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RESEARCH ARTICLE

SYMPTOMATOLOGY OF OKRA VIRAL DISEASES (*ABELMOSCHUS ESCULENTUS*) AND THEIR POTENTIAL IMPACT ON YIELD

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ABSTRACT

Okra is an important food for many populations in Côte d'Ivoire. However, viral diseases are a real threat to this crop. The general objective is to generate scientific knowledge to protect okra production. Phytosanitary surveys were carried out two weeks after planting at the Ahoué (Ayaman-Abidjan) experimental site and until harvest. The incidence and severity of symptoms were observed. The effect of the viral disease on yield was also determined by comparing the different agronomic parameters between 50 apparently healthy and infected plants. These plants were previously labeled after the results of the PCR assays with the universal primers directed against okra-infecting Begomovirus (*Cotton Leaf Curl Gezira Virus* (CLCuGV) and *Okra Yellow Crinkle Virus* (OYCrV) but also simple symptoms (Mosaic, crinkle, leaf curl, leaf discoloration and vein brightening), but complex symptoms have also been observed. The incidence of viral diseases ranges from 50% to 80% throughout The highest yields, 4 on the scale of 1 to 5, were recorded in the complex symptoms of mosaic + crinkle + leaf curl, yield losses by 48.93% were due to Okra viral disease. The use of resistant or tolerant varieties combined with appropriate farming techniques to control effectively the viral disease.

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INTRODUCTION

Okra belongs to the *Malvaceae* family and to the genus *Abelmoschus*. It is currently cultivated in all tropical and subtropical regions; South America (India, Indonesia), South America (Charrier, 1983) and Africa (Nigeria, Sudan, Côte d'Ivoire Ghana and Egypt) (Tiendrébeogo *et al*., 2008). Its fruits and leaves are consumed fresh or dry. In Western Africa, okra ranks in vegetable production after tomato (FAO, 2009).
Okra is rich in vitamins, fiber and mineral salts. It is used in sugar industry and in pharmaceutical industry (Seck, 1991). In Côte d'Ivoire, the fruits of that vegetable are much appreciated in fresh or dried culinary preparations (Seck, 1991). However, the farming of okra is experiencing more and more difficulties that affects its production level. These include control of production techniques, pests and diseases that directly affect production (Mahan, 2005; Sika, 2006). Viral diseases are a real threat to okra cultivation. In Côte d'Ivoire and in the West African region, the presence of several okra-infecting viruses has been reported, among others, *Cotton Leaf Curl Gezira Virus* (CLCuGV) and *Okra Yellow Crinkle Virus* (OYCrV) (N'Guessan *et al.*, 1992; Bigarré *et al*., 2001; Idris *et al*., 2005; Tiendrébéogo *et al*., 2010; Séka *et al*., 2016).

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However, little work has been done to get the relevant information for a satisfactory virus control. The general objective of this work is to generate scientific knowledge to secure okra production in Côte d'Ivoire. Specifically, this study aims to draw up an inventory of the symptoms of such viral disease; determine the incidence and severity of these symptoms and the effect of these symptoms on the yield parameters of okra plant.

MATERIALS AND METHODS

Experimentation and Planting

The trials were conducted in the locality of Ahoué (Anyama-Côte d'Ivoire). A random experimental system consisting of three sub-plots has been established. The area of each sub-plot was 16 m x 12 m. Each sub-plot consisted of 10 rows and 15 columns, giving a total of 150 planting points for each sub-plot. The spacing between two sub-plots was 1.5 m. Within each sub-plot, the distance between two seed hole is 1 m on the line and between the lines. A direct seeding of 3 grains of okra of the Hiré variety per seed hole was carried out, giving a total of 450 planting points. Fifteen days after sowing, three weedings were carried out at two-week intervals. The plants have not undergone any phytosanitary treatment.

Symptomatology and Sample Collection

Phytosanitary surveys were carried out on the site to make an inventory of the observed symptoms. The different types of symptoms have been described. The description took into account the shape, coloring and distribution of the disease on the plot. Young symptomatic and asymptomatic leaves were collected on plants aged at least 45 days. A total of 120 leaves, collected from different plants, were collected for laboratory analysis. Plants which leaves responded positively to PCR tests directed against Begomoviruses, with the universal primer couple Cluster Cluster 4 F342/ R1032 -QIAGEN (5'-TATMATCATTTCCACBCCVG-3'/5'-GCATGAGT ACATG CCATATAC-3'), and apparently healthy plants have been marked.

Incidence of each symptom

The number of okra plants presenting each type of symptom was determined based on the total number of infected plants on the plot. The mean percentage incidence was calculated using the IITA formula (1997).

Severity of each symptom

For each okra plant, a severity score of 1 to 5 was assigned (IITA, 2010) according to the following criteria: 1 = no viral symptom; 2 = 1 to 25% plant with a type of viral symptom; 3 = 26-50% plant with a type of viral symptom; 4 = 51-75% plant with a type of viral symptom; 5 = more than 76% of plant with a type of viral symptom. The symptom severity index was also calculated according to the following formula of Rempel and Hall (1996.

$$\text{Severity Index (\%)} = \frac{(\sum \text{note x number of plants with this score}) \times 100}{(\text{Highest score x total number of plants})}$$

$$\text{Yield loss (\%)} = \frac{\text{MFrs - MFri}}{\text{MFrs}}$$

P (%): Yield loss, MFrs: Mass of fruits of apparently healthy plants, MFri: Mass of fruit of infected plants

Data analysis

The data obtained in this study were analyzed using the Statistica 7.1 software. The Kruskal Wallis rank test was used to compare the mean of the different symptoms observed according to the agronomic parameters measured in okra. This same test was also used to analyze the incidence and severity of the various symptoms observed. The Mann-Whitney U test was used to compare the averages of apparently healthy and infected plants against the parameters measured in okra. In the case of a significant difference at 5% threshold, the Mann-Whitney U test was performed to determine the different homogeneous groups.

RESULTS

Symptoms observed on okra leaves

The symptoms observed on the collected leaves have been described. Two types of symptoms were observed on okra leaves. This involves alteration of the color and alteration of the organs.

The change in color is characterized by mosaics and discolorations. As regards the alteration of the organs, it was characterized by crinkles, curls and dwarfism. These various symptoms showed in the form of symptom complexes (Figure 1).

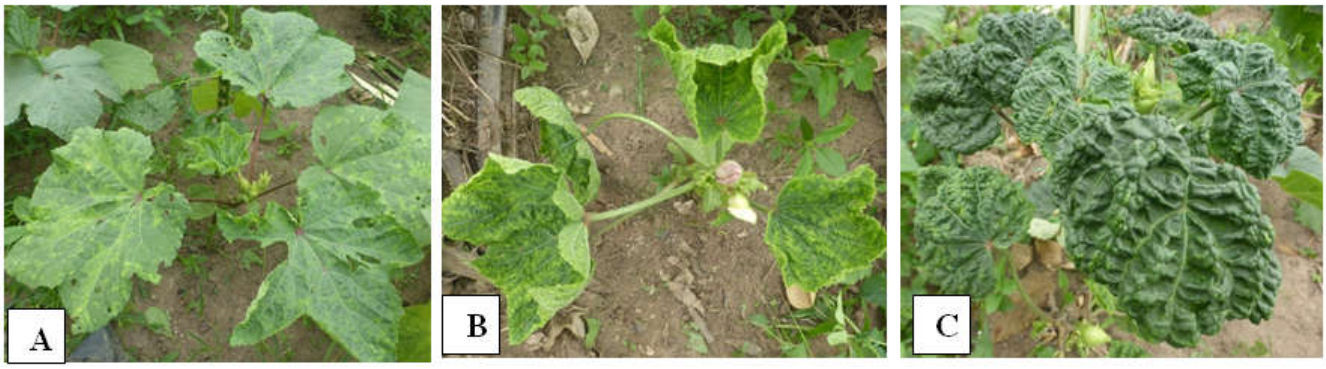

Figure 1. Symptoms observed on okra leaves, A : Mosaic and B: Discoloration + brightening of the veins + upward curl causing by *Okra Yellow Crinkle Virus* (OYCrV) ; C : Crinkle + downward curl (epinasty) causing by *Cotton Leaf Curl Gezira Virus* (CLCuGV)

Determination of the effect of viral disease on yield

To determine the effect of the viral disesase on yield, the vegetative growth characteristics (Leaf size - EFe, leaf length - LFe, plant size - TP) and reproductive (Flower size - EF, Effectiveness and length of fruit - EF and LF, the fruit mass - MF) were studied. Comparative study of these different parameters between 50 apparently healthy and infected plants was carried out two weeks after planting until harvesting at intervals of two weeks. Estimated yield losses were assessed using the formula below.

Incidence of symptoms on okra leaves

Incidence varied from 3.08 % to 26.30 %, depending on the symptoms. With 20.75 % and 26.30 % respectively, mosaic (M) and crinkle with downward curl (C + D) had the highest incidences and discolorations with vein brightening and upward curl (L + V + U), dwarfism (D), mosaic with crinkle and downward curl (M + C + D) had the lowest incidences (3.08 and 5.08 %). Statistical analysis revealed a significant difference (F = 22.93 and P = 0.003) and three homogeneous groups were obtained. The first group of symptoms composed of mosaic (M) and crinkle with downward curl (C + D)

recorded the highest incidences. The second group of symptoms, consisting of mosaic with crinkle and upward curl (M + C + U), crinkle with upward curl (C + U) and crinkle (C) with respective incidences of 11.04 % and 14.52 %. The third group formed of discolorations with vein brightening and upward curl (L + V + U), dwarfism (D) and mosaic with crinkle and downward curl (M + C + D) lower with respectively (3.08 % and 5.08 %) (Table 1).

Table 1. Incidence of symptoms observed on okra infected leaves

Symptom Types	Symptoms	Incidence (%)
Unique	M	20.75 ± 7.83 (66.50) a
	C	14.52 ± 1.19 (59.50) b
	D	4.87 ± 2.15 (24) c
	C+U	11.04 ± 2.64 (46) b
	C+D	26.30 ± 3.61(76) a
	M+C+D	5.08 ± 0.95 (25.50) c
	M+C+U	12.89 ± 7.32 (52) b
Complexes	L+V+U	3.08 ± 2.12 (13) c
	H	22.93
	P	0.003

The values with letters are statistically different at 5% threshold, according to the Kruskal Wallis test, H: Kruskal Wallis value, P: Probability, the values in brackets represent the ranks. M : Mosaic; C : Crinkle; D : Dwarfism; C+U : Crinkle with upward curl; C+D : Crinkle with downward curl; M+C+D : Mosaic with crinkle and downward curl; M+C+U : Mosaic with crinkle and upward curl; L+V+U : Discolorations with vein brightening and upward curl.

ML[ML:Mlo1:04], DQ902715; OYCrV-ML[ML:Bam4:06], EU024119). CLCuGeV and OYCrV implicated in Okra leaf curl disease (OLCD) in Côte d'Ivoire.

Table 2. Severity of symptoms observed on okra leaves

Symptom Types	Symptom	Severities (1 through 5)
Uniques	M	3.67 ± 0.57 (20.50) b
	C	4 ± 0.00 (27) a
	D	3.67 ± 0.57 (20.50) b
	C+U	4.33 ± 0.57 (38.50) a
	C+D	5 ± 0.00 (61.50) a
	M+C+D	5 ± 0.00 (61.50) a
	M+C+U	5 ± 0.00 (61.50) a
Complexes	L+V+U	5 ± 0.00 (61.50) a
	H	19.03
	P	0.01

The values with letters are statistically different at 5% threshold, according to the Kruskal Wallis test, H: Kruskal Wallis value, P: Probability, the values in brackets represent the ranks. M : Mosaic; C : Crinkle; D : Dwarfism; C+U : Crinkle with upward curl; C+D : Crinkle with downward curl; M+C+D : Mosaic with crinkle and downward curl; M+C+U : Mosaic with crinkle and upward curl; L+V+U : Discolorations with vein brightening and upward curl

Effect of the viral disease on different yield parameters

The different symptoms had an effect on certain vegetative parameters, namely the total number of leaves (EFe), and the leaf length (LFe) as well as the reproductive parameters: number of flower (EF), number of fruit (EFr) fruit length (LFr) and fruit mass (MFr) (Table 3).

Table 3. Effect of the viral disease on different yield parameters

Samples	EFe	EF	EFr	LFe	LFr	Tp	M Fr
Infected	5.91±0.53 b (2008.50)	4.14±1.35 b (1402)	2.29±1.27 b (12.18)	11.76±3.74 b (1580)	3.2±2.11b (1645.50)	33.52±9.11a (22855.50)	0.46±0.33 b (1495.50)
Heath	6.27±0.57 a (2647.5)	6.45±1.30 a (3254)	5.52±0.96 a (3438)	15.94±3.04 a (3076)	5.57±1.62 a (3010.50)	34.60±8.66 a (2370)	0.94±0.32 a (3160.50)
U	832.50	226.00	42.00	404.0000	469.50	1109.50	319.50
P	0.00	0.00	0.00	0.00	0.00	0.75	0.00

EFe : Number of leaves, LFe : leaf length, TP : Plant size, EF: Number of flower, EFr Number of fruit, LFr: Fruit length, MFr: Fruit mass

Severity of Symptoms on Okra Leaves

The severity of the various symptoms observed varied from 3.67 and 5 on the scale from 1 to 5. The statistical analysis showed a significant difference (H = 19.03 and P = 0.014) between the different severities associated with Symptoms. Two homogeneous groups were obtained. Mosaic with crinkle and upward curl (M + C + U); mosaic with crinkle and downward curl (M + C + D); crinkle with upward curl (C+U), discolorations with vein brightening and upward curl (L + V + U), crinkle with downward curl (C + D), crinkle (C) recorded the greatest severities (between 4 and 5) and symptoms of mosaic (M) and dwarfism (D), recorded the smallest severities (3.67) (Table 2).

Molecular analyzes

In Côte d'Ivoire, One complete genome sequence (GenBank Accession No. KX100570) showed the highest pairwise sequence identity of 99% (100% coverage) with isolates of the Niger strain of CLCuGeV from Niger (CLCuGeVNE [NE:Sad:NG2FL:Ok:07], FJ469627) and Burkina Faso (CLCuGeVNE[BF:Kap:Ok4:08], FN554524). The other three sequences (KX100571 to KX100573) showed the highest pairwise sequence identity of 98-99% (100% coverage) with isolates of the Mali strain of OYCrV from Mali (OYCrV-

The number of leaves (EFe) in the plants varied from 5.91 to 6.27 on average. In infected plants, it stood at 5.91 against 6.27 in apparently healthy plants. Statistical analysis showed a significant difference (U = 832.50 and p = 0.00) between apparently healthy plants and infected plants. The largest number of leaves was recorded in healthy plants and the smallest number of leaves was recorded in infected plants. The leaf length (LFe) varied from 11.76 cm to 15.94 cm. Statistical analysis showed a significant difference (U = 404.00 and p = 0.00) between apparently healthy plants and infected plants. The largest length was recorded in apparently healthy plants. The shortest length was recorded in infected plants. About plant size (TP), size variation was observed in infected plants (34.33 cm) and apparently healthy plants (34.60 cm). Statistical analysis found no significant difference (U = 1109.50 and p = 0.75) between apparently healthy and infected plants.

The number of flowers (EF) varied on average from 4.14 to 6.45. Statistical analysis showed a significant difference (U = 226.00 and p = 0.00) between apparently healthy and infected plants. Apparently healthy plants produced more flowers than the infected plants. Regarding the number of fruits (EFr), the apparently healthy plants produced more fruits on average (5.52) than the infected plants (2.29). Statistical analysis showed a significant difference (U = 42.00 and p = 0.00)

between apparently healthy and infected plants (Table 3). The average fruit length (LFr) in apparently healthy plants was 5.57 cm while that of the infected plants was 3.2 cm. Statistical analysis showed a significant difference (U = 469.50 and p = 0.00) between apparently healthy and infected plants (Table 3). In terms of length, the longest fruit was recorded in apparently healthy plants and the smallest fruit was recorded in the infected plants. The fruit mass in the apparently healthy plants was 0.94 g while that of the infected plants was 0.46 g. Statistical analysis showed a significant difference (U = 319.50 and p = 0.00) between apparently healthy and infected plants (Table 3). The largest fruit mass was recorded in apparently healthy plants and the smallest fruit mass was recorded in the infected plants. The disease impact on the infected plants was more highlighted in the reproductive and vegetative parameters except the size of the plant compared to apparently healthy plants. 48.93 % yield losses were recorded in the plants showing symptoms alike those caused by the viruses.

DISCUSSION

A variety of symptoms of viral disease were observed on okra leaves. They include mosaic, crinkle, foliar curl, and discoloration of leaves. Similar results were recorded by Barro (1994). This author observed symptoms of mosaic, leaf curl and yellowing on okra leaves in Burkina Faso. The diversity of symptoms could be due to the presence of a virus on the plot. Indeed, during their work on okra, in Burkina Faso, Tiendrébéogo *et al*. (2008) reported that the diversity of symptoms observed may be caused by the presence of different strains of the same virus which, depending on climatic conditions and the virulence of the inoculum strains, would result in different types of symptoms. Complexes involving mosaic, crinkle and curl were the most severe. Barro (1994); Konaté *et al*., 1995 in their okra studies, noted that the mosaic is sometimes associated with curl. Based on the symptoms and the nexted PCR test result, the mosaic is attributed to Okra *Yellow Crinkle Virus* (OYCrV). As for the crinkle and curl of the leaves, it is caused by *Cotton Leaf Curl Gezira Virus* (CLCuGV) (Tiendrébéogo *et al*., 2010, Séka *et al*., 2016). The incidence of viral diseases ranged between 50 % and 80 %. These results are conversant with the work of Tiendrébéogo *et al*. (2010). Indeed, this author recorded an incidence ranging from 68.5 % to 72.5 % in commercial varieties of okra in Burkina Faso. The results are also consistent with those reported in Uganda and Tanzania (Nono-Womdim *et al.,* 1996, Ndunguru and Rajabu, 2004, Sekyewa, 2006). These authors shown that the incidence of viral diseases encountered is often very high, above 80 %. Severity ranging from 3.67 to 5 were observed for the various symptoms. These symptoms were more severe in mixed infection.

These mixed infections usually lead to synergistic interactions, which would increase the severity of leaf symptoms. Diallo and *al*., 2004 and Séka and *al*. (2009) also showed that mixed infections induced synergistic action of the viruses and caused more severe symptoms on the leaves of the infected plants respectively during their respective work on the viruses. Viral disease reduced reproductive and vegetative traits other than plant size on infected plants. This difference could be attributable to the viruses once transmitted to plants negatively influence their physiological and photosynthetic properties (Friess and *al*., 1996; Ryslava and *al*., 2003). However, Baron and *al*. (2012) in their study of the photosynthetic response to biotic stress in environmental change, showed that of virus attack could alter the growth and morphology of the plant. The development of generalized viral particles to the whole plant brings about metabolic disturbances leading to the expression of various symptoms (Kummert and Semal, 1989). Viruses cause different leaf modifications in susceptible plants (Hollings and Brunt, 1981; Astier *et al*., 2001). These remarks were also observed in our study where symptoms of crinkle, curl were observed. Costa (2003) also demonstrated during this work that the virus uses the metabolism of the plant to multiply, causing leaf and fruit malformations. The viral disease reduced the yield of infected okra plants by 48.93 %. These symptoms are therefore a real threat to okra cultivation in Côte d'Ivoire. The use of resistant or tolerant varieties coupled with appropriate cropping techniques will prove effective in combating or controlling such virus.

Conclusion

A variety of symptoms of the viral disease were observed on okra. They include mosaics, discolorations, crinkle, curl and complexes of symptoms. Among the symptoms the mosaic and crinkle + downward curl are the most observed. Symptom complexes involving mosaic and crinkle were the most severe on okra plant leaves. This symptom causes more losses because the fruits of such plants presenting this symptom are aborted. This viral disease caused by *Okra Yellow Crinkle Virus* (OYCrV) and *Cotton Leaf Curl Gezira Virus* (CLCuGV) reduce the yield of okra by 48.93 %. This viral disease is therefore a real threat to okra cultivation in Côte d'Ivoire. The use of resistant or tolerant varieties combined with appropriate cropping techniques will prove effective in controlling this virus.

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