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 centers 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 Côte d'Ivoire (Tiendreëogo 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'-GGRRTDGARGCATGHTACANGCC-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 Etbiyum 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.
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 the 10 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 pepper 2007 (Accession number: FM876848) (Table II).

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

<table>
<thead>
<tr>
<th>Sample Id</th>
<th>PCR diagnosis with AV/AC core primers</th>
<th>PepYVV-Mali identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PM2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PM3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PM4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PM5</td>
<td>+</td>
<td>-</td>
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<tr>
<td>PM6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PM7</td>
<td>+</td>
<td>-</td>
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<td>PM8</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PM9</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PM10</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Virus species</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>PepYVV Mali CI:Kor:Cl157-12</td>
<td>KY271075</td>
</tr>
<tr>
<td>PepYVV Mali CI:Tou:Cl30-12</td>
<td>KY271076</td>
</tr>
<tr>
<td>PepYVV Mali</td>
<td>AY502935</td>
</tr>
<tr>
<td>PepYVV Mali BF-Ouaga-sweet pepper 2-2008</td>
<td>FM876851</td>
</tr>
<tr>
<td>PepYVV Mali BF-Bazega-hot pepper1-2007</td>
<td>FM876848</td>
</tr>
<tr>
<td>PepYVV Mali BF:Po:Hpe:08</td>
<td>FN555171</td>
</tr>
<tr>
<td>TLCV Namakely</td>
<td>FN600540</td>
</tr>
</tbody>
</table>
PM1-Cl and PM2-Cl constitute a new PepYVV Mali clade in Côte d’Ivoire

In order to phylogenetically identify PM1-Cl and PM2-Cl a phylogenetic analysis using PepYVV Mali virus isolates from Côte d’Ivoire and Mali including PepYVV Mali Cl: Kor: Cl57: 12, PepYVV Mali Cl: Tou: Cl30-12, 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-Cl and PM2-Cl 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-Cl and PM2-Cl 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-Cl and PM2-Cl. 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_Cl_Tou_Cl3012 98 100
>KY271075_PepYVV_Mali_Cl_Kor_Cl57_12 98 99 100
>FM876851_PepYVV_Mali_BF_Ouaga_sweet_pepper2008 97 98 98 100
>PM1-Cl 97 96 97 98 100
>PM2-Cl 96 95 96 97 99 100
>FN555171_PepYVV_Mali_BF_Po_Hpe_08 97 97 97 99 99 98 100
>FM876848_PepYVV_Mali_BF_Bazega_hot_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-Cl and PM2-Cl 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 (Séka 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; Lefeuvre 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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