Salaudeen, M. T et al (2016). Performance of Two Locally Adapted Okra Varieties as Influenced by Cucumber Mosaic Virus Disease. NJTR Vol. 11: 1-6.

Performance of Two Locally Adapted Okra Varieties as Influenced by Cucumber Mosaic Virus Disease

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Abstract

The responses of two locally adapted okra varieties (Bokungi and Ikeregi) to Cucumber mosaic virus (CMV) were evaluated under field conditions during the 2014 cropping season. Seedlings were inoculated with the virus at 1 week after emergence. Each genotype was evaluated as inoculated and uninoculated treatments. Disease incidence, severity of infection (scale 1 – 5), yield and yield related parameters were measured. Virus titre was quantified using Enzyme-Linked Immunosorbent Assay (ELISA). Data were subjected to independent t test and significance was determined at 5 % level of probability. One hundred percent infection was found in both varieties but disease severity was higher in "Bokungi" (5) than in "Ikeregi" (3). Higher virus concentration was found in the inoculated leaves of "Bokungi"(ELISA value = 0.89) compared to "Ikeregi" (ELISA value = 0.41). Reductions in plant height (8.8 %), fruit number (33.3 %) and fruit weight (37.6 %) were significantly lower in "Ikeregi" than "Bokungi". The present data reveal that both okra varieties are susceptible to CMV infection but "Ikeregi" appears to be more tolerant. Cultivation of the more CMV-tolerant okra variety would offer some level of insurance against complete crop failure in case of disease outbreak in the study area.

Keywords: *Cucumber mosaic virus*, disease severity, resistance, yield, okra
Email: mtsalaudeen.fut@gmail.com.
Received: 2015/05/29
Accepted: 2016/03/28
DOI: http://dx.doi.org/10.4314/njtr.v11i1.1

Introduction

Okra (*Abelmoschus esculentus*) is an important vegetable crop in tropical and sub-tropical Africa. It is cultivated in all the agro-ecological zones of Nigeria (Adeniji *et. al.*, 2007). It is grown mainly as fruit and leafy vegetable for human consumption. The crop is an annual and erect plant belonging to the family Malvaceae (Naveed *et. al.*, 2009). Okra is a good source of vitamins and mineral salts. Some of its nutrient compositions are water (86.1 %), protein (2.2 %), fat (0.2 %), carbohydrate (9.7 %), fiber (1.0 %), ash (0.8 %) (Saifullah and Rabbani, 2009) and about 20 to 40 % oil (Benchasr, 2012). Several other studies have shown its significant contribution to the development of foetus in pregnant women, proper functioning of the heart and reduction of body's cholesterol. Okra also plays a major role in maintaining good eye sight, managing ulcer, asthma and diabetes (Ngoc *et. al.*, 2008; Sengkhamparn *et. al.*, 2009; Sabitha *et. al.*, 2011; Messing *et. al.*, 2014).

In spite of the numerous uses of okra, productivity is constrained by poor soil fertility and attack by several insects (Echezona *et. al.*, 2010). Some of the most damaging ones include cotton aphid (*Aphis gossypii* Glover), cotton bollworm (*Helicoverpa. armigera* Hubner) and the flea beetle (*Podagrica uniformis* L.). Besides, different pathogens induce stresses on okra plants at various growth stages. Some of the major diseases are damping off (*Pythium* sp., *Rhizoctonia* sp.), Fusarium wilt (*Fusarium oxysporum* f. sp. *vasinfectum*), Powdery mildew (*Erysiphe cichoracearum*), *Okra leaf curl virus* and *Cucumber mosaic virus*.

Cucumber mosaic virus disease is an economically important disease of several crops including okra. It belongs to the genus *Cucumovirus* and family *Bromoviridae*. Symptoms of infection include leaf mottling and curling, chlorosis, and stunting. *Cucumber mosaic virus* produces a systemic infection in most plants (Mih *et. al.*, 1991). Older tissues and organs that develop prior to infection are usually not affected by the virus, but newer cells and tissues that develop after infection may be affected with varying degree of severity (Agrios, 2005). The virus is transmitted by over 60 aphid species in a non-persistent manner and induces severe yield losses in several crops including vegetables, ornamentals and legumes (Palukaitis *et. al.*, 1992). The two okra varieties commonly grown in the study area are "Bokungi" and "Ikeregi". Both are extensively cultivated because they mature early and are high-yielding. However, their performance under CMV infection had not been reported. Therefore, this study determined the resistance status of both varieties to cucumber mosaic virus disease.

Materials and Methods

Source of CMV Inoculum and Multiplication

Cucumber mosaic virus-infected leaves were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan. The virus was maintained on silica gels in plastic vials kept at room temperature. *Cucumber mosaic virus* was recovered from the leaves by grinding (1g/mL) with inoculation buffer (0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M L-cysteine per litre of distilled water, pH 7.2). Carborundum powder (600-mesh) and a drop of β-mercapto ethanol were added to the CMV extract and this was rubbed on the primary leaves of the cv. Ife Brown.

Experimental Site

Two experiments were conducted simultaneously at the Teaching and Research Farm of the Department of Crop Production, School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, during the 2014 cropping season. The actual geographical position of the site is Latitude 6.4474°E, Longitude 9.51824°N, and is about 220 m above sea level. Minna is located in the Southern Guinea Savanna agro-ecological zone of Nigeria with a mean annual rainfall of 1200 mm. The rainfall which peaks in September normally begins in April and ends in the first week of October. The temperature ranges between 35 and 37.5 °C with relative humidity between 60 and 80 % in the month of July and 40 and 60 % in January. The soils of Minna originated from basement complex rocks and are generally classified as Alfisols (Adeboye *et. al.*, 2011).

Treatments and Experimental Design, Sowing and Inoculation

The experimental design was randomised complete block design with three replications. Seeds of the two okra varieties "Bokungi" and "Ikeregi" which are commonly grown in the study area were obtained from an okra farmer in Minna. Each variety was sown in 5-m long ridges. There were six ridges per plot such that data were collected from four inner ones while the outer two served as guards. Just before sowing, seeds were dressed with the chemical (Ciba Plus) for protection against soil-borne pathogens and insects. Sowing was done on 23rd May, 2014 at an intra and inter-row spacing of 0.3 and 0.75 m, respectively. Three seeds were sown per hole, and after emergence, seedlings were thinned to two plants per stand. The CMV-infected leaves collected from the indicator plants (Ife Brown) were ground in inoculation buffer and used for inoculating the test plants. Inoculation was as described above. The inoculated plants were then monitored for symptom development.

Serological test for virus concentration

To estimate the relative virus concentration, the topmost leaves of the plants inoculated with CMV were collected and subjected to Enzyme-Linked Immunosorbent Assay (ELISA) according to Koenig (1981). Leaves were homogenized in carbonate buffer pH 7.4 (0.015 M sodium carbonate plus 0.0349 M sodium bicarbonate per litre of distilled water) at the rate of 100 mg/mL. For each sample 100 µL of the sap was tested in duplicate wells of the ELISA plate.

Sap sample from a healthy okra plant and a known CMV-infected plant were used as negative and positive controls, respectively. After incubating at 37 °C for 1 h the plate was washed three times with phosphate buffered saline-Tween (8 g NaCl, 1.1 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, 0.5 mL Tween – 20, 1 L distilled water, pH 7.4) (PBS-T. This was followed by addition of two hundred microlitres of a blocking solution [3 % (w/v) dried nonfat skimmed milk in PBS – T]. The plate was incubated at 37 °C for 30 min and then tap-dried on a paper towel. One hundred microlitres of the polyclonal antibody diluted (1:10, 000; v/v) with conjugate buffer [half strength PBS-T containing 0.05 % (v/v) Tween-20, 0.02 % (w/v) egg albumin, 0.2 % (w/v) polyvinylpyrolidone] was added to each well. The plate was incubated again at 37 °C for 1 h, washed thrice and 100 µL of the goat anti-rabbit antibody diluted with conjugate buffer (1:15,000) was added to the wells. The plate was incubated at 37 °C for 1h, washed and 100 µL of *p*-nitrophenyl phosphate dissolved in substrate buffer (97mL diethanolamine, 1000 mL H_2O, pH 9.8) was added to the wells. The plate was finally incubated in dark at room temperature (37 °C). Absorbance of virus concentration was read at 405 nm (A_{405}) using a microplate reader (MRX, Dynex Technologies, Inc., USA) after

1h. ELISA values were accepted to be positive when the absorbance readings exceeded two times the negative controls.

Data Collection and Statistical Analysis

Plants were assessed for percentage of CMV infection, disease severity, yield and yield components. Disease severity was rated on a 1 – 5 point scale according to Arif and Hassan (2002) based on the magnitude of symptoms on the leaves and general growth conditions of the inoculated plants. On the scale:

1 = no symptoms (apparently healthy plant);
2 = slightly mosaic leaves (10-30 %);
3 = mosaic (31-50 %) and leaf distortion;
4 = severe mosaic (51-70 %), leaf distortion and stunting;
5 = severe mosaic (>70 %), stunting and death of plants.

Data were subjected to independent *t* test and significance was determined at 5 % level of probability. Statistical analysis was performed using statistical analysis system (SAS, 2008).

Results.

Incidence and Severity of CMV on Okra

All the plants inoculated with CMV showed symptoms of infection. While necrotic rings were observed on the leaves inoculated with the virus, the newly emerging ones exhibited varying degrees of yellow colourations and leaf curling. Foliar symptoms began at seven days after inoculation and it was initially mild but increased over time. Intensity of infection was consistently higher in "Bokungi" than "Ikeregi" except during the first assessment at 2 weeks after inoculation (WAI) when both of them exhibited an average severity of 2 (Fig. 1). In "Bokungi", disease severity of 4 was observed at 3 and 4 WAI and the value rose to 5 for the rest of the period of evaluation. In contrast, a score of 3 was consistently maintained in the plants of "Ikeregi" from 3 WAI onwards. Although all the plants inoculated with the virus tested positive for CMV, virus concentrations were generally higher in "Bokungi" than in "Ikeregi" (Fig. 2).

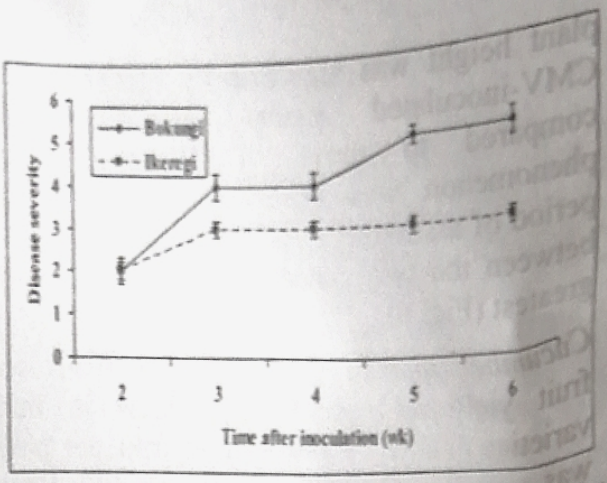

Fig. 1: Progress of disease severity in two okra varieties inoculated with *Cucumber mosaic virus* Vertical bars represent standard deviation.

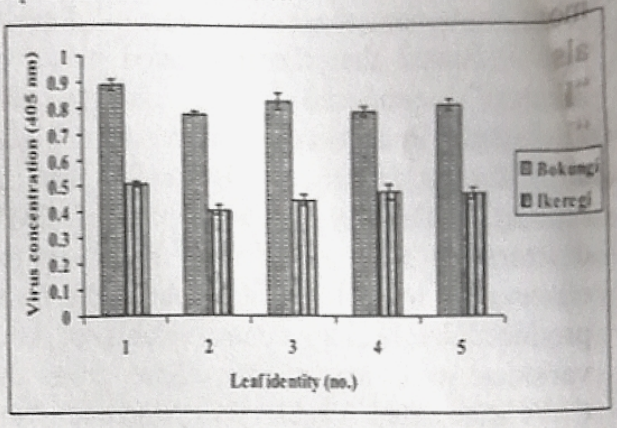

Fig. 2: Virus concentration in the topmost leaves of two okra varieties inoculated with *Cucumber mosaic virus* Vertical bars represent standard deviation

Effect of CMV on Growth and Yield

Cucumber mosaic virus infection significantly affected the number of leaves per plant in both okra varieties. Most of the leaves produced after inoculation were smaller and narrower with a micropattern of alternating wide yellow and green areas in "Bokungi" when compared with those of "Ikeregi". Conversely, the control plants of both varieties were apparently healthy with leaves of normal size, shape and colour. Control plants of the two varieties produced significantly ($p<0.05$) higher leaves per plant than their inoculated counterparts (Fig. 3). More leaves were produced by the CMV-inoculated plants of "Ikeregi" compared to those of "Bokungi" but the difference in leaf reduction between them was not significant ($p>0.05$) (Fig. 4).

Significant height difference was found between the CMV-inoculated and uninoculated plants of both okra varieties (Fig. 5). Some of the severely infected plants developed small stems, short internodes and the plants were markedly stunted. Contrary to these, the control plants were tall and exhibited

plant height was significantly higher in the CMV-inoculated plants of "Bokungi" compared to those of "Ikeregi". This phenomenon was observed throughout the period of assessment and the height difference between the two varieties at 8 WAI was the greatest (Fig. 6).

Cucumber mosaic virus infection depressed fruit yield and yield components in both varieties (Table 1). Number of fruit per plant was significantly higher in the control plants than those inoculated with CMV. However, the uninoculated plants of "Ikeregi" produced more fruits than those of "Bokungi". Results also indicated that the inoculated plants of "Ikeregi" produced more fruits than "Bokungi". Reduction in fruit production was significantly higher in "Bokungi" than in "Ikeregi". The deleterious impact of CMV diseases on fruit length was similar to the observation made for fruit number. The fruits produced by healthy control plants of both varieties were longer than those from the CMV-inoculated (Table 1). Moreover, the fruits produced by the inoculated plants of "Bokungi" were shorter than those of "Ikeregi". Consequently, reduction in fruit length was much more conspicuous on the CMV-inoculated plants of "Bokungi". In contrast, the fruits from uninoculated plants of the two varieties were bigger than those inoculated with the virus (Table 1).

The fruits of CMV-inoculated plants in "Bokungi" were slightly bigger than those of "Ikeregi"; higher reduction in fruit diameter was observed in "Ikeregi" than in "Bokungi". Infection by CMV also had adverse effected on fruit weight in both okra varieties. However, reduction in fruit weight was more severe in the plants of "Bokungi" than those of "Ikeregi" (Table 1).

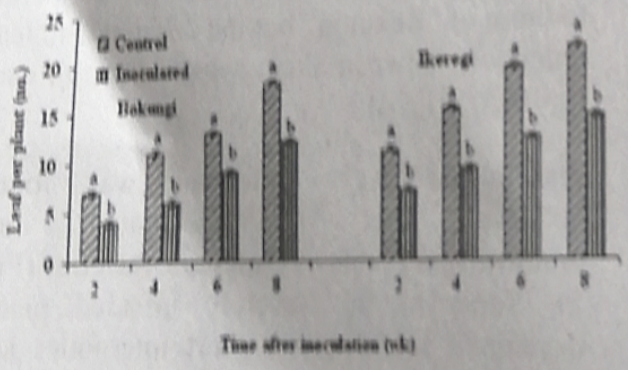

Fig. 3: Average number of leaves per plant in okra plants inoculated with *Cucumber mosaic virus* and of the uninoculated control plants. Bars labelled with dissimilar letter within the same week differ significantly according

to Least Significant Difference (LSD) at $p=0.05$. Vertical line on each bar represents standard deviation

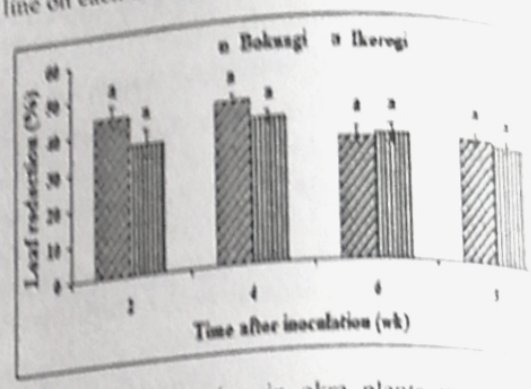

Fig. 4: Leaf reduction in okra plants inoculated with *Cucumber mosaic virus*. Bars labelled with similar letter within the same week do not differ significantly according to Least Significant Difference (LSD) at $p=0.05$; Vertical line on each bar represents standard deviation.

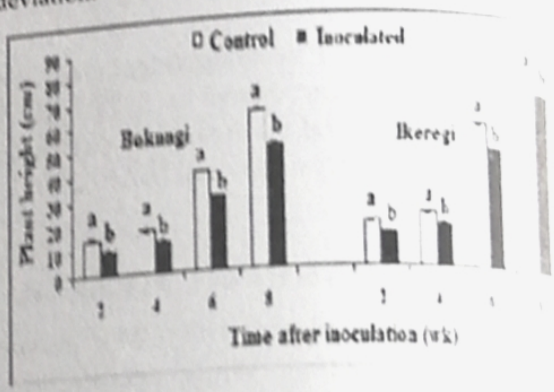

Fig. 5: Average heights of okra plants inoculated with *Cucumber mosaic virus* and of the uninoculated plants. Bars labelled with dissimilar letter within the same week differ significantly according to Least Significant Difference (LSD) at $p=0.05$. Vertical line on each bar represents standard deviation

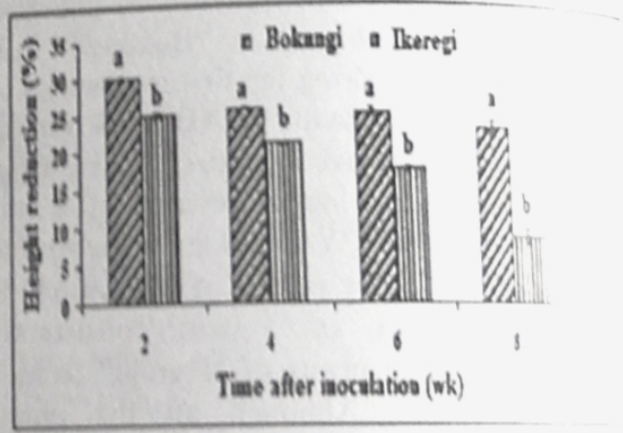

Fig. 6: Height reduction in okra plants inoculated with *Cucumber mosaic virus*. Bars labelled with dissimilar letter within the same week differ significantly according to Least Significant Difference (LSD) at $p=0.05$. Vertical line on each bar represents standard deviation

Table 1: Effect of *Cucumber mosaic virus* infection on the yield and yield components of two okra varieties in Minna during the 2014 cropping season.

Parameter/Treatment	Okra variety	
	Bokungi	Ikeregi
Fruit per plant (no.)		
Control	14[a]	20[a]
Inoculated	8[b]	13[b]
±SE	0.4	0.4
Fruit length (cm)		
Control	9.0[a]	10.5[a]
Inoculated	6.0[b]	8.7[b]
±SE	0.3	0.3
Fruit diameter (cm)		
Control	2.0[a]	2.0[a]
Inoculated	1.2[b]	1.0[b]
±SE	0.2	0.0
Fruit weight (g)		
Control	39.5[a]	58.5[a]
Inoculated	23.5[b]	36.5[b]
±SE	0.3	0.3

Means followed by the same letter within the same column for each parameter are not significantly different according to Least Significant Difference (LSD) at p=0.05

Discussion

The appearance of leaf curling and necrosis on the inoculated plants is in agreement with the earlier report by Palukaitis *et. al.* (1992) that CMV is pathogenic on several vegetable plants. Mild symptoms were observed at the early stage of infection because of low level of virus replication. Some leaves of the inoculated plants turned yellow owing to inhibition of plants' chloroplast. The earlier appearance of CMV disease symptom on the inoculated plants was due to the short incubation period required by the virus to establish in a vulnerable host. A similar result was observed by Agrios *et. al.* (1985) when some pepper plants were challenged with CMV. Appearance of necrotic symptom on the inoculated leaves was occasioned by hypersensitive reaction (HR) elicited by defense elements in the attacked plants. Hypersensitivity is a mechanism operative in some plants in an attempt to restrict virus multiplication and establishment. Goldbash *et. al.* (2003) elucidated HR as an active response to pathogen invasion in which cells surrounding the primary inoculation site of the virus die as a result of a rapidly induced programmed cell death. Fraser (1986, 1990) reported that where host plant is overcome, a series of compatible interactions occur between the host and a limited number of viral gene products.

Disease severity progressed with time because once an invading virus establishes in a susceptible host, the entire physiological activities would be hijacked for further synthesis of the virus particles. Symptom severity of CMV in "Ikeregi" was comparable to that of "Bokungi" soon after inoculation probably because the defense compounds in the former which later exhibited some level of tolerance had not been fully activated. According to Petty *et al.* (1990) and Collmer *et al.* (2000), passive or active resistance can manifest at any stage of the virus life cycle although most known viral resistance mechanisms appear to target virus replication or movement. Several studies have shown that the level of viral accumulation may influence its ability to move systemically. The higher disease severity and virus concentration detected in the inoculated plants of "Bokungi" reveals its vulnerability to CMV. Conversely, it could be argued that the intensity of disease symptom and titre of CMV was mild in "Ikeregi" probably because it possessed resistance gene (s).

Significant leaf discolouration and reduction in leaf size were due to impairment of chloroplast. In a plant susceptible to virus infection, synthesis of chlorophyll is greatly reduced and this ultimately results in chlorosis and necrosis. A plant which cannot produce sufficient chlorophyll is prone to malnutrition as a result of inability to carry out photosynthesis. This probably explains the reduced leaf size and growth of the CMV-inoculated plants. Low level of photosynthesis in the diseased plants also affected plant height. However, the observation that the plants of "Bokungi" were drastically affected by CMV revealed its susceptibility to the pathogen. The result indicating that uninoculated plants were taller corroborates the findings of Balogun *et. al.* (2007) when some okra lines were challenged with CMV.

The fruits from CMV-inoculated plants of "Ikeregi" were heavier than those of "Bokungi"; the latter produced tiny and unmarketable fruits. Plants inoculated with the virus generally produced fewer fruits which were of poor marketing value probably owing to the cumulative effect of disease severity and

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the findings of Balogun and Fawehinmi (2008). The higher fruit number and weight obtained from "Ikeregi" implies that the variety combined CMV tolerance with desirable yield. Fruit weight is one of the important traits for selection in breeding programmes. This is due to the fact that varieties that are capable of producing appreciable yield under disease pressure are more likely to be adopted by farmers. Based on the results of this investigation, the cv. "Ikeregi" which appears to be more tolerant to CMV infection is somewhat reliable in case of disease outbreak.

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