Albanian j. agric. sci. 2014;13 (2): 125-130 *Agricultural University of Tirana*

RESEARCH ARTICLE **(Open Access)**

Virus symptoms and viruses associated with two cucurbit crops grown in a derived savannah agro-ecology in Nigeria

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Abstract

Five watermelon varieties, Sugar Baby, Charleston Grey, Kaolak, Crimson Sweet and Oranaise and three cucumber varieties, Poinsett, Ashley and Royal hybrid were grown on the field in two trials (i) late season between August and October 2008 and (ii) early season between April and June 2012 in Abeokuta, Ogun State to evaluate the cultivars for virus symptoms and viruses under natural tropical conditions. Symptomatic leaf samples were collected from each crop variety and indexed for *Cucumber mosaic virus* (CMV), *Melon necrotic spot virus* (MNSV), *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Cucumber green mottle mosaic virus* (CGMMV) in Double Antibody Sandwich (DAS) Enzyme-linked immunosorbent assay (ELISA). The disease incidence was 100.0% at 6 weeks after planting (WAP) for all the cucurbits crops for both seasons while the highest symptom severity score at 10 WAP was 4 and 5 for watermelon and 3.6 and 4 for cucumber in the 1st and 2nd trials. The viruses detected in late season were CMV, CGMMV and MNSV which occurred in all the cucumber and watermelon varieties. In addition PRSV, WMV and ZYMV occurred in mixed infection in the cucumber varieties Pointsett and Royal Hybrid. In the early season, PRSV was the most prevalent virus infection in the cucumber and watermelon varieties. Also CMV+PRSV, PRSV+WMV, and MNSV+PRSV occurred in mixed infection in Charleston Grey, Oranaise and Sugar Baby respectively. The viruses detected are among the viruses reported to limit the production of cucurbit crops world-wide.

Keywords: Cucumber, watermelon, virus, incidence, severity, cucurbits

1. Inroduction

Cucurbits belong to the family Cucurbitaceae. The family has 130 genera and 825 species [22]. Cucurbits are major vegetables crops in Nigeria. They include Cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), and melon (*Citrullus colocynthis*). They are mainly cultivated in open fields under rain-fed conditions. Cucumber is widely consumed both fresh and as a processed product, when used fresh; they can be shred, diced in salads because it is an indispensable salad recipe. They are fresh pickled with sweet, sour, or herb flavouring in varied ways depending upon ethnic and regional taste preferences [17]. Likewise, watermelon is one of the important horticultural crops worldwide and plays important role in international trade. Watermelon immature fruits are also pickled and either eaten fresh or added to cooked foods. Farmers in Nigeria grow these crops commercially for consumption to improve nutrition, contribute to food security as well as increase revenue of the producers/farmers and create employment opportunities thereby improving on the efficiency of utilization of labour [15].

In Nigeria today, Kano state is the leading watermelon market which has been in existence for many years and it is one highly traded major fruit in Kano [1]. *C. sativus* and *C. lanatus* are now widely grown and used all over Nigeria today because of the crop potentials and benefits. Many varieties of the crop listed in Table 1 are sourced from local seed shops and they are grown all over Nigeria in the different agro ecologies. The varieties grown in the different regions are probably due to mere preference and or availability of seeds and most of the varieties have not been evaluated for the crop suitability for the different environments. For optimum crop production and yield, it is important to determine the responses of the various varieties grown to pest and diseases under the different environmental conditions especially in the southwest of Nigeria where the crop is widely and recently being introduced. Cucurbit virus diseases are a worldwide problem, and in Nigeria they represent one of the most limiting factors for growers. Approximately 35 viruses infect cucurbits worldwide [21], the five most important viruses of cucurbits reported worldwide include the aphid-borne *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Papaya ringspot virus* (PRSV),

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(Accepted for publication 20 June 2014)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

Zucchini yellow mosaic virus (ZYMV) and the whitefly-borne *Cucumber vein yellowing virus* (CVYV) [12]. Despite the importance of cucurbit crops in Nigeria, there is little or no information at the local level on the distribution and relative incidence of these viruses on cucurbit crops. This type of information is essential for the development and the implementation of effective control strategies as well as recommendation of suitable varieties for farmers and growers. This study was carried out to determine the incidence and severity virus symptoms and the viruses associated with cucumber and watermelon in a derived savannah region where the crops is widely being introduced.

Table 1: Sources of Cucumber and Watermelon varieties used for assessment to viral diseases in derived savannah agro ecology

CROP	***VARIETY***	***SOURCE***
Cucumber	Ashley	Product of Italy, marketed by The Seed Project Company Limited, Kano, Nigeria.
Cucumber	Pointsett	Product of France, marketed by The Seed Project Company Limited, Kano, Nigeria.
Cucumber	Royal hybrid F1	Product of France, marketed by The Seed Project Company Limited, Kano, Nigeria.
Cucumber	Biggy Green	Product of Italy, marketed by The Seed Project Company Limited, Kano, Nigeria.
Watermelon	Charleston Grey	Product of Italy, marketed by The Seed Project Company Limited, Kano, Nigeria.
Watermelon	Kaolak	Product of Italy, marketed by The Seed Project Company Limited, Kano, Nigeria.
Watermelon	Crimson Sweet	Product of Italy, marketed by The Seed Project Company Limited, Kano, Nigeria.
Watermelon	Seedless F1 Sunlight	Green Seeds Company Limited, Austria.
Watermelon	Sugar Baby	Ibadan Seminis Agriculture Nigeria Limited.
Watermelon	Oranaise	Product of France, marketed by The Seed Project Company Limited, Kano, Nigeria.
Watermelon	Greybell	Product of Italy, marketed by The Seed Project Company Limited, Kano, Nigeria.

2. Materials and Methods

Field operations

The watermelon varieties Sugar baby, Charleston Grey, Crimson Sweet, Kaolak, Oranaise and the cucumber varieties RoyalhybridF_1, Ashley and Poinsett seeds sourced from local seed shops and distributors were planted on the experimental farm in Federal University of Agriculture, Alabata, Abeokuta (Altitute144 m, N 07˚13' 15.5", E 003˚ 26' 26.1") on a piece of land prepared by ploughing and harrowing and then divided into blocks measuring 7 m x 19 m. The blocks were divided into sub-plots of 3 m by 3 m. There was 1.5 m between each block and 1 m between each sub-plot. This was replicated three times in a Completely Randomized Block Design. Seeds of cucumber and watermelon were sown at the rate of two seeds per hole at a depth of approximately 2.5 cm. The plant spacing was 1 m between the rows and 0.5 m within the rows. The percentage germination for each cucurbit variety was recorded after 10 – 14 days after planting. Hand weeding was done regularly and no fertilizers or pesticides were applied.

Disease assessment

Disease Incidence and severity

Virus disease incidences were assessed in each plot by counting the number of plants showing virus-like symptoms and expressed as a percentage of the total number of plants within the plot. Virus symptom expressions were rated on five middle plants using a modification of the scale developed by Hassan and Al-masri [9] where; 1 - No symptom, 2 - Mild symptom such as mosaic, leaf distortion, chlorosis, yellowing and vein banding e.t.c. (10% of leaves), 3 - Moderate symptom (10-30%), 4 - Severe symptom (30-50%), 5 – Severe + leaf distortion and death (over 75%).

Serological detection of viruses infecting watermelon and cucumber crops

At Six weeks after planting, the crops were visually examined for symptoms of virus disease such as mosaic, leaf distortion, chlorosis and vein banding.

Symptomatic leaf samples were subjected to Double Antibody Sandwich (DAS) ELISA as described by Clark and Adams [6] with specific polyclonal antibodies to the following viruses' *Cucumber mosaic virus* (CMV), *Papaya ring spot* virus (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), *Cucumber green mottle mosaic virus* (CGMMV) and *Melon necrotic spot virus* (MNSV) (Deutsche Sammlung von Mikroorganismen Und Zellkulturen GmbH) (DSMZ), Germany. For the ELISA tests two leaf samples were collected in each plot making a total of six leaf samples for each crop variety. The six leaf samples collected for each crop variety were bulked before homogenizing (1:10 w/v) in extraction buffer (8.0 g NaCl, 0.2 g KH_2PO_4 1.1 g Na_2HPO_4, 0.2 g KCl/L, pH 7.4) containing 0.05% v/v Tween 20, and 2% w/v polyvinylpyrrolidone (Sigma PVP-40). The ELISA plates were coated with one of CGMMV, CMV, WMV, PRSV and MNSV 1gG diluted 1 in 1000 according to manufacturer's specification in coating buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ pH 9.6) into each well of the plates and incubated at 37^0C for 2 hours. The plates were washed three times at three minutes interval with washing buffer (PBS-T) from a wash bottle. Using a pipette, 100 µl of the sap were pipetted into each well and incubated overnight at 4^0C. The plates were decanted and washed as earlier described before adding 100 µl IgG-alkaline phosphatase diluted 1 in 1000 in conjugate buffer (PBS-T, 2% PVP and 0.2% egg albumen (Agdia, U.S.A.) into the wells of the plates and incubated at 37^0C for 2 hours. The plates were washed as described before addition of 200 µl of 1mg/ml P-nitro phenyl phosphate (PNP) in substrate buffer (Agdia, U.S.A). The plates were incubated at room temperature for 60 minutes to obtain clear reactions and the absorbance of the well contents read with Mindray MR-96, China at 405nm. The samples with positive reaction to CGMMV, CMV, WMV, PRSV, MNSV and ZYMV were observed when the absorbance value at 405nm doubled that of the healthy control samples.

3. Results and Discussion

The cucurbit seeds were observed to have low percentage germination although the watermelon variety Kaolak consistently recorded the highest germination of 34.5% followed by Sugar baby with 28.6% (Table 2). These two varieties are the most popular with farmers in Nigeria with Kaolak being common in the northern part and Sugar Baby in the southern part of Nigeria. Others were Charleston Grey and Crimson Sweet with percentage germination of 21.4% and 17.9% respectively (Table 2). Similarly in the second trial (April to June), Kaolak had the highest germination of 84.4%, this was followed by Charleston Grey (30.2%) and Sugar baby 25.0%. In the first trial, the cucumber variety royal hybrid F1 had the highest germination with 33.3% followed by Pointsett 32.1%, and Ashley 13.1%. The variety Pointsett had a percentage germination of 71.9% in the 2nd trial but the other cucumber varieties could not be tested due to non-availability of the cucumber seeds. Of the seven varieties of watermelon grown, the varieties Seedless F1 sunlight, and Greybell did not germinate. Likewise the cucumber variety Biggy green did not also germinate (data not shown). The viability of the seeds was low even though the expiry dates on the seed packages were still far (beyond one year) and there were complete germination failure for some of the varieties. The loss of viability in the seeds in sachets of 10 or 20 g which cost between $3.00 to 5.00 have economic implications for the farmers who have to plant several seeds per hole, thereby spending more to purchase enough seeds in order to achieve high germination. The poor germination and pest and disease problems are part of the major reasons why some cucurbit farmers visited in a recent survey in Ogun state said they were no longer planting the crops. The cucumber and watermelon varieties all showed virus symptoms at six weeks after planting. The disease incidences were 100.0% respectively. The viral symptoms mostly on watermelon crops were mosaic, crinkled deformed leaves, yellowing and stunting. For cucumber it was mosaic and vein banding. Similar symptoms have earlier been reported for cucurbit crops elsewhere [11; 2; 23; 20].

The degree of symptom expressions ranged from moderate to severe. The symptom severity score at 10 weeks after planting was 3.0 and 4.0 in the first and second trials for the cucumber variety Poinsett (Table 3). The highest disease severity score at 10 weeks after planting was 4 and 5 in the 1st and 2nd trials for the watermelon varieties (Table 4). Experience with the cucurbit farmers in southwest Nigeria showed that crop rotation especially in isolated areas where cucurbit crops had not been grown previously recorded better fruit yield. Such areas usually have low pest incidence because previous vegetation may not have been suitable for insect pest feeding (Femi

Pitan personal communication). There is also low inoculum build-up. Although in this study no foliar pesticide were applied but most farmers have to apply pesticides to achieve good yield.

Table 2: Percentage Germination of Cucumber and Watermelon varieties grown in Abeokuta within derived savannah agro ecology

CROP	***VARIETIES***	***Percentage germination (%)***	
		1st trial	**2nd trial**
Cucumber	Ashley	13.1	Not tested
	Royal hybrid F1	33.3	Not tested
	Pointsett	32.1	71.9
	Charleston Grey	21.4	30.2
Watermelon	Kaolak	34.5	85.4
	Sugar Baby	28.6	25.0
	Crimson Sweet	17.9	Not tested
	Oranaise	Not tested	61.5
	Grey bell	Not tested	0.0

Table 3: Virus disease incidences and Symptom severity scores for three Cucumber varieties grown in Abeokuta within derived savannah agro ecology

Time of planting	***Variety***	*Disease Incidence (%)*	*Disease Incidence (%)*	*Disease severity*
		4 WAP	**6 WAP**	**10 WAP**
August to October	Ashley	100.0	100.0	2.3
	Pointsett	100.0	100.0	3.0
	Royal hybrid F1	100.0	100.0	3.7
April to June	Pointsett	75.0	100.0	4.0

Table 4: Virus disease incidence and Symptom severity scores for five watermelon varieties grown in Abeokuta within derived savannah agro ecology

Time of planting	***VARIETY***	***DISEASE INCIDENCE (%)***	***DISEASE INCIDENCE (%)***	***DISEASE SEVERITY***
		4 WAP	**6 WAP**	**10 WAP**
August to October	Kaolak	100.0	100.0	3.7
	Charleston Grey	100.0	100.0	4.0
	Crimson Sweet	100.0	100.0	2.7
	Sugar Baby	100.0	100.0	3.7
April to June	Kaolak	66.0	100.0	5.0
	Charleston Grey	85.0	100.0	4.0
	Sugar Baby	58.0	100.0	3.0
	Oranaise	70.0	100.0	4.0

Table 5: Serological detection of viruses in leaf samples of cucurbit crops grown in Abeokuta

Late season planting							
CROP	**VARIETY**	**CMV**	**CGMMV**	**MNSV**	**PRSV**	**WMV**	**ZYMV**
Watermelon	Charleston Grey	+*	+	+	**–	–	–
	Crimson Sweet	+	+	+	–	–	–
	Kaolak	+	+	+	–	–	–
	Sugar Baby	+	+	+	–	–	–
Cucumber	Ashley	+	+	+	–	–	–
	Pointsett	+	+	+	+	+	+
	Royal hybrid	+	+	+	–	+	+
Early season planting							
Watermelon	Charleston Grey	+	–	–	+	–	–
	Kaolak	–	–	–	+	–	–
	Oranaise	–	–	–	+	+	–
	Sugar Baby	–	–	+	+	–	–
Cucumber	Pointsett	–	–	–	+	–	–

- = leaf sap reacted positively with specific polyclonal antibodies to the virus,
- ** - = leaf sap reacted negatively with specific polyclonal antibodies to the virus in DAS-ELISA

Using enzyme-linked immunosorbent assay, the six viruses, CMV, CGMMV, MNSV, PRSV, WMV and ZYMV indexed for were detected although the distribution varied with the crops. The data presented in this study revealed the identities of viruses infecting commercially grown cucurbits in derived savannah agro ecology. In the late season trial carried out between August and October, the most prevalent viruses were CMV, CGMMV and MNSV which infected the cucumber and watermelon varieties. In addition, PRSV, WMV and ZYMV were also detected in the watermelon varieties. The cucumber variety Pointsett was infected with the six viruses indexed for (Table 5). In the second trial, the most prevalent virus was PRSV which occurred in the cucumber variety Pointsett and the watermelon varieties, other viruses detected were CMV, MNSV and WMV which infected the watermelon varieties Charleston Grey, Sugar Baby and Oranaise respectively. CGMMV and ZYMV were not detected (Table 5). The cucumber

variety Poinsett was not infected with other viruses in the 2nd trial. The previous reports of cucurbit viruses in Nigeria were *Watermelon mosaic virus* isolated from *C. edulis* (*C. manni*) [10] and a strain of PRSV from *Cucumis sativus* [19] which are the potyviruses that have been reported naturally infecting cucurbits in Nigeria [18]. In the first field trial CMV, CGMMV and MNSV were the most prevalent viruses while in the second trial it was PRSV. Yuki et al. (2000) had observed that virus distribution patterns were not static as has been shown for cucurbit viruses in the United States. Studies in California conducted from the 1950s through 1981 indicated that WMV-2 and CMV were the most prevalent viruses [8; 14; 16]. PRSV-W was the most prevalent virus, in samples collected from different cucurbits in the four counties of Oklahoma and the virus was also reported in all cucurbit crops [2].

Also, in 1982, ZYMV was identified for the first time in that state (Nameth et al. 1986). Other viruses reported to infect cucurbit crops in California, were WMV, most prevalent, then CMV and ZYMV [7], other reports were CMV and WMV in Spain [3; 4], [13], PRSV and ZYMV in Brazil [24].

Some samples from symptomatic cucurbit plants tested did not react with the polyclonal antibody against any of the six viruses. The absence of positive reactions may be due to any other mosaic-causing agents, or other viruses for which antibodies were not available. Other factors that might explain negative results in ELISA may have been low concentration of virus in the sample being tested or abiotic agents causing virus-like symptoms [24]. Many peasant farmers in Ogun state whose farms were surveyed for virus diseases did not recognize virus symptoms as been due to disease. PRSV and CMV are aphid transmitted that cause serious diseases on cucurbit crops. Favourable weather conditions for vector populations make aphid transmitted diseases one of the most important problems for growers. The avoidance of aphids responsible for transmitting these viruses should be a primary component of management strategy. The most important strategy for control of virus diseases should include proper land cultivation to eliminate volunteer plants that harbour the virus, effective weed control and or development of resistant varieties. For the seed and soil-borne MNSV soil disinfection and clean seeds may be used where available. CGMMV (genus *Tobamovirus*) is a severe virus of watermelon and other cucurbits in Asia and 15% losses has been reported in cucumber [20]. It is important to avoid infection with CGMMV. The virus can be transmitted by seeds and infects many cucurbit species, causing serious yield losses in cucumber and watermelon plants. In watermelon, CGMMV induces slight leaf mottling and dwarfing, but can cause serious internal discoloration and decomposition of the fruit [5]. Therefore, a good understanding of virus and vector reservoirs, vector population behavior, and vector transmission efficiency are necessary for the development of effective control measurements. One most important strategy for control of virus diseases on cucurbits for farmers should be the elimination of the common practice of starting new crops near old infected plantings. Old crops should be destroyed before starting new plantings. Control of wild species of Cucurbitaceae in the vicinity of cucurbit crops would reduce virus sources. Control of weeds that harbor vectors is also recommended [24].

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