

Full Length Research Paper

Distribution, incidence and severity of viral diseases of yam (*Dioscorea* spp.) in Côte d'Ivoire

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A survey was conducted in major yam cultivation zones in Côte d'Ivoire in 2009 to determine the incidence, severity of viral diseases, and viruses associated with the infected plants. Incidence and severity of the viral diseases were estimated based on symptoms. Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR)-based techniques were used for the detection of *Yam mosaic virus* (YMV), *Cucumber mosaic virus* (CMV), *Dioscorea mottle virus* (DMoV) and yam badnaviruses in the sampled yam leaves. Disease incidence varied from 0 to 90% and symptom severity from 1 to 5. There were significant difference in incidence and severity between different agro-ecological zones ($P < 0.001$). About 36% of the samples tested positive to YMV, ca. 1.5% tested positive to CMV, ca. 39.1% samples tested positive to yam infecting badnaviruses, and none of the samples tested positive to DMoV. This study demonstrated high incidence of virus diseases in all the yam production regions and warrants implementation of virus disease control measures.

Key words: Yam, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), viruses, Côte d'Ivoire.

INTRODUCTION

Yam (*Dioscorea* spp.) is a major source of food and income for millions of people in West and Central Africa (Asiedu et al., 1998; Séka et al., 2009a). Countries such as Nigeria, Côte d'Ivoire, Ghana, Benin, Togo and Cameroon grow 95% of the world production, estimated at 51.95 million tons (FAO, 2010). Côte d'Ivoire is the third largest producer in the world with 5.7 million tons, behind Nigeria (29.148 million tons) and Ghana (5.96 million tons), respectively; first and second producers in the world (FAO, 2010). Despite the importance of this food crop to this region, production potential has been limited by diseases caused by fungi, viruses and nematodes resulting in significant yield losses (Nwankiti

and Arene, 1978; Kenyon et al., 2001; Hughes et al., 1997). Viruses are of particular concern because, apart from causing significant reduction in tuber yield and quality, they restrict international exchange of germplasm (Hughes et al., 1997). Viruses reported to infect yam in West Africa belong to the *Potyvirus*, *Badnavirus* and *Cucumovirus* genera (Seal and Muller, 2007). Previous studies conducted by Séka et al. (2009a) confirmed the presence of *Cucumber mosaic virus* (CMV, genus *Cucumovirus*) and *Yam mosaic virus* (YMV, genus *Potyvirus*) in two towns (Bouaké and Toumodi) in Côte d'Ivoire. These two viruses have been reported to cause significant yield reduction (Thouvenel and Dumont, 1990;

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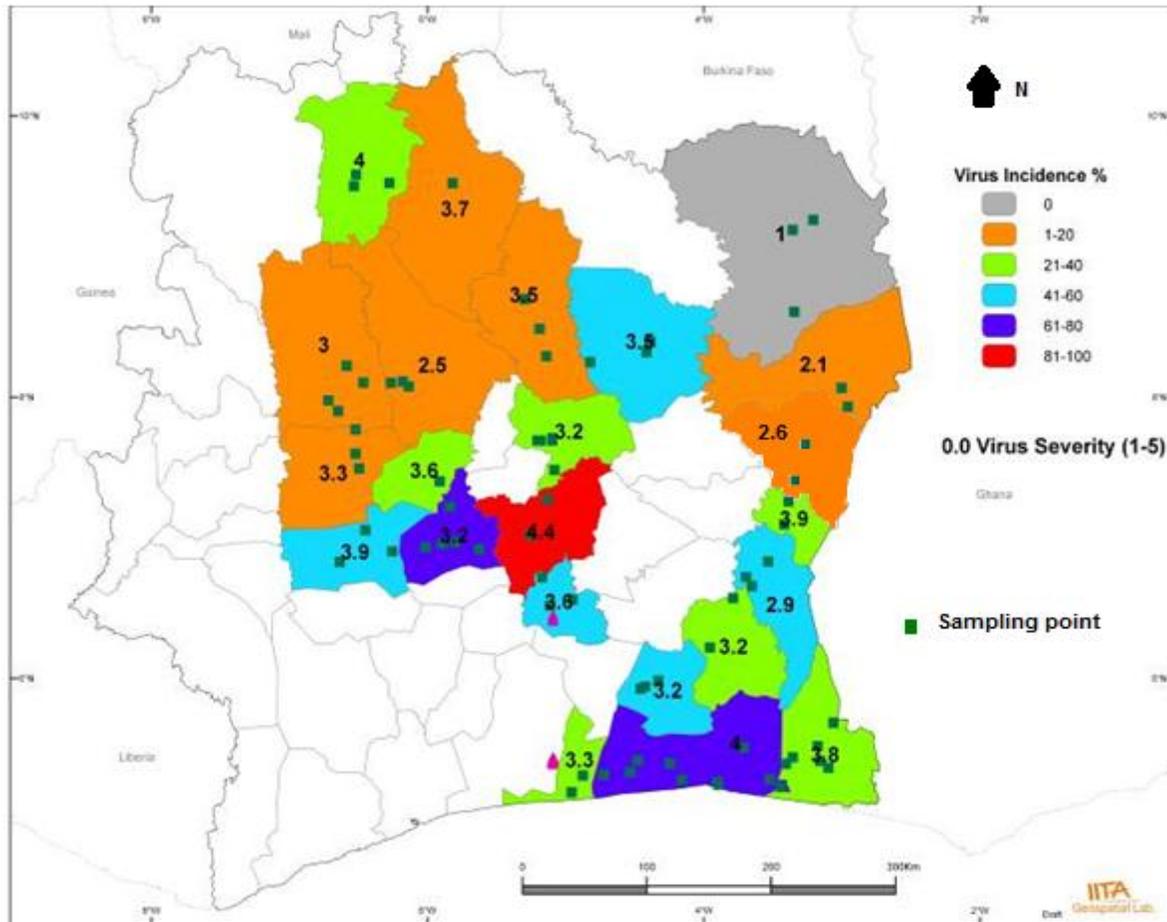


Figure 1. Map of Côte d'Ivoire showing the sampling points, incidence and severity of viral diseases.

Asiedu et al., 1998; Séka et al., 2009b). The effects of virus diseases can be devastating on yams if not prevented (Thottappilly, 1992). Understanding distribution and incidence of viruses associated with yam production in Côte d'Ivoire will further increase our knowledge about these viruses and develop appropriate control measures. Therefore, in this study, comprehensive survey was conducted in all the major yam production zones for determining the incidence, severity and diversity of viruses infecting yam in the major yam production zones in Côte d'Ivoire.

MATERIALS AND METHODS

Survey

Surveys were conducted in 2009 in 79 farmer fields in many locations covering major yam production regions in Côte d'Ivoire (Figure 1). At each survey site, geographic coordinates was taken using a GPS reader, incidence and severity. Disease incidence was estimated in each field by assessing 20 plants by walking in a 'W' shaped path counting five plants per side spaced at an equal

distance from each other. Descriptions of virus symptoms observed on each of the sample collected were recorded at each location. Incidence was estimated using the formula given below:

$$\text{Incidence per field (\%)} = \frac{\text{Number of symptomatic plants}}{20} \times 100$$

Severity of symptomatic plants were assessed by rating plants on 1 to 5 scale, where 1 = plant with no visible symptoms on the leaves; 2 = symptoms on 1-24% of infected leaves; 3 = symptoms on 25-50% of infected leaves; 4 = symptoms on 51-75% of infected leaves and 5 = symptoms on more than 75%-100% of infected leaves. Five samples were collected per field for virus testing in the laboratory. Means were compared using the LSD test at 5% using Statistica 7.1.

Detection of viruses

Enzyme-linked immunosorbent assay

Protein A sandwich-enzyme linked immunosorbent assay (PAS-ELISA) as per the protocol described by Edwards and Cooper (1985) and modified by Hughes and Thomas, (1988) was used for

the detection of YMV, CMV, *Dioscorea mottle virus* (DMoV) and yam infecting badnaviruses in the sampled leaves. Rabbit polyclonal antibodies against these viruses were available at IITA Ibadan, Nigeria. The enzymatic reactions were measured in an ELISA microplate reader (DYNEX MRX) after one hour incubation at room temperature. Leaf sample extracts from healthy and diseased yam were used as negative and positive control, respectively, while buffer was used as blank controls. Samples with absorbance at 405 nm at least twice the value of healthy control was considered as virus positive (infected).

Polymerase chain reaction (PCR)-based methods

Total nucleic acids from sampled leaves were isolated using the Cetyltrimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980). One hundred milligrams (100 mg) of young leaves was ground in 1 mL of CTAB buffer (2% CTAB w/v, 1.4 M NaCl, 20 mM EDTA and 100 mM Tris (pH 8.0) (sterilized by autoclaving) and 0.2% of 2-mercaptoethanol added just before use. The content (about 600 μ L of the extract) of each sample was transferred into a 2 mL sterile microfuge tube. The mixture was vortexed and incubated at 60°C in a water bath for 10 min. The tubes were cooled to room temperature and, 600 μ L of phenol : chloroform : iso-amylalcohol (25:24:1) were added to the mixture. The tubes were vortexed and centrifuged at 12,000 rpm for 10 min. The supernatant (about 450 μ L) was collected into a separate sterile microfuge tube, and 300 μ L of cold iso-propanol was added into the tube and incubated at -20°C for 1 h. The solution was centrifuged at 12,000 g for 10 min to precipitate the DNA. The supernatant was carefully removed and the DNA pellet was washed with 500 μ L of 70% ethanol and tubes were air dried at room temperature or at 37°C to remove final traces of ethanol. The DNA pellet was dissolved in 50 μ L of TE buffer and stored at -20°C until further use. PCR was used for the detection of yam badnaviruses (Badna) and reverse-transcription PCR was used for the detection of CMV and YMV. The following primers were used for the molecular analysis: Badna FP: 5'-ATG CCI TTY GGI ITI AAR AAY GCI CC-3', Badna RP: 5'-CCA YTT RCA IAC ISC ICC CCA ICC-3' (Seal and Muller, 2007); CMV primer 1: 5'-GCC GTA AGC TGG ATG GAC AA-3'; CMV primer 2: 5'-TAT GAT AAG AAG CTT GTT TCG CG-3' (Wylie et al., 1993) and YMV F: 5'-ATC CGG GAT GTG GAC AAT GA-3', YMV R: 5'-TGG TCC TCC GCC ACA TCA AA-3' (Mumford and Seal, 1997).

PCR reaction mixture for the detection of badnaviruses was as follows: 5.94 μ L of sterile distilled water, 2.5 μ L of PCR reaction buffer (5x supplied along with the enzyme by the manufacturer), 0.25 μ L of dNTPs, 0.75 μ L of MgCl₂ (25 mM), 0.5 μ L of Badna forward and reverse primers, and 0.3 U of Taq DNA polymerase (Promega, UK). For CMV and YMV, the RT-PCR mixtures consisted of: 6.36 μ L of sterile distilled water respectively for CMV and YMV, 2.5 μ L of PCR reaction buffer (x5), 0.25 μ L of dNTPs, 0.75 μ L of MgCl₂ (25 mM), 0.25 μ L of CMV and YMV forward and reverse primers, respectively; 0.3 U of Taq DNA, 0.06 μ L of reverse transcriptase. The reaction volume was 12.5 μ L with 10.5 μ L reaction mixture and, 2 μ L 1:100 diluted nucleic acid template.

The PCR profile for badnavirus detection involved an initial denaturation, 94°C for 4 min; 40 cycles, of 94°C for 30 s, 50°C for 30 s and 72°C for 30 s; and a final extension at 72°C for 5 min. The following thermocyclic regime was used in RT-PCR for detection of YMV and CMV: initial incubation for RT reaction at 44°C for 30 min, followed by denaturation at 95°C for 5 min; 35 cycles, of 95°C for 30 s, 55°C for 1 min and 72°C for 1 min; and a final extension at 72°C for 10 min. The PCR/RT-PCR product was electrophoresed on 1% agarose gels for 35 - 40 min at 130 V in 1XTAE buffer. Then, the DNA was stained by ethidium bromide and visualized under UV light.

RESULTS

Symptoms observed on infected samples leaf

A total of 486 yam leaf samples were collected from the 79 localities (farms). Mosaic, mottling and vein banding were the most common symptoms observed, accounting for 68.5% of the field symptoms. Coiled leaf, vein clearing, bleaching, chlorosis, shoe-string, distortion, stunting and reduction of leaf accounted for the remaining 31.5% of the field symptoms (Figure 2). Disease incidence varied from 0 to 90% with an overall mean of 36.2% for the 79 fields surveyed. Incidence exceeded 50% in 38% of the sites visited (Figure 1). The highest incidence of 90% was recorded in Yobouékro in the Central region of Côte d'Ivoire. Incidence was '0' in three locations (Farako, Balamilido and Assoum-2) in the North-East, where symptoms were not visible on the plants. Symptom severity ranged from 2.5 to 5, with an overall mean of 3.3; seventy three percent (73%) of the samples had severity score of 3 to 4; 37% mild symptoms (score = 2) and 4% were asymptomatic (score = 1) (Figure 3).

Detection of viruses

Leaf samples with mean absorbance values at 405 nm (A_{405}) twice or more than those of the healthy leaves in ELISA were considered virus-infected. Leaf samples that showed the expected band sizes of 500 bp for CMV, 586 bp for YMV and 579 bp for yam badnaviruses were considered positive for CMV, YMV and badnavirus, respectively. RT-PCR was not used to detect DMoV. Of 486 samples tested, badnaviruses were detected in 190 samples (39.10%), YMV was detected in 175 samples (36.01%) and CMV was detected in only 7 samples. Mixed infection of CMV and YMV was observed in one sample collected at Yobouékro in the Center region of the country, in a transition zone between the forest and savannah, whereas 64 of the 486 samples analyzed (13.20%) tested positive to both yam badnavirus and YMV. Sixty two per cent (62.1%) of the 486 samples was positive for at least one virus. About 37.8% of symptomatic samples analyzed tested negative to any of the four viruses tested. Negative reaction of apparently symptomatic leaves suggests likely involvement of other viruses especially in the Central zone (Table 1).

The incidence of YMV was 32.5%, 39.3% and 19.4% in West-Central, South and North zones respectively; followed by the Central and the East zones with 10.11 and 1.75% of incidence. YMV was not detected in the North-East. Incidence of badnavirus was high in Central, East and North-East with 11%, 66.7% and 63.64% incidence, respectively. Some asymptomatic leaf samples tested positive to badnavirus and in one of such sample, YMV was also detected.

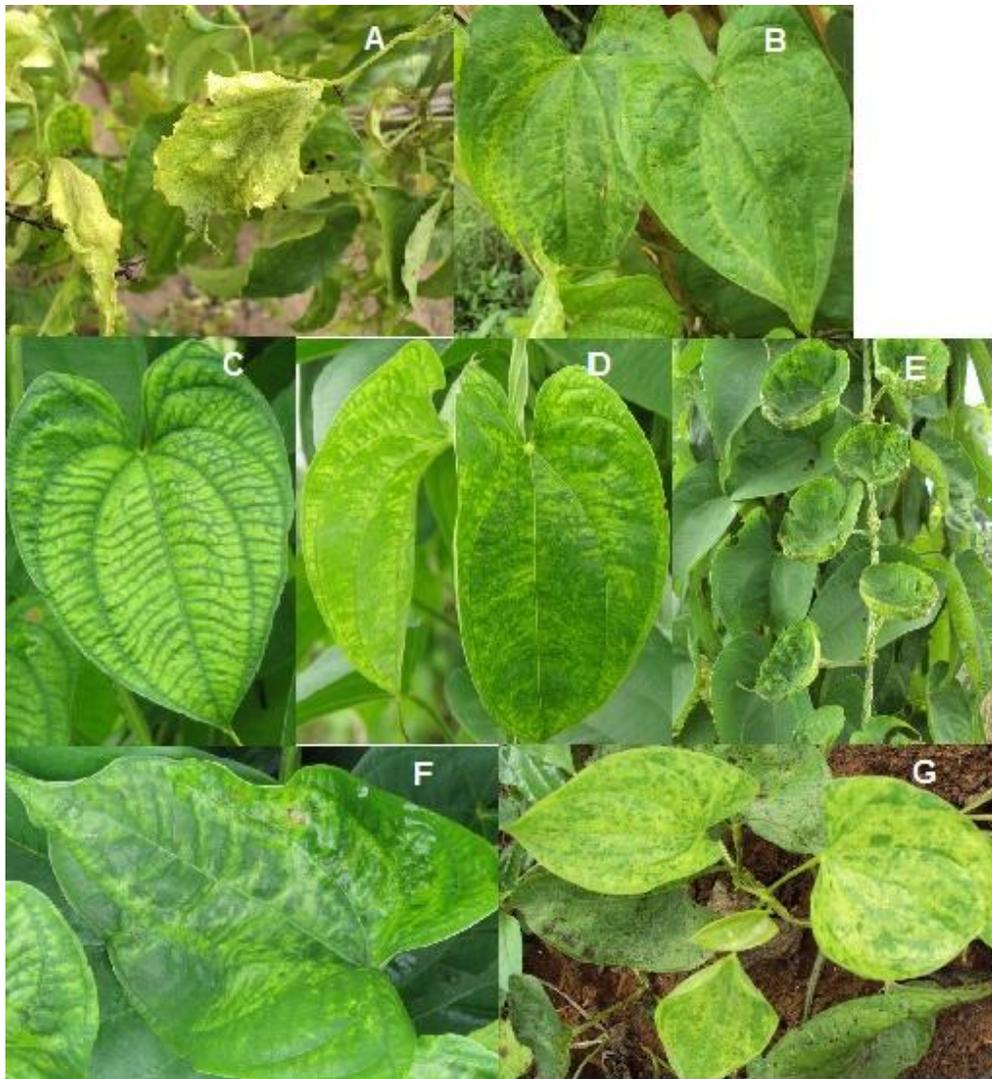


Figure 2. Various symptoms types observed on yam in Côte d'Ivoire. (A) Leaf distortion; (B) Mosaic; (C) Vein banding; (D) Mottling; (E) Coiling; (F) Mosaic and mottling; (G) Mosaic and bleaching.

Badnaviruses and YMV were detected in all the six zones. Mixed infections were highest in the Center, East and in South zones, but CMV incidence was low (Table 2). Virus disease incidence in the six yam production zones varied from 7 to 52% and the severity from 1.7 to 3.6%. Incidence was highest in the Central region and lowest in the Northern region. There seems to be a gradient in the disease incidence, with the highest in the South and the lowest in the North. Viral disease incidence was generally low in the North; however, in one location (Boundiali), the incidence was higher (20%) than in the other areas in the North (Table 3).

DISCUSSION

Diverse symptoms were observed on the yam plants

surveyed in different agro-ecological regions of Côte d'Ivoire. They include leaf coiling, mosaic, mottle, leaf distortion, vein banding, vein clearing and leaf bleaching. Similar symptoms were previously reported in Nigeria, Benin and Côte d'Ivoire (Hughes et al., 1997; Eni et al., 2008; Séka et al., 2009a). Some mixed infections reported in this survey were also observed in previous studies in Benin (Eni et al., 2008) and Côte d'Ivoire (Séka et al., 2009a). Disease incidence and severity varied from North to South with a high rate in the Central region. Analysis of the infected yam leaves confirmed the presence of YMV, CMV and badnaviruses. These viruses have been identified in yam from sub-Saharan Africa (Thouvenel and Fauquet, 1979; Eni et al., 2008; Séka et al., 2009a). Badnaviruses was the most prevalent virus infecting yam in Côte d'Ivoire and in all the zones. There

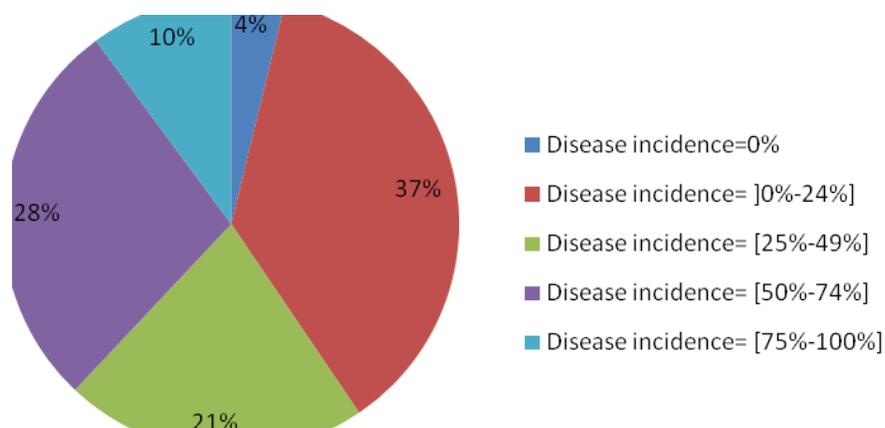


Figure 3. Proportion of symptomatic plants observed in the survey arranged by symptom severity.

Table 1. Incidence and distribution of viruses infecting yam in Côte d'Ivoire.

Zone	Viruses (%)				
	Badnavirus	CMV	DMoV	YMV	Mixed infection
Central	11.3	3.4	0	10.1	18.06
West-central	25.9	0	0	32.5	10.5
East	66.7	0	0	1.7	33.3
North-East	63.6	0	0	0	4.5
North	12.9	0.8	0	19.4	6.4
South	20.5	0	0	39.3	16.2

Table 2. Symptoms and associated viruses in different zones in Côte d'Ivoire.

Zone	Viruses detected	Most symptoms observed
Central	Badnaviruses, CMV, YMV	M, VB, Mo, Mo+VB, VB+C,
West-Central	Badnaviruses, YMV	M, VB, Mo, Mo+VB, VB+C
East	Badnaviruses, YMV	M, VB, Mo, Mo+VB, Mo+VC, VB+C
North-East	Badnaviruses, CMV, YMV	M, Mo, Mo+VB
North	Badnaviruses, CMV, YMV	M, VB, Mo, Mo+VB, VB+C
South	Badnaviruses, YMV	M, VB, Mo, Mo+VB, Mo+VC, VB+C

M= Mosaic; Mo = Mottling; VB= Vein banding; VC = Vein clearing; C = Coiling.

were similar observations in Benin (Eni et al., 2008). It is likely that badnaviruses detected could be endogenous sequences, although no specific studies were done to confirm this (Geering et al., 2010). The fact that some symptomatic plants tested negative in this study indicate the presence of other yam viruses in Côte d'Ivoire or some factors, including poor quality of nucleic acid. Some leaves without symptoms tested positive for viruses. This indicates that the absence of visual symptoms on leaves is not enough to conclude on the absence of virus infection.

High incidence observed in a number of fields suggests

likely origin of infection through planting material. Yam is a clonally propagated crop and traditionally farmers reuse their own seed, which are often infected with viruses. The exchanges of yam seeds in Côte d'Ivoire are more intense in the Central than other zones. The central region represents one of the most important yam production areas in Côte d'Ivoire.

This study reports the presence of several viruses infecting yam in Côte d'Ivoire. The high disease incidence and severity found in the surveyed fields' shows that yam culture is greatly affected by viruses. Badnavirus, followed by YMV and CMV were the most frequently

Table 3. Incidence and severity of virus infections in different yam growing zones in Côte d'Ivoire.

Zone	Mean severity	Mean incidence (%)
Central	3.45 ± 0.10 ^a	52.47 ± 2.32 ^a
West-Central	3.62 ± 0.11 ^a	43.89 ± 2.34 ^b
East	3.01 ± 0.09 ^b	42.79 ± 2.65 ^b
North	3.49 ± 0.08 ^a	22.97 ± 1.96 ^c
North East	1.72 ± 0.21 ^c	7.27 ± 1.88 ^d
South	3.54 ± 0.08 ^a	42.01 ± 2.12 ^b
F	19.56	32.15
P	<0.001	<0.001

Means followed by the same letter are not significantly different from one another at LSD ($\alpha = 0.05$; $P < 0.001$).

encountered viruses in the country. Further work on molecular characterization of these viruses will facilitate the understanding of diversity of viruses in the country. There is a need to promote production and dissemination of virus-free planting materials to reduce virus disease incidence in Côte d'Ivoire.

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