Assessment of Virus Infections Associated with Cucumber and Watermelon Cultivars in Ogun State, Nigeria

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Abstract

In Nigeria, utilization of cucurbit crops such as cucumber (Cucumber sativus L.) and watermelon (Citrullus lanatus (Thunb.) Matsum and Nakai) is on increase. However, viral diseases pose serious constraints to production. Four exotic cultivars each of packaged cucumber and watermelon were assessed to detect and identify viruses infecting them under natural field infestation. Each of these was sown at two seeds per hole in 26m x 17m plot with 840 plant population. At three weeks after planting (WAP), percentage germination and disease severity were evaluated for each variety. Two leaf samples per variety were collected from each sub block for serological indexing using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Six viruses: Cucumber mosaic virus (CMV), Zucchini yellow mosaic virus (ZYMV), Melon necrotic spot virus (MNSV), Papaya ringspot virus (PRSV), Watermelon mosaic virus and Cucumber green mottle mosaic virus (CGMMV) were tested. Disease severity ranged from 2 for watermelon (Kaolak) to 4 for cucumber (Pointsett). Observed viral symptoms include mosaic, necrotic and crinkling. CMV and CGMMV were detected in Pointsett and Royal hybrid which had infection rate of 88.7% and 85.6% respectively. In conclusion, Pointsett and Royal hybrid cucumber cultivars are susceptible to natural infection of viruses.

Keywords: Crinkling, Cucumber, Infection, Severity, Viral symptom, Watermelon.

Introduction

Cucumber (*Cucumis sativus* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) belong to the family Cucurbitaceae, Other members of the family include pumpkins (*Cucurbita moschata* L.), melons (*Cucumis melo* L.) and squash (*Cucurbita pepo* L.) (Maynard, 2001). They are all known for high water content. Cucumber in itself is more commonly thought of and used as a vegetable, though it is botanically a fruit. It is very crispy, with a sweet mild flavour (Zitter, 1984). Cucumbers have a glossy, dark-green skin and tapering ends with the interior generally pale green to white with rows of tender edible seeds down the centre. It is moist and can be eaten fresh, pickled and eaten alone, or added to green salads or sandwiches. When added to yogurt to which raisins and nuts have been included, it makes a cooling condiment for the dwellers in the Mediterranean region (Zitter, 1984). Cucumber enriches the diet of people living in the tropical regions by containing Vitamins such as Vit A, C, K, E, among others; minerals such as magnesium, potassium, manganese, phosphorus, calcium and zinc as well as number of phytonutrients (carotene-β, xantein-β and lutein) (Vimale *et al*., 1999).

Watermelon, which is believed to originate from the Kalahari Desert of Africa is popular due to its succulent, sweet, and cooling flesh. It contains lot of vitamin A and an anti-oxidant lycopene that is very important in cancer prevention and promotion of healthy kidneys (Provvidenti, 1999; Maynard, 2001).

In Nigeria, watermelon grows well both in the humid and drier savanna agroecologies, but foliar diseases are less destructive in the drier climates. The largest production of watermelon comes from the Northern part of Nigeria where the suitable agroecology is found (Adekunle *et al.*, 2007). Watermelon is used as a dessert fruit and a thirst quencher and in the very dry parts of Africa, it is relished by both man and his animals as a source of water (Amadi *et al*., 2009).

Cucurbits in developing countries are affected by at least thirty-nine well characterized viruses. The most important ones are *Cucumber mosaic virus* (CMV) genus *Cucumovirus, Squash mosaic virus* (SqMV) genus *Comovirus, Watermelon mosaic virus-1* (WMV-1) genus *Potyvirus, Watermelon mosaic virus-2* (WMV-2) genus *Potyvirus* and *Zucchini yellow mosaic virus* (ZYMV), genus *Potyvirus.* Some of the induced symptoms include mosaic, leaf reduction, chlorosis, necrosis, fruit deformations and discolouration (Loebenstein and Thottappilly, 2003).

Cultivation of cucurbit crops in Southwestern Nigeria is on increase due to current awareness of importance of fruits in diet. However, viral attack on these crops is one of the major challenges being faced by growers on the field. In addition, farmers depend mostly on packaged seeds for planting, hence the need to identify the probable viruses associated with these important crops during field planting in Nigeria and to determine the rate of viral infection on the field.

Materials and Methods

Seed planting

Four cultivars of cucumber and watermelon each were purchased from a seed distributor at Abeokuta, Ogun State. These seeds were products of France, Italy and Nigeria (Table 1). The cultivars were planted in an experimental field located at Alabata, Abeokuta, Ogun State during the planting season which ranged between June and August, 2008 with two seeds per hole at 2 cm deep. Blocks containing cucumber and watermelon only were intercropped within a replicate. The experiment was laid out as randomized complete block design with three replicates. The total plot size was 26 m x 17 m and it contained plant population of 840.

At three weeks after planting (WAP), percentage germination was recorded for each variety by multiplying the ratio of number of plants germinated to total number of planted seeds by 100. Disease severity was also scored for each variety as described by

Table 1: Sources of Cucumber and Watermelon Seed Planted

Crops	Variety	Source
Cucumber	Ashley	Italy
Cucumber	Pointsett	France
Cucumber	Royal hybrid	France
Cucumber	Biggy Green	Italy
Watermelon	Charleston Grey	Italy
Watermelon	Kaolak	Italy
Watermelon	Crimson sweet	Italy
Watermelon	Sugarbaby	Nigeria

Yuki *et al.* (2000). Percentage incidence was calculated for each cucurbit variety as the ratio of total number of plants infected to total number of plants germinated multiplied by 100 at 10 WAP.

Viral symptoms were recorded at 6 WAP on each cucurbit variety and a leaf sample per plant was collected from two representative plants in a sub block. These leaf samples were put in polythene bags and kept on ice chips in a cool box and thereafter transported to the laboratory at Biotechnology laboratory, FUNAAB for storage at 4°C till serological indexing was done.

Double antibody sandwich ELISA

Wells of ELISA microtitre plates were coated with 100 *ul* of immunoglobulin (specific viral IgG) diluted appropriately in a coating buffer (0.015M Na_2CO_3, 0.035M $NaHCO_3$, in 1 litre distilled water, adjusted to pH 9.6 with 5M NaOH and thereafter incubated at 37°C for 2 hrs. After incubation, plates were washed three times with phosphate buffered saline containing tween-20 (PBS-T). At each wash, wells were left full of buffer for three minutes. Plates were emptied, tap dried on paper towels after which the wells were filled with 100 *ul* expressed leaf sap in PBS-T containing 0.2% polyvinyl pyrollidone (PVP). Plates were incubated overnight at 4°C, washed and dried as described earlier.

Wells were filled with 100*ul* of the detecting antibodies conjugated with alkaline phosphatase in conjugate buffer (half strength PBS containing 0.05% v/v Tween 20 and 0.2% w/v egg albumen) and these were further incubated at 37°C for 2h. Plates were washed three times and 200 *ul* of substrate in substrate buffer (0.5mg/ml para-nitrophenyl phosphate in 10ml/L diethanolamine pH 9.6) was added to each well and incubated at room temperature for 1h, after which the absorbance values of the wells at 405nm (A_{405nm}) were read using a Dynex MRX ELISA plate reader.

Data Analysis

Data obtained for germination were analysed using simple percentage while mean of absorbance of a sample in duplicate wells at 405 nm (A_{405nm}) were compared with mean of absorbance of incorporated healthy controls to determine the ELISA status of each sample tested. A sample was considered positive when the mean A_{405nm} is at least two times greater than the mean value of the incorporated control (Yuki *et al.*, 2000).

Results

Watermelon cultivars performed better than the cucumber cultivars when rates of germination were compared, as all the four varieties planted germinated. Sugarbaby, a watermelon cultivar had the highest germination rate of 71.4% while Charleston grey had 25% (Table 2). Among all the cucumber cultivars planted, Biggy green had a poorest germination rate as it failed to germinate in all the replicates,. Royal-hybrid however had the highest germination rate (60.7%).

Diverse symptoms were observed on the leaves of all the planted cucurbit cultivars. Sole symptom such as necrotic lesion was observed on Royal hybrid cucumber cultivar. Symptom syndromes comprising two to four symptoms on same plants were observed on Ashley, and Pointsett cucumber cultivars and Kaolak which is a watermelon cultivar. Some of these symptoms include mosaic, necrotic lesions, crinkling, greenveinbanding, chlorosis, leaf malformation and reduction (Table 2).

Symptoms of necrotic lesions, mottling and chlorosis were observed on Charleston grey, a watermelon cultivar. Infection rate recorded for

watermelon cultivars ranged from 52.0% in Kaolak to 77.8% in Crimson sweet; and 85.6% in Royal hybrid to 100.0% in Ashley for the cucumber cultivar (Table 3).

Disease severity scores were 2 for Kaolak, a watermelon cultivar and 4 for all the cucumber cultivars (Table 3). Although, visual observation of all the cultivars showed the presence of one or more viral symptoms, only two: Pointsett and Royal hybrid tested positive for virus infection when the leaf samples were serologically tested in the laboratory. The mean of absorbance values were two times greater than the values of incorporated control (Table 4). CMV was detected in leaf samples of Pointsett cucumber cultivar and *Cucumber green mottle mosaic virus* (CGMMV) genus *Tobamovirus* was detected in Royal hybrid cucumber cultivar.

Table 2: Rate of Germination and Symptoms Expressed by Cucumber and Watermelon Cultivars During Field Evaluation at Ogun State, Nigeria

Cultivar	Percentage germination[1]	Symptoms observed					
		Mosaic	Necrotic lesion	Crinkling	Greenvein-banding	Leaf mal.	Chlorosis
Ashley (Cucumber)	32.1	+	+	+	–	–	–
Pointsett (Cucumber)	46.4	+	+	–	–	–	–
Royal hybrid (Cucumber)	60.7	–	+	–	–	–	–
Biggy green(Cucumber)	0.0	ng	ng	ng	ng	ng	ng
Charleston grey (Watermelon)	25.0	–	+	–	–	+	+
Kaolak (Watermelon)	44.0	+	+	–	–	+	–
Crimson sweet (Watermelon)	35.7	–	+	–	–	+	+
Sugarbaby (Watermelon)	71.4	+	–	–	+	+	+

[1]Percentage germination = Number of plants germinated/total number of seeds planted x 100, mal.= Malformation; ng= no germination; += symptoms present; –= symptoms absent

Table 3: Disease severity scores and percentage incidence of viral infection of the germinated cucurbit crops with the viruses detected

Cucurbit variety	*Disease severity score	[1]Percentage incidence ± SE	Virus detected
Ashley (Cucumber)	4	100 ± 0.0	–
Pointsett (Cucumber)	4	88.7 ± 5.9	CMV
Royal hybrid (Cucumber)	4	85.6 ± 4.7	CGMMV
Biggy green (Cucumber)	ng	ng	–
Charleston grey (Watermelon)	3	56.9 ± 13.1	–
Kaolak (Watermelon)	2	52.0 ± 9.9	–
Crimson sweet (Watermelon)	3	77.8 ± 18.2	–
Sugarbaby (Watermelon)	4	77.7 ± 10.2	–

*Disease severity score according to Provvidenti (1999) 1= No symptom, 2 = mild symptom; 3 = moderate; 4 = severe; 5 = dead, ng= No germination

[1]Percentage incidence = Number of plant with symptoms/total number of plants germinated x 100

Table 4: Absorbance value of positive samples detected during enzyme linked immunosorbent assay (ELISA)

Virus detected	Value of control	Value of positive sample
CMV	0.323	1.455
CGMMV	0.353	1.500

Key: CMV= *Cucumber mosaic virus*;
CGMMV= *Cucumber green mottle mosaic virus*

Discussion

The better performance of sugarbaby (a watermelon cultivar) on the field over other cultivars might have probably been due to source of the seeds. While seeds of the former variety were product of Nigeria, others were imported. Seeds of sugarbaby must have been well adapted to existing environmental conditions in the southwestern zone of the country, thereby able to thrive better than others. Some of the symptoms observed were due to viral infection. Sometimes other leaf discolouration might be due to abiotic factors such as nutritional deficiencies, insecticides, insect toxaemia and so on (Matthews, 1991; Tsai *et al.*, 2004). Some of these symptoms that have been previously reported to occur on cucurbits all over the world and which were also detected on cucumber and watermelon plants in this study include mosaic, leaf size reduction and necrotic spots, fruit discolouration and leaf yellowing (Lecoq, 2003; Ali *et al*., 2012). The detection of CMV in this study is similar to the work of Ali *et al*. (2012) who also detected CMV in 1.1 % of 1, 049 samples collected during a field survey at Okhlahoma, USA. While CMV was detected in one pumpkin and 3 watermelon samples, the CMV detected in this study was on cucumber and not watermelon leaf sample. CMV has a worldwide occurrence, having a very large host range and it induces some degree of distortion on plant leaves (Gillaspie, 2001).

The identification of CGMMV in the leaf samples of cucurbit grown from exotic seeds in Nigeria is novel information as this is the first report of such a virus in cucurbit grown from true seeds in Nigeria. The virus CGMMV, is a severe virus of Watermelon and other cucurbits in Asia and 15 % losses had been reported in cucumber (Ali *et al*., 2012). It must have been transmitted through mechanical rubbing of leaves of cucurbit plants during growth or water splash during rainy season as seed transmission of Tobamovirus has not been reported. Provvidenti (1999) observed similar symptoms on growing cucurbits plants during a field survey in the United States of America. Symptoms however are not sufficiently strong factors for the identities of cucurbit viruses on the field as many viruses pose similar symptoms in plants. The high disease severity scores for Pointsett royal hybrid and Ashley varieties suggested their susceptibility to viral infections on the field. This was also confirmed by the ELISA results in which there were positive reactions of the samples from the former two varieties to the antisera against CMV and CGMMV.

The failure of some symptomatic cucurbit plants to give positive ELISA result to any of the antiserum used might probably have been due to the presence of other mosaic causing agent such as *Squash Mosaic Virus* (SqMV) or a yet unidentified virus present (Zitter *et al.,* 1984). Other agents such as abiotic factors or genetic aberration might also have been other reasons for symptom-like appearance of these plants. There is likelihood that these types of plants outgrow these symptoms as they grow to maturity (Hughes, personal communication).

A better understanding of these identified virus in relation to their vector reservoirs, vector population behaviour and vector transmission

efficiency are necessary for the development of overall effective control measures.

References

Adekunle, A.A., Fatunbi, A.O., Adisa, S. and Adeyemi, O.A. (2007). Growing watermelon commercially in Nigeria. An Illustrated Guide. IITA Publication. Pp 16.

Ali, A., Mohammad, O. and Khattab, A. (2012). Distribution of viruses infecting cucurbit crops and isolation of potential new virus-like sequences from weeds in Oklahoma. *Plant Disease* 96: 243-248.

Amadi, J.E., Adebola, M.O. and Eze, C.S. (2009). Isolation and identification of a bacterial blotch organism from watermelon {*Citrullus lanatus* (Thunb.) Matsum and Nakai}. *African Journal of Agriculture Research* 4: 1291-1294.

Gillaspie, A.G. (2001). Resistance to cucumber mosaic virus in cowpea and implications for control of cowpea disease. *Plant Disease* 85:70-100.

Lecoq, H. (2003). Cucurbits In: Virus and virus-like disease of major crops in developing countries. *Loebenstein G, Thottappilly G, (Eds.)* Kluwer Academic Publishers. Dordrecht, Netherlands, pp. 665-687.

Loebenstein, G. and Thottappilly, G. (2003). Virus and virus-like diseases of major crops in developing countries. Kluwer Academic Publishers. Dordrecht, Netherlands, 800pp.

Matthews, R.E.F. (1991). Plant Virology. Third Edition. Academic Press. San Diego, California. 835 pp.

Maynard, D.N. (2001). An introduction to the watermelon characteristics. Production and Marketing. Alexandra, ASHS Press. Pp 9-20.

Provvidenti, R. (1999). Disease caused by viruses. In: *Compendium of cucurbit diseases.* Zitter, T.A., D. Hopkins, C.D. Thomas. (Eds.) , APS Press pp. 347-45.

Tsai, W.S., Shih, S.L., Green, S.K. and Hanson, P. (2004). First report of the occurrence of Tomato chlorosis virus and Tomato infectious chlorosis virus in Taiwan. *Plant Disease* 88: 311.

USDA (2003). United States Developing agency, Agricultural Statistics. http://usda.mannlib.cornell.edu/usda/current/vegesum/pdf

Vimale, P., Ting, C.C., Saibaiah, H., Ibrahim, B. and Ismail, L. (1999). Biomass production and nutrient yield of four green manures and their effect on the yield of cucumber. *Journal of Tropical Agriculture and Food Science* 27: 47-55.

Yuki, V.A., Rezende, J.A.M., Kitajima, E.W., Barroso, P.A.Y., Kuniyuki, H., Groppo, G.A., Pavan, M.A. (2000). Occurrence, Distribution and Relative Incidence of Five Viruses Infecting Cucurbits in the State of Sao Paulo, Brazil. *Plant Diseases* 84: 516-520

Zitter, T.A., Banik, T. (1984). Virus diseases of Cucurbits. *Plant Disease.* 55: 22-25.