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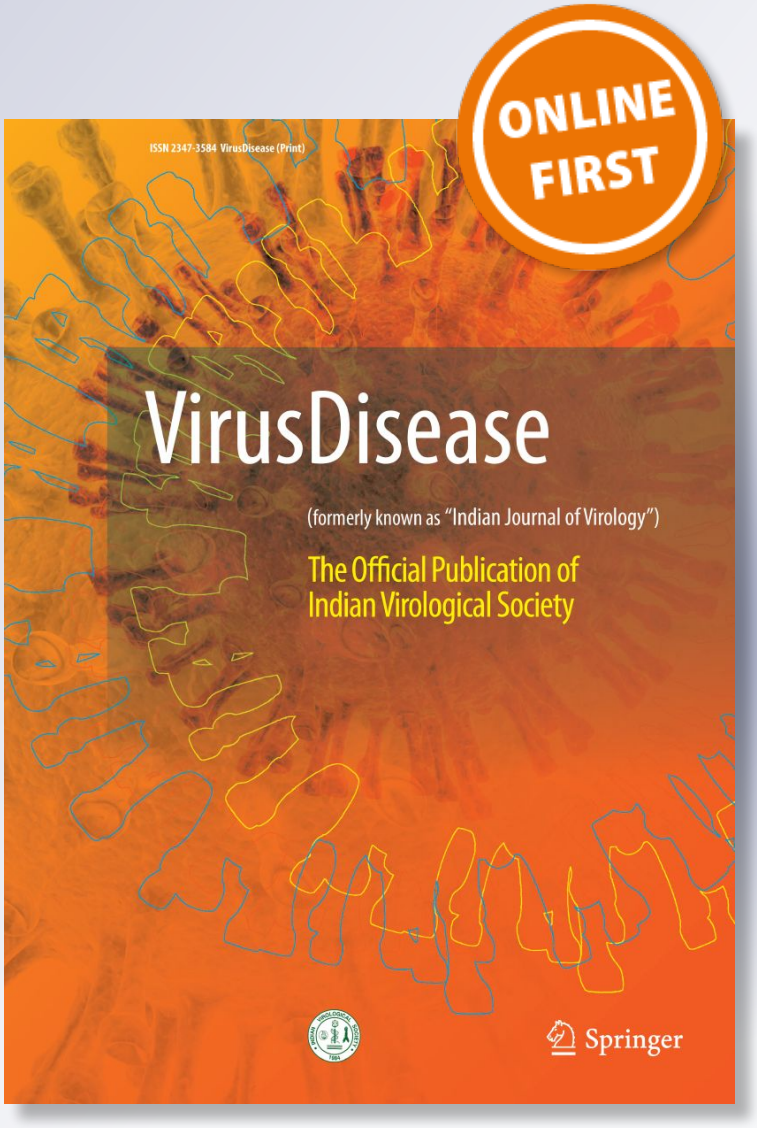

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ORIGINAL ARTICLE

Distribution and diversity of viruses infecting yams (*Dioscorea* spp.) in Cameroon

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Abstract Yam (*Dioscorea* spp.) is an important food crop cultivated for its edible tubers in Cameroon. Surveys were conducted in Cameroon to determine the incidence and severity of yam mosaic disease and associated viruses in 124 yam farms in four agro-ecological zones in 2014 and 2016. *Dioscorea rotundata, D. cayenensis, D. alata, D. Dumetorum* and *D. bulbifera* were most frequently detected yam species in the fields. Symptoms of virus disease were observed on 81.5% of the farms surveyed and the disease incidence ranged from 0 to 96.7%, with an overall mean of 26.5%. Mean symptom severity estimated using a numerical rating scale of 1–5, ranged from 2 to 4.1, with an overall mean of 2.6. Representative set of leaf samples collected from farmers' fields were tested for three viruses known to cause yam mosaic disease in West Africa, viz., *Yam mosaic virus* (YMV), *Yam mild mosaic virus* (YMMV) and *Cucumber mosaic virus* (CMV), using multiplex RT-PCR. YMV and YMMV were detected in 220 (37.2%) of the 591 samples tested and 75% of the farms surveyed. None of the samples tested positive to CMV. Phylogenetic analysis based on the coat protein sequencing of 27 YMV isolates clustered these isolates into three phylogenetic groups. This study demonstrated high prevalence of mosaic disease in yam fields and YMV as main causal agent. Knowledge generated in this study will be useful to augment diagnostic tools and yam mosaic disease control with a view to improve on yam production in Cameroon.

Keywords Yam virus disease surveys · Sequence diversity · Viral diseases · Virus diagnostics

Introduction

Yam (*Dioscorea* spp.) is a multi-species tuber-producing vine belonging to the family *Dioscoreaceae*. The genus includes about 603 domesticated and wild species distributed in tropical and subtropical areas of the world [5]. This vegetatively propagated crop is an important starchy staple and source of income for millions of people in the yam belt extending from Côte d'Ivoire to Cameroon, where over 67.3 million MT of the world's estimated 73 million MT of yams are produced annually [9; FAOSTAT, 2017]. Nigeria is the highest producer with about 47.9 million MT, while Cameroon with its annual production of 648,407 MT is ranked 6th within the yam belt and 7th in the world behind Nigeria, Ghana, Côte d'Ivoire, Benin, Ethiopia and Togo [FAOSTAT, 2017]. Based on production quantities, yam is ranked 3rd after cassava and cocoyam/taro in Cameroon [18]. The most economically important yam species in Cameroon are *D. rotundata, D. cayenensis, D.*

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alata, and *D. dumetorum*, which are cultivated in all agro-ecological zones of the country [18, 20]. Yam cultivation in Cameroon has been increasing due to growing demand for this high value tuberous crop, as reflected in the expansion in surface area from 28,038 ha to 57,612 ha, and production from 262,610 MT to 648,407 MT from 2000 to 2017, respectively [FAOSTAT, 2017]. However, gains in productivity were marginal from 9.4 MT/ha in 2000 to 11.3 MT/ha in 2017. Yam production in Cameroon, like in other yam-producing countries in Sub-Saharan Africa [17], is hampered by many biotic constraints.

Diseases caused by viral pathogens are amongst the most significant as they were reported to reduce tuber yields by up to 40% [2, 17, 24]. In the lack of organized seed sector, farmers continue to reuse their saved seeds which are often infected by viruses. Reuse of the same seeds over generations and informal seed exchanges between farmers over the decades have likely contributed to widespread distribution and increase incidence of viral diseases in yam fields. Viruses infecting yams in the yam belt include, *Yam mosaic virus* (YMV, genus Potyvirus), *Yam mild mosaic virus* (YMMV, genus *Potyvirus*), *Yam mild mottle virus* (YMMV, genus *Carmovirus*), *Cucumber mosaic virus* (CMV, genus *Cucumovirus*), and several species of *Dioscorea*-infecting badnaviruses [6, 9, 13, 23]. Significant losses in yam tuber yields are inflicted by YMV and CMV infections in Côte d'Ivoire and Nigeria [2, 6, 11, 17].

Previous studies have reported the occurrence of YMV and yam badnaviruses in Cameroon [20, 22]; however, knowledge on their distribution and incidence is limited to the western highlands (Zone III) and the humid forest with monomodal rainfall (Zone IV) agro-ecological zones (AEZs). This study was undertaken with an objective to survey all the yam producing zones to generate information on the extent of virus spread and determine virus diversity, to augment diagnostic tools and control measures through 'clean seed' interventions in Cameroon.

Materials and methods

Survey area

Surveys were conducted in two seasons, in October 2014 and July 2016, in four of the five AEZs based on their importance in yam production: High Guinea Savannah (Adamawa Region) or Zone II; Western Highlands (West and North-West Regions) or Zone III; Humid Forest with monomodal rainfall (South-West and Littoral Regions) or Zone IV; and Humid forest with bimodal rainfall (South, East and Centre Regions) or Zone V (Figs. 1, 2). Surveys were not conducted in Sudano-Sahelian (or Zone I) as yam cultivation is limited or non-existent in this AEZ. A total of 124 farms were surveyed; 48 farms (12 per AEZ) in 2014 and 76 farms (19 per AEZ) in 2016 (Table 1; Figs. 1, 2). Geographic coordinates were recorded at each farm location.

Sampling protocol and data recording

In each yam farm, 30 plants were examined by observing along the 'X' transects [25], with 15 plants per diagonal axis. Each plant was assessed for symptom type, and severity, and percent viral disease incidence per field was determined. Viral disease severity was scored using a numerical disease rating scale of 1–5, where: 1 = no visible symptoms; 2 = mild mosaic/mottling on most leaves but no leaf distortion; 3 = mild to moderate mosaic on most leaves, leaf narrowing and leaf distortion on about 1/3 of the plant (mostly in lower portion); 4 = severe mosaic on most leaves in about 2/3 of the plant, leaf distortion and mild to moderate stunting; 5 = severe mosaic, severe leaf distortion and severe stunting [28]. Depending on symptom types, up to 6 leaf samples per field were collected for virus testing. Where symptomatic plants were absent, non-symptomatic leaves were collected. The leaf samples were shipped to laboratory for virus testing.

Detection of viruses on leaf samples

All leaves were tested for YMV, YMMV and CMV using the multiplex-reverse transcription polymerase chain reaction (RT-PCR). Total nucleic acid was extracted from 100 mg of yam leaf tissue from each sample, using modified Cetyltrimethyl Ammonium Bromide (CTAB) method as previously reported by Abarshi et al. [1]. Multiplex RT-PCR was conducted using 100 ng/µl total nucleic acid in 12.5 µl reaction mixture, using a thermal cycler (SeeAmp Thermal Cycler, Seegene South Korea). The reaction mixture contained 2.5 µl of reaction buffer, 1.5 mM $MgCl_2$, 0.3 U of Taq DNA polymerase, 12 U of M-MLV (Promega, USA), 0.2 mM dNTPs mix (New England Biolabs, USA), 0.1 µM of each CMV, 0.2 µM of each YMV, 0.36 µM of each YMMV oligonucleotide (IDT, Belgium) and 2 µl of diluted 1:50 (v/v) (100 ng/µl) total nucleic acid extract. Oligonucleotides used for nucleic acid amplification were YMMV-F: GGCACACATGCAAATGAATGC and YMMV-R: CACCAGTAGAGTGAACATAG for YMMV; CMV-F: GCCGTAAGCTGGATGGACAA and IITA CMV-R: CCGCTTGTGCGTTTAATGGCT for CMV; and YMV-F3x: GACAATGATGGACGGTGCGG and YMVB3x: GTTTGCCATCAAATCCAAACAT for YMV. The thermal cycling condition consisted of reverse transcriptase (RT) phase at 42 °C for 30 min, followed by one cycle of initial denaturation at 94 °C for

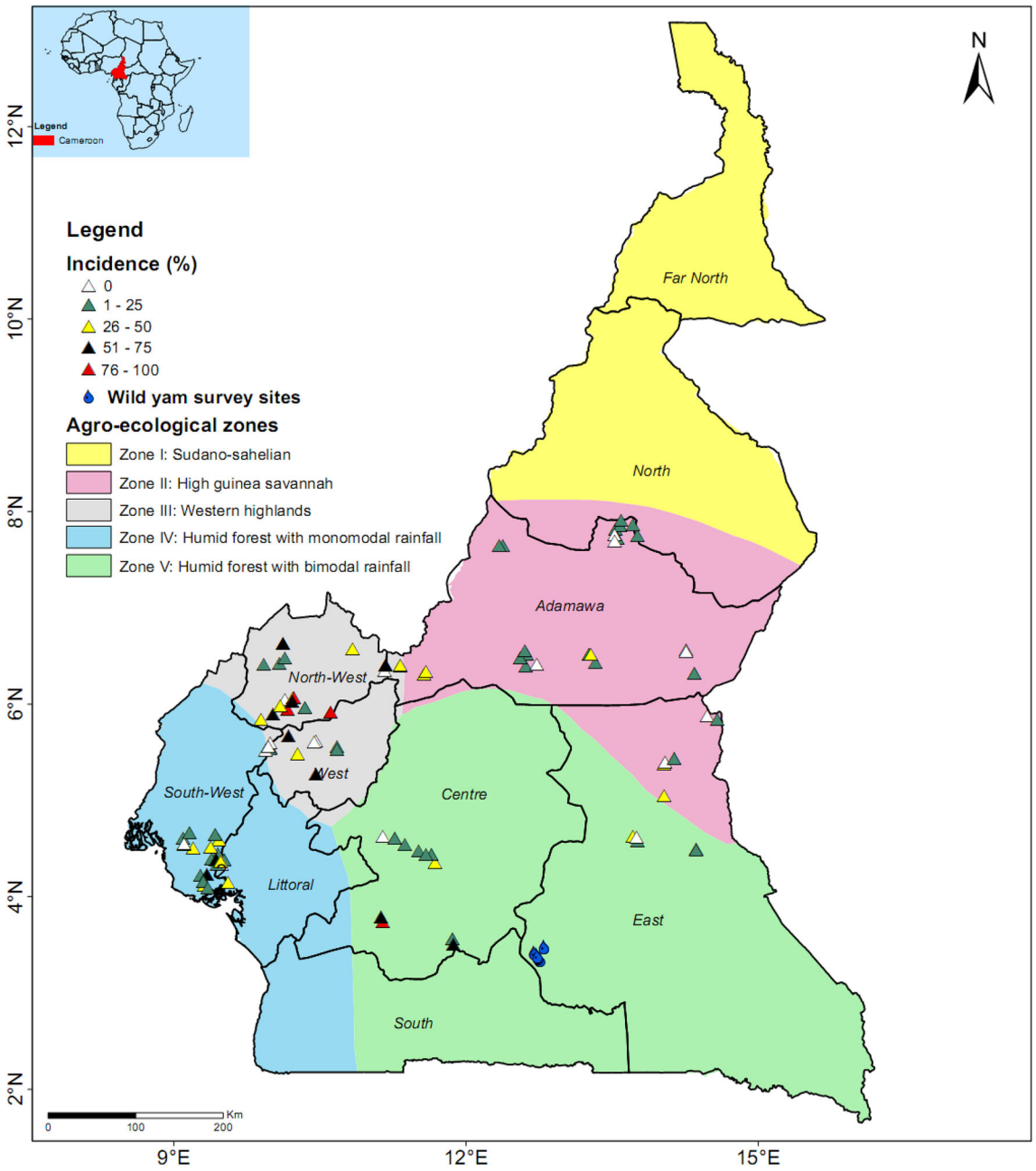

Fig. 1 Percent virus disease incidence in the surveyed yam farms in Cameroon

1 min, annealing at 54 °C for 2 min and extension at 72 °C for 3 min; 35 cycles at 94 °C for 1 min, 54 °C for 2 min, 72 °C for 1 min; and final extension at 72 °C for 5 min. PCR product was resolved in 2% agarose gel, pre-stained with EZ-Vision Bluelight DNA Dye (VWR, USA) and visualized using GelDoc (Biorad, USA). Samples with RT-PCR amplicons showing expected band sizes of 241, 330 and 520 bp, indicated positive results for YMMV, YMV and CMV, respectively (data not shown).

Sequencing and phylogenetic analyses of YMV and YMMV isolates

Viral nucleic acid extracts of 27 samples that tested positive to YMV and 2 samples positive to YMMV were selected for sequencing of full coat protein (CP) encoding segment (Supplementary Table 1). These samples represent wide area coverage and symptom types. RT-PCR reaction was performed in 50 µl reaction mixture as

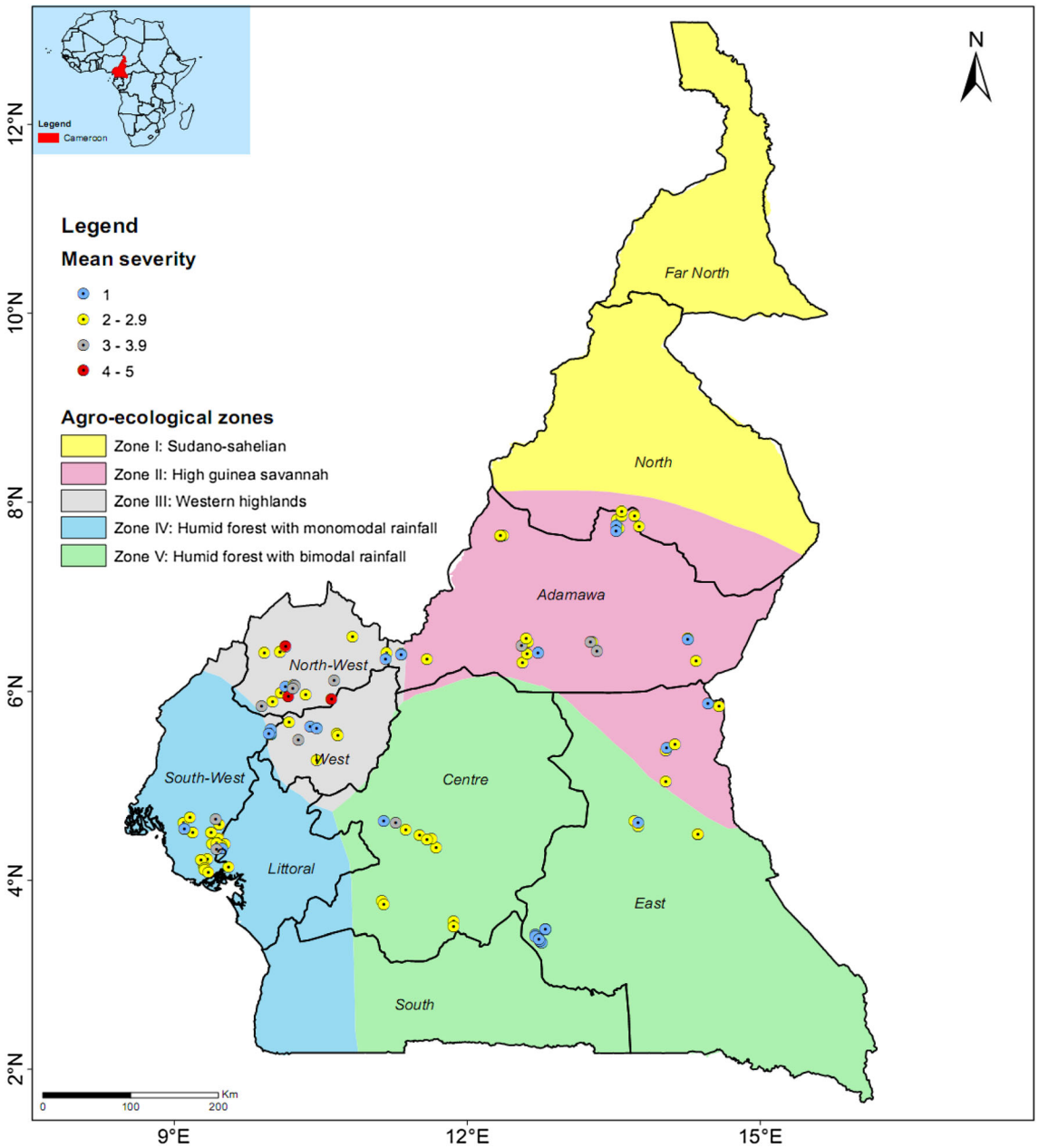

Fig. 2 Mean virus disease severity assessed based on 1–5 rating scale in the order of increasing symptom severity in the surveyed yam fields in Cameroon

detailed before, except for using oligonucleotide primer pairs: YMV CP Bam-FP: AGAGGATCCGCAGATAC ACAGCCAGAT and YMV CP Eco-RP: ATCGAATTCCTA CATACCTCTCATGCCCA, to amplify a 906 bp fragment corresponding to the CP encoding sequence of YMV; and the YMMV CP Bam-FP: CAGAGAGGATCCGCAAGTA AGGAACA and YMMV CP Eco-RP: TTGATCGAATT CCTAGATATTGCGCAC to amplify a 803 bp fragment corresponding to the CP of YMMV. RT-PCR was performed using thermal cycling conditions described earlier, and the amplified products were purified by ethanol precipitation and the products were used for sequencing in both orientations using amplicon-specific primers, at The Iowa State University DNA Facility (Iowa, USA). All the sequences were analyzed and edited manually using BioEdit Software version 7.2.1. The nucleotide sequences were compared with previously published YMV and YMMV CP sequences from Cameroon and other West African countries available in the NCBI GenBank, using the Clustal-W method [8], and phylogenetic relationships were reconstructed using the maximum likelihood (ML) method based on the Tamura–Nei model [26], conducted

Table 1 Yam viral disease incidence and severity across four agro-ecological zones in Cameroon in 2014 and 2016

Agro-ecological zones	Farms surveyed			Mean incidence (%)				Mean severity (1–5 scale)			
	2014	2016	Total	Range	2014	2016	Overall	Range	2014	2016	Overall
Zone II: high guinea savanna	12	19	31	0–56.7	30.0^{b}	3.0^{b}	13.4^{c}	2–3.0	2.5^{ab}	2.4^{b}	2.4^{b}
Zone III: western highlands	12	19	31	0–90.0	54.4^{a}	32.8^{a}	41.2^{a}	2–4.1	2.6^{a}	3.0^{a}	2.8^{a}
Zone IV: humid forest with monomodal rainfall	12	19	31	0–70.0	36.7^{ab}	19.5^{a}	26.1^{b}	2–3.1	2.3^{b}	2.6^{b}	2.5^{b}
Zone V: humid forest with bimodal rainfall	12	19	31	0–96.7	33.3^{b}	20.2^{a}	25.3^{bc}	2–3.0	2.5^{ab}	2.4^{b}	2.5^{b}
F	–			–	2.5	6.1	6.8	–	1.4	4.2	4.5
Total	48	76	124	–	–	–	–	–	–	–	–

Means followed by different letters within each column are significantly different from each other (Fisher's LSD test, $p < 0.05$)

on MEGA7 [15]. Pairwise genetic distance between sequences was calculated using the maximum composite likelihood (MCL) approach.

Data analysis

Analysis of variance (ANOVA) and post hoc tests were conducted on the data with R statistical computing software, using the statistical software package agricolae [12]. Means were compared using the Fisher's Least Significant Difference (LSD) test, at 95% confidence interval. Pearson's product-moment correlation test was used to determine interrelationships among variables (between yam viral disease incidence and severity, viral disease incidence and altitude of sampling location, and viral disease severity and altitude of the sampling location), using the R statistical computing software package ggpubr [14].

Results

Prevalence, incidence and severity of yam viral diseases

In the 124 yam fields surveyed, *D. rotundata* was the most frequently encountered yam species followed by *D. cayenensis, D. alata*, *D. dumetorum* and *D. bulbifera* in both surveys (Table 2). *Dioscorea cayenensis* was the most frequently planted species in Zone III, whilst *D. alata, D. cayenensis* and *D. rotundata* were observed in all the four agro-ecological zones (Table 2). Virus disease symptoms were recorded in 46 (95.9%) of the 48 farms surveyed in 2014, and 55 (72.4%) of the 76 farms surveyed in 2016, with an overall prevalence of 81.5% for two years. A total of 3720 plants (1440 in 2014 and 2280 in 2016) were examined for symptoms. Virus disease incidence in decreasing order was recorded on *D. cayenensis, D. rotundata, D. alata, D. bulbifera* and *D. dumetorum* (Table 2). During the surveys a total of 591 leaf samples (287 in 2014 and 304 in 2016) were collected for virus testing. Eight symptom types were observed, including mosaic, leaf distortion, mottling, leaf bleaching, vein banding, shoe-stringing, stunting and leaf curling (Fig. 3, Table 2). The eight symptom types were observed more frequently on *D. rotundata* and *D. cayenensis*, followed by *D. alata* (Table 2). Only mosaic and leaf distortion were observed on both *D. dumetorum* and *D. bulbifera.*

Virus disease incidence ranged from 0 to 96.7%, with an overall mean of 26.5% (Table 1). Of the 124 farms surveyed, virus disease incidence was 1–25% in 49 farms, 26–50% in 27 farms, 51–75% in 16 farms and > 75% in 9 farms (Fig. 1). Viral disease incidence was least in *D. dumetorum* (3.6%) and highest in *D. cayenensis* (61.1%) with a mean incidence of 28.8%, whilst mean viral disease severity was least in *D. dumetorum* (2.2) and highest in *D. bulbifera* (3.0) with and overall mean of 2.7 (Table 2). Average severity per farm ranged from 2 to 4.1, with an overall mean of 2.6 (Table 1). Average severity scores of ≥ 4 were recorded in four farms (Weh, Bambui, Bamenda upstation and Akum) on *D. cayenensis*, all of which are in Zone III (Fig. 2). Mean viral disease severity per AEZ was highest ($p < 0.05$) in Zone III (2.8), compared with other zones with 2.4–2.5 (Table 1). The disease incidence was highest ($p < 0.05$) in Zone III (41.2%), compared with Zone II (13.4%), Zone IV (26.1%), and Zone V (25.3%) (Table 1). Viral disease incidence scores of 50% or higher were recorded only on *D. cayenensis,* in Zones III (65.1%), IV (50%) and V (53.4%) in both surveys indicating that *D. cayenensis* is most susceptible of the five yam species observed in farms in Cameroon (Table 2).

Pearson's product-moment correlation tests showed a positive correlation between yam viral disease incidence and

Table 2 Viral disease incidence and severity scores recorded in four agro-ecological zones and five yam species, during two surveys conducted in Cameroon in 2014 and 2016

Agro-ecological zone	Yam species	Plants examined	Mean incidence (%)	Mean severity	Associated symptoms
Zone II:	*D. alata*	41	9.4	3	M, Lb, Ld, Sh, St
high guinea savannah	*D. bulbifera*	5	0	0	–
	D. cayenensis	24	41.2	2.9	M, Mo, Ld, Sh, St
	D. dumetorum	1	0	0	–
	D. rotundata	859	15.5	2.4	M, Mo, Cu, Lb, Ld, Sh, St, Vb
	Total	930	16.5	2.5	
Zone III:	*D. alata*	62	3.8	2	M, Mo, Ld, Sh
western highlands	*D. bulbifera*	11	4.6	3	M, Ld
	D. cayenensis	541	65.1	3	M, Mo, Cu, Lb, Ld, Sh, Vb
	D. dumetorum	112	3.8	2	M, Ld, Ln
	D. rotundata	204	32.2	2.5	M, Mo, Cu, Lb, Ld, Sh, St, Vb
	Total	930	43.6	3	
Zone IV: humid forest with monomodal rainfall	*D. alata*	114	13.6	2.1	M, Mo, Ld, St
	D. bulbifera	0	0	0	–
	D. cayenensis	9	50.0	3.3	M, Mo, Ld, St
	D. dumetorum	172	5.0	2.3	M, Ld, St
	D. rotundata	635	37.8	2.6	M, Mo, Lb, Ld, Sh, St
	Total	930	28.2	2.6	
Zone V: humid forest with bimodal rainfall	*D. alata*	103	16.1	2.9	M, Mo, Ld
	D. bulbifera	0	0	0	–
	D. cayenensis	292	53.4	2.7	M, Mo, Cu, Lb, Ld, Sh, Vb
	D. dumetorum	23	00	0	Ld
	D. rotundata	512	18.3	2.4	M, Mo, Cu, Lb, Ld, Sh, Vb
	Total	930	26.8	2.6	
Totals by species	*D. alata*	320	11.2	2.4	M, Mo, Lb, Ld, St
	D. bulbifera	16	4.6	3	M, Ld
	D. cayenensis	866	61.1	2.9	M, Mo, Cu, Lb, Ld, Sh, St, Vb
	D. dumetorum	308	3.6	2.2	M, Ld, St
	D. rotundata	2210	23.7	2.5	M, Mo, Cu, Lb, Ld, Sh, St, Vb
Overall		3720	28.8	2.7	

Yam viral disease symptoms: M = Mosaic, Mo = Mottling, Cu = Curling, Lb = Leaf bleaching, Ld = Leaf distortion, Sh = Shoe-stringing, St = Stunting, Vb = Vein-banding

severity ($r = 0.57$, $p < 0.001$), a weak but marginally not significant correlation between viral disease incidence and altitude ($r = 0.16$, $p = 0.08$) and no significant correlation between viral disease severity and altitude ($r = 0.002$, $p = 0.99$) (Table 3).

Detection of YMV, YMMV and CMV

Of the 591 samples tested, 220 tested positive to YMV or YMMV. In total, 199 of these virus-positive samples showed symptoms and 21 were asymptomatic. YMV was detected in 174 symptomatic and 15 asymptomatic samples, YMMV was detected in 17 symptomatic and 6 asymptomatic samples, and YMV + YMMV was detected in 8 samples, all of which were symptomatic (Table 4). CMV was not detected in any of the samples. YMV and YMMV were detected in all the four agro-ecological zones, while mixed infection of the two viruses occurred only in Zone V (Table 5). In leaf samples from Zone II, III, IV and V, viruses were detected in 32.9, 37.3, 39.6 and 39.2%, respectively. YMV was detected in 29.5, 35.2, 35.6 and 27.7% while YMMV was detected in 3.4, 2.1, 4.0 and 6.1% of the samples from Zone II, III, IV and V, respectively (Table 5). Viruses were detected in 93 (75%) of the 124 farms sampled for testing (Table 5). Based on viruses tested, viral disease prevalence in decreasing order was 83.9% in Zone IV, 77.4% in Zone V, 74.2% in Zone III and 64.5% in Zone II. YMV and YMMV were detected in 88 (70.1%) and 20 (16.1%) farms, respectively. YMV and

Fig. 3 Variety of viral disease symptoms observed on naturally infected yams in farmers' fields in Cameroon. **a** Uninfected, **b** Stunting, mosaic and distortion, **c** Severe mosaic, **d** Shoe-stringing with mosaic, **e** Bleaching, **f** Leaf distortion, **g** Green-vein banding, **h** Mosaic with bleaching, and **i** Mild mottling

YMMV were detected in *D. alata, D. cayenensis, D. dumetorum* and *D. rotundata*, but not in *D. bulbifera* (Table 5). YMV, YMMV and mixed infection of both viruses were frequently associated with samples having mosaic symptoms (Table 4). In the leaves sampled from *D. cayenensis*, viruses were detected in 62.5, 48.9, 75 and 58.4% in Zone II, III, IV and V, respectively (Table 5).

Molecular diversity of YMV and YMMV isolates detected in this study

Phylogenetic analysis and pairwise comparison grouped the 27 YMV isolates sequenced in this study into three major clusters (A1, A2 and A3) as supported by low bootstrap values (< 50%; Fig. 4). However, clustering was

Table 3 Correlation analysis of yam viral disease incidence, severity and altitude of sampling locations for 124 farms assessed in Cameroon

Parameter 1	Parameter 2	Correlation coefficient (r)	*p*-value	Level of significance
Incidence	Altitude	0.16	0.08	ns
Severity	Altitude	0.002	0.99	ns
Incidence	Severity	0.57	$3.7e^{-12}$	**

ns no significant correlation

**Significant correlation at $p < 01$

Table 4 Distribution of 220 virus-positive leaf samples from major yam-growing areas in Cameroon according to symptom types and three viruses tested in 2014 and 2016

Symptoms associated with leaf samples	Virus-positive samples per symptom type				
	YMV	YMMV	YMV + YMMV	CMV	[a]Total
Curling (Cu)	8	1	0	0	9
Leaf bleaching (Lb)	3	0	0	0	3
Leaf distortion (Ld)	6	4	1	0	11
Mosaic (M)	146	8	7	0	161
Mottling (Mo)	5	3	0	0	8
Shoe-stringing (Sh)	5	1	0	0	6
Vein-banding (Vb)	1	0	0	0	1
Total	174	17	8	0	199
Asymptomatic samples	15	6	0	0	21
Overall total	189	23	8	0	220

[a]Total number of virus-positives samples per symptom type

Table 5 Distribution of three viruses detected in cultivated yams within four agro-ecological zones in Cameroon

Agro-ecological zone	Yam species	Farms sampled[a]	Samples tested	Viruses detected (%)				
				YMV	YMMV	CMV	YMV + YMMV	Total
Zone II:	*D. alata*		8	25	12.5	0	0	37.5
high guinea savannah	*D. cayenensis*		8	50	12.5	0	0	62.5
	D. dumetorum		1	0	0	0	0	0
	D. rotundata		132	28.8	2.3	0	0	31.1
	Total	31(64.5)	149	29.5	3.4	0	0	32.9
Zone III: western highlands	*D. alata*		10	20	20	0	0	40
	D. bulbifera		4	0	0	0	0	0
	D. cayenensis		88	47.7	1.1	0	0	48.9
	D. dumetorum		18	0	0	0	0	0
	D. rotundata		25	28	0	0	0	28
	Total	31(74.2)	145	35.2	2.1	0	0	37.3
Zone IV:	*D. alata*		17	11.8	0	0	0	11.8
humid forest with monomodal rainfall	*D. bulbifera*		1	0	0	0	0	0
	D. cayenensis		4	75	0	0	0	75
	D. dumetorum		34	8.8	17.8	0	0	26.5
	D. rotundata		93	48.4	0	0	0	48.4
	Total	31(83.9)	149	35.6	4.0	0	0	39.6
Zone V:	*D. alata*		22	27.3	13.6	0	0	40.9
humid forest with bimodal rainfall	*D. cayenensis*		48	41.7	4.2	0	12.5	58.4
	D. dumetorum		4	50	0	0	0	50
	D. rotundata		74	17.6	5.4	0	2.7	25.7
	Total	31(77.4)	148	27.7	6.1	0	5.4	39.2
Total		124(75)	591	31.9	3.9	0	1.4	37.2

[a]Number of farms sampled (percentage of virus-positive farms given in parenthesis)

not representative of any agro-ecological zone or yam species suggesting wide distribution of various YMV isolates due to inter-zonal movement of planting material and likely spread of viruses between plants by aphid vectors. The 13 YMV isolates in Cluster A1 were from zone II (4 isolates), III (3 isolates), IV (1 isolate) and V (5 isolates) and originated from 10 landraces of *D. cayenensis* and *D. rotundata* (Fig. 4, Supplementary Table 1). The five isolates in cluster A2 were identifiable with zone II (1 isolate), III (3 isolates) and V (1 isolate) and were isolated from *D. cayenensis, D. rotundata* and *D. schimperiana.* Also, the nine isolates in cluster A3 were identifiable with zone II (3

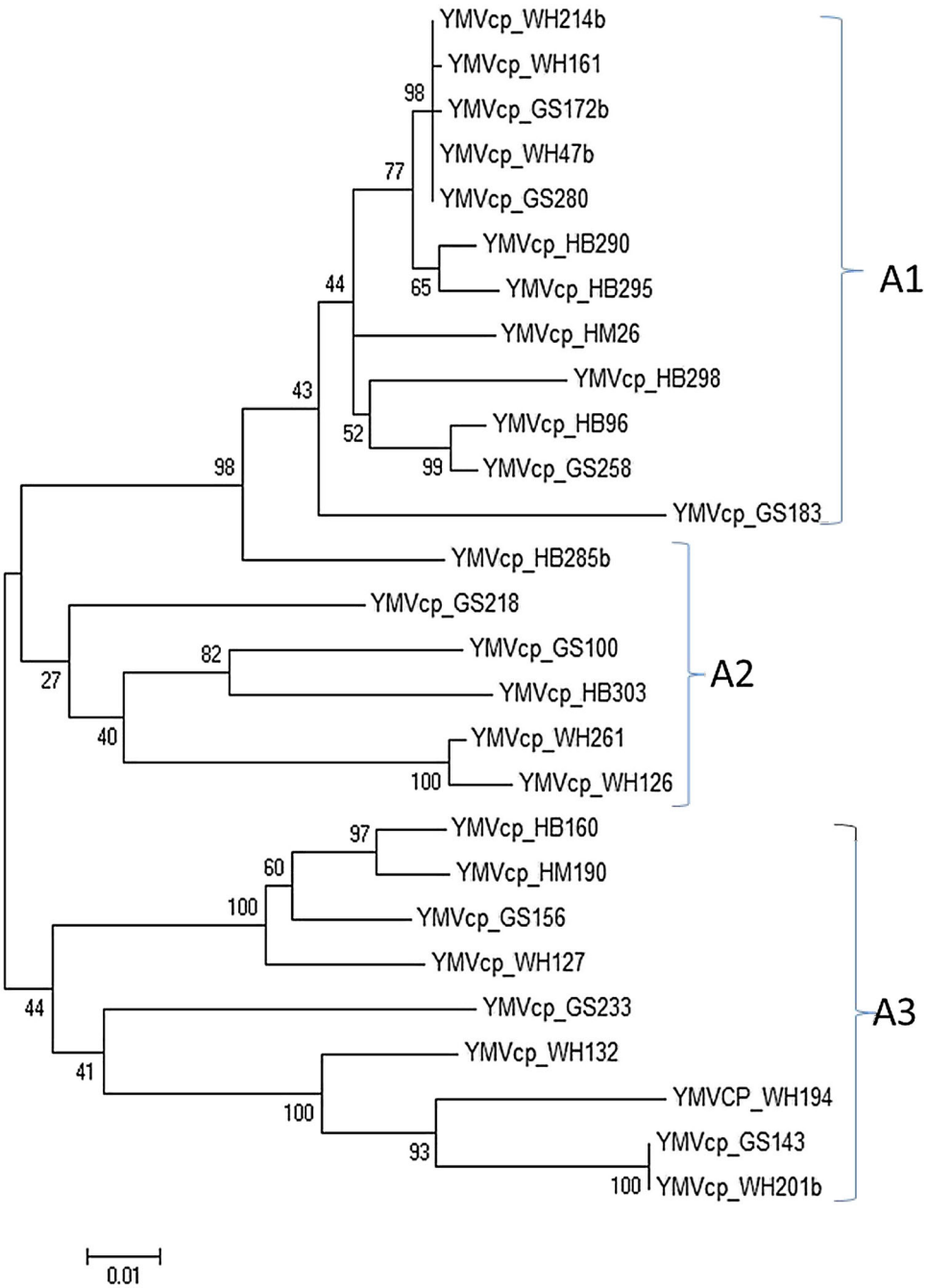

Fig. 4 Unrooted phylogenetic tree constructed using the maximum-likelihood method with the aid of MEGA 7 software. The phylogenetic tree was constructed using the complete coat protein encoding gene of 27 YMV isolates detected in this study in Cameroon. Bootstrap values (1000 replicates) are shown at the branch nodes and branches corresponding to partitions reproduced in < 50% of bootstrap replicates are collapsed. The three groupings are indicated

isolates), III (4 isolates), IV (1 isolate) and V (1 isolate) and were isolated from several landraces of *D. cayenensis, D. rotundata* and *D. esculenta*.

The 27 YMV and 2 YMMV isolates sequenced in this study were compared with 16 sequences retrieved from the NCBI GenBank database representative of Cameroon, and other West African countries. Phylogenetic analyses partitioned all 45 isolates into seven clusters (C1, C2, C3, C4, C5, C6 and C7) (Fig. 5). The topology of the phylogenetic tree did not change significantly, as the 27 YMV and 2 YMMV isolates detected in this study, still occurred in four clusters (C1, C2, C3 and C7). Of the isolates from the NCBI GenBank database, only AJ244049_Benin, AJ244054_Cameroon, and AJ244048_Benin occurred in cluster C1; AJ244047_Nigeria in cluster C2; and YMMV (Nib/CP_region) AB024424.1 in cluster C7 in association with virus isolates detected in this study (Fig. 5). Cluster C3 was exclusively constituted of YMV isolates detected in this study. The remaining 12 virus isolates from the NCBI GenBank separated from isolates detected in this study and were partitioned into three clusters: C4 (AJ244055_Cameroon, AJ244046_Benin, AJ244066 and AJ24406 from Burkina Faso); C5 (AJ244052, AJ244050,

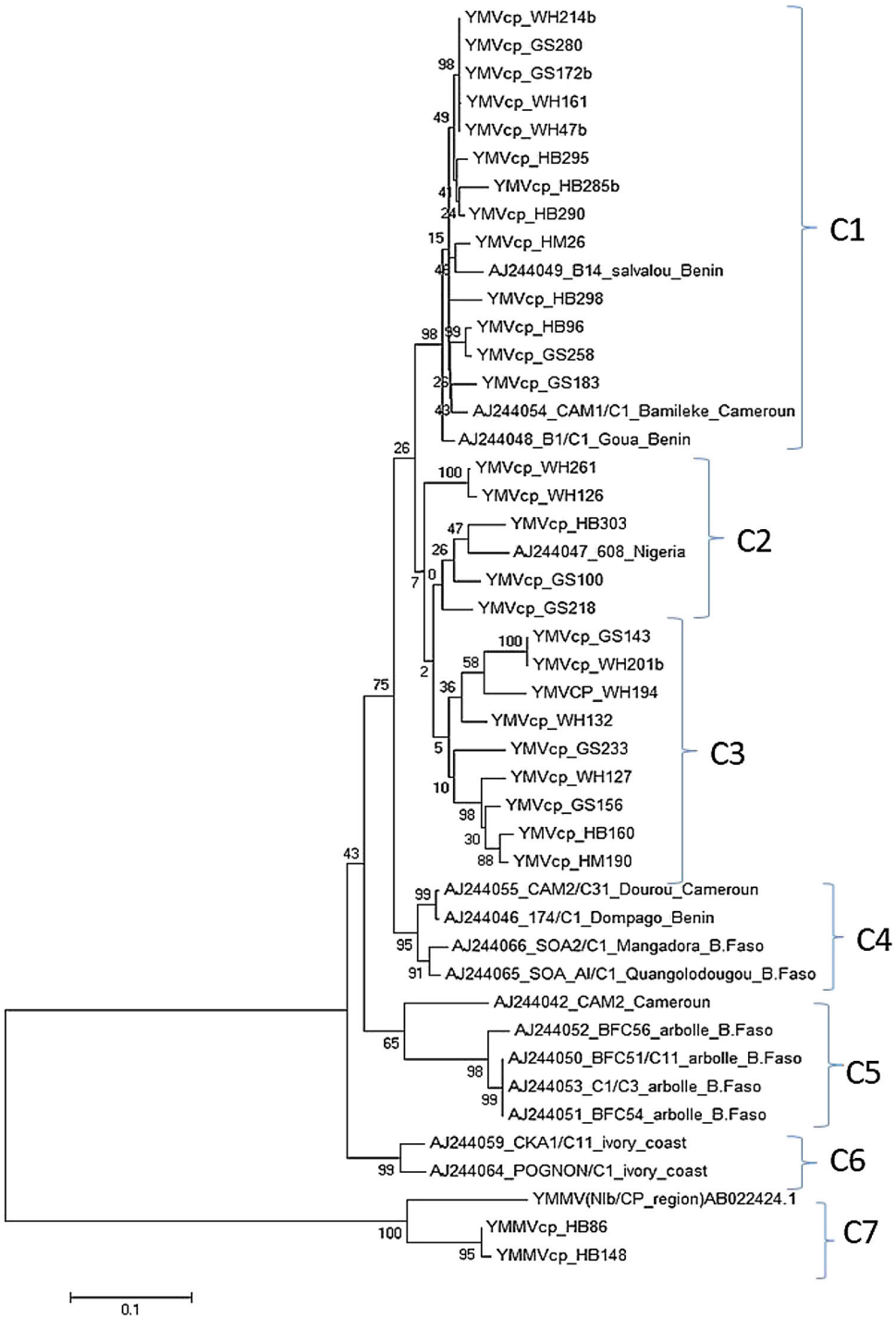

Fig. 5 Rooted phylogenetic tree depicting evolutionary relationships among *Yam mosaic virus* (YMV) and *Yam mild mosaic virus* (YMMV) isolates characterized in Cameroon. The phylogenetic tree was constructed using the complete coat protein encoding gene of 27 YMV isolates, 2 YMMV isolates and 16 YMV isolates from NCBI GenBank and the tree was rooted using YMMV as out-group. The tree was constructed using the MEGA7 [20]. Bootstrap values (1000 replicates) are shown at the branch nodes and branches corresponding to partitions reproduced in < 50% of bootstrap replicates are collapsed

AJ244053 and AJ244051 from Burkina Faso); and C6 (AJ2044059_Côte d'Ivoire) (Fig. 5).

Discussion

Occurrence of YMV in Cameroon was reported previously [20], but YMMV detected in this study is the first report in Cameroon. The occurrence of these two viruses in all countries of the yam belt can be attributed to exchange of infected yam germplasm and transmission by aphid vectors [9]. CMV was not detected in yam, although its occurrence in some weed and vegetable hosts has been reported in Sub-Saharan Africa [10, 28]. YMV and YMMV were detected in all four agro-ecological zones, covering eight of the ten regions of Cameroon. A very high prevalence of YMV and YMMV was observed, confirming previous reports that these viruses are the most frequent potyviruses on yams in

sub-Saharan Africa [10, 16]. Symptoms observed during the surveys in all agro-ecological zones included mosaic, leaf distortion, mottling, stunting, shoe-stringing and leaf bleaching which have been previously reported in Benin, Cameroon, Côte d'Ivoire, Ghana and Nigeria [19, 28]. This indicates that virus infection symptom development on yams is independent of agro-ecology in sub-Saharan Africa.

Viral disease incidence and severity were high across agro-ecological zones and yam species, with the highest incidence in Zone III, dominated by the cultivation of *D. cayenensis* landraces, which are probably more susceptible to yam viral diseases compared with other yam species encountered in the study. The lowest viral disease incidence occurred in Zone II, although the lowest severity did not occur in that agro-ecological zone. This observation suggests that perhaps the variety of *D. rotundata* called *Bakokae* which dominates in Zone II is more tolerant to virus infection, as previous reports have shown that some varieties of this yam species are resistant to viral infections [21]. This observation and the fact that both YMV and YMMV were detected in leaf samples without symptoms, further indicate that some yams in Cameroon are tolerant to virus infections. Extensive screening is needed to identity varieties that are resistant to viral infections in Cameroon, to provide genetic resources for yam breeding, to develop improved varieties and promote production.

None of the three viruses tested in the study was detected in some symptomatic leaves, indicating that the symptoms might have been due to other viruses not tested or other biotic and abiotic factors. Further characterization of such samples by deep sequencing of small RNAs is required to identify the occurrence of any other viruses with the samples. Moreover, some asymptomatic leaves tested positive for both viruses, indicating latent infection. Based on the foregoing we concluded that the absence or presence of symptoms alone cannot be used to accurately predict the occurrence of viral diseases on yams.

There was a significant positive correlation between viral disease incidence and severity, but there was absence of correlation between altitude and viral disease incidence or severity. This indicates that altitude does not have any influence on the incidence and severity of yam viral diseases in Cameroon.

The CP encoding gene sequence of 27 YMV isolates did correlate neither with geographical areas of origin nor their yam host species, as earlier reported [3], as isolates from different agro-ecological zones and yam host species clustered together in the same groups. Except for cluster A2 which contained isolates from Zones II, III, V, the other two clusters (A1 and A3) contained YMV isolates from all the four agro-ecological zones. Furthermore, except for one YMV isolate from *D. esculenta* and another from *D. schimperiana,* all other isolates were from the *D. cayenensis/rotundata* complex. This indicates that the *D. cayenensis/rotundata* complex is infected by several YMV strains as reported in previous studies conducted in Africa [4]. The high genetic variability amongst the YMV isolates in Cameroon may be due to intra-species recombination events [7], mutation caused by virus-yam host interaction, and virus transmission by insect vectors, particularly aphids [4]. The occurrence in the same cluster, of virus isolates from different agro-ecological zones and different landraces of the *D. cayenensis/rotundata* complex may be associated with local exchange of infected planting material amongst the different major yam production areas of the country.

The 15 YMV isolates retrieved from the NCBI GenBank database corresponded to the six African phylogenetic groups (I, II, III, IV, VII and IX) previously reported [4, 7]. When compared with these 15 isolates, phylogenetic analysis revealed that some of the YMV isolates detected in this study are associated to phylogenetic groups III and VII of the six groups previously reported in Africa [7]. Amongst the NCBI isolates two from Benin and one from Cameroon, all being members of phylogenetic group III clustered together with some isolates of this study in group C1. Also, the only member of Phylogenetic group VII, isolated from Nigeria clustered together with some YMV isolates of this study in group C2. This is an indication that YMV isolates of Cluster C1 and C2 are members of phylogenetic groups III and VII, reported in previous studies [7]. Nine YMV isolates formed a unique group together in cluster C3, suggesting a possible new phylogenetic group which may be associated with isolates from other parts of the world, that were not used in this study.

Further studies are needed to continue the improvement of knowledge on yam viral disease ecology and phylogenetic structure of viruses infecting yams in Cameroon. While our study was restricted to Cameroon, the findings could be relevant in all other countries in Central Africa and the Congo Basin, considering that the climate and geography of Cameroon, with its tropical, equatorial and mountain climates, is representative of much of Central Africa and the Congo basin [27].

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