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Virus and virus-like diseases of citrus in West-Africa: an overview

Fabrice Afloukou[1, 2*], Laurence Dossou[2,3], Valérien Zinsou[2]

1, Department of Plant Protection, Faculty of Agriculture, University of Çukurova, Turkey

2, Faculty of Agronomy, University of Parakou, Benin Republic

3, UFR Biosciences, Félix Houphouët Boigny University, Republic of Ivory-Coast

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***Corresponding author:**

Department of Plant Protection, Faculty of Agriculture, University of Çukurova, Turkey.

Email: afloukoufm@gmail.com

ABSTRACT

Purpose: In sub-Sahara Africa, citrus is mainly cultivated in the coastal countries. It plays important nutritional and socio-economical roles by providing vitamins and minerals to consumers and cash to farmers. The crop is being challenged by many constraints. The purpose of this review is to inventory the virus and virus-like diseases known to infect citrus in the West-Africa up today. **Findings**: Less attention is given to citrus in West African countries while the crop is imperiled by biotics as well as abiotic constraints. Then, it is threatened by several diseases. Among them, the updated reported virus and virus-like diseases are tristeza, exocortis, citrus psorosis complex, cachexia, stubborn and greening. There is no recent update regarding the incidence, severity, and distribution of those diseases as well as their impact on yield. The biological or molecular characterization of the diseases' causal agents also still lack. **Research limitations**: There is no effective national or regional plant protection policy; leading to the persistence of citrus diseases and wide-spreading infections. Climate change is enhancing the dilemma. **Directions for future research:** The sustainable production of citrus in the region requires new plant protection policies and investigations regarding citrus diseases and their vectors. Furthermore, rootstocks screening is required in every single agro-ecological zones where citrus is grown for the simultaneous management of citrus viroids, viruses, and soil-born fungi.

INTRODUCTION

The West African region includes countries such as Benin Republic, Burkina Faso, Cape Verde, the Gambia, Ghana, Guinea, Guinea Bissau, Cote-d'Ivoire, Liberia, Mali, Mauritania, the Niger, Nigeria, Senegal, Sierra Leone, and Togo. It covers arid, semi-arid, sub-humid and humid climates, providing opportunities to cultivate a wide range of agricultural items. All efforts are given to staple and cash crops while fruits are almost neglected. Thus, most West-Africans like Africans in general, consume less than one serving of fruits per day; leading to some nutritional gaps (Pretty & Bharucha, 2014).

In 2017, the West-African region supplied close to 6 million metric tons of citrus fruits with an average yield of 10.47 $t.ha^{-1}$ whereas 146.60 million tons were produced with a yield of 16.71 $t.ha^{-1}$ worldwide (FAOSTAT, 2018). These regional trends may be explained by a myriad of reasons. Virus and virus-like diseases play important roles worldwide. Here are the earlier reports, current situation and distribution of virus and virus-like diseases of citrus in the West-African region.

Tristeza

Globally, tristeza is one of the most destructive viral diseases in citriculture. The causal agent, *Citrus tristeza virus* (CTV) (genus *Closterovirus*, family *Closteroviridae*), is a single-stranded, positive-sense, flexuous filament RNA (2000 × 11 nm in size) of 20 kb with 12 open read frames (ORFs) coding for 17 proteins products and two untranslated regions (Dawson, 2010). *Aegle marmelos*, *Aeglopsis chevalieri*, *Afraegle paniculata*, *Citropsis gilletiana*, *Microcitrus australis*, *Pamburus missionis*, and most of citrus species are CTV' hosts (EPPO, 2004). The virus is transmitted to citrus in a semi-persistent manner by aphids species (*Toxoptera citricida* Kirkaldy, Aphis *spiraecola*, *Aphis gossypii* Glover, *Aphis citricola* Van der Goot, *Toxoptera aurantii)* or by grafting (EPPO/CABI, 1996). Depending on the virus strain, scion/rootstock combination, and the existence of vectors in the diseased area, biological activities such as "quick decline", "stem pitting", and "yellow seedling" may be observed on the infected citrus trees (Barzegar et al., 2005). Grapefruit [*C. paradisi* Macf.], mandarin [*C. reticulata* Blanco], and sweet orange [*Citrus sinensis* (L.) Osb.] grafted on sour orange [*C. aurantium* (L.)] rootstocks exhibit "quick decline"; "seedling yellows" show up in grapefruit, lemon[*C. limon* (L.) Burn. f.], and sour orange [*C. aurantium L.*] (Fraser, 1959; McClean, 1960) while stem pitting arises in all citrus varieties regardless of rootstocks (Bar-Joseph & Dawson, 2008). Reduction of productivity and fruit quality may also be recorded in infected trees (Bar-Joseph et al., 1989).

In West-Africa, a survey conducted by Mendel (1968) in 1961 revealed the non-infected citrus by CTV in Guinea, Mali, Upper Volta (Current Burkina-Faso), Cote-d'Ivoire, and Dahomey (Current Republic of Benin), while Ghana and Nigeria were infected. This reality can be explained by the fact that the African Commonwealth countries while establishing the citrus industries, imported the tristeza disease alongside their bud-woods from South Africa. Meanwhile, former French colonies in West Africa received CTV-free bud-woods almost exclusively from the Central research station founded by the "Institut des Fruits et Agrumes Coloniaux" (I.F.A.C) at Foulaya in Guinea (Mendel, 1968). The mode of later infection of citrus in the West-African former French colonies is still unknown.

Ghana was the first West African country to experience the infection. Shepherd (1954) observed vein flecking on lime. The decline has been proven to be caused by a virus and not fungi, bacteria or malnutrition disorders (Lister, 1947; 1948). Symptoms included twig die-back, vein flecking of the young leaves and severe stem pitting of lime' trunks and branches (Hughes & Lister, 1953). Both authors started indexing experiments. Successful trials of graft

inoculation and *Toxoptera citricida* transmission of both diseases enabled investigators to associate a couple of symptoms to one virus: *Citrus tristeza virus*. Subsequent researches in California, Brazil, and South-Africa confirmed this finding. Currently, the disease is widespread in Southern and Central Ghana (Hughes & Lister, 1953; Kumi et al., 2013). The disease was also reported in the southern and center Republic of Benin (Brun, 1971; Cassin, 1966; Montargut, 1971; Vogel, 1972a) where citrus brown aphid (*Toxoptera aurantii*) has been observed feeding in citrus orchards (Lokossou et al., 2009). This CTV vector is also reported in Cote-d'Ivoire, the Gambia, Ghana, Nigeria, and Mauritania (CABI, 2019). The polyphagous cotton aphid (*Aphis gossypii* Glover), recognized as one the most efficient CTV' vector is widespread in the West African region (CABI, 2019). Three hundred (300) infected citrus seedlings were shipped from the Benin Republic CTV outbreak center (Bohicon) to Niger in the 1970s (Vogel, 1972b). There is no updated information about those plants.

The CTV disease occurred in Cote-d'Ivoire (Vogel, 1972a) and Togo (Hughes & Lister, 1953). Regarding the rest of the region, Guinea and Senegal were out of CTV infection until the end 1950s (Jamoussi, 1955). The same situation was observed in Mali until 1974 (Darthenucq & Rey, 1974). The current situation of CTV disease is still unknown in most of the former French West-African colonies.

In Nigeria, the CTV disease was first reported by Varma (1981) and is thought to be wide-spread on sweet orange, tangerine (*C. reticulata* Blanco), grapefruit and lemon and this, regardless rootstocks in Ogun, Oyo, Osun and Lagos state in the south-western part of the country (Adedapo et al., 2015; Kareem et al., 2013).

Classic diagnostic methods such as direct tissue blot immunoassay (DTBIA), double antibody sandwich-indirect-enzyme-linked immunosorbent assay (DAS-ELISA), direct antibody coating enzyme linked immunoassay (*DAC-ELISA*), reverse-transcription polymerase chain reaction (RT-PCR), and real-time PCR promote the disease diagnostic whereas standardized biological indexing methods allow the determination of the virus isolate. Indeed, sweet orange grafted on sour orange rootstock monitors for decline on sour orange; Duncan grapefruit seedlings monitor for stem pitting on grapefruit; Madame vinous sweet orange seedlings monitor for stem pitting; and sour orange or Duncan grapefruit as an indicator for seedling yellows; Mexican lime is a universal indicator (Lee, 2015).

Certification of bud-wood stock, use of resistant rootstocks (trifoliate orange and their hybrids), control of aphids, inoculation of citrus seedlings with the CTV mild form, the production of virus free-plants through shoot-tip grafting and heat treatment, and the disinfection of grafting and cutting tools may help to control the disease.

Citrus psorosis complex

The pathogen associated with the disease, *Citrus psorosis virus* (CPsV), is classified in the genus O*phiovirus* within the family O*phioviridae* (Milne et al., 2000) and is spread through propagating material. CPsV is a tripartite virus of three small single-stranded RNA with negative polarities. Two forms of psorosis were outlined: psorosis A and psorosis B. Psorosis A, the more common form of the disease is characterized by the presence of bark-scaling in the trunk and the staining of interior wood; psorosis B, the more aggressive form causes rampant bark scaling of the trunk and fine twigs in field trees, chlorotic blotching in old leaves with brownish gummy pustules in the leaf underside and in some rare cases ringspots on fruits (Martin et al., 2004; Velázquez et al., 2012). The disease takes generally a long time (10–12year) to exhibits symptoms in the field (Roistacher, 1991). It has been reported at Bohicon in the central part of the Benin Republic (Vogel, 1972a; 1975). Unfortunately, its form was not specified. In Nigeria, psorosis A was thought to occur in lemon, Carrizo and Troyer citrange [*C. sinensis* Osbeck × *Poncirus trifoliata* L. Raf.] in Kaduna, Rivers, Oyo,

Ogun, and Lagos state states (Varma, 1981). Psorosis B was also present in the country but not common (Varma & Atiri, 1993). Reports in the rest of the West-African countries still lack.

Various methods of CPsV detection are available (Martin et al., 2004). DAS-ELISA, triple antibody sandwich-indirect-enzyme-linked immunosorbent assay (TAS-ELISA), and direct tissue blot immunoassay (DTBI) have been developed for the rapid detection of the virus. Alternatively, molecular tests like RT-PCR are available for the diagnostic (Garcia, 1997).

Cross protection, certification and quarantine program, combined shoot tip grafting and thermotherapy, and resistant transgenic plants based on post-transcriptional gene silencing (PTGS) are available for the management of citrus psorosis complex.

Exocortis

Citrus exocortis viroid (CEVd) was the first citrus viroid to be described (Roistacher, 1991). It is an RNA of 366-475 nucleotides with rod-like secondary structure allocated to the genus *pospiviroid* in the pospiviroidae family (Flores et al., 2005; Tabler & Tsagris, 2004). CEVd is known to infect Rutaceae family species mostly; some species in Solanaceae (potato, tomato, petunia) and Compositae family are also hosts to the viroid (Sastry, 2013). The pathogen infects citrus in everywhere susceptible rootstocks [Citrange (*Poncirus. trifoliata* x *C. sinensis*), Citrumelos (*C. paradisi* x *P. trifoliata*), *P. trifoliata*, Rangpur lime] are being used. It was demonstrated to be graft and mechanical transmissible (Barbosa et al., 2005). Most of the time CEVd' hosts are symptomless. High temperatures and light intensities favor the pathogen accumulation and symptoms expression (Carbonell et al., 2008). The latter include epinasty, stunting, and necrosis of the leaf mid-vein; back shelling of the rootstock; dwarfing of scions grafted on the rootstock; and fruit yield losses (Lee, 2015). A yield loss up 59.1 kg per tree was recorded on Hamilton orange grafted on Rangpur lime infected with the severe strain of the viroid in Brazil (Rodriguez et al., 1974).

With regards to West-Africa, the symptoms of CEVd were reported on Valencia sweet orange (*C. sinensis* L. Osbeck), Bergamot sour orange (*C. bergamia* Risso), Temple tangor (Tangerine x Sweet orange), Duncan grapefruit (*C. paradisi* Macf), sweet oranges (*C. sinensis* L. Osbeck), grafted on Rangpur lime (*C. reticulata* × *medica*) in the Benin Republic (Vogel, 1972b; Lokossou et al., 2009). CEVd was also reported on sweet orange and grapefruit grafted on Rangpur in the Agricultural station of Asuansi, Bunso and Aiyinasi in Ghana and is thought to be well distributed in citrus orchards in Ogun, River and Lagos state in Nigeria (Opoku, 1972; Varma & Atiri, 1993; Wallace, 1959).

Biological indexing using Arizona 861-S1 Etrog citron (*C. medica*) and gynura (*Gynura aurantiaca*), and molecular tests [Polyacrylamide gel electrophoresis (PAGE), dot-blot hybridization, hybridization of tissue imprints, RT-PCR] are the available methods of detection of the viroid. Its management requires quarantine and certification programs; clean stock and sterilization of clippers, saws, and other cutting or pruning tools (Lee, 2015; Roistacher, 1991).

Cachexia

Hop stunt viroid (HSVd) also known as *Citrus viroid* II (CVd-II) is the only member of the genus *Hostuviroid* within the *Pospiviroidae* family (Flores & Owens, 2008). The HSVd is an RNA of 295 to 303 nucleotides of length that owns five domains, a central conserved region (CCR) and a "terminal conserved hairpin" (TCH) (Palacio-Bielsa et al., 2004; Serra et al., 2008). Two individual strains of the viroid are available. The non-pathogenic strain (CVd-IIa) and the pathogenic strains (CVd-IIb and CVdIIc). Both strains induce disease on the host but the first does not exhibit symptoms contrary to the pathogenic strains. Only six nucleotides

appearance in the "variable domain" (V) located at the right of the C domain in the viroid genome distinguish and determine symptom expression on a citrus host (Reanwarakorn & Semancik, 1998). Although minor differences have been identified, the six nucleotides are thought to be highly conserved (Velázquez et al., 2002; Palacio-Bielsa et al., 2004).

The pathogenic strains of the viroid also known *as Citrus cachexia viroid* (CCaVd) can spur discoloration, severe gumming, and wood pitting symptoms in mandarins (*C. reticulate* Blanco), mandarin's hybrids, Clementines (*C. clementina* Hort. Ex. Tan), satsumas (*C. unshiu* (Macf.) Marc.), alemow (*C. macrophylla* Webster), Rangpur lime (*C. limonia* Osb.) and kumquats (*Fortunella* spp.) (Eiras et al., 2013). The affected citrus trees are stunted, may decline and die (Serra et al., 2008).

In West-Africa, less is known about the occurrence of the cachexia disease although it causes serious damage in every single citrus growing area of the globe. It was only reported on mandarin grafted on Rangpur lime in the southern-East Benin Republic (Vogel, 1972a; 1975).

Scrapping or removing the outer bark layers of susceptible citrus cultivars allows the detection of the disease in the field. In addition, biological indexing using Parson's Special mandarin (*C. reticulata* Blanco) and Orlando tangelo (Duncan grapefruit × Dancy mandarin) (Roistacher, 1991) or molecular biology tests like RT-PCR or PAGE may contribute to the detection of cachexia disease. The management of cachexia requires clean stock and sterilization of clippers, saws, and other tools used to cut or prune citrus trees.

Stubborn

Described for the first time in California in 1944, the stubborn disease is caused by a wall-less bacterium arrange in the class of mollicutes and called *Spiroplasma citri* (Bove et al., 2002; Saglio et al., 1973). *S. citri* is transmitted by grafting or from plant to plant by phloem-feeding leafhoppers in a propagative manner. *Circulifer haematocepts* is the known vector of Stubborn in the Mediterranean region while *Circulifer tenellus* and *Scaphytopius nitidusare* are the available ones in the USA (Calavan & Bove, 1989). The infected citrus trees, especially the younger are stunted (Roistacher, 1991). Tree canopy is often localized into sectors, especially when larger trees become infected. Leaves are mottled, smaller, upright and cupped. Fruit are small in size, lopsided, with aborted seeds. The fruit stylar end remains green and the peduncle end shows color (Lee, 2015). Fruit yield losses up to 53 % may be recorded (Mello et al., 2010). Although Lee (2015) admit that the disease does not occur in the tropical and subtropical region, it would be reported in Cote d'Ivoire in 1972 and symptoms associated to the disease observed on Washington Navel orange (*C. sinensis* L. Osbeck) in the Center-Benin (Vogel, 1972a; 1975). Updates still lack until now.

The detection of stubborn disease necessitates molecular methods such as PCR and real-time PCR. Indexing of Madame Vinous sweet orange seedlings through inoculation with bud chips or side shoot grafts and maintaining at 37°C/27°C (day/night) is an alternative method of detecting the disease (Roistacher, 1991). With regard to the management, the control of vectors, clean stock, quarantine, and certification programs are required. In addition, the use of trap plants, more attractive to the vectors than citrus has been reported to provide a good result (Schwarz, 1965).

Citrus greening

Also called "Huanglongbing (HLB)", greening disease is caused by a phloem-limited uncultivable gram-negative bacterium named *Candidatus* Liberibacter (Garnier, 1983; Garnier et al., 1984). Worldwide three species of the bacterium are associated with the disease: *Candidatus* L. americanus (Lam) in American continent; *Candidatus* Liberibacter

asiaticus (Las) in Asia and *Candidatus* L. africanus (Laf) in Africa (Jagoueix et al., 1994; Teixeira et al., 2005). Greening affected trees show the stunting, open growth, sparse yellow foliage, twig dieback, huge fruit drop leaves mottling resembling zinc deficiency symptoms, poorly colored and under-developed, and lopsided fruits (EPPO/CABI, 1996). *Candidatus* L. africanus (Laf) especially make fruits to remain green, immature and, seeds stained and aborted (EPPO/CABI, 1996). Sensitive to temperature, it induces moderate to severe symptoms under 22-24°C and suppresses symptoms above 27°C contrary to *Candidatus* L. asiaticus (Las) which exhibits symptoms under both temperature regimes (Halbert & Manjunath, 2004). *Candidatus* L. africanus (Laf) cause severe symptoms in high altitude and latent disease in lower hot areas (Batool et al., 2007).

Graft transmissible, the greening disease is spread in nature by two psyllid vectors namely *Trioza erytreae* (Del Guerico) and *Diaphorina. citri* (Kuwayama) (Bove, 2006). *T. erytreae* transmits *Candidatus L.* africanus (Laf) and *Candidatus L.* asiaticus (Las) while *D. Citri*, the most efficient vector of the disease, transmits every single species of the pathogen (Bove, 2006; Lopes et al., 2010). Dodder (*Cuscuta* spp.) is also reported to transmit the disease (Batool et al., 2007). Significant yield losses due to greening are reported in Asia and Africa. In West-Africa, the disease was only reported in Nigeria where it is thought to be wide-spread (Varma & Atiri, 1993). Further investigations are required in order to get the current occurrence and distribution of the disease in every single citrus growing area of West-Africa.

Regarding the management of the disease different strategy has been used across the globe. Among them, the testing of citrus psyllids for the presence of the bacterium on indicator plants [Sweet orange, Orlando tangelo and Ponkan mandarin (*C. reticulata* Blanco)] (Roistacher, 1991) or with molecular tests are very common. Thermotherapy, chemotherapy, control of vector, quarantine, and certification programs are other ways to cope with the disease.

CONCLUSION

Citrus is being challenged by several viruses and virus-like diseases in West-Africa where less attention is given to the crop. Effective plant protection policies and recent research-based papers of the pathological aspects of the crop greatly lack. The updated status, distribution and molecular characterization of the occurring and potential citrus diseases along with their vectors in the whole region are much-needed.

Conflict of interest

The authors have no conflict of interest to report.

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