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Viral Diseases of Papaya in Côte d'Ivoire: Distribution and Molecular Characterization of *Ringspot Virus* and Other Viruses

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Abstract In Côte d'Ivoire, it has been almost impossible to grow papaya for local consumption and export due to the resurgence of the viral disease of papaya since 2003. It is therefore required to make an inventory, distribution and characterization of papaya virus in the country. To this end, leaf and fruit samples with or without viral disease symptoms were collected in 37 localities from Côte d'Ivoire for laboratory analysis. DAS-ELISA diagnostic tests with a detection kit of *Papaya ringspot virus* (PRSV) were carried out on 218 leaf samples collected. Ten dried leaf samples showing symptoms of viral diseases including three tested positive for PRSV were selected for study on electron microscopy and molecular analysis. Various symptoms similar to those caused by the virus were observed, including: discoloration, arabesques, mosaic, vein clearing, leaf crinkling, shoestring and ringspot of different sizes and shapes on fruits. Of the 218 leaf samples tested, 63 (or 28.90 %) were PRSV-positive and 147 (or 67.43 %) showing symptoms similar to those caused by the viruses did not react positively to PRSV. Viral particles of isometric shape and sizes ranging from 33-50 nm in diameter were observed in 9 out of 10 samples observed. Comparison of the nucleotide sequences of the PRSV gene portion shows homology ranging from 97 to 99% among the 7 isolates from different localities of Côte d'Ivoire. In addition of PRSV, *Papaya meleira virus* (PMeV) could be present en Côte d'Ivoire iven if the primary test are not successuf.

Keywords: *Carica papaya, Côte d'Ivoire, papaya ringspot virus, distribution, molecular characterization*

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1. Introduction

Papaya (*Carica papaya L.*) originated from South and Central America. With a production of 12.4 million tons in 2013, papaya is the third most cultivated tropical fruit in the world [1]. In Côte d'Ivoire, papaya is grown for local consumption and exportation. Papaya cultivation is almost impossible in the southern area of the country. The localities of Azaguié and Anyama used to be the main production areas. Several producers have abandoned their plantations to create others in new areas or have switch to other crops following the outbreak and resurgence of a papaya virus disease in 2003.

Several virus genera are responsible for diseases on papaya. These include *Potyvirus*, *Rhabdovirus*, *Begomovirus*, *Potexvirus* and *Tospovirus* [2]. A number of viral species infecting papaya worldwide have been identified and include *"Papaya ringspot virus"* (PRSV) [3], *"Papaya droopy necrosis and Papaya apical necrosis"* [4] *"Papaya mosaic virus"* (PapMV) [5], *"Tomato spotted wilt virus"* (TSWV) [6], *"Papaya lethal yellowing virus"* (PLYV) [7], *"Papaya leaf distortion mosaic virus"* (PLDMV) [8,9,10], *"Papaya leaf curl virus"* (PLCV) [11], *"Moroccan watermelon mosaic virus"* [12] and *"Chickpea chlorotic dwarf virus"* (CpCDV) [13]. These viruses are transmitted by vectors mainly aphids and white flies. In Côte d'Ivoire, phytosanitary surveys conducted in South in February 2006, in 7 papaya producing regions, helped describe various symptoms potentially associated with viruses [14,15]. These symptoms included vein clearing, mosaics and shoestring on the leaves. The serological diagnostic tests carried out with antibodies to *Papaya ringspot virus* (PRSV) and *Papaya mosaic virus* (PapMV) revealed the presence of PRSV in tested leaf samples. However, the detection ratio was low (9 out of 60 samples tested positive). The presence of PRSV was confirmed by electron microscopy and molecular analysis data [14,15]. However, for some samples with mild symptoms to severe symptoms of mosaic with vein clearing on the leaves, flexible particles of about 800 nm were observed. However, these samples did not react to PRSV antibodies. The possibility of viruses other than PRSV being present in Cote d'Ivoire was suggested [15]. This study updates the diversity of viruses present on papaya in Côte d'Ivoire.

2. Materials and Methodology

2.1. Plant Materiel

In March and April 2011, 218 samples of leaves, fruits and trunk/stem with or without symptoms were collected in 37 localities belonging to 14 regions throughout the Côte d'Ivoire. These samples were collected, dried in newspapers and stored for future laboratory analysis.

2.2. ELISA Serological Test

Double antibody sandwish - Enzyme-Linked Immunosorbent Assay (DAS-ELISA) diagnostic test described by Clark and Adams [16] was applied.

This test is based on the affinity between antigen and antibody. For these tests, polyclonal antibodies were used to detect the presence of the viruses. The ELISA kits [17] (Switzerland) were used for detecting *Papaya ringspot virus* (PRSV), as recommended by the manufacturers. Hundred milligrams of leaves were crushed in a sterilized mortar and then recovered in 5 ml PBS Tween buffer. Antibodies to PRSV were used for the DAS-ELISA test. Absorbance values were read at 405 nm using an ELISA plate reader (Multiskan EX Labsystems version 1.1, Finland), after one hour of incubation of the preparation in the dark at 25°C. The positive threshold was double the average of the absorbance of non-infected samples.

2.3. Electron Microscopy

Ten samples of dried leaves previously showing characteristic symptoms from other localities were selected for electron microscopy studies. Three of the samples were tested positive to PRSV by ELISA. For each sample, 0.1 g of leaves were crushed in 1 ml of distilled water. After centrifugation, the supernatant was transferred into a tube in which an equal volume of phosphotungstic acid (2%) was added. Viral particles were collected on formvar-coated grids and then observed under a transmission electron microscope JEM 2011 Fastem (Jeol UK) operating at 200 kV. The images were taken with the camera US1000 CCD operating through Digital Micrograph software (Gatan UK). These analyzes were performed at the electron microscopy Laboratory of Rothamsted Research (Harpenden, UK).

2.4. PCR detection

Five papaya leaf samples showing symptoms under the transmission electron microscope (Abengourou (14.4), Abengourou (14.5) N'zianouan (25) Miagui (37.2), Lakota (46.4)) and five other samples of dried papaya leaves from Bingerville (70.7), Abidjan (90), Toumodi (133), Azaguié (141), Tiassalé (158) were selected for the molecular analysis. These samples were selected based on their symptoms and their serological reactions. A pair of PRSV-specific primers PRSV-F (5′-TAACACACTGATGGTTTTAATC-3′) and PRSV-R (5′-ATTGCGCATACCCAGGAGAGAG-3′) has been used for the PCR. The resulting PCR products were sequenced directly using appropriate primers which are expected to provide a portion of the gene for the protein capsid of the *Papaya ringspot virus* (PRSV) of about 790 bp. Other primers were used for detection of the *Papaya meleira virus* (PMeV). These analyzes were conducted at Plant Virus Collection DSMZ (Braunschweig, Germany).

2.5. Sequence Analysis

Phylogenetic analyses were carried out to determine the genetic relationship of PRSV isolates from Cote d'Ivoire with isolates from other regions. Construction with publicly available complete DNA-A sequences (MEGA 6) [18], the maximum-likelihood (ML) phylogenetic tree (with 1000 boostrap replicates) were constructed from alignment DNA-A nucleotides sequences with PHYML v.3. Tree was visualized using FigTree v1.3.1

3. Results and Discussion

3.1. Distribution of Papaya Ringspot Virus

Various symptoms of viral diseases was observed on the papaya leaves and fruit in all production areas of Côte d'Ivoire. The symptoms on the leaves included: discoloration, yellowing, mosaic, vein clearing, leaf crinkling, over-coloring of veins, discoloration and shoestring (Figure 1). On the fruits, ringspot of various sizes and forms were observed (Figure 1). Diallo and collaborators had observed similar symptoms in 2007 and 2008 in the localities of Abidjan, Anyama and Yamoussoukro. Out of the 218 leaf samples collected in the present study , 63 were tested positive for PRSV. Out of the 155 samples no infected by PRSV, 147 had symptoms similar to those caused by the viruses (Figure 1 and Table 1). Similar results were also obtained in the study conducted by [19] and in Côte d'Ivoire by [15].

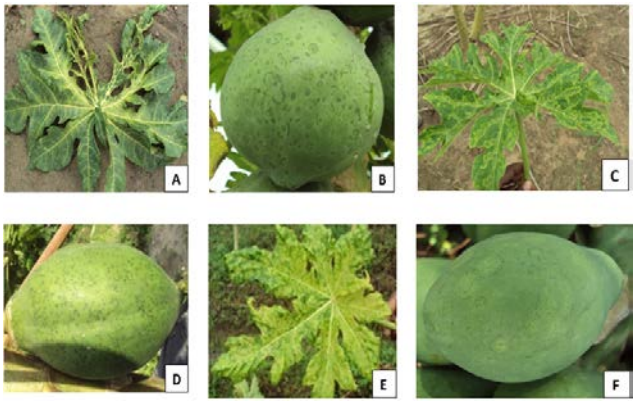


Figure 1. PRSV symptoms (A : Vein cleraing, shoe string of the leaf (A) and ringspot of the fruit (B)) and Symptoms of virus disease not tested positive for PRSV (C : Arabesque, D: small ringspot , E: yellowing + shoestring, F: large clearer ringspot on the fruit)

3.2. Characteristics of Papaya Ringspot Virus

Based on serological test ELISA (Enzyme-Linked Immuno sorbent Assay) carried out, *Papaya ringspot virus* (PRSV) was identified in 19 out of the 37 localities visited (Tables 1 and 2).

Table 1. Geographical distribution of PRSV in Côte d'Ivoire

Regions	Localities	Occurrence of PRSV
Lagune	Abidjan	-
	Anyama	+
	Azaguié	+
	Bingerville	+
Agneby	Agboville	+
	N'Zianouan	+
	Tiassalé	+
Indenié-Djuablin	Abengourou	+
	Agnibilékro	-
Gontougo	Bondoukou	-
Gboklè	Sassandra	+
Belier	Toumodi	+
	Yamoussoukro	+
Gbekè	Bouaké	-
Poro	Korhogo	-
San-Pedro	Grand-Béréby	-
	San-Pedro	-
Guemon	Duekoué	-
Cavally	Guiglo	+
Tonkpi	Man	-
Gôh	Gagnoa	-
Sud-Comoé	Bonoua	+
	Aboisso	+

+: Presence of PRSV, - : Absence of PRSV

Table 2.Characteristics of samples collected from localities and PCR-PRSV test result

Localities	Samples collected	Samples no symptoms collected	Samples with symptoms collected	Samples tested positive for PRSV	Samples tested negative for PRSV
Abidjan	30	2	28	16	12
Agneby	41	2	39	14	25
Indenié-Djuablin	24	1	23	10	13
Gontougo	6	0	6	1	5
Gboklè	11	0	11	1	10
Belier	20	0	20	8	12
Gbekè	14	1	13	4	9
Poro	7	0	7	1	6
San-Pedro	12	0	12	2	10
Guemon	10	0	10	2	8
Cavally	8	0	8	1	7
Tonkpi	6	0	6	1	5
Gôh	11	0	11	1	10
Sud Comoé	18	1	17	4	13

The 19 localities where samples were tested positive are distributed across the country. The incidence of viral diseases varies from one region to another. Similar results obtained in Philippines [20] and in Pakistan [21]. In some regions, no symptom like resembling those caused by the viruses or other pathogens was observed. It was the case in the Savanes and Zanzan regions. The higher disease prevalence was obtained in the regions of Lacs, Agnéby, Moyen Comoé and Sud Comoé (Figure 2) [21].

Observations of the ten papaya leaf samples showing symptoms under the transmission electron microscope revealed no viral particle characteristic of Potyviruses. However, viral particles of isometric form and sizes ranging from 33 to 50 nm in diameter were observed in 9 of the 10 samples (Figure 3). A virus different from the PRSV would be present in papaya orchards in Côte d'Ivoire. The same conclusion was reach in a previous study conducted in Mexico [22], in Cote d'Ivoire [15] and in China [23].

Eight out of ten samples showing symptoms collected from different localities and tested positive by ELISA for the presence of PRSV. Comparison of the nucleotide sequences of the PRSV gene portion shows homology ranging from 97 to 99 % between 7 isolates from Agboville, Azaguié, Bingerville, Bonoua, Tiassalé, Toumodi and Soubré (Table 3 and Figure 4).

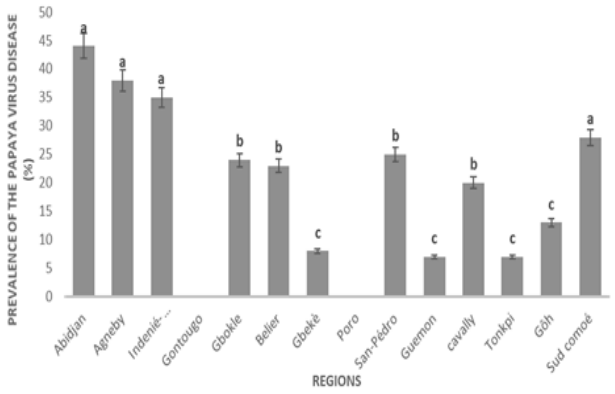
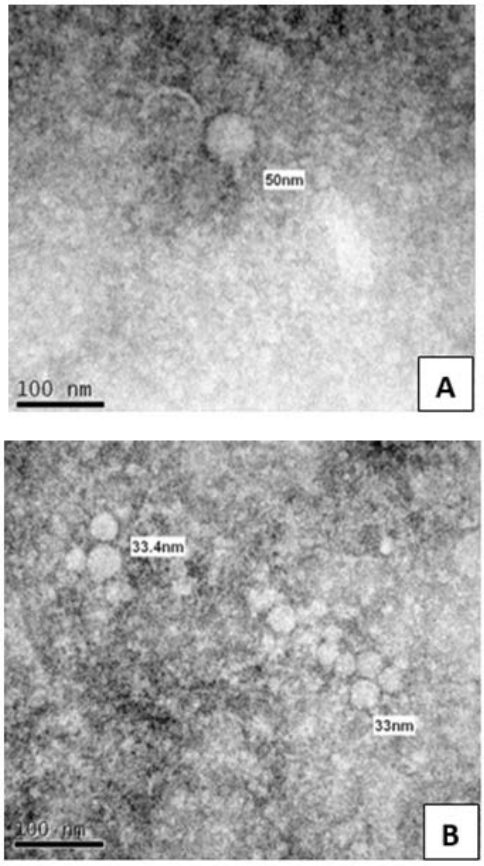


Figure 2. Prevalence of the papaya virus disease in the different regions of Cote d'Ivoire



A: Particle observed from a leaf sample in Figure 2.A B: Particle observed from a leaf sample in Figure 2.C

Figure 3. sometric virus particles of varying sizes observed from infected leaves samples which were not test positive for PRSV by ELISA

Table 3. Name, acronym and accession numbers of virus reported in this study

Virus names	Acronym	Accession number
Papaya ringspot virus-[Côte d'Ivoire : Soubré : 38.3: 2011]	PRSV-[CI:Sou:38.3:11]	KF289933
Papaya ringspot virus-[Côte d'Ivoire : Bingerville : 70.7: 2011]	PRSV-[CI:Bin:70.7:11]	KF289934
Papaya ringspot virus-[Côte d'Ivoire : Bouna : 115: 2011]	PRSV-[CI:Bou:115:11]	KF289935
Papaya ringspot virus-[Côte d'Ivoire : Toumodi : 133: 2011]	PRSV-[CI:Tou:133:11]	KF289936
Papaya ringspot virus-[Côte d'Ivoire : Azaguié : 141: 2011]	PRSV-[CI:Aza:141:11]	KF289937
Papaya ringspot virus-[Côte d'Ivoire : Agboville : 150: 2011]	PRSV-[CI:Agb:150:11]	KF289938
Papaya ringspot virus-[Côte d'Ivoire : Tiassalé : 158: 2011]	PRSV-[CI:Tia:158:11]	KF289939
Papaya ringspot virus-W-[Venezuela : VE08-57: 2008]	PRSV-W [VE:VE08-57:08]	KC345575
Papaya ringspot virus-W-[Venezuela : VE08-58: 2008]	PRSV-W [VE:VE08-58:08]	KC345576
Papaya ringspot virus-W-[Venezuela : VE10-200: 2010]	PRSV-W [VE:VE10-200:10]	KC345591
Papaya ringspot virus-W-[Venezuela : VE06-44: 2006]	PRSV-W [VE:VE06-44:06]	KC345597
Papaya ringspot virus-W-[US : Oklahoma : A5: 2008]	PRSV-W [US:Okl:A5:08]	JN132413
Papaya ringspot virus-W-[US : Oklahoma : A13: 2008]	PRSV-W [US:Okl:A13:08]	JN132421
Papaya ringspot virus-[US : Floride : 2001]	PRSV-[US:Flo:01]	D00594
Papaya ringspot virus-[US : Hawaï : 2001]	PRSV-[US:Haw:01]	X67672
Papaya ringspot virus-[US : Hawaï : 2001]	PRSV-[US:Haw:01]	X67673

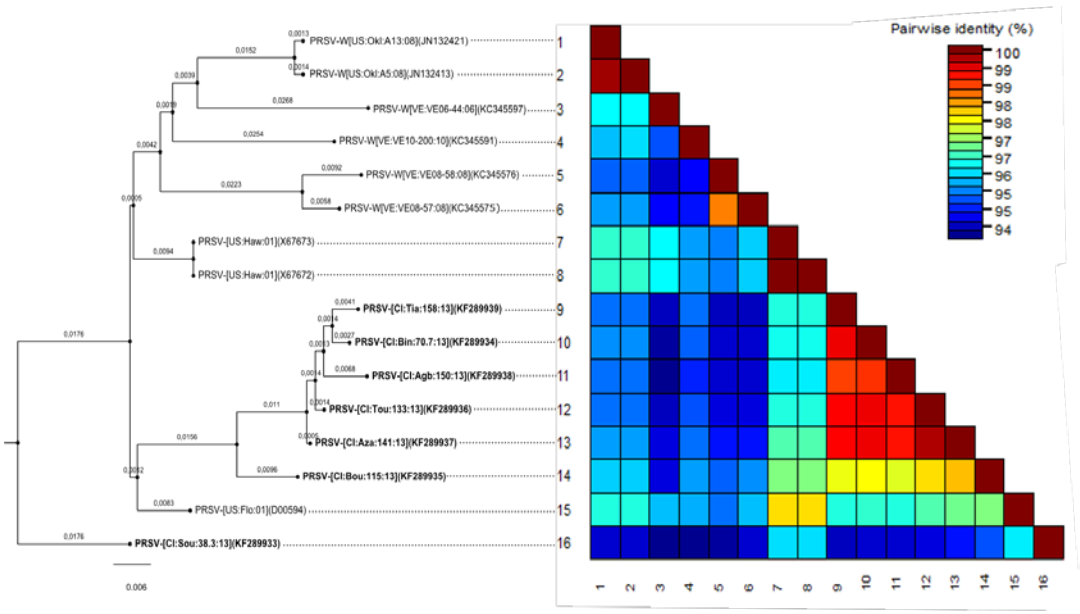


Figure 4. Maximum-likelihood phylogenetic tree and color-coded matrix of pairwise nucleotide identity inferred from alignments of complete sequences of PRSV nucleotide sequences of Cote d'Ivoire (in bold).

Seven complete DNA-A genome sequence (EMBL-Geenbank-DDBJ accession Nos. KF289933 to KF289939 (table)) showed the highest pairwise sequence identity of 94-97 % (BLASTn, NCBI) with isolates of PRSV-W from Venezuela (PRSV-W [VE:VE08-57:08], KC345575; PRSV-W [VE:VE08-58:08], KC345576; PRSV-W [VE:VE10-200:10], KC345591) and USA (PRSV-W[US:Okl:A5:08], JN132413; PRSV-[US:Flo:01], D00594; PRSV-[US:Haw:01], X67672). Analysis of the maximum-likelihood (ML) phylogenetic tree confirmed the genetic relationship of PRSV isolates from Cote d'Ivoire with isolates from Venezuela and the USA (Figure 4). The isolate PRSV of Soubré would have experienced recombinations and mutations of gene in its genome [24]. It is very different from the other Ivory Coast PRSV sequences. The molecular test for detecting *Papaya meleira virus* (PMeV), a potential virus, was inconclusive. It is therefore recommended to pursue the work on the identification of papaya viruses other than PRSV present in Côte d'Ivoire. Thus, the study on the inventory, distribution and characterization of papaya viruses should cover the entire country.

4. Conclusion

The aim of this study is to update the viruses present on papaya. PRSV has been detected on papaya in various production localities in Côte d'Ivoire. Some samples showing virosis symptoms did not react positively to PRSV primers. However, electron microscopy revealed virus structures close to those of Papaya meleira virus. Viruses other than PRSV could be present in papaya in Côte d'Ivoire.

GenBank Accession Numbers

KF289933 to KF289939

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Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval No studies involving human participants or animals performed by any of the authors are described

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