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RESEARCH ARTICLE

CHARACTERIZATION OF *CUCUMBER MOSAIC VIRUS* (CMV) ASSOCIATED WITH *CUCUMIS SATIVUS* L. DISEASE IN PERIURBAN AREAS IN SOUTHERN CÔTE D'IVOIRE

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ABSTRACT

Côte d'Ivoire produces cucumber (*Cucumis sativus* L.) in peri-urban areas. The cultivation is threatened by a disease which symptoms are similar to a virus disease despite the use of varieties so-called resistant to virus diseases. In order to implement a control method, a comprehensive diagnosis was made. After assessing the extent of the disease in two large cucumber production areas on the outskirts of the city of Abidjan, two direct and indirect diagnoses were made. The first one was made on the basis of observation and description of the characteristic symptoms of viral infection. As for the second one, samples of symptomatic and asymptomatic leaves were randomly collected from infected plots. Molecular analyses were done using PCR followed by reading on an electrophoresis gel. 92.42% of cucumber plants were infected in the plots with a high average severity of 4. The direct diagnosis led to conclude that the cucumber was CMV-infected. PCR products showed the presence of CMV virus responsible for cucumber mosaic in infected leaves. The symptoms developed by cucumber plants and the identification of the viral agent associated with the symptoms justified an infection of cucumber plots in peri-urban areas of Abidjan by CMV with a much stronger pressure than 31 years earlier.

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INTRODUCTION

Cucumber is a market garden crop originating from the Asian continent, more specifically, from India (Bisognin, 2002). Cucumber is one of the most consumed fruits in the world among market garden crops (Holland *et al*., 1991). Peri-urban market garden crops are developing significantly in developing countries. In Côte d'Ivoire, cucumber (*Cucumis sativus* L.) is produced throughout the country and in all seasons (Nouza, 2010). The yields contribute to supply the markets of urban municipalities, places of high consumption (Kouakou, 2009). Cucumber cultivation is a source of income for smallholders. The world cucumber yield is estimated at about 75 million tons to meet the demand of populations. In Côte d'Ivoire, the yield was 19 800 tons and sold between 200 and 500 francs CFA per kilogram. In less than three months, the yields could be estimated at 7 million CFA francs per hectare (Nouza, 2010). However, the yields are declining because of high parasite pressure for producers whom not have any effective means of control during the last three years in Côte d'Ivoire. Virus-resistant varieties of cucumber have been successfully used by producers against virus attacks in other countries (Bisognin 2002, Krístková *et al*., 2003).

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In Côte d'Ivoire, phytosanitary treatments are carried out with the use of the variety Mydas resistant to viral infections, but symptoms similar to viral attacks have been constantly observed on leaves and fruits with a drastic drop in yields by farmers. Infections recur in every cropping season of Cucumber and symptoms persist on plants in all peri-urban production areas. The development of this disease has been a major constraint to cucumber production in recent times in Côte d'Ivoire due to the fact that the virus responsible for such viral symptoms is not identified.

A variety of viruses cause infections in cucumber with various symptoms. Cucumber reproduction is done by pollinator or sucker and biter insects very often vectors of viral phytopathogenic agents. Aphids are vectors of *Cucumber Mosaic Virus* (CMV), *Papaya Ring spot* Virus (PRSV), *Zucchini Yellow Mosaic Virus* (ZYMV) (Baire *et al*., 2010). Moreover, *Cucumber Vein Yellowing Virus* (CVYV), *Cucumber Leaf Spot Virus* (CLSV), *Cucumber Toad Skin Virus* (CTSV) are also found on cucumber. The identification of the viral strain responsible for cucumber infection in infected plots becomes an important step in establishing an effective control method. This work aims at making a direct and indirect diagnosis of the cucumber infection disease in peri-urban areas of Côte d'Ivoire.

MATERIALS AND METHODS

Direct diagnosis of cucumber infection: Direct diagnosis was made on the basis of the disease symptoms in plots during surveys. The level of infection of plots was assessed. The foci of the disease as well as the symptoms were observed and described.

Infection status of cucumber plots surveyed: The status of the disease was assessed in two large peri-urban cultivation areas of geo-referenced cucumber with GPS1: N1 5° 19′ 43″ l; W1 3° 56′ 75″ and GPS2: N2 5° 12′ 00″; W2 3° 44′ 00″ coordinates. The extent of cucumber infection in infected plots was assessed by disease prevalence. Out of 60 cucumber plants selected randomly per plot, the ratio of the number of infected plants to the total of selected plants was determined (Bernardo 2014):

$$\mathbf{P} = Ni/Nt \quad (1)$$

P: prevalence of infection, Ni: number of infected plants, Nt: total number of plants sampled.

The activity of the causative pathogen was estimated by the severity of the symptoms caused per plant. Out of the 60 plants selected, the number of infected leaves was counted per plant. The percentages of infected leaves were calculated and divided into intervals. Severity scores were assigned to each plant based on the percentage of infected leaves on the Lava Scale (2009). 1: No visible symptoms; 2: 1-25% infected leaves; 3: 26-50% infected leaves; 4: 51-75% infected leaves; 5: more than 75% infected leaves.

Symptomatology: The foci of the disease were observed in the plots. On infected plants, symptoms were described on the leaves during site surveys. To this end, 60 infected cucumber plants of the variety Mydas RZ F1 were randomly selected. The characteristic and specific symptoms of a viral infection were identified according to the shape, color of leaves and fruits.

Indirect diagnosis of cucumber infection

Sampling: Fresh leaves of cucumber variety "Mydas", were collected randomly in the most infected plots observed during surveys. In each plot, 30 non-senescent mature leaves, symptomatic or not, were collected per 35 day-old plant. The collected leaves were packaged separately in sterile labeled plastic and transported for storage at -20°C before being used for laboratory analyses.

Molecular identification of the virus associated with cucumber symptoms

DNA extraction: A composite sample of derived leaves was prepared per area and 33 were randomly selected for nucleic acid extraction. The leaves selected were disinfected using blotting paper soaked in 70% ethanol. The cleaned leaves were disinfected from the blade while keeping the veins. The veins of each leaf were cut and 200 mg were weighed per sample. Extraction of nucleic acids from leaf veins was done according to the method of Doyle and Doyle (1990). The DNA obtained was eluted in 50 µl of sterile distilled water and then kept in the freezer at -20°C for PCR.

PCR amplification by Reverse Transcriptase (RT-PCR)

RT-PCR was performed with CMV 1 (5'-GCCGTA AGCTGGATGGACAA-3') and CMV 2 (5'-TATGATA AGAAGCTTGAAACGCG-3") primer pair (Wylie *et al*., 1993) in a reaction volume of 12.5 µl for CMV detection in 33 samples according to the method of Wylie *et al*. (1993). The Mix consisted of: 6.38 µl of sterile distilled water, 2.5 µl of 5X green buffer, 0.25 µl of dNTPs, 0.75 µl of $MgCl_2$ (25mM), 0.25 µl of CMV1 and CMV2 primers, 0.06 µl of Taq polymerase and 0.06 µl of reverse transcriptase.

To the total volume of 10.5 µl obtained, 2 µl of nucleic acid diluted 1:50 was added to each reaction micro-tube, thus giving a final reaction volume of 12.5 µl. RT-PCR reactions were performed in the thermocycler (BIO-RAD T100) according to the program: an initial incubation at 44°C for 30 min, followed by a denaturation cycle of 5 min at 95°C, then by 35 cycles each comprising a denaturation of 45 sec at 95°C, a hybridization of 45 sec at 54°C and an elongation of 45 s at 72°C. Finally a final amplification at 72°C for 7 min ended the RT-PCR. The products amplified by RT-PCR were separated by 1.5% agarose gel electrophoresis incorporated from Syber Safe in a 1X TAE solution for 25-30 min at 100 V and visualized under UV light.

Statistical analysis

The STATISTICA 7.1 software was used. Two one-way analyzes of variance (ANOVA 1) were performed to compare the prevalence and average severity of symptoms developed by cucumber plants in both production areas. The different homogeneity groups were determined by a post ANOVA test of Newman and Keuls in case of significant difference between averages at 5% threshold. A classification of the different homogeneity groups was made so as to determine the highest average groups.

RESULTS

The symptoms observed on cucumber plants at both collection sites were organ deformation and color change. Cucumber plants developed a variety of symptoms ranging from the simplest to the most complex on the leaves observed. Organ deformation was characterized by convex leaf curling (Fig. 1), leaf blistering (Figure 2), mosaic of infected plant leaves (Fig. 3), the fruits showed chlorosis along the fruits (Fig. 3 d) and fruit malformation or distortion and size reduction (Fig. 4). The infected leaves showed the different simple and complex symptoms. Convex leaf curling was associated with vein banding and mosaic. Likewise, leaf curling was associated with blistering and/or mosaic.

Serious foliar infection of cucumber

A high prevalence of foliar infection was noted on cucumber plants from the different plots surveyed (Table 1). Disease prevalence on both sites was 92.42% infected plants. Symptom complexes of blistering, mosaic, vein banding and curling were more observed on the leaves of the plants of infected plots at 45.45%. Mosaic alone was estimated at 15.15%. The statistical analysis revealed a very highly significant difference ($F = 15.20$, $p = 0.00$) between the average prevalence of symptoms found on infected plants.

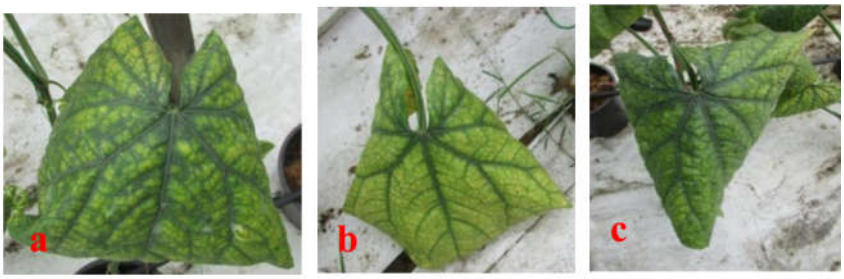

Fig. 1. Infected leaves of cucumber. a: Convex curling of infected leaves. b: Convex curling and vein banding.c: Convex curlingand mosaic

Fig. 2. Blistering of infected leaves. a: Blistering plus leaf reduction. b: Blistering plus mosaic. c: Blistering plus margin stricture

Fig. 3. Cucumber plants. a: Apparently healthy plant. b: Plants with chlorotic leaves. c: Plants with mosaic-infected leaves

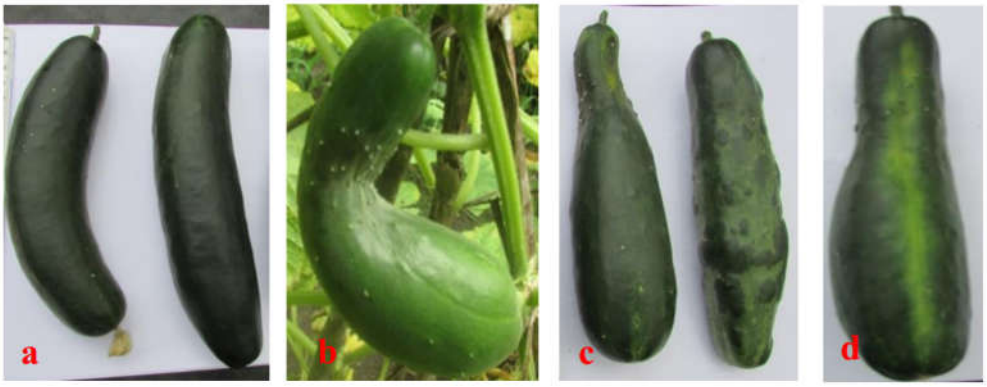

Fig. 4. Cucumber fruits. a: Apparently healthy fruit. b: Deformed reduced fruit. c: Atrophied fruit faded at the end with blisters d: Chlorosis in longitudinal strip of the fruit

Table 1. Average prevalence of foliar infections depending on symptoms in surveyed plots in peri-urban areas

Types of sample	Foliar symptoms		Average prevalence
Symptomatic	Simple symptoms	Mosaic	15.15 ± 0.36^{bc}
		Blistering	4.54 ± 0.21^{e}
		Convex curling	3.03 ± 0.17^{cd}
	Complex symptoms	Blistering and Mosaic	1.51 ± 0.12^{d}
		Blistering, Mosaic and Vein banding	21.21 ± 0.41^{b}
		Blistering, Mosaic, Vein banding and Convex curling	45.45 ± 0.50^{a}
Asymptomatic		Apparently healthy	9.09 ± 0.28^{cd}

Development of severe foliar symptoms: Cucumber plants infected have developed a variety of symptoms with a high average severity. The statistical analysis indicated a very highly significant difference ($F = 16.96$, $P = 0.002$) between the severity levels of disease symptoms on cucumber plants. Disease severity index ranging from 1 to 5 was used to group average severities of symptoms on plants infected. 40% of the plants showed infection with a severity of 4 while only 3.33% were affected with severity 1. Cucumber plants were infected with the disease with a high severity of symptoms in the surveyed plots.

Table 2. Average severities of cucumber foliar infection in peri-urban areas

Severity ratings	Average severities
1	3.33 ± 0.18^{e}
2	6.67 ± 0.25^{d}
3	26.67 ± 0.45^{b}
4	40 ± 0.50^{a}
5	23.33 ± 0.43^{c}

CMV virus associated with foliar symptoms of cucumber infection: The electrophoresis gel of PCR products revealed the presence of cucumber virus (CMV) in seven infected leaf samples out of 30 tested (Fig. 5), that is, 21.21% tested leaves. Depending on the symptoms on tested leaves (Table 3), sample 5 showed a complex of mosaic and vein banding while 8; 9; 10 and 11 showed mosaic. Sample 29 was a leaf showing a complex of blistering, mosaic, vein banding and convex curling. In contrast, sample 33 was asymptomatic. A variety of symptoms is caused by CMV virus on cucumber leaves. However, the changes of the limb and vein color materialized by mosaic and vein banding, respectively, are the most common symptoms associated with CMV virus in cucumber.

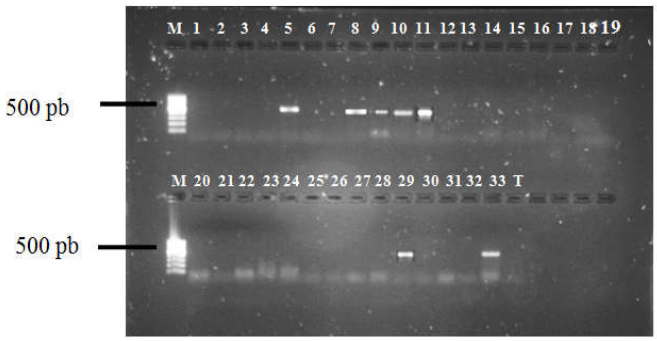

Figure 5. Electrophoresis gel of PCR products of cucumber leaf samples for detection of CMV on a 1.5% agarose gel

M: indicated fragment size (in base pair) by the 100 bp DNA Ladder molecular weight marker (Thermo Scientific), T: Negative control (viral nucleic acid-free sample), bp: base pair, 1 -33: samples extracted from nucleic acid of cucumber leaves. The molecular weight of CMV DNA fragments is 500 bp.

Table 3. State of infection of leaves with CMV_I virus depending on observed symptoms

Samples	Symptoms	Presence of CMV virus
5	Mosaic and Vein banding	+
8		+
9	Mosaic	+
10		+
11		+
29	Mosaic, Vein banding and blistering	+
33	Asymptomatic	+

DISCUSSION

Cucumber plants have developed a variety of symptoms ranging from the simplest to the most complex ones on leaves showing features of viral infection. The leaves of infected cucumber plants developed deformation and discoloration of leaves and organ fruits showing features of viral infection. Leaf blistering, curling and mosaic caused by virus infection have been reported by Septariani *et al*. (2014) and Supyani *et al*. (2017) on several cucurbitaceae including cucumber. The first objective of this study is to identify and characterize the virus responsible or associated to specific symptoms noted on those cucumber plants. Indeed, according to the mechanism of infection of phytopathogenic viruses, the modification of the host genome could lead to a modification of the protein synthesis in the host, hence the deformation and discoloration of vegetative and reproductive organs in the plant infected. The interruption of chlorophyll synthesis in limb chloroplasts might be the first pathogenicity developed by the virus before any pathogenic activity of leaves. Mosaic might be one of the first symptoms developed on leaves in case of viral attack in cucumber. The same symptom has also been reported in the research works of Zitter and Murphy (2009) and Supyani *et al*. (2017) on viral infections of cucurbitaceae. According to Lecoq and Desbiez, (2012), CMV causes the same symptoms of mosaic on leaves and sometimes on fruits when the disease is quite serious in cucumber as observed.

Cucumber mosaic is a viral disease that is expressed on cucumber by mosaic, vein banding and malformations according to Zitter and Murphy (2009). Direct diagnosis of this cucumber disease might lead to conclude that the cucumber was infected with mosaic virus. However, the symptoms observed might vary depending on the infective CMV strain (Ben Hassena, 2009). Moreover, in cucurbitaceae, many viruses cause the appearance of mosaic symptoms (Sydanmetsa and Mbanzibwa, 2016); hence the interest of indirect diagnosis. CMV causes symptoms depending on the host, the environment and the physiological condition. Thus, in addition to the symptoms mentioned above, viruses could cause several abnormalities that can cause stunted growth, leaf reduction, leaf and fruit malformations (Astier *et al*., 2001). Symptoms appear on younger leaves, which curl down and become spotted, deformed and reduced in size (Jarvis and Nuttal, 1979). The combined pathogenic activities of several viruses could justify the diversity as well as the complexity of the symptoms observed in infected plots. Indeed, the diversity of symptoms characteristic of viral infection observed could be the result of a mixed infection with several viruses according to Lecoq and Desbiez (2012), Septariani *et al*., (2014), or a synergistic action of different CMV strains (Lecoq and Desbiez, 2012). The use of run of seed by producers could increase infection rates in plantations and justify the high prevalence of CMV foliar infection noted on plants infected with high average severity. The very high average prevalence and severities of this viral infection in cucumber production areas in Côte d'Ivoire is a threat to this peri-urban crop. The variety "Mydas" so-called resistant used by producers might have lost its resistance to the virulence of the CMV viral strain found in infected cucumber organs. Cucumber leaves revealed the presence of CMV in symptomatic and asymptomatic leaves. They might be very sensitive to the CMV strain identified in this work and develop metabolic disturbances leading to the expression of symptoms that are in some cases masked or not visible (Kummert and Semal, 1996).

This phytovirus is considered as the one that infects several plants of agricultural importance in the world (Palukaitis and Garcia-Arenal, 2003, Scholthof *et al*., 2011). Kouadio *et al*. (2015) mentioned that CMV was identified in Côte d'Ivoire in 1987 by Fauquet and Thouvenel on a range of host plants including cucumber. Subsequently, the recent works of Séka *et al*. (2009), Sorho *et al*. (2014) as well as those of Toualy *et al*. (2014) confirmed the presence of CMV in Côte d'Ivoire respectively on yam and certain plants of the Solanaceae family. In 1987, *Cucumber mosaic virus* (CMV) was reported in Côte d'Ivoire on a range of host plants including cucumber by Fauquet and Thouvenel (1987). 31 years later the virus is still more present in several cultivation areas with strong pressure marked by a high severity of symptoms and especially a reduction in yield. Environmental conditions as well as cultivated cucumber varieties are still favorable for the spread of CMV in Côte d'Ivoire. The development of cucumber cultivation could not be possible without the establishment of suitable CMV control methods.

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