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Research Article
Molecular Characterization of Cassava Mosaic Viruses and Current Mosaic Disease Concern in Three Major Cassava Production Areas in Côte D'ivoire

[1]F. Yao, [1]M.Koffi, [1,2]I. Abe, [1]M. N'Djetchi, [1]T. Konan and [1]T.A. Sanogo

[1]Research Unit in Genetics and Molecular Epidemiology (URGEM), UFR Environment,
Laboratory of Biodiversity and sustainable Management of Tropical Ecosystems, Jean Lorougnon Guédé University, BP 150 Daloa, Ivory Coast
[2]Laboratory of Genetics, UFR Biosciences, Félix Houphouët Boigny University, 22 BP 582 Abidjan 22, Ivory Coast

Abstract

Background and Objective: Since its introduction in Côte d'Ivoire in the 1980s, Cassava Mosaic Disease has so far continued to cause damage to cassava production in the country. This study aimed to characterize cassava mosaic disease's pathogens and emphasize current concerns in three major cassava-producing regions for better disease control. **Materials and Methods:** Two hundred cassava leaf samples comprising symptomatic infection and healthy characteristics from improved and traditional varieties were collected in the departments of Bouaké, Yamoussoukro and Daloa from 2019-2020 in rainy and dry seasons. These leaves were subjected to molecular analyses. Cassava Mosaic Disease's prevalence and severity were evaluated. All data were analyzed with software R, version 3.3.1. **Results:** Cassava Mosaic Disease overall phenotypic prevalence was 43.37% and almost equally distributed in all investigated departments. After the molecular diagnostic, the infection rate reached 77.7% for symptomatic plants and 34.28% for asymptomatic plants. The severity scores in traditional and improved varieties were S2, S3 and S4 but S2 were the most frequent. ACMV strains were the most detected while EACMV and co-infections showed the highest injury. **Conclusion:** The Cassava Mosaic Viruses largely present in all surveyed regions. Traditional varieties seem to be more resistant to infections and therefore require particular attention for viruses' control.

Key words: Cassava production, cassava mosaic disease, prevalence, severity, molecular characterization

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Corresponding Author: M. Koffi, Research Unit in Genetics and Molecular Epidemiology (URGEM), UFR Environment,
Laboratory of Biodiversity and sustainable Management of Tropical Ecosystems, Jean Lorougnon Guédé University, BP 150 Daloa, Ivory Coast

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a euphorbiaceous plant with 36 chromosomes[1]. Since its introduction in Africa, this plant has been adopted in almost the whole continent and has become one of the leading foods for populations[2]. It is grown mainly for human consumption and animal feed but also industries used[3]. Yet, cassava has a strategic place in food security because it is one of the main subsistence crops in several African countries, including Côte d'Ivoire[4]. However, for decades, Ivorian cassava production has been threatened by Cassava Mosaic Disease (CMD), which reduces considerably its yield. The pathogen which causes the disease, Cassava Mosaic Virus (CMV), is a begomovirus[5] and it is mainly transmitted by infected cuttings or by whiteflies, *Bemisia tabaci* is a polyphagous biting-sucking insect[6]. Viral strains impact differently cassava plants, depending on their virulence[7]. The main symptoms of CMD are deformation and discolouration of plant, chlorosis, plant stunting through stunting are non-specific to a virus type[8,9]. In Côte d'Ivoire, according to Djaha *et al.*[10], production losses due to CMD are enormous. Given the damage and spread of CMD, the current study presents the outcome of country-wide survey conduct across three major cassava-producing regions with the objective to genotype CMV and map their distribution.

MATERIALS AND METHODS

Survey areas: The sampling was carried out in Côte d'Ivoire in departments of Bouake (7°69N, 5°03W), Yamoussoukro (6°48N, 5°17W) and Daloa (6°53N, 6°27W). These regions are important cassava-growing regions in Côte d'Ivoire where the rainy seasons stretch from April-June and from September to November while the dry seasons stretch from July-August and from November-March. In each department, five villages were surveyed according to the diversity of cassava varieties.

Field sampling and sample collection: In each village, 5-12 small-holder farmer fields were randomly selected for the surveyed according to the diversity of cassava varieties. A total of 183 cassava farms were visited. A questionnaire was used to collect information such as department, village, cultivar, crop age, sampling season, cuttings origin and field coordinates recorded using the Global Positioning System (GPS). Sampling was carried out during the dry season, from November, 2019 to February, 2020 and in the rainy season from May-August, 2020. In each field, leaves were collected from 30 plants along two diagonals of the sampling farms following Sseruwagi's method[11]. The CMD prevalence per farm was obtained by the per cent (%) of the ratio of the number of infected plants (n) to the total number of plants sampled (N)[12] and the severity degrees are chlorotic symptom expression on leaves assessed using a scale of 1-5 according to Bakuzezia[13] as described in Table 1. A total of 200 CMD symptomatic and healthy leaf samples were collected per field and preserved like herbarium specimen[14] including 65 in Bouaké, 70 in Yamoussoukro and 65 in Daloa.

DNA Extraction from cassava leaf sample: Total DNA were extracted from about 50 mg of leaf sample using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) protocol as detailed by Abarshi *et al.*[15]. For each sample, CTAB lysis buffer was replaced with a buffer containing PBS (400 µL) and ASL (400 µL). Extracted DNA was suspended again in 100 µL of Tris-EDTA (TE) elution buffer for each sample in a labelled 1.5 mL Eppendorf tube and stored at -20°C before PCR.

DNA quality test: After each extraction, 10 out of 30 extracted DNA samples were randomly selected for the quality test. DNA were visualized after electrophoresis in 1% agarose gel stained with ethidium bromide and run at 100 volts for 30 min in Tris-borate-EDTA (TBE 0,5 X). The gel was visualized under a UV photographic trans-illuminator. When the staining obtained is of high intensity, the DNA is of good quality and can be used for PCR.

CMD virus diagnosis: Cassava leaf samples were analyzed by PCR using primers JSP001/F and JSP002/R or ACMV-AL1/F and ACMV-AR0/R for ACMV identification and JSP001/F and JSP003/R primers for EACMV identification in Table 2. The PCR

Table 1: Grading scale of CMD severities

Score	Symptom description
1	No symptoms on leaves or stems
2	Mild vein yellowing, chlorotic blotches on leaves No brown streaks, lesions on green stem or leaves
3	Mild vein yellowing, chlorotic blotches on leaves, Mild brown streaks, lesions on green stem portions
4	Sever, extensive vein yellowing, chlorotic blotches on leaves, Severe brown streaks, dark lesions on green stem portions, No defoliation, stem die-back and stunting
5	Severe/extensive vein yellowing, chlorotic blotches on leaves, Severe brown streaks, dark lesions on green stem portions, Defoliation, stem die-back and stunting

Table 2: Primers of CMD virus's identification

Primers	Sequence (forward, reverse)	Expected size (pb)	Virus
JSP001	5'ATGTCGAAGCGACCAGGAGAT 3'	783	ACMV
JSP002	5'TGTTTATTAATTGCCAATACT 3'		
JSP001	5'ATGTCGAAGCGACCAGGAGAT 3'	780	EACMV
JSP003	5'CCTTTATTAATTTGTCACTGC 3'		
ACMV-AL1/F	5'GCGGAATCCCTAACATTAT 3'	1030	ACMV
ACMV-AR0/R	5'GCTCGTATGTATCCTCTAAGGCCTG 3'		

mix contains 5 µL of DNA extract, 5 µL of amplification buffer 10X with $MgCl_2$, 3.2 µL of dNTP (200 µM), 2.6 µL of each primer (10 pM), 0.1 µL Taq polymerase (5U μL^{-1}) and 31.5 µL of molecular water, for a total volume of 50 µL. Amplification reactions were performed in a PCR thermal cycler (BioRad T100™, SINGAPOUR). This amplification used the standard thermal whose conditions are as follows: Initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec and initial extension at 72°C for 55 sec. The PCR reaction ends with a final extension at 72°C, for 10 min. The amplified DNA fragments were electrophoresed in 2% agarose gel stained with ethidium bromide and run at 100 volts for 45 min in Tris-borate-EDTA (TBE 0,5 X). Gels were visualized under a UV photographic trans-illuminator. Amplified products were scored (+) for CMVs positive and (-) for CMVs negative.

Statistical analysis: Comparison of phenotypic prevalence between departments was performed by the pairwise comparison test using the pairwise prop function. Factors likely to influence the disease development were determined by the Odds Ratio at 95%. These factors were compared using Fisher's exact tests according to the Cochran rule or independence Chi-square test, Heat map was carried out to evaluate the rate of each severity degree in departments. The reduction of the colour intensity in a block reflects the percentage increase of the severity score. And the rate of infected plants compared between symptomatic and asymptomatic plants as well as the rates of each virus detected in the different departments using the Chi-square test. The relationship between CMD's severity degrees and virus strains was carried out using the generalized linear model (glm). All statistics tests were performed using R software version 3.3.1 and differences were considered to be significant when $p \leq 0.05$.

RESULTS

Phenotypic prevalence and severity of CMD: Cassava mosaic disease occurred in all the three departments surveyed in Côte d'Ivoire with an average phenotypic prevalence of 43.37%. No significant difference was observed in CMD prevalence between the three study sites ($p = 0.23$), although higher in Daloa (44.74%) followed by Bouake (43.37%) and Yamoussoukro had the lowest prevalence (42.04%) in Table 3. The CMD severity scores observed were S1, S2, S3 and S4 with S2 being the most dominant severity score in all the departments followed by S1 and S3. The score 4 which was the highest severity score was observed only in Bouake and Yamoussoukro with low proportions as 3.08 and 1.43%, respectively in Fig. 1.

Risk factors associated with cassava mosaic disease: Table 4 shows risk factors associated with cassava disease occurrence in this study. Fields wild cuttings are around 6 times more likely to be infected than cuttings originate from the breeder of the National Centre for Agronomic research or the National Agency for Support to Rural Development (ANADER) (OR = 6.53, $IC_{95\%}$ [3.34-12.76]). Significant differential infection was observed among field age intervals ($p<0.05$). The highest field susceptible age interval was 3-6 months followed by fields which ages were $\geq$7 months. The infection risk is around 6 times more prevalent in the dry season than in the rainy season (OR = 5.86, $IC_{95\%}$[2.82-12.17]).

Molecular detection of CMV and virus variants distribution: Viral strains identified in this study are African Cassava Mosaic Disease (ACMV) and East African Cassava Mosaic Disease (EACMV). The ACMV was detected by the size 783 and 1030 Pb while EACMV was characterized by size 780 Pb. Out of the 200 samples tested for viral infection,133 (66.5 %) were positive in Fig. 2. The global infection rates were 70.71, 62.86 and 66.15% in Daloa, Bouake and Yamoussoukro, respectively. There was no significant difference between these rates ($p = 0.93$) in Table 5. Among the 133 infected, 91 (68.42%) were only ACMV positives, 25 (18.80%) were EACMV positives and 17 (12.78%) were positive to both virus strains. ACMV was significantly more prevalent than EACMV in all departments ($p = 0.03$).

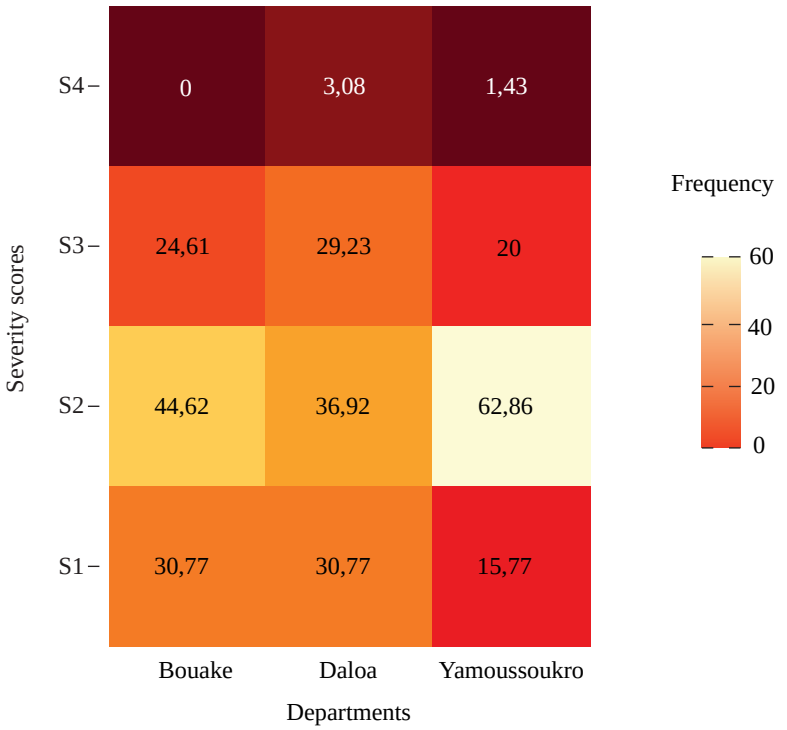

Fig. 1: Proportions of CMD severity degrees in each department

Table 3: Phenotypic prevalence observed in all the departments

		Visual observation				
Departments	N	Symptomatic	Asymptomatic	F (%)	X^2	p-value
Bouake	1946	844	1102	43.37	2.93	0.23
Yamoussoukro	2010	845	1165	42.04		
Daloa	1940	868	1072	44.74		
Total	5896	2557	3339			

N: Total number of samples, F: Frequency, X^2: Chi-square constant, p: Probability associated at x^2 test

Table 4: Risk factors associated with CMD

Factors	N	Symptomatic (%)	Asymptomatic (%)	p-value	OR ($IC_{95\%}$)
Cutting origin					
CNRA	58	20 (34.48)	38 (65.52)		
Village	142	110 (77.46)	32 (22.54)	<0.001[a]	6.53 (3.34-12.76)
Age (month)					
1-2	28	4 (14.28)	24 (85.71)		
3-6	100	78 (78)	22 (22)	<0.001[b]	6.38 (2.58-15.80)
≥7	72	42 (58.33)	30 (41.77)	0.04[a]	2.52 (1.02-6.22)
Season					
Rainy	43	14 (32,56)	29 (67.44)		
Dry	157	116 (73.88)	41 (26.12)	<0.001[a]	5.86 (2.82-12.17)
Total	200	130 (65)	70 (35)		

N: Total number of samples, p: Probability associated at x^2 test, OR: Odds Ratio, (IC95%), 95% Confidence Interval, a: Independence Chi-square approximation, b: Fisher's exact test

Table 5: Comparison of infection rates between departments

		Virus test				
Departments	N	+	-	T (%)	X^2	p-value
Bouake	65	46	19	70.71	0.14	0.93
Yamoussoukro	70	44	26	62.86		
Daloa	65	43	22	66.15		
Total	200	133	67	66.5		

N: Total number of samples, T: Infection rate, X^2: Chi-square constant, p: Probability associated at X^2 test, +: Virus presence, -: Virus absence

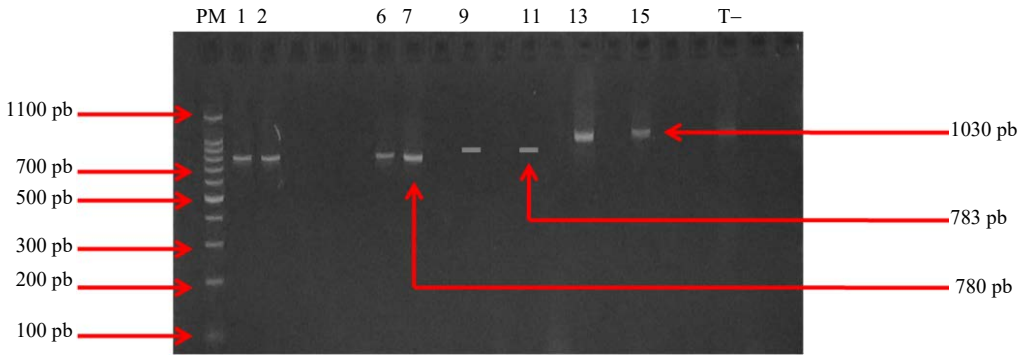

Fig. 2: Electrophoretic profile encoding genes of cassava mosaic virus strains

1, 2, 6 and 7 Samples tested positive to EACMV; 13, 15 and 18 Samples tested positive to ACMV; T-: Negative control (no amplification); PM: Molecular weight marker (100 bp, Invitrogen)

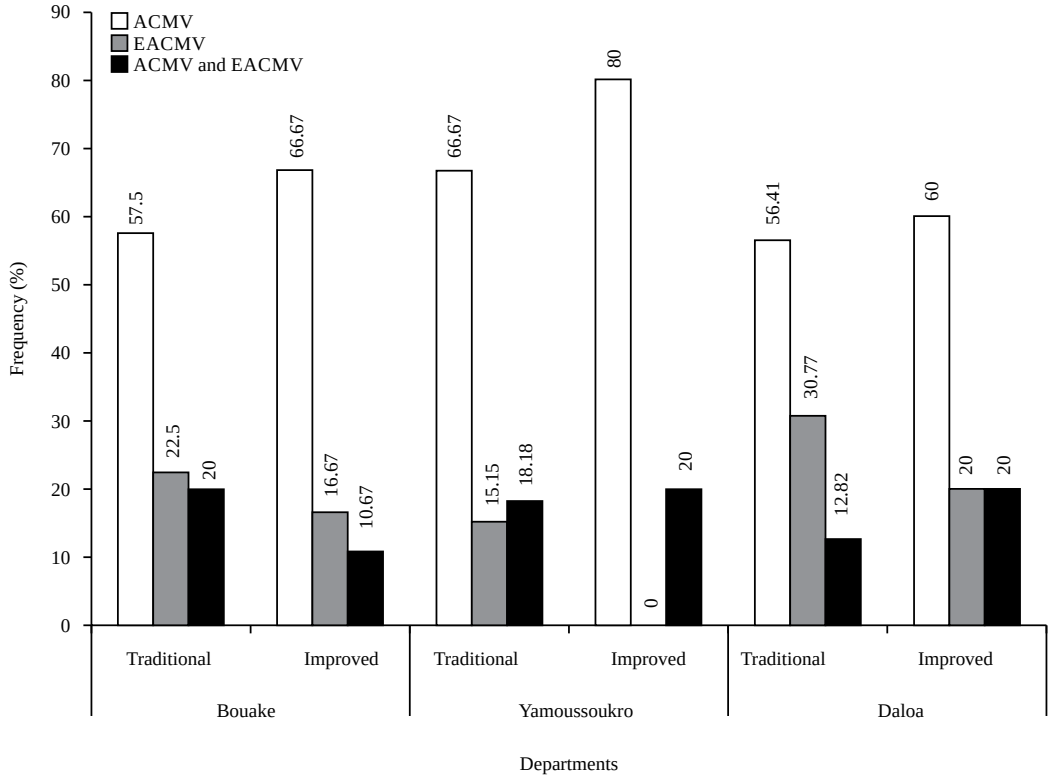

Fig. 3: Virus infection rate in traditional and improved varieties for each department

Infection rates were compared within the department between traditional and improved varieties and it comes out that ACMV strains were more detected than EACMV in both traditional and improved cassava varieties in Fig. 3. The improved varieties involved in severe infections were TMS, Bocou5 and Bocou8.

Infection rate between symptomatic and asymptomatic samples: From the 130 symptomatic DNA analyzed, 101(77.7%) were CMV's positive. This infection rate didn't differ significantly between departments ($p>0.05$) in Fig. 4. Also, the infection rate of asymptomatic plants was evaluated. The results show that from the 70 asymptomatic DNA analyzed, 24 (34. 28%) were CMV's positive and this rate didn't differ significantly between departments ($p>0.05$). However, the infection rate of symptomatic plants is higher than asymptomatic plants ($p<0.01$).

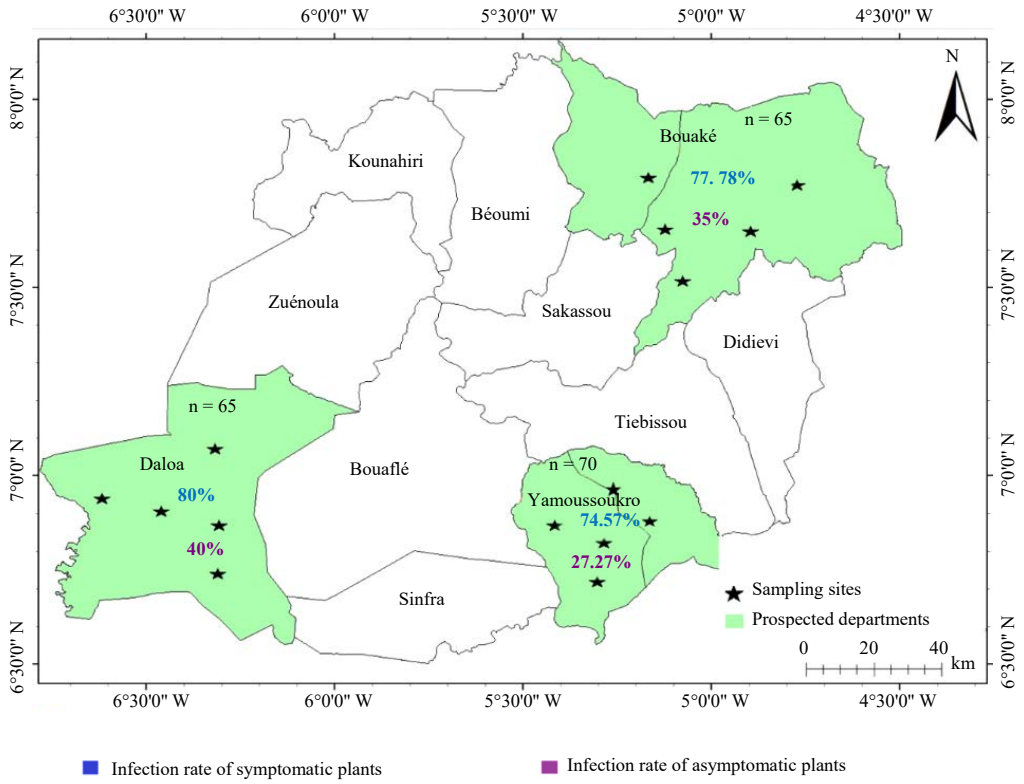

Fig. 4: Distribution of infection rate in symptomatic and asymptomatic plants

DISCUSSION

This study showed that Cassava Mosaic Disease (CMD) is equally distributed in all the three departments surveyed with an overall prevalence of 43.37%. The most observed severity scores were S2 and S3 and the score S4 was observed only in Daloa and Yamoussoukro with a low proportion. Cuttings origin, plants age and season are factors that increase CMD prevalence in Ivorian sites visited. Farms whose plants are aged from 3-6 months old were most infected. At this stage, cassava plants are actively growing, so they are more vulnerable to pathogens and in case of infection, they provide infected leaflets[16]. Moreover, the dry season is a suitable period for virus development and propagation in fields[17]. Correlation between virus development and dry season could be explained by the water stress in the young plants, which increases their vulnerability to pathogens, particularly viruses[18].

Cuttings cultivated very often come from other fields of the same village or the same department or another department or even other neighbouring countries. According to Toualy *et al.*[19], this cassava growing method is the primary means to perpetuate disease in the plant population. Indeed, cutting is the most widely practised method in developing countries by cutting exchanging between farmers[20]. However, many times, these recycled cuttings come from infected village plantations where cuttings are not controlled. This promotes the development and spread of cassava mosaic disease[21]. Virus development could also be explained by the maintenance of the poor field observed during sampling. Some research has shown that CMV is associated with factors such as plant age, climatic conditions, susceptibility of the infected cassava variety, the field maintenance, the virus strain responsible for the infection and co-infection[9]. The current study revealed the existence of the two viral strains in the three cassava production areas surveyed with some co-infection cases which can be very unsafe depending on the EACMV variants present in these co-infections. EACMV variants can be more perilous and result from recombination causing considerable yield losses[6]. However, ACMV was the most detected virus with a less aggressive severity score. These results are similar to Toualy *et al.*[19], findings, which showed the presence of ACMV and EACMV in the North-East of Côte d'Ivoire with ACMV identified as the most widespread virus in the cassava growing areas. Indeed, the symptoms caused by ACMV alone are moderate while plants infected with EACMV

or co-infection between ACMV and EACMV present high severity scores[22] even if some samples that have expressed the presence of both strains of virus ACMV and EACMV showed a low severity score (S2). That could mean that the variants of EACMV involved in these co-infections were less severe or the particular genotype of the plant is tolerant to the virus. On other hand, some authors reported that infection due to EACMV is necessarily associated with the presence of ACMV, which was not the case in this study in which for some plants, only the EACMV virus was detected[23]. Most of the improved varieties were infected by viruses while only these varieties have been popularized for several years in Côte d'Ivoire at the expense of the large local cultivars which are disappearing. The large prevalence of cassava mosaic disease could be due to the reduction of virus tolerance in most improved cultivars whose genetic structures are very homogeneous compared to traditional varieties and therefore would have caused a great vulnerability to diseases[24]. Indeed, the abandonment of traditional varieties would have caused a loss of genetic diversity[25]. Some authors argue that traditional varieties can maintain or even increase genetic diversity in the field through gene flow[26,27]. Therefore, the permanent contact between insect-virus-host would have turned the improved varieties into favourable hosts for the viruses and facilitate their spread[28]. On the other hand, some traditional varieties (22%) weren't infected and these could contain resistance genes to CMV. Resistant genes study could confirm or not this assertion to better appreciate these varieties use for possible improvements of cassava in Côte d'Ivoire. The virus infection rate was significantly greater in symptomatic plants than in asymptomatic plants. Thirty-four symptomatic samples (22.82%) were CMV's negative meaning that these symptoms could be related to other factors or others CMV variants not tested in this study. Indeed, CMD evolves rapidly and this evolution is largely due to recombination between existing strains resulting in new viruses with the same symptoms but sometimes more aggressive. According to Bisimwa *et al.*[29], the severe epidemic that affected Uganda in the 1990s was caused by the recombinant virus EACMV-UG2, resulting from the recombination between ACMV and EACMV. Also, recombination can occur between CMV and other viruses such as cassava brown steak virus (CBSV)[9].

Some asymptomatic samples (35.29%) were CMV positive. The absence of symptoms in these leaves tested positive could be justified by the possibility that these plants contained CMD resistance genes. Indeed, the resistant character in these plants would have reduced viruses' manifestation. This result shows that PCR diagnostic is necessary to confirm whether plants are infected or not[30].

CONCLUSION

This study shows that Cassava Mosaic Disease is still active and equally distributed in all the three departments surveyed in Côte d'Ivoire with an overall prevalence of 43.37%. The risk factors associated were cutting materials used, field age and dry season. Although severity scores 3 and 4 were observed, 'score 2' was the most frequent in both traditional and improved varieties. The improved varieties were infected the same as some traditional varieties and the most severely infected improved varieties were TMS, Bocou 5 and Bocou 8. This study noticed a resistance loss in improved varieties. The viruses ACMV, EACMV and co-infection were present in both variety types. However, ACMV was the most viruses detected while EACMV and co-infection showed the highest aggressive scores. The 22% of ignored traditional varieties seem to be more CMV's resistant and therefore require particular attention. The virus infection rate was greater in symptomatic plants than asymptomatic plants but PCR is necessary to assess if plants are affected or no by mosaic disease.

SIGNIFICANCE STATEMENT

This study highlights a high prevalence of Cassava mosaic disease in Côte d'Ivoire and a resistance loss in improved cassava varieties. These results suggest significant control measures to delay the spread of the pathogen. Resistant or tolerant character in some plants would have reduced viruses' manifestation. This result shows that PCR diagnostic is very useful to confirm whether plants are infected or not. A control approach based on the combination of traditional varieties tolerant to viruses might help.

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