Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2023, 14, 429-438
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Molecular Identification and Characterization of a Begomovirus Associated with Okra Enation Leaf Curl Disease in Mali

Gaoussou K. Keita[1], Laya Kansaye[1], Lassina Doumbia[2*], Ibrahim Keita[2], Mariam Sangare[2], Tidiani Sinayoko[2], Boubacar Macalou[2], Moussa Noussourou Maiga[3], Nadou Paul Sanogo[1], Ousmane Koita[2]

[1]Institut Polytechnique Rural de Formation et de Recherche Appliquée (IPR/IFRA) de Katibougou, Koulikoro, Mali
[2]Université des Sciences, des Techniques et des Technologies de Bamako (USTTB) de Bamako, Laboratoire de Biologie Moléculaire Appliquée (LBMA), Bamako, Mali
[3]Institut Economie Rural (IER), Bamako, Mali
Email: *dolslassina@yahoo.fr

How to cite this paper: Keita, G.K., Kansaye, L., Doumbia, L., Keita, I., Sangare, M., Sinayoko, T., Macalou, B., Maiga, M.N., Sanogo, N.P. and Koita, O. (2023) Molecular Identification and Characterization of a Begomovirus Associated with Okra Enation Leaf Curl Disease in Mali. *Advances in Bioscience and Biotechnology*, **14**, 429-438.
https://doi.org/10.4236/abb.2023.1410029

Received: August 4, 2023
Accepted: October 23, 2023
Published: October 26, 2023

Abstract

Okra is one of the most widespread vegetable crops in the world, particularly in West Africa. However, several factors influence okra crops as biotic and abiotic factors. Among the diseases affecting its culture, okra leaf curl disease is a major threat. This study aims to assess begomoviruses from okra plants with symptoms like leaves curl by molecular approach. A total of thirteen serologically positive samples were tested by PCR assay, and one sample was sequenced among them. The begomovirus was found in six isolates, *Bhendi yellow vein mosaic virus* (BYVMV) in three isolates, and *Okra enation leaf curl virus* (OELCuV) in three isolates, respectively. The begomovirus isolate sequences shared 90% identity with the *Cotton leaf curl Gezira virus*. Mixed infections between these viruses were found. Thus, these results highlight the need to monitor the spread of these disease-causing viruses for okra crops in Mali. In addition, they can also lead to a considerable loss in okra fields in Koulikoro, which is an agricultural region by excellence.

Keywords

Okra, PCR, Begomovirus, BYVMV, OELCuV, Mali

1. Introduction

Okra or *Abelmoschus esculentus* L. Moench, is a flowering plant composed of edible green seed pods [1]. The Malvaceae Familly, okra is a vegetable native to

West and Central Africa [2].

The Geminiviridae family is composed of small circular ssDNA viruses, encapsidated within virions, with geminate morphology, and known to cause diseases in crop plants across the world [3].

Cultivated okra is recognized to be susceptible to a multiple number of begomovirus-like mosaic virus diseases and others. These others could be responsible viruses such as Yellow vein mosaic disease (OYVMV), Okra leaf curl disease (OLCV), and Okra enation leaf curl disease (OELCuV), CLCuGV, and CYCrV which are potentially transmitted in the field by the whitefly [4]-[8].

Most geminiviruses are in the genus *Begomovirus* [9].

Begomovirus infects primarily dicotyledonous plants and the transmission is done by the whitefly *Bemisia tabaci* [10] [11]. Transmission is mainly by whitefly [12], and infection increases as the number of whiteflies increases. Symptoms appear 8 to 12 days after infection [3].

OLCD is an important viral disease characterized by leaf curling either upward or downward, mostly associated with plant stunting. Caused by viruses of the genus *Begomovirus*, and affects the culture of Okra in terms of yield and fruit quality in tropical and subtropical areas [13] [14].

In most African countries, Okra leaf curl disease (OLCD) is considered the most serious disease menacing okra production [14]. Infection usually occurs at an early stage in the crop [15]. The age of the seedlings plays an important role in the acquisition of infection. It is highest between 7 and 15 days of seeding [3].

OYVMV is one of the most devastating and widespread viruses in the okra fields, transmitted by whitefly (*Bemisia tabaci* Genn.), which is the most troublesome pest of many crops in the world [16]. Kulkarni initially reported the virus in Mumbai, India in 1924. Yadav *et al.* reported that the okra Leaf curl disease can cause significant yield losses. The losses of yield are more important among plants infected at an early stage [17]. The virus is responsible for 80% - 90% of yield losses and it affects all the developmental stages of okra plants. In Ghana, Africa, more than 50% of disease incidence in okra farms has been reported [16].

Usually, viral infections are controlled by vector control. Controlling whitefly populations and/or treating seedlings with phytopesticides are just some of the ways to avoid infection by the okra Leaf curl virus [18]. It has been reported that controlling whitefly populations is the best measure against okra Leaf curl disease [18].

Near Bamako, Mali, typical symptoms of begomovirus infection, such as yellow veining, leaf yellowing, crinkling, and cupping, were observed in okra (*Abelmoschus esculentus*) variety trials. After sequence analysis, the disease was caused by a distinct begomovirus named Okra Yellow Vein Crinkle Disease (OYVCrD) [19]. This study aimed to identify and characterize begomoviral infection by molecular method from okra plants with OLCD plus the mosaic symptoms in Koulikoro, Mali.

2. Materials and Methods

2.1. Sample Collection

Leaves of okra showing symptoms were collected from nine (9) different field locations of Koulikoro (IPR Diakitebougou, IPR boarding garden, IPR garden near bridge, IPR garden, Kolebougou, Koulikoro Downtown, and Niarebougou). It was previously 52 samples from which 13 samples have tested positive for the Okra Mosaic virus (OkMV) serologically.

2.2. DNA Isolation

Approximately 100 mg of okra leaves frozen at −20˚C were used for DNA extraction. Virus DNA was extracted using a DNeasy Plant Mini kit (Qiagen, Maryland, USA) according to the manufacturing's instructions. The concentration and purity of DNA were evaluated at 260 to 280 nm with a spectrophotometer (Eppendorf AG, Germany) by absorbance at (260/280, and 260/230 ratio) respectively. DNA was stored at −20˚C when it was not used immediately.

2.3. PCR Amplification

All DNA isolates were screened for begomovirus detection, *Bhendi yellow vein mosaic virus* (BYVMV), and *Okra enation leaf curl virus* (OELCuV). The primers used were "Okra-F318/Okra-R1004, and Okra-1469/Okra-R2338" for begomovirus, "BY" for BYVMV, and "OE" for OELCuV (Table 1).

Polymerase chain reactions (PCR) were carried out at the Laboratoire de Biologie Moléculaire Appliquée (LBMA) in Bamako. Primers (Table 1) were used to identify begomoviruses, BYVMV, and OELCuV in diseased plant leaf samples. PCR was performed following the protocol modified from studies conducted by Naresh *et al.* (2019) in southwest India, and Tiendrebeogo (2014) in Burkina Faso [20] [21].

Amplification was carried out using the thermocycler PTC 200. The total volume of the reaction was 25 µl containing different concentrations: 1 X Buffer, 3

Table 1. List of primers.

Primer name	Sequence 5' → 3'	Amplified fragment size bp	References
OE For	CGCTATAAGTACTTGCGCACTAAG	327	(Naresh *et al.* 2019)
OE Rev	CATTCGTGATTTTGTGACGCGG		
BY For	GGCATGGACAAACAGGCCTATG	470	
BY Rev	CCAACGCCTGTTCCCTCGCTG		
Okra-F318	AATTATGTCGAAGCGACCAG	700	(Tiendrebeogo *et al.* 2010; Tiendrebeogo, 2014)
Okra-R1004	GCATTCTCCGTATGATTCTC		
Okra-1469	CAGTGATGAGTTCCCCTGTG	900	
Okra-R2338	GTCAAGTCCTACATCGACAAGG		

mM $MgCl_2$, 0.4 mM dNTPs, 0.4 µM each primer, 1.25 U GoTaq® DNA Polymerase, and 5 µl of DNA. The program was conducted using the thermocycler PTC 200:1 cycle of denaturation at 94˚C for 5 minutes followed by 35 cycles of 94˚C for 1 min (denaturation), 52˚C for 30 sec (hybridization), 72˚C for 1 min (initial elongation), 72˚C for 10 min (final elongation).

The product amplifications were visualized on 1% and 1.5% agarose gel according to primer size with ethidium bromide using UV-light with UV® light transilluminator and Doc-ItLS Analysis Software.

2.4. Sequencing

One sample positive with Okra-F318/Okra-R1004 primers selected based on product quality PCR was subject to the Sanger sequencing technique using the CEQ™ 8000 DNA analyzer (Beckman Coulter). The sequences were analyzed using the NCBI BLAST Search Tool (BLAST, https://www.ncbi.nlm.nih.gov/) consulted on 31 July 2023. Following amplification, the PCR products were purified and re-amplified using the GenomeLab DTCS Quick Start Kit (Beckman Coulter, USA) according to the manufacturer's instructions. Finally, sequencing products were purified by ethanol precipitation.

2.5. Phylogenetic Analysis

The Basic Local Alignment Search Tool (BLAST) of NCBI (National Center for Biotechnology Information) was used to confirm the identity of the generated sequences in the GenBank nucleotide database. The sequences were aligned with BioEdit software version 7.7 using sequences retrieved on GenBank alignments. The GenBank accession numbers, strain, country of origin, district of origin, date, and the plant of isolation were given for each virus when available.

The phylogenetic tree was performed using Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0. The Maximum likelihood statistical method based on the Tamura-Nei model was performed [22]. The robustness of the phylogenetic tree was evaluated with 1000 bootstrap replicates.

3. Results

Identification of Okra Leaf Curl Virus

At the left, the PCR product shows Okra-F318/Okra-R1004 which amplified 700 bp with samples B1, C3, D3, E3, E4, and E5 positive; B2, B3, and B4 were negative; NC negative control and PC positive control to the assay; Marq-100 molecular weigh (100 bp). At the right, Okra-1469/Okra-R2338 which amplified 900 bp with B1, D3, E3, and E5 positive, B2, B3, B4, C1, and C3 were negative respectively. NC negative control and PC positive control to the assay; Marq-100 molecular weight (100 bp).

Symptoms were mainly mosaic and leaf curl. We found mixed infections.

The genome highlighted in red corresponds to the sequence of this study. The phylogeographic analysis of the sequences using BLAST tools showed that the

viruses isolated in Mali are mostly similar to those from Burkina Faso (Figure 2).

4. Discussion

Among the samples collected from different areas of Koulikoro, 13 samples have been positive for serological tests. PCR was successfully applied for begomoviral detection in these symptomatic serological positive samples with fragments of approximately 900 bp, 700 bp (see Figure 1), 470 bp, and 327 bp (the figure does not show). The result obtained was expected, the band of products PCR was properly visible on the agarose gel after electrophoresis (Figure 1).

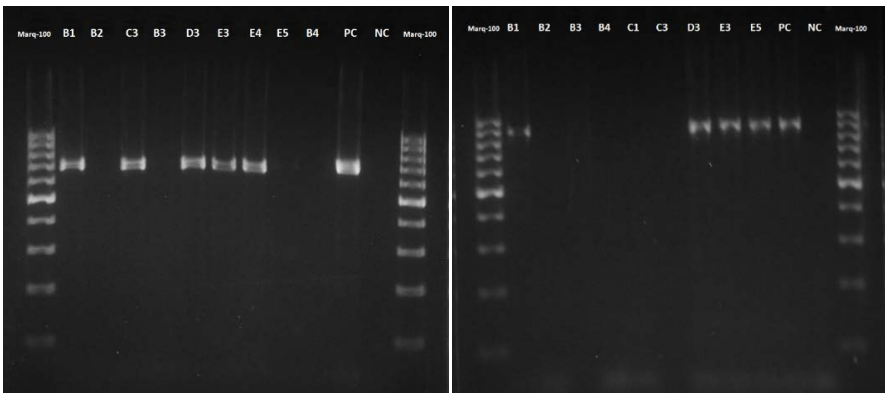
These PCR product fragments found in this study corroborate those of Tiendrebeogo *et al.* (2010) [14]; Tiendrebeogo, (2014) [21], and Naresh *et al.* (2019) [20] who have carried out similar work on okra begomoviruses.

In this study, we have evaluated the possibility of okra leaves being infected by several types of viruses. We first tested okra leaves serologically for okra mosaic virus. PCR was used to identify the viruses responsible for okra leaf disease. In the present study, PCR was used to identify the begomoviruses BYVMV and OELCuV.

This study showed that 46% (6/13) of samples were positive for begomovirus-specific primers, 23% (3/13) for BYVMV, and 23% (3/13) for OELCuV respectively (Table 3).

In the identification process of the viruses infecting okra leaves, we found in the same DNA extracts begomovirus plus BYVMV and OELCuV. This is the first time that these two viral species infecting okra leaves (BYVMV and OELCuV) have been reported in Mali, and they may be responsible for a major threat to okra production.

Tiendrébéogo *et al.* (2010) [14] reported that the effect of okra leaf curl disease is greater in local okra seed crops with an overall considerable yield loss, ranging from 68.5% to 72.5% in Burkina Faso compared to commercial seed crops from 8.7% to 16.2%. The effect of the disease on local okra seed crops was



Okra-F318/Okra-R1004 Okra-1469/Okra-R2338

Figure 1. Gel electrophoresis analysis of the products from PCR of begomovirus.

observed by a 58% reduction in growth compared to 39.6% for commercial ones [14].

We found mixed infections with the virus responsible for enrolment and yellow vein mosaic of leaves, with the other viruses investigated in this study accounting for 23% (3/13) (**Table 3**). Tiendrébéogo *et al.* (2010) [14], reported major symptoms such as leaf curl found in this study.

The sequenced sample was 90% identified with 5% gaps using the NCBI BLAST tool with a higher similarity to Cotton leaf curl Gezira virus from Burkina Faso. Thus, we used BLAST NCBI, which showed similarity with Cotton leaf curl Gezira virus from different geographical areas Burkina Faso, Côte d'Ivoire, and Niger (**Figure 2**).

Table 2. Results of PCR by samples symptoms and primers used.

ID	Symptoms	PCR			
		BY	OE	Okra318	Okra1469
B1	Mosaic + leaf curl	POS	POS	POS	POS
B2	Mosaic + leaf curl	NEG	NEG	NEG	NEG
B3	Mosaic + leaf curl	NEG	NEG	NEG	NEG
B4	Mosaic + leaf curl	NEG	NEG	NEG	NEG
C1	Mosaic + leaf curl	NEG	NEG	NEG	NEG
C2	Mosaic + leaf curl	NEG	NEG	NEG	NEG
C3	Mosaic + leaf curl	POS	POS	POS	NEG
C4	Mosaic + leaf curl	NEG	NEG	NEG	NEG
D2	Mosaic + leaf curl	NEG	NEG	NEG	NEG
D3	Mosaic + leaf curl	NEG	NEG	POS	POS
E3	Mosaic + leaf curl + yellow spots	NEG	NEG	POS	POS
E4	Mosaic + leaf curl	NEG	NEG	POS	NEG
E5	Mosaic + leaf curl	POS	POS	POS	POS

NEG: Negative POS: Positive.

Table 3. Number of positive samples detected by PCR.

PCR Positive	Fréquency n = 13	Percentage
BY	3	23.1
OE	3	23.1
Okra-F318/R1004	6	46.2
Okra-F1469/R2338	4	30.8
Mix infection*	3	23.1

*: begemovirus + BYVMV + OELCuV.

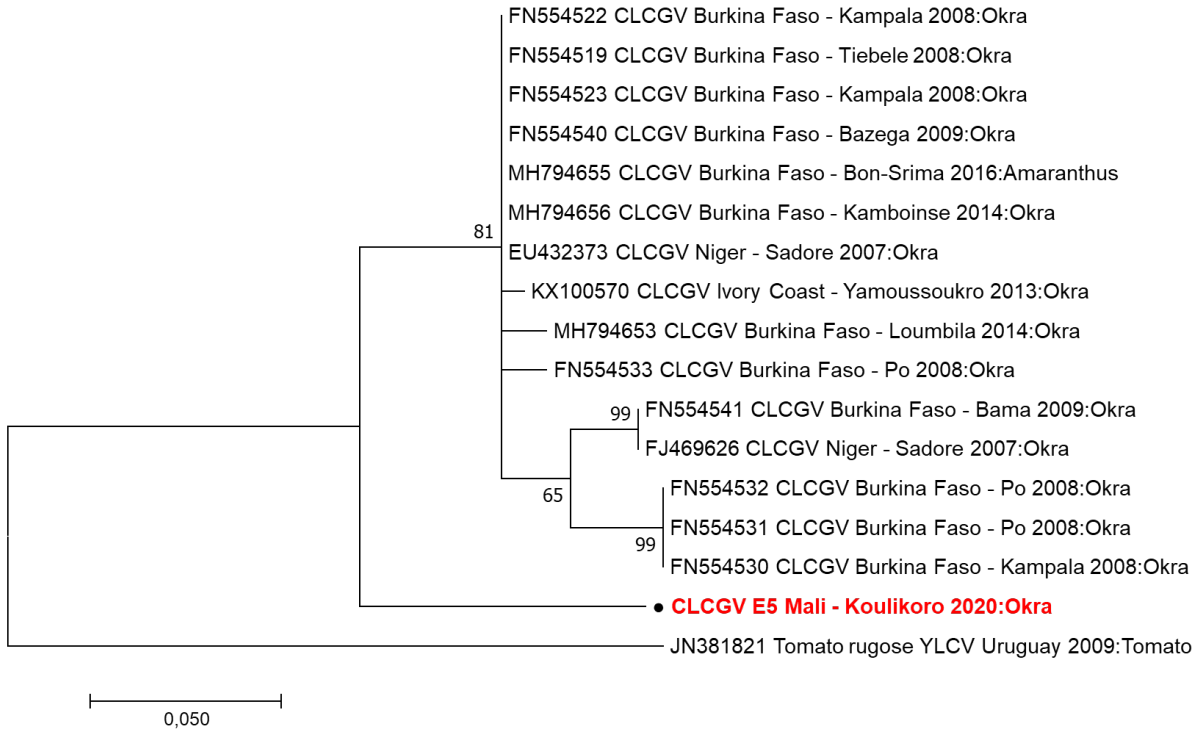


Figure 2. Maximum likelihood (ML) phylogenetic tree based on generated sequence and 16 begomovirus sequences retrieved by BLAST on NCBI.

The bogomovirus was also found associated with the Okra curl disease and CLCuGV (*Cotton leaf curl Gezira virus*) in Burkina Faso [14].

In this study, the sequenced sample showed a very high similarity to CLCuGV. This suggests a permanent circulation in the fields where the samples were collected. It is also important to note that cotton is also well-grown in these fields during the rainy season.

Cotton leaf curl Gezira virus has been reported in several African countries, including Burkina Faso [23], Niger [24], Côte d'Ivoire [25], and Sudan [26].

5. Conclusion

This study is the first of its kind, with all activities from collection to sequencing analysis carried out in Mali. The okra leaf curl disease (OLCD) is present in the agroecological zone of Koulikoro, Mali. These molecular results highlight the complex causes of okra leaf curl disease in Mali and suggest future investigations.

Acknowledgements

Thank you to Valérie Verdier of IRD—France and Professor Daouda Koné of UFHB in Abidjan—Côte d'Ivoire for their support.

Funding

This research didn't receive grants from any funding agency in the public, com-

mercial or not-for-profit sectors.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Adelakun, O.E. and Oyelade, O.J. (2011) Potential Use of Okra Seed (Abelmoschus esculentus Moench) Flour for Food Fortification and Effects of Processing. In: Preedy, V.R., Watson, R.R. and Patel, V.B., Eds., *Flour and Breads and Their Fortification in Health and Disease Prevention*, Academic Press, Cambridge, 205-212. https://doi.org/10.1016/B978-0-12-380886-8.10019-4

[2] Schippers, R.R. (2000) African Indigenous Vegetables: An Overview of the Cultivated Species.

[3] Venkataravanappa, V., Kodandaram, M.H., Lakshminarayana Reddy, C.N., Shankarappa, K.S. and Krishna Reddy, M. (2017) Comparative Transmission of Bhendi Yellow Vein Mosaic Virus by Two Cryptic Species of the Whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). 3 *Biotech*, **7**, Article No. 331. https://doi.org/10.1007/s13205-017-0970-8

[4] Hameed, S., Khalid, S., Ehsan-ul-Haq, S. and Hashrni, A.A. (1994) Cotton Leaf Curl Disease in Pakistan Caused by a Whitefly-Transmitted Geminivirus. *Plant Disease*, 78, 529. https://doi.org/10.1094/PD-78-0529H

[5] Konaté, G., Barro, N., Fargette, D., Swanson, M.M. and Harrison, B.D. (1995) Occurrence of Whitefly-Transmitted Geminiviruses in Crops in Burkina Faso, and Their Serological Detection and Differentiation. *Annals of Applied Biology*, **126**, 121-129. https://doi.org/10.1111/j.1744-7348.1995.tb05008.x

[6] Vegetable Seeds (2023) Disease Management: Okra Disease Management. https://www.nunhems.com/in/en/disease-management/okra-disease-management.html

[7] Venkataravanappa, V., Reddy, C.N.L., Jalali, S., Briddon, R.W. and Reddy, M.K. (2015) Molecular Identification and Biological Characterisation of a Begomovirus Associated with Okra Enation Leaf Curl Disease in India. *European Journal of Plant Pathology*, **141**, 217-235. https://doi.org/10.1007/s10658-014-0463-0

[8] Venkataravanappa, V., Reddy, C.N.L., Jalali, S. and Reddy, M.K. (2013) Molecular Characterization of a New Species of Begomovirus Associated with Yellow Vein Mosaic of Bhendi (Okra) in Bhubhaneswar, India. *European Journal of Plant Pathology*, **136**, 811-822. https://doi.org/10.1007/s10658-013-0209-4

[9] Fiallo-Olivé, E., *et al*. (2021) ICTV Virus Taxonomy Profile: *Geminiviridae* 2021. *Journal of General Virology*, **102**, Article ID: 001696. https://doi.org/10.1099/jgv.0.001696

[10] Morales, F.J. and Anderson, P.K. (2001) The Emergence and Dissemination of Whitefly-Transmitted Geminiviruses in Latin America. *Archives of Virology*, **146**, 415-441. https://doi.org/10.1007/s007050170153

[11] Varma, A. and Malathi, V.G. (2003) Emerging Geminivirus Problems: A Serious Threat to Crop Production. *Annals of Applied Biology*, **142**, 145-164. https://doi.org/10.1111/j.1744-7348.2003.tb00240.x

[12] Venkataravanappa, V., Lakshminarayana Reddy, C.N., Jalali, S. and Krishna Reddy, M. (2012) Molecular Characterization of Distinct Bipartite Begomovirus Infecting

bhendi (*Abelmoschus esculentus* L.) in India. *Virus Genes*, **44**, 522-535. https://doi.org/10.1007/s11262-012-0732-y

[13] Inoue-Nagata, A.K., Lima, M.F. and Gilbertson, R.L. (2016) A Review of Geminivirus Diseases in Vegetables and Other Crops in Brazil: Current Status and Approaches for Management. *Horticultura Brasileira*, **34**, 8-18. https://doi.org/10.1590/S0102-053620160000100002

[14] Tiendrébéogo, F., *et al*. (2010) Impact of Okra Leaf Curl Disease on Morphology and Yield of Okra. *Crop Protection*, **29**, 712-716. https://doi.org/10.1016/j.cropro.2010.02.007

[15] Senevirathna, H.M.S.I., Wasala, S.K., Senanayake, D.M.J.B., Weerasekara, D., Wickamasinghe, H.A.M. and Deepal, P.K.G.A. (2016) Characterization and Detection of Yellow Vein Disease of Okra (*Abelmoschus esculentus* (L.) Moench) in Sri Lanka. *Tropical Agricultural Research*, **27**, 360-369. https://doi.org/10.4038/tar.v27i4.8213

[16] Mubeen, M., *et al*. (2021) Yellow Vein Mosaic Disease in Okra (*Abelmoschus esculentus* L.): An Overview on Causal Agent, Vector and Management. *Phyton*, **90**, 1573-1587. https://doi.org/10.32604/phyton.2021.016664

[17] Yadav, Y., *et al*. (2018) Enation Leaf Curl Virus (ELCV): A Real Threat in Major Okra Production Belts of India: A Review. *Journal of Pharmacognosy and Phytochemistry*, **7**, 3795-3802.

[18] Asare-Bediako, E., Addo-Quaye, A. and Bi-Kusi, A. (2023) Comparative Efficacy of Plant Extracts in Managing Whitefly (*Bemisia tabaci* gen) and Leaf Curl Disease in Okra (*Abelmoschus esculentus* L). *American Journal of Agricultural Science and Technology*, **2**, 31-41.

[19] Kon, T., Rojas, M.R., Abdourhamane, I.K. and Gilbertson, R.L. (2009) Roles and Interactions of Begomoviruses and Satellite DNAs Associated with Okra Leaf Curl Disease in Mali, West Africa. *Journal of General Virology*, **90**, 1001-1013. https://doi.org/10.1099/vir.0.008102-0

[20] Naresh, M., *et al*. (2019) Occurrence and Variability of Begomoviruses Associated with Bhendi Yellow Vein Mosaic and Okra Enation Leaf Curl Diseases in South-Western India. *VirusDisease*, **30**, 511-525. https://doi.org/10.1007/s13337-019-00551-4

[21] Tiendrébéogo, F. (2014) Caractérisation et aspects épidémiologiques des Begomovirus infectant les plantes maraîchères et le manioc au Burkina Faso. Master's Thesis, Université de Ouagadougou, Ouagadougou.

[22] Tamura, K. and Nei, M. (1993) Estimation of the Number of Nucleotide Substitutions in the Control Region of Mitochondrial DNA in Humans and Chimpanzees. *Molecular Biology and Evolution*, **10**, 512-526.

[23] Tiendrébéogo, F., *et al*. (2010) Molecular Diversity of Cotton Leaf Curl Gezira Virus Isolates and Their Satellite DNAs Associated with Okra Leaf Curl Disease in Burkina Faso. *Virology Journal*, **7**, Article No. 48. https://doi.org/10.1186/1743-422X-7-48

[24] Shih, S.L., Kumar, S., Tsai, W.S., Lee, L.M. and Green, S.K. (2009) Complete Nucleotide Sequences of Okra Isolates of Cotton Leaf Curl Gezira Virus and Their Associated DNA-β from Niger. *Archives of Virology*, **154**, 369-372. https://doi.org/10.1007/s00705-008-0304-6

[25] Sék, K., *et al*. (2016) First Reports of Cotton Leaf Curl Gezira Virus and Okra Yellow Crinkle Virus Associated with Okra Leaf Curl Disease in Cote d'Ivoire. *New Disease Reports*, **34**, 8. https://doi.org/10.5197/j.2044-0588.2016.034.008

[26] Idris, A.M. and Brown, J.K. (2002) Molecular Analysis of Cotton Leaf Curl Virus-Sudan Reveals an Evolutionary History of Recombination. *Virus Genes*, **24**, 249-256. https://doi.org/10.1023/A:1015380600089