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Identification and Distribution of Some Viral Diseases of Solanaceous in Côte D'ivoire

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ABSTRACT: Tomato, pepper and eggplant belong to the forty vegetable species most produced in the world (FAO, 2008). These solanaceous plants, which are sources of vitamins, serve as a source of nutritional supplements especially among low income populations, that subsist on diets of cereals and starchy foods. They are a source of income and employment for small rural and peri-urban farmers. As a result of their tolerance to various climates, they are cultivated in all agro-ecological zones of tropical and subtropical areas, throughout the year. However, their production is inhibited by many constraints, some of which are biotic involving fungi, bacteria and viruses. In May 2013, sampling of leaves of the three solanaceous plants was carried out in various zones of production in Côte d'ivoire (Songon, Divo, Sinfra and Djèbonoua). On the basis of viral symptoms observed on the leaves, 117 samples were collected. Serological tests, DAS-ELISA, TAS-ELISA and ACP-ELISA, were used to detect viruses using specific antibodies. The presence of *Cucumber mosaïc virus* (CMV) and *Pepper veinal mottle virus* (PVMV) was confirmed in Côte d'Ivoire. On the other hand, no sample was found positive with *Tomato Yellow Leaf Curl Virus* (TYLCV), using the TAS-ELISA method. With the ACP-ELISA method, a new virus which could be *Potato virus Y* (PVY-n) was highlighted for the first time in Côte d'Ivoire. On the whole, the production of solanaceous plants in Côte d'Ivoire is threatened by viral diseases. Adequate support should be given to farmers to guarantee a healthy and durable production of solanaceous food crops.

Keywords: Solanaceous, Tomato, Pepper, Eggplant, Virus, CMV, PVMV, TYLCV, PVY-n

RESUME : La tomate, le piment et l'aubergine font partie des 40 espèces légumières les plus produites dans le monde (FAO, 2008). Ces solanacées qui sont des sources de vitamines constituent une source de suppléments nutritionnels par rapport au régime alimentaire basé sur les céréales et les féculents, notamment parmi les populations à faibles niveaux de revenus. Elles sont une source de revenus et d'emplois pour les petits exploitants agricoles ruraux et péri-urbains. Du fait de leur tolérance à divers climats, elles sont cultivées dans toutes les zones agro-écologiques dans les régions tropicales et subtropicales tout au long de l'année. Cependant, leur production est confrontée à de nombreuses contraintes biotiques, en particulier fongiques, bactériennes et virales. Au mois de mai 2013 une collecte d'échantillons de feuilles des trois solanacées a été effectuée dans différentes zones de production de Côte d'Ivoire (Songon, Divo, Sinfra et Djèbonoua). Sur la base des symptômes virales observés sur les feuilles, 117 échantillons ont étés collectés. Les tests sérologiques DAS-ELISA, TAS-ELISA et ACP-ELISA ont été utilisés pour détecter les virus à l'aide d'anticorps spécifiques. La présence du *Cumcumber Mosaïc Virus* (CMV) et du *Pepper veinal mottle virus* (PVMV) a été confirmée en Côte d'Ivoire. Par contre, aucun échantillon n'a été positif au *Tomato Yellow Leaf Curl Virus* (TYLCV) avec la méthode TAS-ELISA. Avec la méthode ACP-ELISA un nouveau virus qui pourrait être le *Potato virus Y* (PVY-n) semble avoir été mis en évidence pour la première fois en Côte d'Ivoire. Au total la production des solanacées en Côte d'Ivoire est également menacée par les viroses. Des conseils et mesures adéquats, devraient être prodigués aux agriculteurs afin de garantir une production saine et durable des solanacées.

Mots clés : Solanacées, Tomate, Piment, aubergine, Virus, CMV, PVMV, TYLCV, PVY-n

Introduction

The family, solananceae, is one of the most important food crops among angiosperms. This family represents the third taxon of economic importance to share the diversity of crops. It includes hundreds of genus and about 2500 species (Olmstead et al., 2008), half of which belong to the genus Solanum (Weese and Bohs, 2007). The Solanaceae family includes economically important food crops such as tomato (*Lycopersicum esculentum* Mill.), Eggplant (*S. melongena* L.), and pepper (*Capsicum* sp.) (Daunay

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and Lester, 1989). These three are among the 40 solanaceous vegetable crops most produced in the world (Lebeau, 2012).

In Côte d'Ivoire, gardening is a main income generating activity and has a strong socio-economic impact on the population (Coulibaly and Bly, 2000; Adama, 2004). These vegetable crops (tomato, eggplant, pepper, etc) are a source of nutritional supplements in relation to a diet based on starchy foods, especially among people with low levels of income (Nono-Womdim, 2001). These crops also increase the income of small farmers and are important sources of vitamins and minerals required in a balanced diet (Ildefonso, 1995; Coulibaly and Bly, 2000). These crops are subject to various attacks due to pests and diseases. Among the diseases, viruses cause significant yield losses on farmers' fields. Studies by Fauquet and Thouvenel (1987) identified the Cucumber Mosaic Virus (CMV) and Pepper veinal mottle virus (PVMV) on plants grown in Côte d'Ivoire.

However, since that time, few studies have been conducted to confirm the presence of both viruses in the Solanaceae. To our knowledge, no study has been conducted to identify PVY-n in culture of Solanaceae. To improve the productivity of solanaceous plants, it is necessary to study these viruses. Hence, the need to address the serological diagnosis of viruses infecting solanaceous food crops in Côte d'Ivoire. The objective of this study was to serologically identify and to know the distribution of the four viruses infecting tomato (*Lycopersicon esculentum* Mill.), Eggplant (*Solanum melongena* L.) and pepper (*Capsicum* sp.) in four departments (Songon, Divo, Sinfra and Djébonoua) of vegetable production in Côte d'Ivoire.

1.MATERIALS AND METHODS

1.1 Exploration and Collection of samples

A survey was conducted in four major Solanaceae producing areas (Songon, Divo, Djébonoua and Sinfra) in Côte d'Ivoire. Leaf samples of tomato, eggplant and pepper were collected on the basis of viral symptoms such as mosaic, deformations, chlorosis, bleaching and stunting. A total of 117 samples were collected, 65 of which were symptomatic tomato leaves, 33 pepper and eggplant in 19 different departments surveyed (Table 1). After observing the symptoms, two to four leaves of three cultures were collected per plant. The samples were placed in plastic, labeled and stored in the cooler until the bags were collected in the evening. To avoid loss of viral infective power, the samples were kept by the method of Bos (1977). The samples were cut in the evening with a collection of sample blade and placed in pots containing 4 g of calcium chloride ($CaCl_2$), which was separated from the sample by a sterile cotton. The sealed jars were stored in the cooler until the end of the collection and the samples were refrigerated at 4°C in the Laboratory of Plant Physiology, University Felix Houphouet Boigny of Cocody-Abidjan. The samples were then subjected to serological ELISA (Enzyme Linked Immunosorbent Assay) for the detection of viruses.

Table 1. Number of samples collected by department.

Department	Tomato	Eggplant	Chili Pepper	Total
Songon	11	06	05	22
Divo	14	03	02	19
Sinfra	16	10	23	49
Djèbonoua	24	00	03	27
Total	65	19	33	117

1.2 Serological Tests

All samples were assayed using Double Antibody Sandwich (DAS), Antigen Coated Plate (ACP) and Triple Antibody Sandwich (TAS) ELISA tests, as described by Clark and Adams (1977). The tests were conducted in the Plant Pathology Laboratory at University of Parakou in Benin. The samples were tested for four viruses namely PVMV virus, CMV, PVY-n and TYLCV with antibodies and positive controls provided by DSMZ, Germany. The various dilutions and washes of ELISA plates at different stages of the protocol were in accordance with the manufacturer's ELISA kits.

1.2.1 Detection of virus by DAS ELISA

The DAS ELISA method was used for the detection of CMV and PVMV. Dried sample leaves of the solanaceous plants were diluted (1:40 g / v) in extraction buffer (PBST + 2% PVP (PVP-15 Serva polyvinyl pyrrolidone) at pH 7.4 for 5 min. Samples were then ground and the supernatant was removed and stored at -20°C for 16 h before use. Antibodies of CMV and PVMV were diluted (1: 1000) into the binding buffer (pH 9.6) and used to cover different ELISA plates of 100 µl solution of the mixture, placed in each well and the plates were incubated at 37°C for 4 h. Then, the plates were washed in washing buffer (PBS-T) and 100 µl of extract diluted in the sample extraction buffer was placed in wells of the plate. Two consecutive wells were used for each sample, with positive and negative controls. The plates were incubated overnight at 4°C, followed by

three successive washes of three (3 min) with washing buffer (PBS-T). Two types of conjugated antibodies were diluted (1: 1000) in the buffer and the conjugate of this mixture (100 µl) was deposited in each well of the plate. The plates were again incubated at 37°C for 4 h and washed. The tablets of the p-substrate components (nitrophenil phosphate) were dissolved in proportions of 10 mg in 10 ml of substrate buffer (pH 9.8) and 100 µl of this mixture was placed in each well. The plates were placed at room temperature for the reading of optical density (OD) at 30 min, 1h and 2 h after incubation. The optical density readings were made with an ELISA plate reader at 405 nm absorbance. The spectrophotometer was connected to a computer (HP, Pentium 4) for visualization of the results using the KC Junior and the data was exported to Excel software.

1.2.2 Detection of virus by ACP ELISA

The ACP ELISA was used to detect PVY-n. Leaf samples were soaked in the extraction buffer (binding buffer + 0.05 M DIECA) after diluting at 1:40 (g / V)) for 5 min. The samples were then ground and 100 µl of supernatant was removed and placed in the wells of the ELISA plate. The plates were incubated at 37°C for 16 h, then washed with wash buffer (PBS-T). 100 µl mixture of 2% skimmed milk dissolved in PBS-T were placed in the wells and incubated at 37°C for 30 min. Then, the blocking solution was poured and the plates were tapped dry using absorbent paper. The MAb was diluted (1: 1000) in the conjugate pad and 100 µl of the mixture was removed and placed in the wells. The plates were then incubated at 37°C for 4 h and washed with PBS-T. The conjugate of the antibody (RaM-AP) was diluted (1: 1000) in the conjugate and buffer; 100 µl of this mixture was collected and put in the wells. The plates were incubated at 37°C for 2 h, then washed with PBS-T. Tablets of P-nitrophenyl phosphate (PNPP) were dissolved in the substrate buffer and 100 µl of this mixture was removed and placed in the wells. The plates were then incubated at 37°C and the optical density (OD) was read in the spectrophotometer for 30 min, 1 h and 2 h after substrate deposition.

1.2.3 Detection of viruses by TAS ELISA

TAS ELISA was used for the detection of TYLCV. Leaf samples were soaked in the extraction buffer (PBST + 2% PVP) at a dilution of 1:40 (g / V) for 5 min. The samples were then ground and the supernatant was removed and placed in Eppendorf tubes and stored at -20°C for 16 h before utilisation. The antibody (IgG) diluted (1: 1000) in binding buffer (pH 9.6) and 100 µl of the mixture solution was put in each well. The plates were then incubated at 37°C for 4 h, followed by three successive washes of three (3) minutes with wash buffer (PBS-T). 100 µl mixture of 2% skimmed milk dissolved in PBS-T were placed in the wells and the plates were incubated at 37°C for 30 min. Then, the blocking solution was poured and the plates were tapped dry on absorbent paper. 100 µl of the supernatant samples extracted using the extraction buffer was placed in the wells of the plates. Each sample was placed in two successive wells and the plates were incubated at 4°C for 16 h, followed by three successive washes of three (3) minutes with PBS-T. The MAb was diluted (1: 1000) in the conjugate pad. Then, 100 µl of this mixture was placed in the wells and the plates were incubated at 37°C for 4 h. Then, the plates were washed with PBS-T and 100 µl antibody RaM-ap diluted (1: 1000) in the conjugate buffer were placed in the wells and the plates incubated at 37°C for 2 h. The plates were then washed and 100 µl mixture tablets of P-nitrophenyl phosphate (PNPP) dissolved in substrate buffer was removed and placed in the wells. The plates were then incubated at 37°C and the optical density (OD) was read with a spectrophotometer after 30 min, 1 h and 2 h.

1.2.4 Analysis of optical densities

The threshold of positivity (SP) was determined using optical densities (OD) of the wells containing the negative control of the second reading, after 1 h of incubation according to the formula:

SP = 2 X the mean absorbance at 405 nm (in wells containing the negative control).

A sample is said to be positive for DAS-ELISA, ACP-ELISA and TAS ELISA, if the average OD exceeds the threshold of positivity (SP) test. A sample is positive if it contains the virus for which it was tested. A sample is negative if the average OD is less than SP.

2-RESULTS

2.1 Rate of viral infections by culturing

Table 2 shows viral infections present in cultivated plots in the different departments visited. For cultivated tomato, 55 samples were positive for the virus sought with occurrence at 86.61%. 15 and 29 samples, were positive for eggplant and peppers with a percentage of 78.95 and 87.88, respectively. The Department in Sinfra had the highest percentage of infection (93.75%) for growing tomato followed by that in Divo (85.71%). For the cultivation of eggplant, the department of Songon had the highest percentage (83.33%), followed by that of Sinfra (80%). For growing chili, Djébonoua and Divo departments had the highest percentage (100%) followed by that of Sinfra (86.96%).

Table 2. Rates of viral infections and culture department.

Department	Tomato				Eggplant				Chili Pepper			
	No. of samples	Positive	Negative	Percentage	No. of samples	Positive	Negative	Percentage	No. of samples	Positive	Negative	Percentage
Songon	11,00	8,00	3,00	72,73	6,00	5,00	1,00	83,33	5,00	4,00	1,00	80,00
Divo	14,00	12,00	2,00	85,71	3,00	2,00	1,00	66,67	2,00	2,00	0,00	100,00
Sinfra	16,00	15,00	1,00	93,75	10,00	8,00	2,00	80,00	23,00	20,00	3,00	86,96
Djébonoua	24,00	20,00	4,00	83,33	0,00	0,00	0,00	0,00	3,00	3,00	0,00	100,00
Total	65,00	55,00	10,00	84,61	19,00	15,00	4,00	78,95	33,00	29,00	4,00	87,88

2.2 Distribution of virus by culture in the departments

The percentages of viral infection detected in different departments are shown in Table 3. CMV was detected in tomato samples as follows: 54.54% in Songon, 50% in Divo, 68.75% in Sinfra, and 62.50% in Djébonoua. In eggplant samples, CMV was not detected in any of the departments visited. In samples of chili, 20 and 13.04% of CMV were identified in Songon and Sinfra, respectively. The PVMV was identified in tomato samples: 28.57% Divo, 18.75% Sinfra, and 12.50% for Djébonoua tomato. The PVMV was detected in eggplant samples as 33.33 and 20% in Songon and Sinfra, respectively. However, it has neither been identified in Djébonoua nor Divo. The PVMV was also detected in samples of chili, 40% in Songon, 100% in Divo, with 65.22 and 66.67% in Sinfra and Djébonoua, respectively. PVY-n was identified in samples of tomato such that 18.18% were in Songon, 7.14% in Divo, in addition to 6.25 and 8.33%, in samples from Sinfra and Djébonoua, respectively. In eggplant samples from Songon, Divo and Sinfra, PVY-n was detected as 50, 66.67 and 60%, respectively. However, in samples of chili from Songon, Sinfra and Djébonoua, PVY-n was detected as 20, 8.7 and 33.33%, respectively. In the tomato samples assessed, CMV was detected in 60%, PVMV in 15.38% while 9.23% were positive for PVY-n. In samples of chili, CMV and PVY-n were detected in 12.12% while PVMV was detected in 63.64%. In eggplant samples, PVMV was detected in 21.05%, PVY-n accounted for 57.89% and CMV was absent (Figure 1).

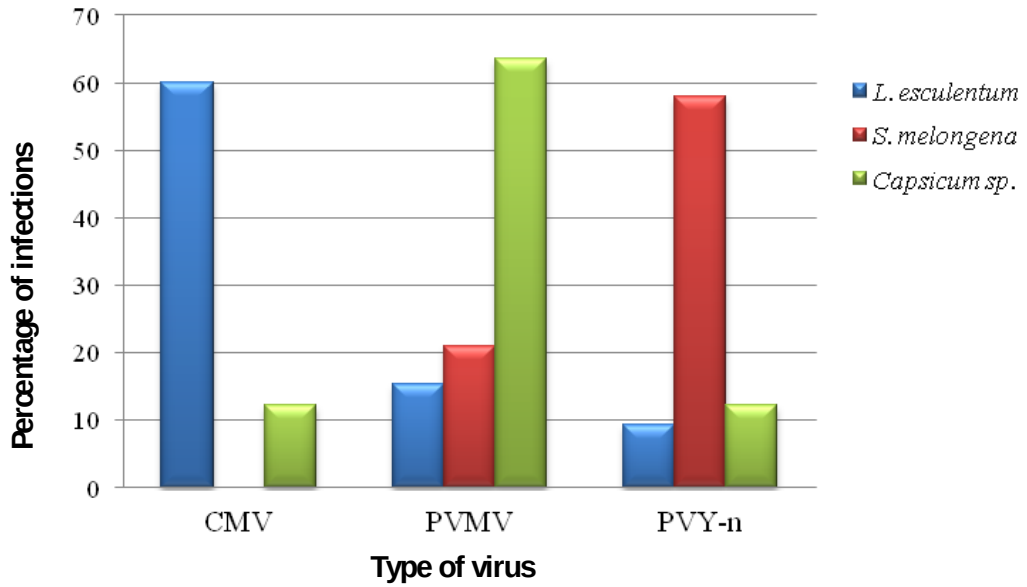

Figure 1. Percentage of viral infections in different cultures.

Table 4. Percentages of samples infected by viruses to each culture in the departments.

Department	Tomato						Eggplant						Chili Pepper					
	CMV		PVMV		PVY-n		CMV		PVMV		PVY-n		CMV		PVMV		PVY-n	
	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%	No. of sample	%	No. of sample	%	No. of sample	%	No. of sample	%
Songon	6,00	54,54	0,00	0,00	2,00	18,18	0,00	0,00	2,00	33,33	3,00	50,00	1,00	20,00	2,00	40,00	1,00	20,00
Divo	7,00	50,00	4,00	28,57	1,00	7,14	0,00	0,00	0,00	0,00	2,00	66,67	0,00	0,00	2,00	100	0,00	0,00
Sinfra	11,00	68,75	3,00	18,75	1,00	6,25	0,00	0,00	2,00	20,00	6,00	60,00	3,00	13,04	15,00	65,22	2,00	8,70
Djébonoua	15,00	62,50	3,00	12,50	2,00	8,33	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,00	66,67	1,00	33,33
Total	39,00	60,00	10,00	15,38	6,00	9,23	0,00	0,00	4,00	21,05	11,00	57,89	4,00	12,12	21,00	63,64	4,00	12,12

Nb. Sample .: sample number,%: percentage.

2.3 Different types of viral infections

Figure 2 shows different types of infections in culture. In cultivated tomato plots, simple infections occurred at the rate of 39.39, 10.10 and 6.06% for CMV, PVMV and PVY-n, respectively. Double infections recorded 49.49, 45.45 and 16.16% for CMV+PVMV, CMV+PVY-n and PVMV+PVY-n, respectively; while triple infections (CMV+ PVMV+PVY-n) accounted for 55.55%. In eggplant, simple infections were recorded as 4.04 and 11.11% for PVMV and PVY-n, respectively. In addition, double infections (PVMV+PVY-n) accounted for 15.15%. In pepper, simple infections occurred at the rate of 4.04, 21.21 and 4.04% for CMV, PVMV and PVY-n, respectively. 25.25% represent double infections for CMV+PVMV and CMV+PVY-n and 8.08% for n-PVY+PVMV. Triple infections (CMV+PVMV+PVY-n) occurred in 29.29% of pepper samples. In the four departments, three types of infections were detected namely single, double and triple (Figure 3). In Songon, simple infections of 5.12% were recorded for CMV and PVY-n and 3.42% for PVMV. Double infections of 8.55% were recorded for CMV+PVMV and PVMV+PVY-n, and 10.26% for CMV+PVY-n. Triple infections (CMV+PVMV+PVY-n) represent 11.96% in Songon. In Divo, simple infections were recorded as 5.98, 5.12 and 2.56% for CMV, PVMV and PVY-n, respectively. Double infections of 9.40, 8.55 and 7.70% were recorded for CMV+PVMV, CMV+PVY-n and PVMV+PVY-n, respectively. Triple infections (CMV+PVMV+PVY-n) were recorded as 13.67%. In Sinfra, the percentage of single infection was 11.97% for CMV. The percentage of double infections recorded was 29.06, 17.95 and 24.79% for CMV+PVMV, CMV+PVY-n and PVMV+PVY-n, respectively. Triple infections (CMV+PVMV+PVY-n) accounted for 31.62%. In Djébonoua, the percentage of infection was about 12.82% for CMV. The percentages of double infections were 17.09, 16.24 and 6.84% for CMV+PVMV, CMV+PVY-n and PVMV+PVY-n, respectively. Triple infections (CMV+PVMV+PVY-n) were recorded in 19.66%.

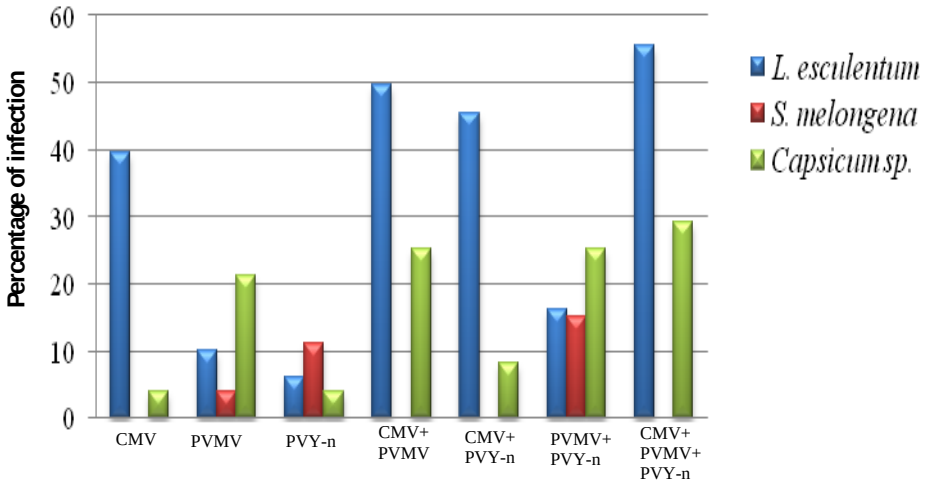

Figure 2. Percentages of different types of viral culture-nfections.

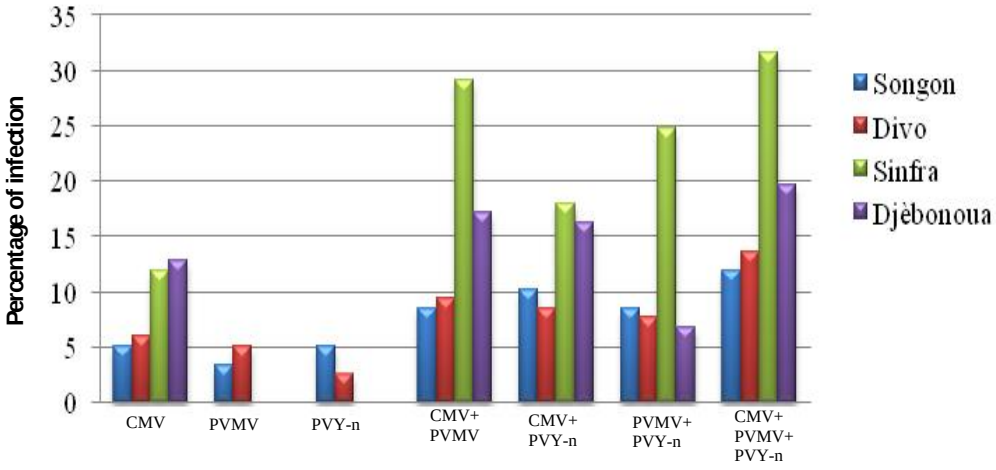

Figure 3. Percentages of different types of viral nfections by department.

In this study, two tomato weeds summers positive for PVY-n were developed by Traore et al. Thus, in 2014 it was shown that they are alternative hosts of the virus

3 DISCUSSION

DAS ELISA serology and ACP ELISA detected three viruses (CMV, PVMV and PVY-n) whose percentages vary depending on the culture. No virus was detected with the TAS ELISA. The three detected viruses infect three cultures of solanaceous plants (*L. esculentum, S. melongena* and *Capsicum* sp.) and were found in all surveyed departments. The results of this first study showed that CMV, PVMV and PVY-n infect Solanaceae in Songon, Divo, Sinfra and Djébonoua. To our knowledge, this is the first time of detecting PVY-n in solanaceous plants of Ivory Coast.

Of 117 samples tested, 84.61% were positive for the three sought viruses (CMV, PVMV and PVY-n). However, no sample was positive for TYLCV. Yet, Begomovirus (TYLCV) is the most important virus of tomato and pepper (Fauquet et al., 2005) and has been reported in several African countries. TYLCV was described in tomato by Walter et al. (1980) in Cote d'Ivoire. Studies in Benin on pepper and tomato crops identified CMV, PVMV and PVY-n (Afouda et al., 2013). These three viruses are the most important pathogens causing viral diseases in solanaceous plants of Divo, Sinfra and Djébonoua. Information is lacking on the main vectors and alternative hosts of these viruses, in the surveyed departments. However, many insects especially aphids were observed during the collection of samples and it is known that many of these species are vectors of these viruses (Afouda et al., 2013; Hobbs et al., 2000; Sikora et al., 1998). Most parcels visited are characterized by the presence of weeds, it is likely that the high incidence of CMV, PVY-n and PVMV could be due to these hosts that constitute a primary source of inoculum and contribute to the spread of these viruses. In tomato (*S. lycopersicon*), CMV is the largest followed by the

virus PVMV. CMV has many cultivated or spontaneous host plants in Ivory Coast (Agneroh et al., 2012; Koné et al., 2010; Aka et al., 2009; Thouvenel and Fauquet, 1987). The PVMV and PVY-n viruses are the most predominant in pepper (*Capsicum* sp) and eggplant (*S. melongena)*, respectively. The PVMV was first found in Ghana (Brunt and Kenten, 1971) and Nigeria (Lana et al., 1975) on *Capsicum* sp. and Ivory Coast on *N. fabacum* (De Wijs, 1973) and *Capsicum* sp. (Fauquet and Thouvenel, 1987). Proximity to Ghana and exchange of plant material could contribute to the spread of these viruses. PVY-n was identified in *Solanum tuberosum* in the United States by Smith (1931); however, it appears all over the world and can cause huge damage to other solanaceous plants beside potato. PVY-n was observed in every department visited, confirming its ability to infect most of the Solanaceae and vegetables. In these departments, producers utilized repetitive spray on their crops because CMV, PVMV and PVY-n are transmited in the non-persistent mode indicating that this type of spraying is inappropriate (Afouda et al., 2013).

Three types of infections were identified (single, double and triple) in the four departments. Detection of single and mixed infections in the same plot can be explained by the fact that the symptoms observed are not necessarily due to just one but may involve several viruses. The double and triple infections are most important in these departments. In Djébonoua and Sinfra, these infections are more predominant in the departments of Divo and Songon. The high infection rates (triple) identified in Sinfra could be explained by intercropping. Some plants may be alternative hosts and constitute sources of virus for propagation.

CONCLUSION

Serological characterization of viruses infecting four members of the Solanaceae family in four departments of Cote d'Ivoire, detected three viruses (CMV, PVMV and PVY-n) as the main causes of viral infections in tomato crops (*S. lycopersicon*), eggplant (*Solanum melongena*) and pepper (*Capsicum* sp.). The samples that tested positive for the virus were 84.61%. PVMV and CMV were detected in all departments prospected. This confirms their presence in Côte d'Ivoire. PVY-n was detected for the first time on the Solanaceae in Côte d'Ivoire. The viral impact is most important in Sinfra and Djébonoua, because of the high incidence (percentages) of mixed infections. Faced with severe economic impact of viral diseases, it is necessary to adopt control strategies to improve yields of the three members of the Solanaceae family. The study of these viruses by sequencing of viral genomes will help in the design of breeding programs necessary for the development of viral resistant crops.

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