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Detection of Pepper Yellow Vein Mali Virus associated with pepper in Yamoussoukro, Côte d'Ivoire

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ABSTRACT

A field survey was conducted in Yamoussoukro Côte d'Ivoire in order to collect pepper leaves harboring the Pepper Yellow vein disease (PYVD). Five local farmers pepper fields located around the lakes of Yamoussoukro were surveyed. Ten samples were PCR- amplified using universal degenerated core-Cp primers and sequenced. Blast analyses showed that the PepYVV-Mali was responsible of the PYVD symptoms within two samples. Phylogenetic analyses showed that the two identified PepYVV-Mali formed a new clade different from the previous PepYVV-Mali isolated in Burkina Faso and Côte d'Ivoire. The pairwise analysis confirmed this discrepancy by a sequence similarity of 95-96%.

INTRODUCTION

In Côte d'Ivoire, there is a huge population growth and changes in urban eating habits. Consequently, market gardening needs are constantly growing in both urban and rural areas as elsewhere (Satterthwaite *et al.,* 2010). As such, many market gardening vegetables including okra, eggplant, pepper, tomato, onion, shallot, carrot and various leafy vegetables (amaranth, cabbage, sorrel, potato, cassava, eggplant… etc.) are the most cultivated common species in dietary habits (Grubben *et al*., 2014). These vegetables are very important due to richness in vitamins, minerals, and proteins. As such these vegetable products play a dual role in food security and fight against malnutrition (FAO 2004). In Côte d'Ivoire, many vulnerable populations, including women, rely on the production and trade of vegetable and protein crops, mainly in urban and peri-urban areas like the Yamoussoukro district. Yamoussoukro the capital city in the center of Côte d'Ivoire is one of the important districts where vegetables are cultivated because of the presence of lake waters (Adama et Michel, 2004; ANADER, 2004; Tano *et al.,* 2011). However, many constraints limit the development of vegetables and protein crops that cover 60% of the needs of the country. One of the most important cultivated vegetables in the Yamoussoukro district is pepper. However, pepper gardening is subject to biotic stresses due to viruses, bacteria, and fungi, that cause various symptoms from nursery to storage after harvest (Bolou *et al.,* 2016). Several begomoviruses infecting vegetable crops have been detected in West and Central Africa (Leke *et al.,* 2015). In Côte d'Ivoire, there are a few reports on the incidence of begomovirus infecting market garden in general, and pepper in particular. These included Cotton leaf curl Gezira virus and Okra yellow crinkle virus associated with okra leaf curl disease in Côte d'Ivoire or Pepper yellow vein Mali virus associated with pepper yellow vein disease in Mali and Cote d'Ivoire (Tiendreogo *et al.,* 2008; Seka *et al.,* 2016; Seka *et al.,* 2017). The Pepper Yellow vein disease (PYVD) is a major threat to pepper in West Africa (Tiendrébéogo *et al*., 2008). The PYVD causes significant yield losses that reduce the income of market gardeners in the district of Yamoussoukro. The Yellow vein virus Mali was initially identified as the PYVD causing agent in Mali (Tiendrébéogo *et al*., 2008). Moreover, evidence that PYVD is caused by the PepYVV-Mali in Côte d'Ivoire was also demonstrated (Seka *et al*., 2017). However, the same authors did not detect the PepYVV-Mali implicitly in the symptomatic pepper leaves they collected in 2012 and 2013. Considering the importance of pepper cultivation in the district of Yamoussoukro, it was therefore important to implement a PYVD surveillance. It was then urgent to investigate whether or not the PepYVV-Mali prevails in the Yamoussoukro district. The aim of this study was the launch of a survey in order to detect the PepYVV-Mali disease on symptomatic pepper leaves in Yamoussoukro.

MATERIAL AND METHODS

Pepper leaves samples showing PYVD symptoms were collected around the lakes in Yamoussoukro market garden fields, between June and August 2018 (figure 1). 100mg of leaf tissue was used to extract total genomic DNA according to a modified CTAB method (Doyle and Doyle, 1990). PCR amplification of the viral CP gene was carried out with the degenerated primers Av core (5'-GCCHATRTAYAGRAAGCCMAGRAT-3') and Ac core (5'-GGRTTDGARGCATGHGTACANGCC-3') (Brown *et al.,* 2001). Each PCR reaction was performed in a final volume containing 100ng of extracted DNA, 0.25 mM dNTPs, 2.5 µM of each primer, 1X enzyme buffer and 0.5 U of *Taq* DNA polymerase. The PCR reaction conditions were 94°C for 2 min of initial denaturation, followed by 35 cycles of 94°C for 30 sec, 55°C for 2min and 72°C for 40 sec and a final extension of 72°C for 10 min. The amplified products were visualized on 0.8 % agarose gel in TAE buffer (Tris-acetate-EDTA) and stained with Etybium Bromide. The amplified products were sequenced by INQABA Biotech SA.

The sequences obtained were subjected BLAST analyses. Pairwise distances were determined using Standard Demarcation Tool (SDTv1.2) software (Muhire *et al.,* 2014) with selected sequences from GenBank. Sequence alignment was carried out in MUSCLE (Edgar, 2004), implemented in CLC Sequence Viewer 7.5 software. Phylogenetic analysis with 500 bootstrapped replicates was performed using the Neighbor-Joining algorithm implemented in MEGA6. Sequences selected for pairwise and phylogenetic analysis were based on the top BLASTn hits in GenBank for each virus.

Figure 1 : Pepper plants with Pepper Yellow Vein Virus symptoms

RESULTS

Evidence of PepYVV Mali associated to pepper in Yamoussoukro

The degenerated primers used in this study allowed the amplification of a 575 bp expected fragment from the10 samples that exhibited PYVD diseases (Table I). In order to accurately identify the virus involved in the disease, a BLAST analysis was performed. The BLAST analyses allowed the identification of two samples PM1-CI and PM2-CI that were highly similar (95-97%) to the PepYVV Mali BF-Bazega-hot pepper1-2007 (Accession number: FM876848) (Table II).

Table I: PYVD disease diagnosis and PepYVV Mali identification using Blast analysis

Sample Id	PCR diagnosis with AV/AC core primers	PepYVV-Mali identification
PM1	+	+
PM2	+	+
PM3	+	-
PM4	+	-
PM5	+	-
PM6	+	-
PM7	+	-
PM8	+	-
PM9	+	-
PM10	+	-

Table II: Begomovirus sequences used for Pairwise and phylogenetic analysis of the core CP gene

PepYVV : Peper Yelow Vein Virus
TLCV : Tomato leaf curl

Virus species	Accession number
PepYVV Mali CI:Kor:CI57:12	KY271075
PepYVV Mali CI:Tou:CI30-12	KY271076
PepYVV Mali	AY502935
PepYVV Mali BF-Ouaga-sweet pepper 2-2008	FM876851
PepYVV Mali BF-Bazega-hot pepper1-2007	FM876848
PepYVV Mali BF:Po:Hpe:08	FN555171
TLCV Namakely	FN600540

PM1-CI and PM2-C1 constitute a new PepYVV Mali clade in Côte d'Ivoire

In order to phylogenetically identify PM1-CI and PM2-CI a phylogenetic analysis using PepYVV Mali virus isolates from Côte d'Ivoire and Mali including PepYVV Mali CI: Kor: CI57: 12, PepYVV Mali CI:Tou: CI30-12, PepYVV Mali, PepYVV Mali BF-Ouaga-sweet pepper 2-2008, PepYVV Mali BF-Bazega-hot pepper1-2007, PepYVV Mali BF:Po:Hpe:08 was performed. It was shown that both PM1-CI and PM2-CI formed a clade that was different from the clade formed with the PepYVV Mali previously isolated from Mali and Côte d'Ivoire (Figure 2). The pairwise analysis between the Cp sequences PM1-CI and PM2-CI and the sequences used for phylogenetic analysis, gave an identity (95%-96%) with the Pepper Yellow Vein Viruses (KY271076 and KY271075) previously isolated in Côte d'Ivoire (Figure 3).

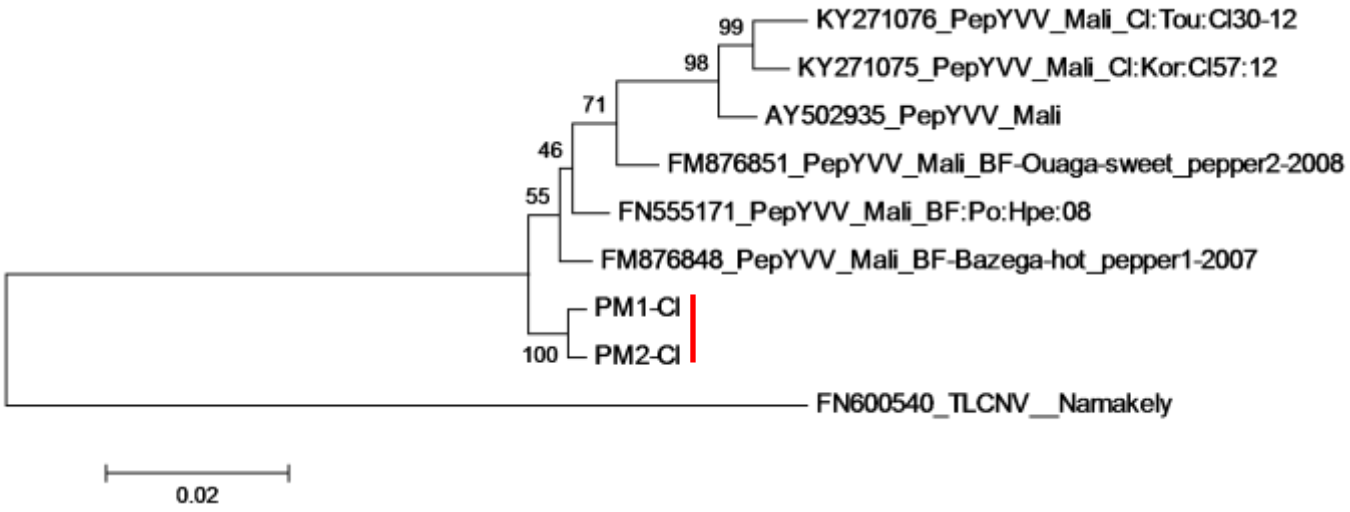

Figure 2: Phylogenetic analysis using Neighbor-Joining method in MEGA 6. The reliability of the tree was assessed by 500 bootstrap replications. The sequences downloaded from GenBank are indicated by their Accession number. Each Cp sequence identified in this study is indicated by PM1-CI and PM2-CI. The sequence alignment was carried out using MUSCLE, implemented in MEGA 6. Total of 02 Cp sequences, obtained after experiments and 07 from NCBI was used as a basis for phylogenetic analysis.

>AY502935_PepYVV_Mali	100								
>KY271076_PepYVV_Mali_CI_Tou_CI3012	98	100							
>KY271075_PepYVV_Mali_CI_Kor_CI57_12	98	99	100						
>FM876851_PepYVV_Mali_BFOuagasweet_pepper22008	97	98	98	100					
>PM1-CI	97	96	97	98	100				
>PM2-CI	96	95	96	97	99	100			
>FN555171_PepYVV_Mali_BF_Po_Hpe_08	97	97	97	99	99	98	100		
>FM876848_PepYVV_Mali_BFBazegahot_pepper12007	97	97	97	99	99	98	99	100	
>FN600540_TLCNV__Namakely	85	86	86	86	86	85	86	86	100

Figure 3 : SDT analysis with Cp sequences ~~from~~ PM1-CI and PM2-CI and 07 NCBI begomovirus Cp sequences.

DISCUSSION

The aim of this study was to investigate whether or not the pepper yellow vein disease (PYVD) observed in Yamoussoukro was caused by the PepYVV Mali. For this purpose, we used degenerated primers to amplify the Cp region. The degenerated primers used in this study were robust enough to amplify the cp region of PepYVD symptoms. Two PepYVV Mali were detected at a rate of 20% in Yamoussoukro. This confirms that pepper yellow vein disease is associated with the African begomovirus *Pepper yellow vein Mali virus* in Côte d'Ivoire (Seka *et al.,* 2017). These authors detected the PepYVV Mali in Toumodi and Korhogo in samples collected in 2012 and 2013. The study by Séka et al. (2017) showed that the PepYVV Mali evolved very quickly over time. Indeed while the virus was present in Toumodi in 2012, it could not be detected in 2013 in the same region. This could be explained by the fact that begomoviruses are subject to intense recombination rates, leading to a quick evolution and huge molecular diversity in order to respond to changes in the environment and to invade new ecological niches (Padidam *et al.*, 1999; Lefeuvre *et al.*, 2009; Duffy and Holmes 2009; Lefeuve *et al.,* 2011). This could also explain why the PepYVV Mali was not detected within the samples surveyed in Yamoussoukro in 2012 and 2013 (Séka *et al.,* 2017). The phylogenetic analyses showed that the two PepYVV Mali present in Yamoussoukro formed a clade different from the one previously isolated in Côte d'Ivoire and Mali. This was confirmed by the pairwise analyses that showed that the two viruses had 95-96% similarity with the previously isolated PepYVV Mali. This level of variation may indicate a possible recombination that has occurred. Since the Cp region is a very conserved region, a further analysis of the whole genome should allow a clear indication of this event. The result of this study is the first report on the evidence that PepYVV Mali is the causing agent of PYVD in Yamoussoukro, Côte d'Ivoire. This study clearly highlights the need to constantly make a surveillance of the PepYVV disease. Indeed while the previous study did not allow the detection of this virus in Yamoussoukro (Séka *et al.,* 2017), our study clearly indicates its presence at a frequency of 20%.

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