Original Research Article

Diagnosis of Pepper (*Capscium sp.*) Viral Diseases in Bouaké and Abidjan and Characterization of Associated Begomoviruses

Comment [u1]: common Viral Diseases in

Comment [u2]: localities

ABSTRACT

Objective: This study aims at gaining a better understanding of Pepper Begomovirus diseases in order to improve yield in a sustainable way.

Study design :

Comment [u3]: Include design

Location : Abidjan and Bouaké (Côte d'Ivoire); Study duration: 12 months

Methodology: A phytosanitary survey was carried out in Abidjan and Bouaké, and symptomatic samples were collected. A total of 60 samples with single and complex viral disease symptoms were observed and collected from the two study areas. Results: The severity of symptoms observed was virtually identical in both localities, in contrast to prevalence, which was higher in the Bouaké locality. Two of the samples from Bouaké reacted positively to the PCR test for Begomovirus. The *Pepper Yellows Vein Mali Virus* was thus identified as a potential cause of the viral diseases observed. The sequences of this virus recorded a similarity rate of 97.16 and 99.42% with its homologues found on GenBank under accession numbers MH460532 and MH778694, respectively.

Conclusion: A clear identification of pepper pathogenic viruses could better guide the control of these viral diseases.

Keywords: Capsicum sp., viral diseases, diagnosis, molecular characterization

1. INTRODUCTION

Pepper (*Capsicum* sp.) is a vegetable of the Solanaceae family. It is one of the 40 most widely produced vegetable species worldwide [1]. Today, pepper is grown all over the world and is one of the most widely consumed vegetables, for its several virtues. It contains a yellowish oil with a hot taste, and is rich in minerals and vitamins A and C. Worldwide yield of pepper is estimated at over 31 million tons [2]. The African continent is the 3rd producer with a rate of 8.8% behind the Asian and American continents [2]. The world's main pepper producers are: China with 14 million tons, Mexico with 1.8 million tons and Turkey with 1.7 million tons [3]. In Africa, the top five producers are Nigeria, Ghana, Algeria,

Comment [u4]: Include authority

Ethiopia and Tunisia. With 500 000 and 110 000 tons per year respectively, Nigeria and Ghana are the biggest pepper producers in West Africa [2]. In Côte d'Ivoire, pepper is grown all over the country and sold fresh or dried on national and international markets. Pepper price per kilogram varies from 1000 to 1100 francs, depending on the season and the Ivorian markets [3]. The crop generates substantial income for many families, with a yield estimated at 32 900 tons a year [2]. The increase in pepper yield nationwide since 2015 is partly due to the increase in acreage [4, 5]. This is because several factors, such as crop cycle staggering, climatic disturbances, technical itineraries that have remained traditional and the presence of pests and diseases, contribute to the decline in its yield [6]. Among all these diseases, the most damaging are viral diseases, as some viruses cause a total yield loss. They occur as blistering, mosaics, necroses, fruit malformation and discoloration, viral complexes [7]. The spread of these viruses is carried out mechanically or by several vectors, including insects [8]. As the symptoms of Begomovirus diseases resemble those of other viral diseases, this study aims at gaining a better understanding of pepper Begomovirus diseases in order to improve its yield in a sustainable way. More specifically, it aims at determining the symptomatology of pepper viral diseases, assess epidemiological parameters (prevalence and severity) and characterize the Begomoviruses associated with the symptoms.

2. MATERIAL AND METHODS

2.1 Phytosanitary survey and sample collection

In the survey areas, symptomatology was determined by visual diagnosis based on observation of symptoms on pepper leaves. The different virus symptoms observed on the leaves were described. The description took into account the shape, coloration and appearance of infected leaves. Sampling was carried out in 3-month-old fields in each of Abidjan and Bouaké localities. Samples were taken from plants showing symptoms of viral infection. Sampling was carried out randomly in the fields. Diseased plants were photographed and collected. Each leaf sample collected was stored in a paper envelope and identified by a code (number specifying the origin and order of the sample). Sixty samples were collected. The samples were transported to the laboratory and oven-dried at 45°C for 48 hours for further processing.

2.2 Determining the prevalence and severity of viral disease symptoms

2.2.1 Determining the prevalence of viral disease symptoms

The percentage of plants exhibiting viral disease characteristic symptoms was determined in relation to the total number of infected plants in the plot. The average prevalence was calculated using the following formula [9]:

$$Pm\ (\%) = \frac{Pis}{Pti} \times 100 \quad (1)$$

Pm : mean prevalence

Pis : number of plants infected by viral diseases

Pti : total number of plants visited in the locality

2.2.2 Determining the severity of viral disease symptoms

A severity score was assigned according to the scale of [10] ranging from 1 to 5, where (1) no visible symptoms and 5: more than 75% of leaves attacked. Severity index was determined as follows

$$IS = \frac{\Sigma(Xi \times Ni \times 100)}{5 \times Nt} \quad (2)$$

IS: Severity index of the symptom considered

Xi: Score attributed to a symptom

Ni: Number of plants of the same species exhibiting the symptom

Nt: Total number of plants exhibiting symptoms or not

2.3 Molecular identification of the main viruses associated with symptoms

2.3.1 DNA Extraction and amplification using the PCR technique

Extraction was carried out using the viral DNA extraction protocol kit DNeasy plant (Qiagen) version 14 April 2019. PCR (Polymerase Chain Reaction) makes it possible to obtain a very large number of copies of a DNA segment and an exact volume. The principle consists in using a pair of primers (short DNA sequences of 18 to 24 nucleotides) which hybridize to complementary sites located in inverted orientation on the two strands of the target DNA and which frame the region to be amplified [11]. Two primers Cluster 4 F342 (5'-TATMATCATTTCCACBCCVG-3') and Cluster 4 R1032 (5'- GCATGTACATGCCATATAC-3') were used for universal PCR detection of Begomoviruses. The solution mix consisted of water (5.9 µl x n), primer pair ("forward" 0.5 µl x n and "reverse" 0.5 µl x n) and Taq (3.6 µl x n). The solution thus prepared was dispensed into the 1µl, that is, 10.5µl per well. Next, 2µl of DNA were added for a final

volume of 12.5µl. The whole set was placed in QIAXCEL (QIAGEL) and the positive samples migrated at a molecular weight ranging from 690 to 750 Pb. PCR amplicons obtained with the digested primers (Cluster 4 Cl) were sequenced using the Sanger sequencing method by the company Macrogen (Europe).

2.3.2 Phylogenetic analysis

The sequences were edited and assembled using Mega 10 software. Nucleotide sequences were subjected to a BLAST search for preliminary species assignment. Alignments of query sequences with representative Geminivirus sequences available from Genbank were performed using the alignment tool available in Geneious Mega 10 and edited with the naked eye. In order to assign sequences to known viral species and strains, pairwise nucleotide sequence identity comparisons were performed with pairwise gap deletion. The maximum likelihood (ML) phylogenetic tree was constructed using this software.

2.4 Statistical analysis

The data obtained in this study were analyzed using Statistica 7.1 software. The Kruskal wallis rank test was used to compare the prevalence and mean severity of single and complex symptoms observed in each locality. In the event of a significant difference at 5% threshold, the Mann- Whitney test was used to determine the different homogeneous groups.

Comment [u5]: Statistical

3. RESULTS

3.1 Observed symptoms

A variety of viral disease symptoms, including color changes and organ alterations, were observed in the areas surveyed. Color changes were characterized by interveinal discoloration and mosaics, while organ alterations were characterized by blistering and leaf curling. These symptoms were referred to as single symptoms. The combination of two or more single symptoms was referred to as complex symptoms. Thus, complex symptoms such as blistering+curling, blistering+mosaic, mosaic+curling, mosaic+discoloration and interveinal discoloration + curling were recorded (Fig. 1).

3.2 Prevalence and severity of viral disease symptoms

3.2.1 Prevalence of symptoms in surveyed localities

3.2.1.1 Prevalence of single symptoms

The prevalence of pepper viral disease single symptoms varied not only from one locality to another, but also from one symptom to another ($P < 0.05$). Thus, blistering and leaf curl symptoms were more prevalent in Abidjan (16.66% and 13.36%) than in Bouaké (10.05% and 6.66%). However, the prevalence of mosaic and interveinal discoloration were statistically similar in both localities ($P > 0.05$).The prevalence of interveinal discoloration was the lowest in both localities (Table 1).

3.2.1.2 Prevalence of complex symptoms

As with single symptoms, the prevalence of complex symptoms varied depending on the localities and symptoms ($P < 0.05$). In Abidjan, the highest prevalence (10.07%) was noted for Blistering + leaf curl symptoms. In Bouaké, in contrast, the highest prevalence (30.02%) of pepper viral symptoms was noted with the blistering+mosaic complex. However, symptoms of interveinal discoloration+curling showed the lowest prevalence (3.33%) in both localities. All complex symptoms recorded the highest prevalence in Bouaké, unlike interveinal discoloration + curling symptoms (Table 2).

Overall, the prevalence of single symptoms was statistically similar in the two localities surveyed, while that of complex symptoms was higher in Bouaké (16.68 %) than Abidjan (6.68%) (Table 3). A significant difference in both localities with regard to these complex symptoms ($P < 0.05$).

A and B: Single symptoms **C and D:** Complex symptoms

Fig. 1. Viral disease symptoms observed in both study localities

Table 1. Prevalence of single symptoms in surveyed areas

Single symptoms		Mean prevalence ± Standard error (%)		Statistics	
		Abidjan	Bouaké	H	P
Blistering		16.66 ± 2.46^{a1}	10.05 ± 0.31^{a2}	3.86	0.04
Curling		13.36 ± 1.17^{ab1}	6.66 ± 0.66^{b2}	3.86	0.04
Mosaic		10.03 ± 0.59^{b1}	10.03 ± 0.53^{a1}	0.04	0.83
Interveinal discoloration		3.33 ± 0.68^{c1}	3.33 ± 0.53^{c1}	0.04	0.83
Statistics	H	9.67	9.36		
	P	0.02	0.02		

In the columns and lines, the values topped by the same letters and numbers respectively are not statistically different at 5% threshold according to the Kruskal walis ANOVA test.

Table 2. Prevalence of complex symptoms in surveyed areas

Complex symptoms		Mean prevalence ± Standard error (%)		Statistics	
		Abidjan	Bouaké	H	P
Blistering + curling		10.07 ± 0.88^{a2}	23.33 ± 1.29^{b1}	3.86	0.04
Curling + mosaic		6.67 ± 0.61^{b2}	10.04 ± 0.92^{c1}	3.86	0.04
Blistering + mosaic		6.67± 1.72^{b2}	30.02 ± 2.36^{a1}	3.86	0.04
Interveinal discoloration + curling		3.33± 0.31^{c1}	3.33 ± 0.21^{d1}	0.04	0.83
Statistics	**H**	8.74	9.97		
	P	0.03	0.01		

In the columns and lines, the values topped by the same letters and numbers respectively are not statistically different at 5% threshold according to the Kruskal walis ANOVA test.

Table 3. Prevalence of viral disease symptoms on pepper in Bouaké and Abidjan localities.

Localities		Mean prevalence of single symptoms ± error Standard (%)	Mean prevalence of complex symptoms ± Standard error (%)
Bouaké		7.52 ± 1.61^{a}	16.68 ± 0.80^{a}
Abidjan		10.85 ± 0.87^{a}	6.68 ± 3.24^{b}
	F	3.32	9.00
Statistics	P	0.08	0.00

In the columns, the values topped by the same letters are not statistically different at 5% threshold according to Fisher's LSD test.

3.2.2 Severity of symptoms observed

Symptom severity was ranked from 1 to 5 in both localities. In Abidjan locality, the mean severity of single symptoms, more precisely blistering, was the highest, with a score of 5, and the lowest, with a score of 2, was obtained with leaf curl. In terms of complex symptoms, the lowest mean severity was obtained with leaf curl + interveinal discoloration symptoms, with a score of 3, while it was highest with leaf curl + blistering symptoms, with a score of 5. Similarly, in the Bouaké locality, the lowest mean severity of single symptoms was obtained with blistering, while it was higher with blistering, with scores of 2 and 4, respectively. In terms of complex symptoms, the lowest mean severity (score 2) was obtained for leaf curl+mosaic symptoms, while it was higher (score 5) with blistering+mosaic symptoms. In both localities, the mean symptom severity index ranged from 12.10 in Abidjan to 13.26 in Bouaké (Table 4). However, statistical analyses revealed no significant difference between the symptoms observed ($P > 0.05$).

Table 4. Symptom severity index in Bouaké and Abidjan localities

Localities	Mean Severity index ± Standard error	Statistics	
		F	P
Bouaké	13.26 ± 1.14[a]	0.03	0.51
Abidjan	12.10 ± 1.32[a]		

In the columns, the numbers bearing the same letters are not statistically different at 5% threshold according to Student's t-test

3.3 DNA amplification using the PCR technique

Among a total of 60 samples tested, DNA from two samples showed a fragment of the expected size (750 bp) after PCR amplification (Fig. 2). These included samples with laboratory codes CI 250 and CI 251, collected in the Bouaké areas (Fig. 3). Symptoms of infection occurred by two complex symptoms which were mosaic + blistering for sample CI 250, and interveinal discoloration + curling for sample CI 251. These symptoms were observed on almost all the leaves of infected plants.

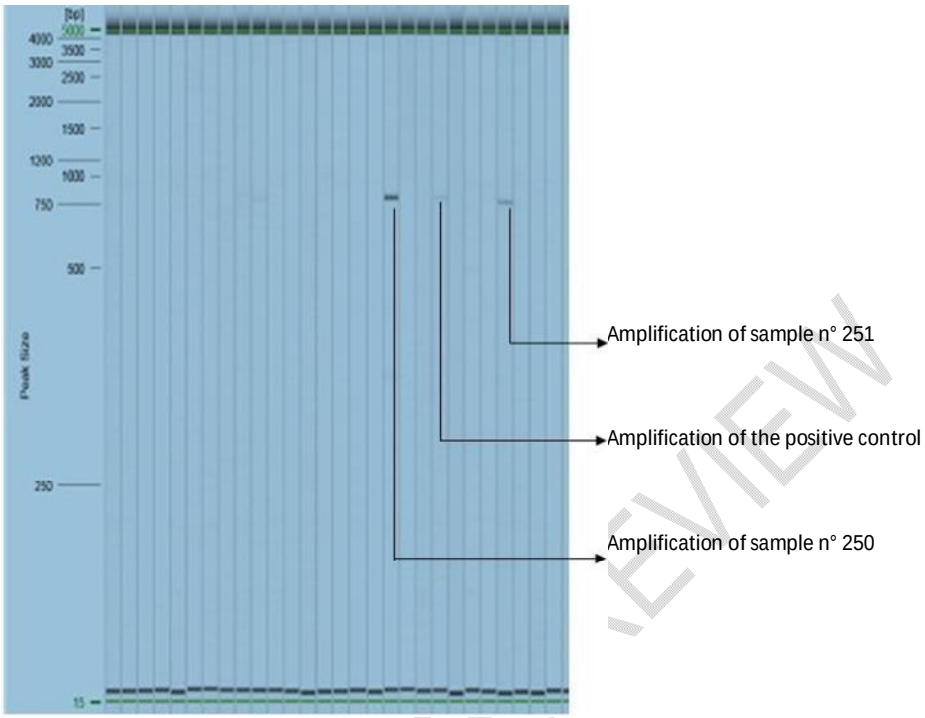
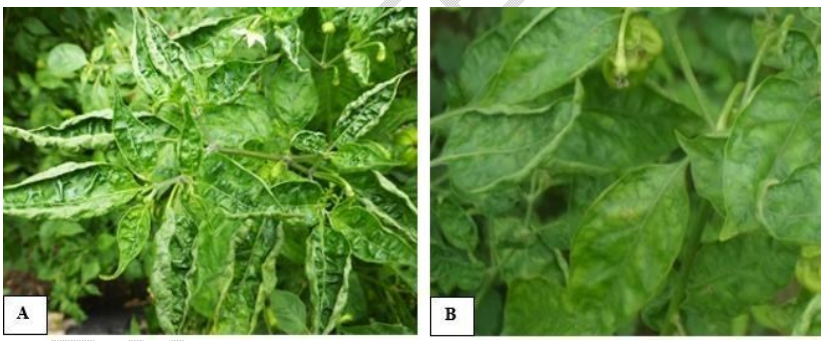


Fig. 2. Amplicons of samples that reacted positively to PCR for Begomovirus detection



A: Sample CI 250 (mosaic + blistering) B: Sample CI 251 (interveinal discoloration + curling)

Fig. 3: Symptomatic pepper samples that reacted positively to a pair of primers directed against Begomoviruses.

3.4 Results of phylogenetic analysis

Two symptomatic samples collected in Bouaké (CI 250 and CI 251) reacted positively to the PCR test for Begomoviruses. The characteristics of the *Pepper Yello Vein Mali Virus* (PepYVMLV) sequences detected in these two samples are shown in Table 5. The two virus sequences were grouped together on a tree rooted on ToLCV [AU: 08] identified by code S53251 on Genbank. Thus, the

PepYVMLV sequence from sample CI251, slightly closer to the root, was related to the PepYVMLV sequence detected at Ferkessédougou in Côte d'Ivoire and found on Genbank with code MH460532. In contrast, the other PepYVMLV sequence from sample CI250, a little further from the root, is more closely related to the PepYVMLV sequence detected in Burkina Faso and found on Genbank under code MH778694 (Fig. 4).

Table 5: Sequence characteristics of *Pepper Yellow Vein Mali Virus* found on pepper in Bouaké.

Code	CI250	CI251
Name of similar sequences	*Pepper yellow vein Mali virus* [Burkina Faso:Sakabi:pepper:72B4:2013]	*Pepper yellow vein Mali virus* [Côte d'Ivoire:Ferkessedougou:CI11_1:2017]
Acronyms for similar sequences	PepYVMLV-[BF:Sak:Pep:72B4:13]_MH778694	PepYVMLV-[CI:Fer:CI11:1:17]_MH778694
% identity	99.42 %	97.16 %
Similar accession	MH77694	MH460532

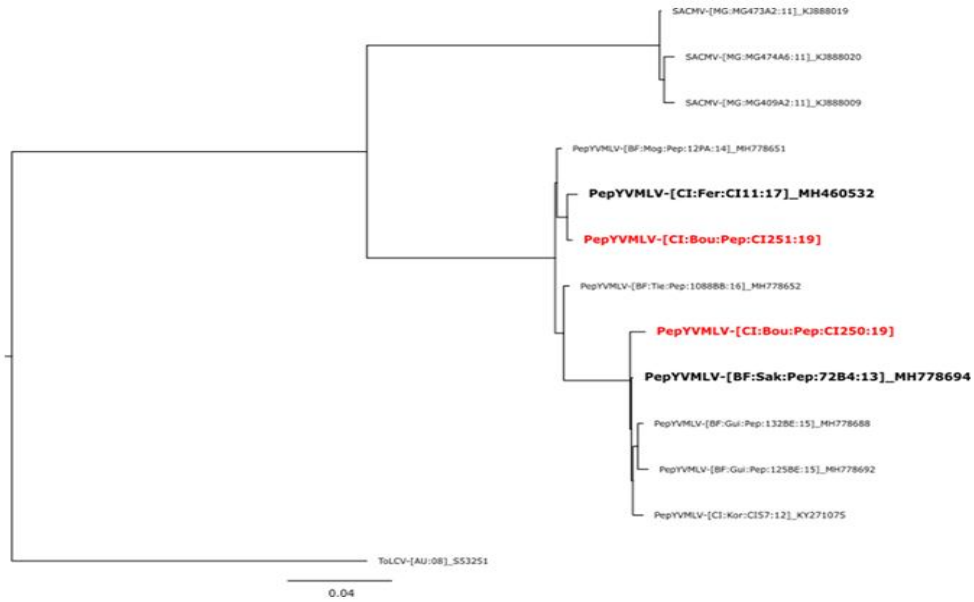


Fig. 4: Phylogenetic clustering of *Pepper Yellow Vein Mali Virus* sequences found on pepper in the Bouaké locality in relation to some sequences existing on Genbank.

4. DISCUSSION

A variety of symptoms caused by viruses have been observed on pepper plants in Bouaké and Abidjan localities. These included single and complex symptoms. This variability of viral disease symptoms was also recorded by [12] during his work on viruses infecting pepper in Burkina Faso. Prevalence showed that single viral disease symptoms were more recurrent in Abidjan locality than in Bouaké. However, in terms of complex symptoms, the most recurrent were in Bouaké locality. However, statistical analyses revealed no significant difference between the different types of viral disease symptoms in both localities. This may be explained by the presence of the same insect vectors, the period of visit, the surface area of the plots which are too small, or by the fact that environmental conditions have no influence on viral disease evolution. This contradicts the assertion [13] made about plant viral diseases that symptoms vary with environmental conditions. As for severity, in terms of single and complex symptoms, the value of the severity index was virtually the same in both localities, except that in Bouaké locality the severity was slightly higher than in Abidjan. This higher level of infection in Bouaké locality is thought to be due to the existence of numerous reservoir hosts in the form of weeds and diseased plants. From these sources of primary inoculum, the insects will spread the viruses to the plots. Thus, a large-scale attack by these insects will increase disease severity.

Among 60 samples collected, only two samples collected from Bouaké were tested positive by molecular analysis in the presence of universal Begomovirus primers. These included samples CI 250 exhibiting blistering + mosaic symptoms and CI 251 exhibiting curling + internervial discoloration symptoms. These results are in line with the work of [12] and [14], who assert that these symptoms are specific to Begomoviruses. However, according to [15], the presence of a symptom complex on the same pepper plant would be due to the antagonistic action of at least two viruses, since the way in which these symptoms occur suggests that they would be caused by the presence of one or more viruses. These pepper plants showed several symptoms at the same time, which would mean that other viruses or environmental factors were interacting with the virus, and that the latter was not solely responsible for the symptoms observed on pepper plants. The PepYVMLV sequences detected in Bouaké are closer to those detected in Côte d'Ivoire in Ferkessédougou and Burkina Faso. These sequences exist on Genbank under codes MH460532 and MH778694, respectively. The presence of this virus on both sides of the Ivorian-Burkinabé border could be explained by

Comment [u6]:

exchanges of plant material between these two countries.

However, some samples showing symptoms tested negative. In fact, the symptoms observed on thesesamples were similar to those caused by Begomoviruses, but molecular analyses detected no presence of Begomovirus. These results are justified by the fact that these viruses are caused by other groups of viruses, causing symptoms similar to those caused by Begomoviruses. These results contradict those obtained by [12], who were able to identify the Begomoviruses responsible for pepper diseases in Burkina Faso, and those obtained by [16] on the discovery of new Begomovirus species in Madagascar and Mayotte.

5. CONCLUSION

The phytosanitary survey carried out in Abidjan and Bouaké localities recorded a variety of single and complex symptoms of viral diseases on pepper plants. A high prevalence of these symptoms was noted in Bouaké, in contrast to Abidjan. However, the level of disease or severity of symptoms was identical in both localities. Molecular analyses using Polymerase Chain Reactions confirmed the presence of Begomovirus in samples CI250 and CI251 collected in Bouaké locality. Several viruses, including *Pepper Yellow Mali Virus* (PepYMLV), are now attacking peppers in Côte d'Ivoire. A complete diagnosis of pepper viral diseases could help to better guide the fight against this pathology.

REFERENCES

1. Lebeau A. Resistance of tomato, eggplant and pepper to Ralstonia solanacearum: interaction between resistance progenitors and bacterial diversity, characterization and mapping of genetic factors involved in eggplant. PhD thesis, University of Reunion, Maisonneuve, France. 2010;183-193.

2. FAO. FAOSTAT Database. Food and Agriculture Organization, Roma, Italy. Available online at URL: www.fao.org. 2014;1-5.

3. FAO. Food and Agricultural Organisation. Http: //faostat.fao.org/faostat. 2012;2-4.

4. Kouassi C. Bioactive potentials and antimicrobial activity of pepper varieties (Capsicum) grown in Côte d'Ivoire. PhD thesis; Nangui Abrogoua University, Abidjan, Côte d'Ivoire. 2012;161.

5. Koffi AC, Koffi-N, Kouassi K, Loukou Y. Activity of extracts of six varieties of pepper (Capsicum) used in Côte d'Ivoire. Journal. Applied. Biosciences. 2014;82:7379-7388.

6. Djinet AI, Nguinambaye MM, Ganon F, Kanga L. Effect of composted manure of cow dung, sheep excrement, poultry droppings (chickens and pigeons), dead straw and residues of local drink "Bilibili" on the agromorphological parameters of peppers (Capsicum annuum L.) grown on the sandy soil of Bongor in Chad. African Journal of Environment and Agriculture. 2023;6(4):70-80.

7. Kouadio P. Diagnosis of pepper virus (Capsicum spp.) in the localities of Abidjan and

Yamoussoukro. 2016;10-11.

8. Ryckebusch F. Characterization of the transmission of capulaviruses, geminiviruses of a new genus whose originality is transmission by aphids (Doctoral dissertation, Montpellier SupAgro). 2020;45-100.

9. Ouattara A. Molecular epidemiology of geminiviruses responsible for emerging diseases on market garden crops in Burkina Faso. Thesis, University of Reunion; University Ouaga 1, Ouagadougou, Burkina Faso. 2017;229.

10. Lava KP. Methods for the diagnosis of plant virus diseases. Laboratory manual. 2009;94.

11. Harry WJ., Bing-Yuan C. PCR CloningProtocols. In: molecular biology. 2nd ed. 2002;192:19.

12. Tiendrébéogo F, Traoré V, Lett J-M, Barro N, Konaté G, Traoré AS, Traoré O. Impact of okra leaf curl disease on morphology and yield of okra. Crop Protection. 2010;29(7):712-716.

13. Ouffroukh A. (2014). Contribution to the knowledge of biotic stresses affecting winter cereals: Identification and approach to the epidemiological study of barley yellow dwarf viruses (BYDV) or barley yellow dwarf virus (BYDV) affecting cereal crops in eastern Algeria. Mentouri-Constantine University. 2014;1:22.

14. Séka K, N'cho JA, Assiri KP, Yao KF, Diallo HA. Medicinal plants of Côte d'Ivoire and viral infections: diagnosis of Begomovirus. International Journal of Agronomy and Agricultural Research. 2017;12(2):78-86.

15. Diallo HA, Gergerich RC, Anderson EJ. Partial molecular characterization of cowpeast isolates of CMV and BlCMV from Arkansas and Georgia. African Agronomy. 2004;16:59-69.

16. Delatte H, Reynaud B, Lett J M, Peterschmitt M, Granier M, Ravololonandrianina J, Goldbach R. First molecular identification of a begomovirus in Madagascar. Plant Disease. 2002;86:1404.