.00c	0.0a	HR
TMS 94/0270	1.6bcd	1.00c	0.0a	HR
Boborola	0.5f	1.00c	0.0a	HR
Nouhao	0.7ef	1.02c	0.97a	R
Santidougou	2.4a	1.49b	26.3c	R
Local varieties	1.8bc	2.54a	91.6b	S
Mean	1.5	1.4	24.5	
p-value	***	***	***	

WN = mean number of whiteflies per plant; SEV = mean CMD severity score; INC = Incidence of CMD (%); RS = Resistance status; HR = highly resistant; R = resistant; S = susceptible; *** = $p < 0.001$

Table 4. Whitefly number, CMD severity and disease incidence of cassava varieties at Léo, Gourpouo and Savili

Varieties	Léo			Gourpouo			Savili		
	WN	SEV	INC	WN	SEV	INC	WN	SEV	INC
TMS 30572	1.4^{de}	1.00^{c}	0.0^{a}	2.8^{a}	1.00^{c}	0.0^{a}	1.4^{a}	1.00^{c}	0.0^{a}
TMS 4(2)1425	1.2^{def}	1.00^{c}	0.0^{a}	1.0^{cd}	1.00^{c}	0.0^{a}	0.4^{b}	1.00^{c}	0.0^{a}
TMS 91/02312	2.7^{bc}	1.00^{c}	0.0^{a}	1.2^{cd}	1.00^{c}	0.0^{a}	0.5^{b}	1.00^{c}	0.0^{a}
TMS 92/0067	1.7^{d}	1.00^{c}	0.0^{a}	1.2^{cd}	1.00^{c}	0.0^{a}	0.5^{b}	1.00^{c}	0.0^{a}
TMS 92/0325	2.1^{cd}	1.00^{c}	0.0^{a}	0.6^{d}	1.00^{c}	0.0^{a}	0.6^{b}	1.00^{c}	0.0^{a}
TMS 92/0427	3.3^{b}	1.00^{c}	0.0^{a}	2.0^{ab}	1.00^{c}	0.0^{a}	0.7^{b}	1.00^{c}	0.0^{a}
TMS 94/0270	2.8^{bc}	1.00^{c}	0.0^{a}	1.1^{cd}	1.00^{c}	0.0^{a}	0.7^{b}	1.00^{c}	0.0^{a}
Boborola	0.3^{f}	1.00^{c}	0.0^{a}	0.7^{cd}	1.00^{c}	0.0^{a}	0.3^{b}	1.00^{c}	0.0^{a}
Nouhao	0.7^{ef}	1.00^{c}	0.0^{a}	1.1^{cd}	1.03^{c}	2.8^{a}	0.3^{b}	1.02^{c}	1.1^{a}
Santidougou	4.5^{a}	1.39^{b}	22.8^{b}	1.6^{cd}	1.48^{b}	26.6^{c}	0.4^{b}	1.68^{b}	31.7^{c}
Local varieties	3.3^{b}	2.42^{a}	88.5^{c}	1.0^{cd}	2.80^{a}	92.8^{b}	0.8^{b}	2.34^{a}	94.5^{b}
Mean	2.3	1.38	31.5	1.2	1.50	35.3	0.6	1.34	30.4

WN = mean number of whiteflies per plant; SEV = mean CMD severity score; INC = Incidence of CMD (%)

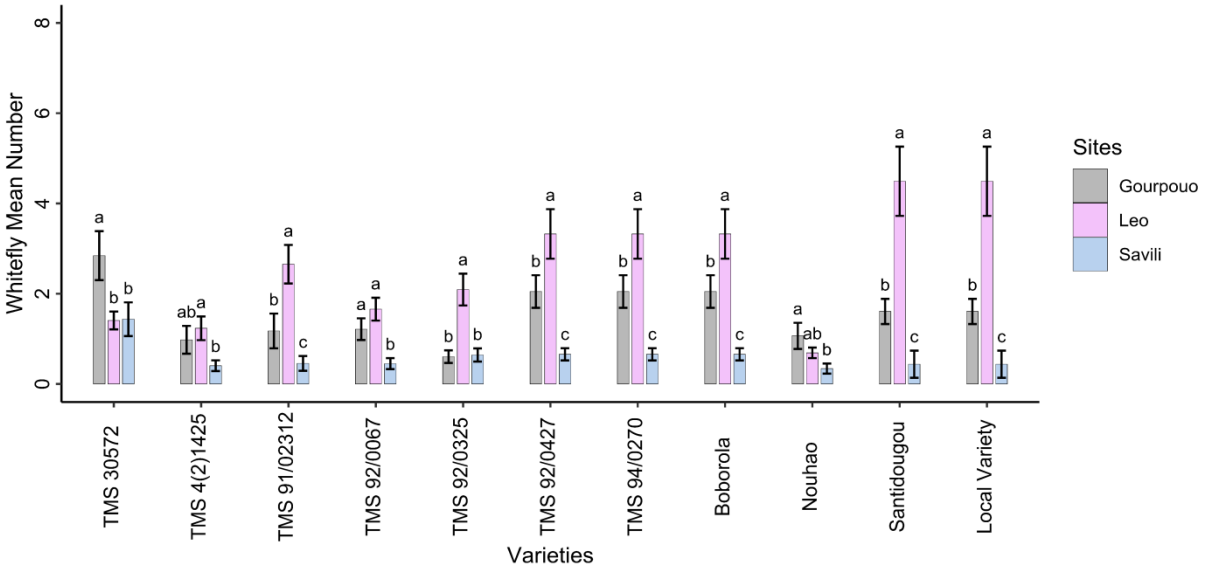

Figure 2. Comparison of whitefly mean number per variety in the three experimental locations. Mean number of whiteflies followed by the same letters are not significantly different between locations

3.2. Cassava Mosaic Disease Symptoms Severity and Disease Incidence

The mean CMD symptom severity score observed during this study was very moderate (1.4). The highest mean CMD symptom severity score (2.5) was found on the local varieties, used as inoculum (Table 3). The mean CMD severity score of these varieties was moderate in all experimental locations (Table 4). A significant difference was found between mean CMD symptom severity score of the local variety of Gourpouo (2.8) and those of Léo (2.4) and Savili (2.3) ($p < 0.001$). The disease incidence on the local varieties was 88.5%, 92.8% and 94.5% respectively at Léo, Gourpouo and Savili. Only the variety Santidougou showed significant difference of mean CMD symptom severity score compared to others in all the experimental locations (Table 4). The highest incidence among the 10 varieties was also found on the variety Santidougou in all the experimental locations. Incidences of 2.8% and 1.1% were found on the variety Nouhao respectively at Gourpouo and at Savili. No CMD symptom was found on the remaining 8 varieties in any of the experimental site (Table 4).

3.3. Classification of Cassava Varieties for Resistance to CMD

Based on disease mean symptoms severity scores, the cassava varieties were classified into three groups: highly resistant (HR), resistant (R) and susceptible (S). Group HR contained the varieties with a mean severity score of 1, group R had mean severity scores from 1.01 to 2 and group S mean severity score was ranging to 2.01 to 3 (Table 3).

3.4. Molecular Screening for Presence of CMD Resistance Genes

Molecular markers linked to genes of resistance to CMD were detected by PCR using SSR and SCAR primers. The results revealed the presence of *CMD1* gene in all the varieties using SSRY40 primer (Table 5). *CMD2* gene was detected in all varieties using SSRY28, in 11 varieties using the primer NS158, in 10 varieties using the primer RME1 and in 9 varieties using the primer NS169. At least two of the four markers linked to the *CMD2* gene have been detected in all the varieties and all these markers have been detected in six varieties (TMS 92/0067, TMS 92/0325, TMS 92/0427, Nouhao, Local Léo and Local Savili) (Table 5). The genotypic coincidence, relative to the presence of markers linked to the *CMD2* gene, was higher (0.85) for the markers NS158 × NS169 and NS158 × SSRY28. The RME1 × NS169 combination presented the lowest genotypic coincidence (0.46), while the NS169 × SSRY28, SSRY28 × RME1, and NS158 × RME1 combinations showed intermediate coincidences (ranging from 0.62 to 0.77) (Table 6). *CMD3* gene was found in 8 varieties using NS198 primer (Table 5). In summary, the markers linked to *CMD1*, *CMD2* and *CMD3* genes were detected in 8 varieties (TMS 91/02312, TMS 92/0067, TMS 92/0325, TMS 92/0427, TMS 4(2)1425, TMS 94/0270, TMS 30572 and Boborola). The markers linked to *CMD1* and *CMD2* genes were detected in Nouhao, Santidougou, Local Léo, Local Gourpouo and Local Savili varieties (Table 5).

Table 5. Molecular screening for CMD resistance genes

Varieties	CMD1	CMD2				CMD3
	SSRY40	SSRY28	NS158	NS169	RME1	NS198
TMS 30572	+	+	+	+	-	+
TMS 4(2)1425	+	+	+	-	+	+
TMS 91/02312	+	+	-	-	+	+
TMS 92/0067	+	+	+	+	+	+
TMS 92/0325	+	+	+	+	+	+
TMS 92/0427	+	+	+	+	+	+
TMS 94/0270	+	+	+	+	-	+
Boborola	+	+	-	-	+	+
Nouhao	+	+	+	+	+	-
Santidougou	+	+	+	+	-	-
Local Léo	+	+	+	+	+	-
Local Gourpouo	+	+	+	-	+	-
Local Savili	+	+	+	+	+	-

Table 6. Coincidence of varieties for the presence of markers that are linked to CMD2 gene in varieties

Markers	NS169	SSRY28	NS158
SSRY28	0.69		
NS158	0.85	0.85	
RME1	0.46	0.77	0.62

4. Discussion

Cassava mosaic disease is the most important biotic constraint to cassava production. The deployment of resistant cassava varieties offers a potentially effective means of addressing the problem [25], [26], [45]. An important step in the implementation of this control strategy, is the evaluation of the responses to CMD of some of the most cultivated cassava varieties in Burkina Faso. A field screening of 10 cassava varieties for CMD resistance based on the score of CMD symptom severity and classification according to Lokko *et al* (2005) [29] and Houngue *et al* (2019) [46] was carried out in three locations (Léo, Gourpouo and Savili) in 2017/2018 cassava growing season. All the seven improved varieties were found to be highly resistant (HR) using morphological markers. The results of some of them are at variance with past studies [47], [48] who found that TMS 4(2)1423, TMS 30572, TMS 92/0325 and TMS 92/0067 are either susceptible or moderately susceptible. This could be explained by the low number of whiteflies in our experimental locations but also by the low level of the inoculum. In addition, the presence of no symptom on the leaves of highly resistant or resistant varieties does not mean that they are not infected with the virus. Indeed, Asare *et al* (2014) [49], using CMGs strain-specific primers showed through PCR amplification that some varieties displayed no symptom on field were infected with ACMV. This suggests that these varieties are tolerant to ACMV infection whereas those with no PCR amplification band were resistant. Thus, field selection of resistance should be complemented with virus detection and virus quantification methods.

The pressure of whiteflies populations was different in the three experimental locations. This agrees with results of Zinga *et al* (2016) [1], who found that populations of whiteflies mainly change with environmental conditions. No relationship was found between whiteflies abundance and severity of the CMD symptoms, nor between whitefly abundance and incidence of the disease. This result agrees with some previous studies [1], [50] where no clear association between whitefly abundance and CMD symptom severity was observed.

The results of molecular screening for CMD resistance genes using *CMD1*, *CMD2* and *CMD3* genes linked markers (SSRY40, SSRY28, NS158, NS169, RME1, and NS198) agree with the data of field screening for resistance to CMD. The flanking markers of the three resistance genes to CMD were detected in all highly resistant varieties and the markers linked to *CMD1* and *CMD2* genes were detected in the resistant varieties. The similar results were reported by previous studies for the flanking markers *CMD2* gene [34], [49], [51]. Although *CMD3* confers very high levels of resistance to CMD with little or no expression of disease on the leaves [34], some *CMD1* and *CMD2*-type plants become infected with CMGs and develop typical mosaic symptoms [52], [53] but this result depend on CMGs strains used as inoculum. According to Kuria et al. (2017) [53], plants of all genotypes (*CMD1*, *CMD2* and *CMD3*) inoculated with EACMV developed more severe CMD symptoms, compared to those challenged with ACMV. Subsequently,

all plants of varieties carrying *CMD1* and *CMD3* resistance had produced non-symptomatic new leaves. In contrast, *CMD2*-type varieties showed partial recovery and continued to display mild CMD symptoms [53]. In addition, some studies reported the presence of markers linked to CMD resistance genes in certain susceptible varieties [51], [53]. This could explain the detection of markers linked to resistance to CMD (*CMD1* and *CMD2*) in the susceptible varieties used as source of inoculum in our study, or the fact that these varieties displayed a moderate symptom severity score of CMD.

The genotypic coincidence, relative to the presence of markers linked to the CMD2 gene, was higher (0.85) for the NS158 × NS169 and NS158 × SSRY28 markers. This could be explained by the fact that the distance between the NS158 × SSRY28 and NS158 × NS169 markers is relatively small, i.e., 2 cM and 9 cM respectively [54], which certainly contributes to providing a lower recombination rate between these markers. In addition, the NS158 and NS169 markers are anchored in the same scaffold [26], which reinforces the physical connection between these markers in the *M. esculenta* genome. The RME1 × NS169 combination presented the lowest genotypic coincidence (0.46). The great genetic distance between the NS169 × RME1 markers (20 cM) tend to result in less genotypic coincidence due to the possibility of historical occurrence of crossing over between these markers.

5. Conclusions

In this study, ten cassava varieties from Burkina Faso were screened for CMD resistance. All the seven improved and one local cassava varieties screened against CMD were morphologically highly resistant and two local varieties were resistant. A subsequent molecular screening showed that the 8 highly resistant varieties possessed *CMD1*, *CMD2* and *CMD3* genes while the resistant varieties possessed *CMD1* and *CMD2* genes. The low number of whiteflies (vector of the disease) and the low pressure of viruses found during the study, comfort the idea that a better assessment of resistance to CMD of cassava varieties in Burkina Faso could be obtained by combining agro-inoculation and molecular screening.

Acknowledgements

This work was supported, in whole or in part, by the Bill & Melinda Gates Foundation and The United Kingdom Foreign, Commonwealth & Development Office (FCDO) under grant number OPP1082413 to the Central and West African Virus Epidemiology (WAVE) Program for root and tuber crops—through a subgrant from Université Félix Houphouët-Boigny (UFHB) to the Institut de l'Environnement et de Recherches Agricoles (INERA). Under the grant conditions of the Foundation, a Creative Commons Attribution 4.0 Generic License has already been assigned to the Author Accepted Manuscript version that might arise from this submission.

REFERENCES

[1] I. Zinga, F. Chiroleu, A. Valam Zango, C. S. A. Ballot, M. Harimalala, E. Kosh Komba, P. S. Yandia, S. Semballa, B. Reynaud, P. Lefeuvre, *et al.*, "Evaluation of Cassava Cultivars for Resistance to Cassava Mosaic Disease and Yield Potential in Central African Republic," *J Phytopathol*, vol. 164, pp. 913–923, 2016, doi: 10.1111/jph.12511.

[2] K. M. Olsen and B. A. Schaal, "Evidence on the origin of cassava: Phylogeography of Manihot esculenta," *Proc Natl Acad Sci U S A*, vol. 96, no. 10, pp. 5586–5591, 1999, doi: 10.1073/pnas.96.10.5586.

[3] G. Léotard, A. Duputié, F. Kjellberg, E. J. P. Douzery, C. Debain, J. J. de Granville, and D. McKey, "Phylogeography and the origin of cassava: New insights from the northern rim of the Amazonian basin," *Mol Phylogenet Evol*, vol. 53, pp. 329–334, 2009, doi: 10.1016/j.ympev.2009.05.003.

[4] C. Fauquet and D. Fargette, "African Cassava Mosaic Virus: Etiology, Epidemiology, and Control," *Plant Dis*, vol. 74, no. 6, pp. 404–411, 1990, doi: 10.1094/PD-74-0404.

[5] M. A. El-Sharkawy, "Cassava biology and physiology," *Plant Mol Biol*, vol. 56, no. 4, pp. 481–501, 2004, doi: 10.1007/s11103-005-2270-7.

[6] H. Ceballos and C. H. Hershey, "Cassava (Manihot esculenta Crantz)," in *Genetic Improvement of Tropical Crops*, 2017, pp. 129–180. doi: 10.1007/978-3-319-59819-2.

[7] FAOSTAT, "Food and agriculture organization of the united nations," *Crop Prod domain*, 2021.

[8] B. L. Patil and C. M. Fauquet, "Cassava mosaic geminiviruses: Actual knowledge and perspectives," *Mol Plant Pathol*, vol. 10, no. 5, pp. 685–701, 2009, doi: 10.1111/j.1364-3703.2009.00559.x.

[9] P. Ntawuruhunga, A. G. O. Dixon, E. Kanju, G. Ssemakula, R. Okechukwu, J. B. A. Whyte, H. Obiero, S. Bigirimana, G. Gashaka, S. Lukombo, *et al.*, "Successful innovations and lessons learnt in cassava improvement and deployment by IITA in the Eastern African Region," *African J Root Tuber Crop*, vol. 10, no. 1, pp. 41–54, 2013.

[10] H. Saediman, M. A. Limi, Rosmawaty, P. Arimbawa, and Y. Indarsyih, "Cassava consumption and food security status among cassava growing households in southeast sulawesi," *Pakistan J Nutr*, vol. 15, no. 12, pp. 1008–1016, 2016, doi: 10.3923/pjn.2016.1008.1016.

[11] H. M. Ally, H. El Hamss, C. Simiand, M. N. Maruthi, J. Colvin, C. A. Omongo, and H. Delatte, "What has changed in the outbreaking populations of the severe crop pest whitefly species in cassava in two decades?," *Sci Rep*, vol. 9, no. 1, pp. 1–13, 2019, doi: 10.1038/s41598-019-50259-0.

[12] G. Byju and G. Suja, "Mineral nutrition of cassava," *Adv*

Agron, vol. 159, pp. 169–235, 2020, doi: 10.1016/bs.agron.2019.08.005.

[13] R. Pushpalatha and B. Gangadharan, "Is Cassava (Manihot esculenta Crantz) a Climate 'Smart' Crop? A Review in the Context of Bridging Future Food Demand Gap," *Trop Plant Biol*, vol. 13, pp. 201–211, 2020, doi: 10.1007/s12042-020-09255-2.

[14] F. Tiendrébéogo, P. Lefeuvre, M. Hoareau, M. A. Harimalala, A. De Bruyn, J. Villemot, V. S. Traoré, G. Konaté, A. S. Traoré, N. Barro, *et al.*, "Evolution of African cassava mosaic virus by recombination between bipartite and monopartite begomoviruses," *Virol J*, vol. 9, no. 67, pp. 1–7, 2012, doi: 10.1186/1743-422X-9-67.

[15] J. Stanley and M. . Gay, "Nucleotide sequence of cassava latent virus DNA," *Nature*, vol. 301, pp. 260–262, 1983.

[16] V. N. Fondong, J. S. Pita, M. E. C. Rey, A. De Kochko, R. N. Beachy, and C. M. Fauquet, "Evidence of synergism between African cassava mosaic virus and a new double-recombinant geminivirus infecting cassava in Cameroon," *J Gen Virol*, vol. 81, pp. 287–297, 2000.

[17] S. E. Bull, R. W. Briddon, W. S. Sserubombwe, K. Ngugi, P. G. Markham, and J. Stanley, "Genetic diversity and phylogeography of cassava mosaic viruses in Kenya," *J Gen Virol*, vol. 87, no. 10, pp. 3053–3065, 2006, doi: 10.1099/vir.0.82013-0.

[18] X. Zhou, D. J. Robinson, and B. D. Harrison, "Types of variation in DNA-A among isolates of East African cassava mosaic virus from Kenya, Malawi and Tanzania," *J Gen Virol*, vol. 79, no. 11, pp. 2835–2840, 1998, doi: 10.1099/0022-1317-79-11-2835.

[19] J. S. Pita, V. N. Fondong, A. Sangaré, G. W. Otim-Nape, S. Ogwal, and C. M. Fauquet, "Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda," *J Gen Virol*, vol. 82, no. 3, pp. 655–665, 2001, doi: 10.1099/0022-1317-82-3-655.

[20] M. N. Maruthi, S. Seal, J. Colvin, R. W. Briddon, and S. E. Bull, "East African cassava mosaic Zanzibar virus - A recombinant begomovirus species with a mild phenotype," *Arch Virol*, vol. 149, no. 12, pp. 2365–2377, 2004, doi: 10.1007/s00705-004-0380-1.

[21] L. C. Berrie, E. P. Rybicki, and M. E. C. Rey, "Complete nucleotide sequence and host range of South African cassava mosaic virus : further evidence for recombination amongst begomoviruses," *J Gen Virol*, vol. 82, pp. 53–58, 2001.

[22] G. Konaté, N. Barro, D. Fargette, and M. M. Swanson, "Occurrence of whitefly-transmitted geminiviruses in crops in Burkina Faso and their serological detection and differentiation," *Ann Appl Biol*, vol. 126, pp. 121–130, 1995.

[23] F. Tiendrébéogo, P. Lefeuvre, M. Hoareau, V. S. E. Traoré, N. Barro, B. Reynaud, A. S. Traoré, G. Konaté, O. Traoré, and J. M. Lett, "Occurrence of East African cassava mosaic virus -Uganda (EACMV-UG) in Burkina Faso," *Plant Pathol*, vol. 58, p. 783, 2009.

[24] M. Soro, F. Tiendrébéogo, J. S. Pita, E. T. Traoré, K. Somé, E. B. Tibiri, J. B. Néya, J. M. Mutuku, J. Simporé, and D. Koné, "Epidemiological assessment of cassava mosaic disease in Burkina Faso," *Plant Pathol*, vol. 00, pp. 1–10, 2021, doi: 10.1111/ppa.13459.

[25] A. O. Akano, A. G. O. Dixon, C. Mba, E. Barrera, and M. Fregene, "Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease," *Theor Appl Genet*, vol. 105, no. 4, pp. 521–525, 2002, doi: 10.1007/s00122-002-0891-7.

[26] I. Y. Rabbi, M. T. Hamblin, P. L. Kumar, M. A. Gedil, A. S. Ikpan, J. L. Jannink, and P. A. Kulakow, "High-resolution mapping of resistance to cassava mosaic geminiviruses in cassava using genotyping-by-sequencing and its implications for breeding," *Virus Res*, vol. 186, pp. 87–96, 2014, doi: 10.1016/j.virusres.2013.12.028.

[27] R. F. W. Nichols, "Breeding Cassava for Virus Resistance," *East African Agric J*, vol. 12, no. 3, pp. 184–194, 1947, doi: 10.1080/03670074.1947.11664554.

[28] J. P. Legg and C. M. Fauquet, "Cassava mosaic geminiviruses in Africa," *Plant Mol Biol*, vol. 56, pp. 585–599, 2004.

[29] Y. Lokko, E. Y. Danquah, S. K. Offei, A. G. O. O. Dixon, and M. A. Gedil, "Molecular markers associated with a new source of resistance to the cassava mosaic disease," *African J Biotechnol*, vol. 4, no. 9, pp. 873–881, 2005, doi: 10.5897/AJB2005.000-3175.

[30] R. E. C. Mba, P. Stephenson, K. Edwards, S. Melzer, J. Nkumbira, U. Gullberg, K. Apel, M. Gale, J. Tohme, and M. Fregene, "Simple sequence repeat (SSR) markers survey of the cassava (Manihot esculenta Crantz) genome: Towards an SSR-based molecular genetic map of cassava," *Theor Appl Genet*, vol. 102, pp. 21–31, 2001, doi: 10.1007/s001220051614.

[31] M. Fregene and J. Puonti-Kaerlas, "Cassava Biotechnology," in *Cassava: Biology, Production and Utilization*, Wallingford, UK.: CABI, 2002, pp. 179–207. [Online]. Available: https://www.cabi.org/isc/FullTextPDF/2007/20073012663.pdf

[32] M. Fregene, D. Danforth, P. Science, and N. Morante, "Molecular markers for the introgression of useful traits from wild Manihot relatives of cassava ; marker-assisted selection of disease and root quality traits," *J Root Crop*, vol. 32, no. 1, pp. 1–31, 2006.

[33] E. Okogbenin, M. C. M. M. Porto, C. Egesi, C. Mba, E. Espinosa, L. G. Santos, C. Ospina, J. Marín, E. Barrera, J. Gutiérrez, *et al.*, "Marker-assisted introgression of resistance to cassava mosaic disease into latin American germplasm for the genetic improvement of cassava in Africa," *Crop Sci*, vol. 47, no. 5, pp. 1895–1904, 2007, doi: 10.2135/cropsci2006.10.0688.

[34] E. Okogbenin, C. N. Egesi, B. Olasanmi, O. Ogundapo, S. Kahya, P. Hurtado, J. Marin, O. Akinbo, C. Mba, H. Gomez, *et al.*, "Molecular marker analysis and validation of resistance to cassava mosaic disease in elite cassava genotypes in Nigeria," *Crop Sci*, vol. 52, no. 6, pp. 2576–2586, 2012, doi: 10.2135/cropsci2011.11.0586.

[35] A. G. O. Dixon, R. U. Okechukwu, M. O. Akoroda, P. Ilona, F. Ogbe, C. N. Egesi, P. Kulakow, G. Ssemakula, B. Maziya-Dixon, P. Iluebbey, *et al.*, *Improved Cassava*

Variety Handbook. IITA Cassava Project, Ibadan, Nigeria. 2010.

[36] F. Guira, K. Some, D. Kabore, H. Sawadogo-Lingani, Y. Traore, and A. Savadogo, "Origins, production, and utilization of cassava in Burkina Faso, a contribution of a neglected crop to household food security," *Food Sci Nutr*, vol. 5, no. 3, pp. 415–423, 2017, doi: 10.1002/fsn3.408.

[37] MAAH, "Plan d'action de la filière manioc du Burkina Faso." 2019.

[38] R. Dabiré and J. Belem, "Les plantes à tubercules et racines au Burkina Faso," *WASNET*, vol. 8, pp. 12–16, 2003.

[39] D. Diancoumba, "Diagnostic actualisé de la filière manioc pour une analysede chaines de valeur ajoutée (CVA)," *PDA*, pp. 1–26, 2008.

[40] E. R. Terry, "Description and evaluation of cassava mosaic disease in Africa," in *The International Exchange and Testing of Cassava Germplasm in Africa*, E. R. Terry and R. MacIntyre, Eds. Ibadan, Nigeria: IITA, 1975, pp. 53–54.

[41] P. Sseruwagi, W. S. Sserubombwe, J. P. Legg, J. Ndunguru, and J. M. Thresh, "Methods of surveying the incidence and severity of cassava mosaic disease and whitefly vector populations on cassava in Africa : a review," *Virus Res*, vol. 100, pp. 129–142, 2004, doi: 10.1016/j.virusres.2003.12.021.

[42] J. A. Houngue, J. S. Pita, H. B. Ngalle, M. Zandjanakou-Tachin, A. F. Kuate, G. H. Todjro Cacaï, J. M. Bell, and C. Ahanhanzo, "Response of cassava cultivars to African cassava mosaic virus infection across a range of inoculum doses and plant ages," *PLoS One*, vol. 14, no. 12, pp. 1–14, 2019, doi: 10.1371/journal.pone.0226783.

[43] H. R. Permingeat, M. V Romagnoli, I. Juliana, and R. H. Vallejos, "A Simple Method for Isolating DNA of High Yield and Quality from Cotton (Gossypium hirsutum L .) Leaves," *Plant Mol Biol Report*, vol. 16, p. 89, 1998.

[44] Y. Benjamini and D. Yekutieli, "The control of the false discovery rate in multiple testing under dependency," *Ann Stat*, vol. 29, no. 4, pp. 1165–1188, 2001, doi: 10.1214/aos/1013699998.

[45] J. M. Thresh, L. D. C. Fishpool, G. W. Otim-Nape, and D. Fargettey, "African cassava mosaic virus disease: an underestimate and unsolved problem.," *Trop Sci*, vol. 34, pp. 3–14, 1994, doi: 10.1016/j.jviromet.2006.05.031.

[46] J. A. Houngue, M. Zandjanakou-Tachin, H. B. Ngalle, J. S. Pita, G. H. T. Cacaï, S. E. Ngatat, J. M. Bell, and C. Ahanhanzo, "Evaluation of resistance to cassava mosaic disease in selected African cassava cultivars using combined molecular and greenhouse grafting tools," *Physiol Mol Plant Pathol*, vol. 105, pp. 47–53, 2019, doi: 10.1016/j.pmpp.2018.07.003.

[47] C. N. Egesi, F. O. Ogbe, M. Akoroda, P. Ilona, and A. Dixon, "Resistance profile of improved cassava germplasm to cassava mosaic disease in Nigeria," *Euphytica*, vol. 155, no. 1–2, pp. 215–224, 2007, doi: 10.1007/s10681-006-9323-0.

[48] O. A. Ariyo, A. G. O. Dixon, G. I. Atiri, E. W. Gachomo, and S. O. Kotchoni, "Disease resistance characterisation of improved cassava genotypes to cassava mosaic disease at different ecozones," *Arch Phytopathol Plant Prot*, vol. 48, no. 6, pp. 504–518, 2015, doi: 10.1080/03235408.2015.1045233.

[49] P. A. Asare, I. K. A. Galyuon, E. Asare-Bediako, J. K. Sarfo, and J. P. Tetteh, "Phenotypic and molecular screening of cassava (Manihot esculentum Crantz) genotypes for resistance to cassava mosaic disease," *J Gen Mol Virol*, vol. 6, no. 2, pp. 6–18, 2014, doi: 10.5897/JGMV2014.0056.

[50] M. Tembo, M. Mataa, J. Legg, P. C. Chikoti, and P. Ntawuruhunga, "Cassava mosaic disease: Incidence and yield performance of cassava ultivars in Zambia," *J Plant Pathol*, vol. 99, no. 3, pp. 1–28, 2017, doi: 10.4454/jpp.v99i3.3955.

[51] E. Parkes, M. Fregene, A. Dixon, E. Okogbenin, B. Boakye-Peprah, and M. Labuschagne, "Developing Cassava Mosaic Disease resistant cassava varieties in Ghana using a marker assisted selection approach," *Euphytica*, vol. 203, no. 3, pp. 549–556, 2014, doi: 10.1007/s10681-014-1262-6.

[52] G. Beyene, R. D. Chauhan, H. Wagaba, T. Moll, T. Alicai, D. Miano, J. C. Carrington, and N. J. Taylor, "Loss of CMD2-mediated resistance to cassava mosaic disease in plants regenerated through somatic embryogenesis," *Mol Plant Pathol*, vol. 17, no. 7, pp. 1095–1110, 2016, doi: 10.1111/mpp.12353.

[53] P. Kuria, M. Ilyas, E. Ateka, D. Miano, J. Onguso, J. C. Carrington, and N. J. Taylor, "Differential response of cassava genotypes to infection by cassava mosaic geminiviruses," *Virus Res*, vol. 227, pp. 69–81, 2017, doi: 10.1016/j.virusres.2016.09.022.

[54] C. D. do Carmo, M. S. da Silva, G. A. F. Oliveira, and E. J. de Oliveira, "Molecular-assisted selection for resistance to cassava mosaic disease in manihot esculenta crantz," *Sci Agric*, vol. 72, no. 6, pp. 520–527, 2015, doi: 10.1590/0103-9016-2014-0348.