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Detection of Cassava Mosaic Disease and Assessment of Selected Agronomic Traits of Cassava (*Manihot esculenta*)

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Abstract: A study was conducted in Sierra Leone to identify cassava plants that are asymptomatic and symptomatic to cassava mosaic disease (CMD) and collect planting materials for field trial establishment; determine the prevalence of CMD caused by African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) using the Nuru App and virus indexing techniques; and assess selected agronomic traits in cassava. A total of 80 cassava farms spanning four provinces (Southern, Eastern, Northern, and North-West) were surveyed in April 2022. Findings showed that the cassava variants of the experiment and locations significantly ($p < 0.001$) affected CMD incidence, severity, growth, and fresh storage root yield traits. The CMD incidence (87.0%) and whitefly abundance (144.8) were highest, and the CMD severity was moderate (4.0) for the plants derived from cuttings obtained from symptomatic Cocoa mother plants, while plants derived from cuttings of improved mother plants exhibited no visible symptoms of the disease and the lowest population (45.1) of whiteflies. The Nuru app is inefficient for phenotypically detecting CMD at 3 months after planting (MAP), while at 6, 9 and 12 MAP, the app efficiently detected the disease using a molecular analysis technique. Resistant, non-diseased plants derived from cuttings obtained from SLICASS 4 mother plants produced the highest fresh storage root yield (54.9 t ha⁻¹). The highest storage root yield loss was recorded in the plants obtained from cuttings of symptomatic variety Cocoa mother plants harvested at Matotoka grassland ecology, Bombali District (90.2%), while those harvested from cuttings of asymptomatic variety Cocoa mother plants grown at the four test environments had a similar storage root yield loss ranging from 40.3 to 46.2%. Findings suggest the importance of genetic variability, environmental adaptation, utilization of diseased-free materials, and phytosanitation as disease management strategies for increased production. These findings provide important insights into the distribution, impact, and spread of CMD and whitefly abundance in the studied areas in Sierra Leone that could be exploited for cassava production, productivity, conservation, and population improvement.

Keywords: disease detection; whitefly; agronomic traits; yield loss; cassava genotypes



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1. Introduction

Cassava (*Manihot esculenta* Crantz) is the world's sixth most economically important storage root crop for food, feed, and industrial applications [1]. Cassava is the third most important source of carbohydrates in Africa [2] and the second most important staple crop in Sierra Leone [3]. The crop meets the food needs of more than 800 million people worldwide [4], accounting for about 500 calories daily for over 70 million people [5].

Despite the importance of cassava, increased production and productivity are constrained by both biotic and abiotic factors [6]. Cassava mosaic disease (CMD) is one of cassava's most important biotic constraints [7]. CMD is caused by 11 Begomovirus species globally [8,9], of which 8 have been detected in sub-Saharan Africa including the African Cassava Mosaic Virus (ACMV) [10], the East African Cassava Mosaic Virus (EACMV) and EACMV-like strains [7], the East African Cassava Mosaic Cameroon Virus (EACMCV) [11], the East African Cassava Mosaic Malawi Virus (EACMMV) [12], the East African Cassava Mosaic Zanzibar Virus (EACMZV) [9], the South African Cassava Mosaic Virus (SACMV) [13], the Indian Cassava Mosaic Virus [14], and the South East African Cassava Mosaic Virus (SEACMV) [15]. The phenotypic symptoms of some of the CMD-causing CMDs include leaf chlorosis, distortion, mottling, and stunting. twisted leaflets, misshapen leaves, reduction in leaf size, and plant and root size [16]. The causal agents of CMD have been described and recognized in what is now known as African Cassava Mosaic Virus (ACMV) and East African Cassava Mosaic Virus (EACMV), both from the Family Geminiviridae and Genus Begomovirus [7]. The ACMV was found in all cassava-growing areas in Africa, while the EACMV was mainly detected in Eastern Africa. During the 1990s, consequent to the CMD pandemic observed in Uganda, detection and diagnosis studies revealed a new virus, the East African Cassava Mosaic Virus-Uganda (EACMV-UGV), a recombinant progeny obtained from the cross between the ACMV and EACMV [17]. The rapid spread of EACMV-UGV was associated with dual infections with ACMV and a high vector population, which are considered the main factors driving the severe epidemic disease in several African countries [7].

In DR Congo, Neuenschwander et al. [18] and Monde et al. [19] reported that only ACMV and EACMV UGV are found in single or mixed infections. The epidemic spread of CMD has caused serious crop failure and fresh storage root yield losses, ranging between 25% and 95%, which have seriously affected the livelihood of local farmers in sub-Saharan Africa [20]. In the Ivory Coast, the epidemic spread of CMD led to a greater fresh storage root yield decrease of 40% when plants were infected from cuttings than when whiteflies infected them.

In Uganda, CMD infection accounted for 82% of the fresh storage root yield decrease due to double infections of ACMV-EACMV-UG2 'severe'. In contrast, ACMV alone, EACMV-UG2 'mild' and ACMV-UG2 'severe' accounted for 42%, 12%, and 68% of storage root yield losses, respectively [21]. In Tanzania, 72 to 90% of storage root yield loss was noted on the three most cultivated local varieties in different locations [7], whereas in Kenya, Mallowa et al. [22] recorded 68% of fresh storage root yield loss.

In Sierra Leone, there is a dearth of well-articulated information on fresh storage root yield loss caused by CMD using advanced phenotypic and molecular detection techniques. One of the advanced phenotypic detection techniques, the PlantVillage Nuru App (AI), is an innovative technique utilized for its high-accuracy detection of diseases in various crops [23]. It is a smartphone-based AI-powered app utilized to alert farmers and researchers and expedite disease diagnosis, potentially preventing or limiting pest and disease outbreaks by selecting a disease-free cutting or symptomless cutting for the

next planting season [24–26]. The Nuru App has been utilized to identify CMD symptoms caused by different plant pathogenic virus species [24–27]. Thus, the current study was aimed at identifying cassava plants that are asymptomatic and symptomatic to cassava mosaic disease (CMD) and collecting planting materials for field trial establishment, determining the prevalence of CMD caused by African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV), using the Nuru App and virus indexing techniques; and assessing selected agronomic traits in cassava.

2. Materials and Methods

2.1. Identification and Collection of Cassava Planting Material

The identification and collection of asymptomatic and symptomatic cassava mosaic disease planting materials of the local variety Cocoa and the improved variety SLICASS 4 were conducted in April 2022 on 80 cassava fields. The identification of these set of planting materials was conducted using the Nuru App. The Nuru App is a smartphone-based AI-powered app utilized to alert farmers and researchers and expedite disease diagnosis, potentially preventing or limiting pest and disease outbreaks by selecting a disease-free cutting or symptomless cutting for the next planting season [24–26]. The working principles of the Nuru app involve scanning, diagnosing, and detecting disease infection and pest infestation on crops. The Nuru app captures images of plants affected by diseases and pests to predict their health status. Thus, the Nuru App might be inefficient in detecting plants lacking symptom expression of disease(s) or that are at the latent phase of disease infection. The identification and collection of asymptomatic and symptomatic cassava mosaic disease planting materials was carried out in four major cassava-producing districts of Sierra Leone, including Bonthe District in the southern province, Kenema District in the eastern province, Bombali District in the northern province, and Port Loko District in the Northwestern province; the districts were selected based on their history of CMD hotspots, large volume of crop production, and varying agroecology favorable for cassava production (Figure 1). The precise location of the sampled plants was determined using a global positioning system (Compass Deluxe Navigation, a free application) [28].

2.2. Experimental Material, Layout, Design, and Management

A field experiment was conducted at Njagbahun, Bonthe District in the Southern province, Kpai, Kenema District in the Eastern province, Matotoka, Bombali District in the Northern province, and Kangbatanma, Port Loko District in the Northwestern province. The experimental materials were stem cuttings obtained from asymptomatic and symptomatic mother plants of the local variety Cocoa, and improved released genotype SLICASS 4. Genotype SLICASS 4 possesses high tolerance to CMD with a high storage root yield, whereas the local variety Cocoa is susceptible to CMD with a low fresh storage root yield. The planting materials utilized in this study were obtained from the farmers' fields during the survey and cassava planting material collection. Cuttings were planted at 1 m × 1 m in a randomized complete block design with three replications spaced 2 m apart. Severely diseased plants of Cocoa were planted around the plots to reinforce the inoculum pressure. Each replication comprised three plots measuring 5 m × 10 m (50 m²) spaced 1 m apart. The trial was established in late May 2020. About two-thirds of each cutting measuring 30 cm were planted slantwise at about 45° on the crest of ridges. All routine agronomic practices were performed, except that no agrochemicals were applied.

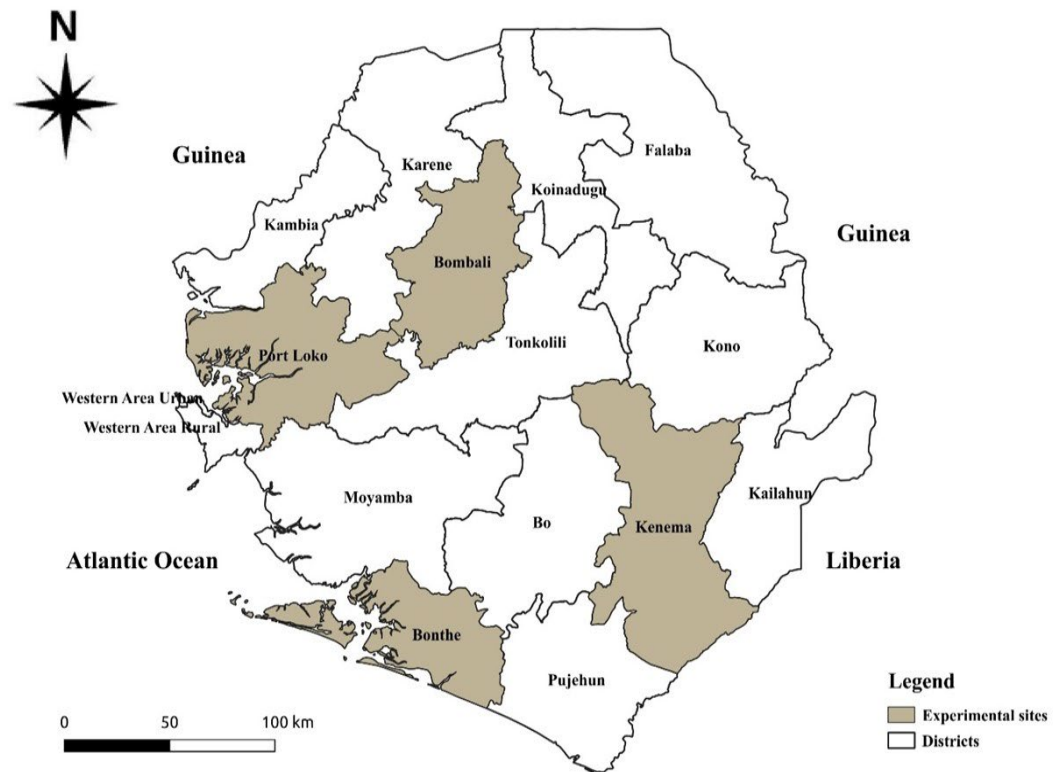


Figure 1. Location of sampling sites of symptomatic, asymptomatic, and symptomless cassava mosaic disease plants in Sierra Leone.

2.3. Phenotypic Data Collection and Analysis

The data collected during this study included the severity of CMD scored at 3, 6, 9, and 12 months after planting (MAP) using a scale of 1–5, where 1 represents a plant with no symptoms, and 5 represents very severe symptoms, including chlorosis, leaf distortion and stunting [29]. The incidence of CMD and the number of whiteflies were scored at 3, 6, 9, and 12 MAP. The size of whitefly populations was determined by counting the number of adult whiteflies on the five topmost leaves of each sampled plant [30]. At harvest (12 MAP), the number of nodes per stem, storage root length, diameter, and weights were measured. The diameter measurements were conducted using a vernier caliper, length and height measurements by meter rule, weight measurements by electronic hanging scale, and the numbers of whiteflies and nodes by counting. The fresh storage root yield loss was expressed as a percentage of the diseased plants' yield compared to the yields of the healthy and improved variety. The phenotypic data collected were subjected to analysis of variance (ANOVA) using the GENSTAT statistical program (GENSTAT, 15th release, Rothampstead, UK). A Duncan's Multiple Range Test (DMRT) was used to compare variants of experiment means using a significance level of $\alpha = 0.05$.

2.4. Genotyping, Virus Indexing, and Analysis

For the genotyping of samples from the variant of experiment, total genomic DNA was isolated from lyophilized young and fully expanded leaves. Deoxyribonucleic acid (DNA) was extracted from the leaf samples using a slightly modified cetyltrimethylammonium bromide (CTAB) method by Permingeat et al. [31]. The DNA quality and concentration were assessed using agarose gel and the nanodrop method following Aljanabi and Martinez [32].

Virus indexing using the Polymerase Chain Reaction (PCR) method was done to detect the ACMV and EACMV strains causing the mosaic symptoms in leaves of cassava genotypes. The DNA samples of the cassava genotypes were tested for the presence or

absence of CMD using specific primers that could detect the strains of ACMV and EACMV. Two pairs of primer sequences, as described by Pita et al. [33], Matic et al. [34], Alabi et al. [35], and Fondong et al. [11], were used (Table 1).

Table 1. List of pair of primers used to confirm the presence of ACMV, EACMV, and EACMVCM.

Primer	Sequence (5'-3')	Target Region	Expected Size (bp)	Virus Species	Reference
JSP001	ATGTCGAAGCGACCAGGAGAT	DNA-A (CP)	783	ACMV	[33]
JSP002	TGTTTATTAATTGCCAATACT				
ACMBVF	TCGGGAGTGATACATGCCAAGGC	DNA-B (BV1/BC1)	628	ACMV	[34]
ACMBVR	GGCTACACCAGCTACCTGAAGCT				
JSP001	ATGTCGAAGCGACCAGGAGAT	DNA-A (CP)	780	EACMV	[33]
JSP003	CCTTTATTAATTTGTCACTGC				
CMBRepF	CRTCAATGACGTTGTACCA	DNA-A(AC1)	650	EACMV	[35]
EACMVRepR	GGTTTGCAGAGAACTACATC				
VNF031F	GGATACAGATAGGGTTCCCAC	AC2/AC3	≈560	EACMV-CM	[12]
VNF032R	GACGAGGACAAGAATTCCAAT				

The PCR analysis of the DNA of each sample was performed in a SimpliAmp™ Thermal Cycler (Cat. no. A24811) (Life Technologies Holdings Pte Ltd., Marsiling Industrial Estate, Marsiling, Singapore) for 35 cycles of 45 s at 94 °C, 45 s at 55 °C and 60 s at 72 °C, and an additional 10 min at 72 °C. Reactions were carried out in a total volume of 25 µL using 20.9 µL of molecular biology grade water, 2.5 µL of 10× reaction buffer, 0.5 µL of 10 mM dNTPs, 0.5 µL of 10 µM of each primer, 0.1 µL of 5 U/µL of Maximo Taq DNA polymerase (GeneON, SibEnzyme Ltd., Academtown, Novosibirsk City, Russia), and 150 ng DNA template of each sample. Reactions were carried out twice to evaluate the consistency of the banding patterns for all isolates studied. Products were separated on a 1% (*w/v*) agarose gel in 1x TBE buffer at 75 V for about 3 h when the bands reached the red line. The gels were stained with 1 mL ethidium bromide, visualized with UV light using a Cole Palmer FLUO-LINK FLX apparatus, and photographed for later assessment.

3. Results

3.1. Phenotypic Trait Assessment

Cassava mosaic disease (CMD) percentage incidence, severity, and whitefly abundance significantly ($p < 0.001$) varied between plants derived from stem cuttings of symptomatic, asymptomatic, and improved mother plants assessed at various sampling regimes (Table 2). The plants from cuttings of asymptomatic Cocoa and improved SLICASS 4 mother plant varieties exhibited no visible CMD expression at 3 MAP, while those from symptomatic mother Cocoa had an incidence of 61%. At 6, 9, and 12 MAP, a progressive increase in CMD percentage incidence was detected for the symptomatic and asymptomatic mother Cocoa plants, except for symptomatic mother plants, in which a slight decrease from 98.3 to 93.5% occurred in the plants derived from the symptomatic mother plants from 9 to 12 MAP, respectively. The plants from cuttings of the improved mother plant variety consistently had no symptom expression of CMD throughout the sampling regimes. Similarly, plants from cuttings of asymptomatic Cocoa and improved SLICASS 4 mother plant varieties exhibited no visible CMD expression at 3 MAP, while those from symptomatic Cocoa mother plants had a low (3.0) attack of the disease. At 6, 9, and 12 MAP, a progressive increase in CMD severity was detected for the plants obtained from cuttings of symptomatic and asymptomatic mother Cocoa plants. The severity was high (4.6) for the plants obtained from cuttings of symptomatic Cocoa mother plants, while for the plants derived from asymptomatic mother ones, it progressed from mild (2.2) to low (2.9). The plants from

the improved mother plant variety consistently had no symptom expression of CMD throughout the sampling regimes. Generally, the plants obtained from the symptomatic mother plant Cocoa variety had the highest number of whiteflies across all sampling regimes, followed by those from other asymptomatic Cocoa plants. At the same time, the plants obtained from cuttings of improved SLICASS 4 mother plants exhibited the lowest abundance of the pest.

Table 2. Cassava mosaic disease (CMD) percentage incidence, severity and whitefly abundance of symptomatic, asymptomatic, and improved cassava plants assessed at various sampling regimes.

Variant of Experiment	Sampling Regime			
	3 MAP	6 MAP	9 MAP	12 MAP
Percent Incidence of CMD				
Improved	0 ± 0.00 ^b	0.0 ± 0.00 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c
Asymptomatic	0 ± 0.00 ^b	28.9 ± 10.87 ^b	41.08 ± 20.98 ^b	51.17 ± 25.11 ^b
Symptomatic	61.25 ± 29.63 ^a	95.0 ± 11.60 ^a	98.33 ± 5.77 ^a	93.50 ± 8.67 ^a
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001
Significance	***	***	***	***
Severity of CMD				
Improved	1.00 ± 0.00 ^b	1.0 ± 0.00 ^b	1.00 ± 0.00 ^c	1.00 ± 0.00 ^c
Asymptomatic	1.00 ± 0.00 ^b	2.2 ± 0.00 ^b	2.73 ± 0.55 ^b	2.87 ± 0.50 ^b
Symptomatic	3.01 ± 1.00 ^a	4.1 ± 0.60 ^a	4.58 ± 0.51 ^a	4.17 ± 0.83 ^a
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001
Significance	***	***	***	***
Whitefly abundance				
Improved	51.3 ^{gh}	40.4 ^h	50.2 ^{gh}	38.6 ^h
Asymptomatic	70.2 ^f	80.2 ^e	71.1 ^f	59.8 ^g
Symptomatic	142.9 ^c	120.0 ^d	155.3 ^b	160.8 ^a
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001
Significance	***	***	***	***

Variants of experiment with the same letter are not significantly different at $\alpha = 0.05$; MAP = months after planting; *** = significant at $p < 0.001$.

Generally, the cassava variant of experiment and locations significantly ($p < 0.001$) affected CMD incidence, severity, growth, and fresh storage root yield traits (Table 3). The CMD incidence (87.0%) and whitefly abundance (144.8) were significantly highest, and the CMD severity was moderate (4.0) for the plants from cuttings derived from the symptomatic Cocoa mother plant variety. In contrast, the plants derived from cuttings obtained from the improved mother plant variety exhibited no visible symptoms of the disease and the lowest population (45.1) of the whitefly pest. Cassava grown in the Bonthe environment had the lowest whitefly abundance and a mild attack of CMD compared to those cultivated in the Kenema, Bombali, and Port Loko environments. For the growth and yield traits, the plants derived from cuttings obtained from improved variety mother plants exhibited the highest number of nodes per stem (20.7), widest storage root diameter (5.2 cm), longest storage root length (87.34 cm), and fresh storage root yield (54.9 t ha⁻¹), whereas the plants from cuttings of symptomatic Cocoa mother plants had the lowest of 11.9, 2.7 cm, 36.06 cm and 10.5 t ha⁻¹, respectively. The Bonthe environment exhibited the highest number of nodes per stem (16.2), widest storage root diameter (4.1 cm), and fresh storage root yield (38.9 t ha⁻¹), whereas Port Loko (27.8 t ha⁻¹) and Bombali (30.0 t ha⁻¹) had the lowest fresh storage root yields.

Table 3. Impacts of symptomatic, asymptomatic, and improved cassava variant of experiment and locations on cassava mosaic disease (CMD) incidence, severity, growth, and fresh storage root yield traits.

Variant of Experiment	CMD Incidence	CMD Severity	Whitefly Abundance	Number of Nodes per Stem	Storage Root Diameter (cm)	Storage Root Length (cm)	Fresh Storage Root Yield (t ha ⁻¹)
Improved	0.0 ^c	1.0 ^c	45.1 ^d	20.7 ^a	5.2 ^a	87.34 ^a	54.9 ^a
Asymptomatic	30.3 ^b	2.2 ^b	70.3 ^c	14.8 ^b	4.0 ^b	54.13 ^b	32.5 ^b
Symptomatic	87.0 ^a	4.0 ^a	144.8 ^a	11.9 ^c	2.7 ^c	36.06 ^c	10.5 ^c
Location							
Bombali	36.2 ^b	2.4 ^a	88.1 ^b	15.6 ^a	3.8 ^{ab}	57.9 ^b	30.0 ^c
Bonthe	34.4 ^b	2.3 ^{ab}	80.2 ^c	16.2 ^a	4.1 ^a	55.5 ^b	38.9 ^a
Kenema	38.2 ^b	2.4 ^a	92.2 ^a	15.3 ^{ab}	4.0 ^a	61.6 ^a	33.7 ^b
Port Loko	47.7 ^a	2.5 ^a	86.4 ^b	16.0 ^a	4.0 ^a	61.6 ^a	27.8 ^c

Variants of experiment with the same letter are not significantly different at $\alpha = 0.05$.

The interactive effects of the variant of experiment, location, and sampling regime on cassava mosaic disease incidence and severity are presented in Table A1. Interactive effects revealed that the improved variety had no visible symptoms across all sampling regimes and test environments. In contrast, the plants from cuttings derived from the symptomatic mother plant variant of the experiment had significant interaction across all sampling regimes and test environments for both incidence and severity of CMD. Similar patterns were also detected for the interactive impacts of the variant of experiment and location on CMD incidence, severity, and whitefly abundance of the plants from cuttings of symptomatic, asymptomatic, and improved cassava mother plants (Table A2). Accordingly, the plants from cuttings derived from the improved variety significantly recorded the lowest whitefly population compared to the plants from cuttings of the symptomatic and asymptomatic Cocoa cassava mother plant variants of the experiment. The interactive impacts of variants of the experiment, location, and sampling regime significantly influenced whitefly abundance of the plants from cuttings of the symptomatic, asymptomatic, and improved cassava mother plant variants of the experiment (Table A3).

Leaf area significantly ($p < 0.001$) varied among the plants from cuttings of symptomatic, asymptomatic, and improved cassava mother plants assessed at various sampling regimes (Table 4). The plants from cuttings of the improved SLICASS 4 mother plant variety significantly and consistently produced the largest leaf area (3.68–10.65 cm²) throughout the sampling regimes, followed by the plants from cuttings of the asymptomatic Cocoa mother plants (3.10–7.63 cm²). In contrast, those from symptomatic Cocoa had the lowest (1.99–3.26 cm²). Findings indicate that the leaf area growth and development variance are probably attributable to decreased CO₂ fixation and carbohydrate accumulation due to CMD infection. Findings generally indicate a negative correlation between leaf area (LA) and disease severity or incidence trait and a positive association between LA and LA traits and between disease and disease traits.

Table 4. Leaf area of cassava variants of experiment sampled at different sampling regimes.

Variant of the Experiment	Leaf Area at 3 Months After Planting	Leaf Area at 6 Months After Planting	Leaf Area at 9 Months After Planting	Leaf Area at 12 Months After Planting
Improved	3.68 ± 0.28 ^a	4.52 ± 0.64 ^a	9.45 ± 0.49 ^a	10.65 ± 0.22 ^a
Asymptomatic	3.10 ± 0.09 ^b	4.05 ± 0.18 ^b	7.30 ± 0.23 ^b	7.63 ± 0.32 ^b
Symptomatic	1.99 ± 0.10 ^c	3.08 ± 0.22 ^c	3.20 ± 0.52 ^c	3.26 ± 0.49 ^c
DMRT	0.15	0.33	0.36	0.30
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001
Significance	***	***	***	***

Variants of experiment with the same letter are not significantly different at $\alpha = 0.05$; *** = significant at $p < 0.001$.

The number of nodes per stem, storage root diameter, length and yield significantly ($p < 0.001$) varied among the plants from cuttings of symptomatic, asymptomatic, and improved cassava mother plants assessed across all experimental locations (Table 5). The plants from cuttings of the improved SLICASS 4 mother plant variety significantly and consistently produced the highest number of nodes per stem (19.0–22.0), widest storage root diameter (5.1–5.4 cm), longest storage root length (80.23–93.3 cm), and heaviest fresh storage root yield (46.7–66.4 t ha^{−1}) across all experimental locations, whereas those from the symptomatic Cocoa mother plant variety had the lowest number of nodes per stem (11.0–12.4), narrowest storage root diameter (2.5–3.4 cm), shortest storage root length (34.53–36.57 cm), and heaviest fresh storage root yield loss (5.2–14.7 t ha^{−1}). The highest storage root yield loss was recorded in the plants obtained from cuttings of symptomatic variety Cocoa mother plants harvested at Matotoka grassland ecology, Bombali District (90.2%), while those harvested from cuttings of asymptomatic variety Cocoa mother plants grown at the four test environments had a similar storage root yield loss ranging from 40.3 to 46.2% (Table 5).

Table 5. Mean number of nodes per stem, storage root diameter, length and yield measured in symptomatic, asymptomatic, and improved cassava plants.

Variant of Experiment	Location			
	Bombali	Bonthe	Kenema	Port Loko
	Number of nodes per stem			
Improved	20.7 ^a	20.7 ^a	19.0 ^b	22.0 ^a
Asymptomatic	14.3 ^c	15.5 ^c	14.0 ^c	15.0 ^c
Symptomatic	11.7 ^d	12.4 ^d	12.0 ^d	11.0 ^d
	Storage root diameter (cm)			
Improved	5.1 ^a	5.1 ^a	5.4 ^a	5.3 ^a
Asymptomatic	3.8 ^c	3.8 ^c	4.1 ^{bc}	4.3 ^b
Symptomatic	2.5 ^e	3.4 ^d	2.5 ^e	2.5 ^e
	Storage root length (cm)			
Improved	82.27 ^b	80.23 ^b	93.43 ^a	93.43 ^a
Asymptomatic	54.9 ^c	51.83 ^c	54.9 ^c	54.9 ^c
Symptomatic	36.57 ^d	34.53 ^d	36.57 ^d	36.57 ^d
	Fresh storage root yield (t ha ^{−1})			
Improved	53.1 ^b	66.4 ^a	46.7 ^c	46.7 ^c
Asymptomatic	31.7 ^d	35.7 ^d	27.3 ^e	27.3 ^e
Symptomatic	5.2 ^h	14.7 ^f	9.5 ^g	9.5 ^g
	Fresh storage root yield loss (%)			
Asymptomatic	40.3 ^a	46.2 ^a	41.5 ^a	41.5 ^a
Symptomatic	90.2 ^c	77.9 ^b	79.7 ^b	79.7 ^b

Variants of experiment with the same letter are not significantly different at $\alpha = 0.05$.

3.2. Correlation Analysis for Trait Associations

The pairwise relationship based on selected disease, growth, and yield traits of cassava is shown in Figure A1. The R-values 0, +1, and −1 indicate no linear relationship, a perfect positive linear relationship, and a negative linear relationship, respectively, based on the standard guidelines of the color intensity in the correlograms. The R-values ranging between 0 and 0.3, 0.3 and 0.7, and between 0.7 and 1 indicate low, moderate, and strong positive linear relationships, respectively. In contrast, those ranging between 0 and −0.3, −0.3 and −0.7, and between −0.7 and −1 indicate low, moderate, and strong negative linear relationships, respectively. The pairs of traits with significantly strong positive associations were between severity at 3 MAP and incidence at 3 MAP; between incidence at 6 MAP and disease traits (severity at 6, 9, and 12, incidence at 9 and 12); between severity at 6 MAP and disease traits (severity at 9 and 12, incidence at 9 and 12); between incidence at 6 and

incidence at 3; between incidence at 6 and severity at 3; between LA3 and LA9; between LA6 and LA9; between LA6 and LA3. The pairs of traits with significantly strong negative associations are between LA9 and disease traits (incidence3, severity3, incidence6, severity6, incidence9, severity9, incidence12, severity12); between LA3 and disease traits (incidence3, severity3, incidence6, severity6, incidence9, severity9, incidence12, severity12); between LA6 and disease traits (incidence6, severity6, incidence9, severity9, incidence12, severity12).

3.3. Molecular Detection of Cassava Mosaic Virus Strains Through Virus Indexing

A total of 120 leaf samples were collected from the four experimental fields, with 10 leaf samples collected per variant of experiment per site. Findings on virus indexing revealed that 30.0% (36/120) of the plants from cuttings of symptomatic and asymptomatic mother plant samples were positive for ACMV. At the same time, ACMV and EACMV mix infections were more predominant in the plants derived from cuttings obtained from symptomatic variants of the experiment than the plants from cuttings of the asymptomatic variant of the experiment (Table 6). The negative disease status of virus-indexed leaf samples of plants from cuttings of improved variety SLICASS 4 mother plants confirmed with the Plant Village Nuru app that they were disease-free materials. However, the plants derived from cuttings obtained from asymptomatic local Cocoa mother plants were negative based on the Nuru app at 3 MAP possibly due to an inability of the Nuru app to detect early stages of infection known as the latent infection phase of the disease. However, the Nuru app detected the disease expression beyond 3 MAP and progressed to harvesting.

Table 6. Virus indexing of symptomatic, asymptomatic, and improved cassava plants for ACMV and EACMV.

Variant of Experiment	Status of Samples for CMD Infection	Detection of ACMV Single Infection	Detection of EACMV Single Infection	Detection of ACMV/EACMV Mix Infection	Negative Samples
Improved	40	0	0	0	40
Asymptomatic	40	26	4	10	0
Symptomatic	40	10	10	20	0
Total	120	36 (30.0%)	14 (11.7%)	30 (25.0%)	40 (33.3%)

CMD = cassava mosaic disease; ACMV = African cassava mosaic virus; EACMV = East African cassava mosaic virus. Total positive samples = 80 (66.7%) and total negative samples = 40 (33.3%).

4. Discussion

Profiling of cassava genotypes for disease, growth, fresh storage root yield, and related root yield loss traits involves analysis of the existing divergence among genotypes and relationships in the traits that constitute the genotypes. This knowledge guides the selection process to match the selection decisions to the end-user's needs. The phenotypic studies detected tolerant plant samples. Tolerant plants are plants that endure an infection by a particular pathogen, without exhibiting symptoms and expressing serious disease [36]. Such plants are infected by the pathogen without showing any symptoms, attributable to a latent infection. Tolerance is referred to as a stable equilibrium between the virus and its host, an interaction where the individual partner accommodates trade-offs for survival and receives other benefits including protection of the plant against super-infection by virulent viruses; virus invasion of meristem tissues that allow vertical transmission [37]. Resistant plants prevent or limit viral disease development or replication [38]. Disease diagnosis limited to the visual or phenotypic assessment of symptoms on plants is insufficient for determination of its resistance. This explains the utilization of two techniques in our study for the selection of plants derived from cuttings obtained from symptomatic and asymptomatic mother plants based on visual assessment; then, using molecular analysis we confirmed the real status of each evaluated sample for CMD.

The plants from the cuttings of symptomless SLICASS 4 mother plants were resistant to CMD. Indeed, 100% (40/40) of the symptomless samples exhibited resistance to CMD at both phenotypic and molecular detection levels. The released improved variety used in this study was part of the resistant CMD genotypes introduced from IITA Nigeria for testing in Sierra Leone, whereas the local variety is widely cultivated in the country, but susceptible to the disease. This suggests genotypic variations to disease response as varieties differ in their natural abilities to resist CMD in farmers' fields. This finding corroborates the view of Fauquet and Fargette [38] and Amoakon et al. [39] who found some varieties to be naturally resistant to CMD. The molecular analytical technique detected ACMV and EACMV in the plants from cuttings of symptomatic and asymptomatic mother plant samples of the local variety Cocoa indicating that the absence of symptoms on the leaves does not exclude the presence of virus infection. This is in agreement with Soko et al. [40], who opined that the expression of plant symptoms is a function of virus accumulation. Thus, the plants from cuttings of the symptomatic and asymptomatic mother plant infected with ACMV and EACMV are healthy carriers (tolerant varieties) and could therefore constitute an important plant reservoir that contributes to the spread of the diseases. The tolerant plants from cuttings of asymptomatic Cocoa should not be used as a solution for the management and/or control of CMD. Thus, the findings suggest regular sanitation by tissue culture and routine virus indexing of planting materials for their usefulness in breeding for other important traits including high yield, dry matter, processing traits, etc.

The disease incidence appeared earlier on the leaves when the plants needed more photosynthetic products to carry out their physiological processes. Findings of the present study agree with the view that a wide range of physiological and biochemical disorders are triggered by cell infection, including relocation of photo-assimilates, redox imbalance, and premature senescence, with significant economic losses [41–43]. The viral infections lead to a decline in CO₂ fixation, which could be directly related to a decrease in carbohydrate accumulation, reducing plant growth and development [44,45]. In contrast, other studies have demonstrated that soluble sugars and starch accumulate in the infected leaves, where photosynthesis is reduced [41,46]. The abnormal accumulation or depletion of starch in systemically infected tissues was reported in a cucumber mosaic virus (CMV)–marrow interaction [47].

Poor cassava root yields are known to be partly due to diseases caused by Africa's cassava mosaic disease, which accounts for many losses [48]. Cassava mosaic disease (CMD) is sub-Saharan Africa's most severe and common disease [49]. Neuenschwander et al. [18], Monde et al. [19], Legg et al. [7], Mallowa et al. [22], and Owor et al. [21] also found that CMD infection significantly contribute to fresh storage root yield loss in cassava. The correlation coefficients were interpreted based on the standard guideline by Ratner [50], depicted by the color intensity in the correlograms. Correlation coefficients in the trait associations displayed in the correlogram guide regarding the direct or indirect selection of traits and the consequences thereof for other traits [51].

The Nuru phenotype tool was inefficient in detecting CMD in plants derived from cuttings obtained from asymptomatic mother plants at the latent phase of development, but efficiently detected CMD similar to the molecular analytic technique for plants derived from cuttings obtained from symptomatic mother plants. These findings partly agree with Johansen et al. [52], Arakpogun et al. [53], and Cuellar et al. [27], who opined that phenotype tools such as the Nuru app are efficient for the phenotypic detection of plant diseases like molecular analysis techniques.

5. Conclusions

This study identifies asymptomatic and symptomatic cassava mosaic disease (CMD) in cassava plants and utilizes them for determination of the prevalence of CMD caused by

African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV). It demonstrates that the Nuru phenotype tool has limitations in detecting latent infections of the cassava mosaic disease, but performs comparably to molecular tools at later stages of 6, 9, and 12 months after planting sampling regimes. The Cassava variant of the experiment and locations significantly influences CMD infection, growth, and fresh storage root yield traits. Plants obtained from the symptomatic mother plant Cocoa variety exhibit the highest disease infection and number of whiteflies across all sampling regimes. Resistant, non-diseased plants derived from cuttings obtained from SLICASS 4 mother plants produce the highest fresh storage root yield that can be exploited for the increased production and productivity of the crop. The fresh storage root yield losses are higher in the plants obtained from cuttings of the symptomatic variety Cocoa mother plants compared to those harvested from cuttings of the asymptomatic variety Cocoa mother plants grown across the four test environments. The findings reveal the importance of cassava-resistant genotype diversity and the plants' adaptation to the environment for disease management strategies. Utilizing disease-free materials and phytosanitation of local genotypes is also important when new and improved genotypes are unavailable.

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Data Availability Statement: Data from this research can be made available on request from the corresponding author due to ethical reasons.

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Abbreviations

ACMV	African Cassava Mosaic Virus
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
EACMV	East African Cassava Mosaic Virus
CMD	Cassava Mosaic Disease
IITA	International Institute of Tropical Agriculture
MAP	Months After Planting
SEACMV	South East African Cassava Mosaic Virus
SLICASS	Sierra Leone Improved Cassava
WAVE	Central and West African Virus Epidemiology

Appendix A

Table A1. Interactive impacts of variant of the experiment $\times$ location $\times$ sampling regime on cassava mosaic disease (CMD) incidence and severity of symptomatic, asymptomatic, and improved cassava plants assessed at various sampling regimes.

Variant of the Experiment	Location	Sampling Regime			
		3 MAP	6 MAP	9 MAP	12 MAP
		Incidence of CMD			
Asymptomatic	Bombali	0.0 ^f	27.7 ^e	36.3 ^d	36.3 ^d
	Bonthe	0.0 ^f	30.0 ^e	41.7 ^d	44.0 ^d
	Kenema	0.0 ^f	28.0 ^{de}	28.0 ^{de}	37.7 ^d
	Port Loko	0.0 ^f	30.3 ^e	58.3 ^c	86.7 ^{ab}
Improved	Bombali	0.0 ^f	0.0 ^f	0.0 ^f	0.0 ^f
	Bonthe	0.0 ^f	0.0 ^f	0.0 ^f	0.0 ^f
	Kenema	0.0 ^f	0.0 ^f	0.0 ^f	0.0 ^f
	Port Loko	0.0 ^f	0.0 ^f	0.0 ^f	0.0 ^f
Symptomatic	Bombali	38.3 ^d	100.0 ^a	100.0 ^a	95.7 ^a
	Bonthe	41.7 ^d	80.0 ^b	93.3 ^a	81.7 ^b
	Kenema	65.0 ^c	100.0 ^a	100.0 ^a	100.0 ^a
	Port Loko	100.0 ^a	100.0 ^a	100.0 ^a	96.7 ^a
Severity of CMD					
Asymptomatic	Bombali	1.0 ^f	2.0 ^e	2.8 ^{cd}	2.8 ^{cd}
	Bonthe	1.0 ^f	2.2 ^e	2.7 ^d	2.7 ^d
	Kenema	1.0 ^f	2.3 ^e	2.4 ^{cde}	2.7 ^d
	Port Loko	1.0 ^f	2.3 ^e	3.0 ^c	3.3 ^c
Improved	Bombali	1.0 ^f	1.0 ^f	1.0 ^f	1.0 ^f
	Bonthe	1.0 ^f	1.0 ^f	1.0 ^f	1.0 ^f
	Kenema	1.0 ^f	1.0 ^f	1.0 ^f	1.0 ^f
	Port Loko	1.0 ^f	1.0 ^f	1.0 ^f	1.0 ^f
Symptomatic	Bombali	2.0 ^e	4.7 ^a	4.7 ^a	4.3 ^a
	Bonthe	2.7 ^d	3.8 ^{bc}	4.7 ^a	3.3 ^c
	Kenema	3.4 ^c	4.0 ^b	4.7 ^a	4.7 ^a
	Port Loko	4.0 ^b	4.0 ^b	4.3 ^{ab}	4.3 ^{ab}

Variants of the experiment with the same letter are not significantly different at $\alpha = 0.05$; MAP = months after planting.

Table A2. Interactive impacts of variant of the experiment $\times$ location on cassava mosaic disease (CMD) incidence, severity, and whitefly abundance of symptomatic, asymptomatic, and improved cassava plants.

Variant of the Experiment	Location			
	Bombali	Bonthe	Kenema	Port Loko
	CMD Incidence			
	CMD severity			
Asymptomatic	25.1 ^f	28.9 ^f	23.4 ^f	43.8 ^e
Improved	0.0 ^g	0.0 ^g	0.0 ^g	0.0 ^g
Symptomatic	83.5 ^c	74.2 ^d	91.3 ^b	99.2 ^a
Asymptomatic	2.2 ^c	2.1 ^c	2.1 ^c	2.4 ^c
Improved	1.0 ^d	1.0 ^d	1.0 ^d	1.0 ^d
Symptomatic	3.9 ^{ab}	3.6 ^b	4.2 ^a	4.2 ^a
Asymptomatic	70.2 ^f	80.2 ^e	71.1 ^f	59.8 ^g
Improved	51.3 ^{gh}	40.4 ^h	50.2 ^{gh}	38.6 ^h
Symptomatic	142.9 ^c	120.0 ^d	155.3 ^b	160.8 ^a

Variants of the experiment with the same letter are not significantly different at $\alpha = 0.05$; CMD=cassava mosaic disease.

Table A3. Interactive impacts of variant of the experiment × location × sampling regime on whitefly abundance of symptomatic, asymptomatic, and improved cassava plants.

Variant of the Experiment	Location	Sampling Regime			
		3 MAP	6 MAP	9 MAP	12 MAP
Asymptomatic	Bombali	79.3 ^f	76.3 ^{fg}	72.7 ^{f g}	52.3 ^{gh}
	Bonthe	82.0 ^f	96.7 ^e	81.0 ^f	61.0 ^g
	Kenema	74.7 ^{fg}	65.0 ^g	74.7 ^{fg}	70.0 ^{fg}
	Port Loko	68.3 ^{fg}	56.7 ^g	62.7 ^{gh}	51.3 ^{gh}
Improved	Bombali	51.3 ^{gh}	53.7 ^{gh}	53.3 ^{gh}	47.0 ^h
	Bonthe	75.3 ^{fg}	34.3 ⁱ	28.3 ⁱ	23.7 ⁱ
	Kenema	60.0 ^{gh}	51.0 ^h	43.7 ^h	46.0 ^h
	Port Loko	47.7 ^h	34.3 ⁱ	39.0 ^{hi}	33.3 ^{hi}
Symptomatic	Bombali	136.7 ^d	176.7 ^b	180.0 ^b	78.3 ^f
	Bonthe	100.0 ^{ef}	133.3 ^d	156.7 ^c	90.0 ^f
	Kenema	130.3 ^d	195.0 ^a	189.3 ^b	106.7 ^e
	Port Loko	157.0 ^c	207.0 ^a	180.0 ^b	99.3

Variants of the experiment with the same letter are not significantly different at $\alpha = 0.05$; MAP = months after planting.

Appendix B

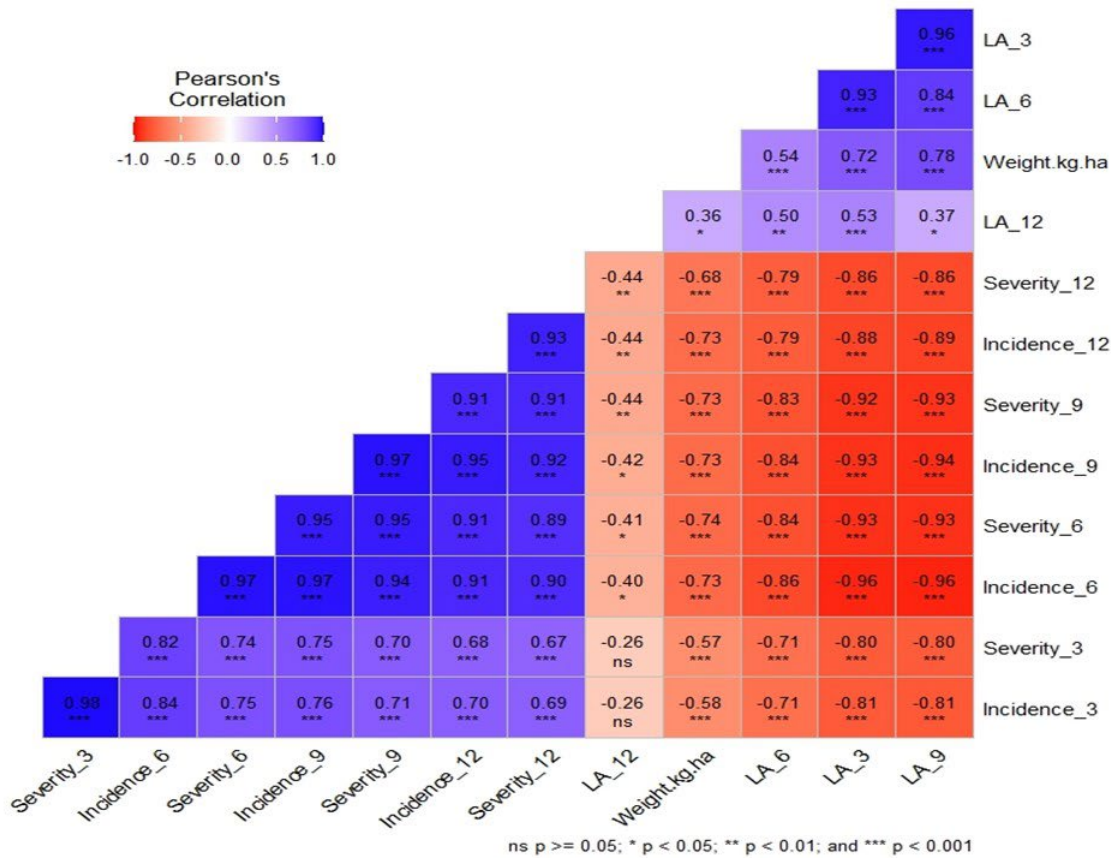


Figure A1. Correlogram of disease, growth, and yield traits of cassava. Positive and negative correlations are displayed in blue and red, respectively. High color intensity is proportional to high correlation coefficients and significance. Very light and blank colors are insignificant.

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