

Full Length Research Paper

Incidence of viruses and virus-like diseases of watermelons and pumpkins in Uganda, a hitherto none-investigated pathosystem

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Common impromptu observations between 2010 and 2012 of apparent virus-like disease symptoms in watermelons and pumpkins in Uganda prompted this study. However, there was no recorded evidence of virus infection in these crops anywhere in Uganda or eastern Africa as a region. Thus, 374 and 522 watermelon and pumpkin plants, respectively, growing in 13 fields were surveyed to record virus-like disease symptoms in Uganda's four districts of Mbale and Kamuli (eastern region), Mpigi and Masaka (central region) during August to November 2013, and January to March 2014. Leaf samples were also collected and tested for four viruses known to commonly infect watermelons and pumpkins worldwide. Symptom severity was assessed using a scale of 0 to 5, while virus incidence was determined by serological methods. The four viruses tested were *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Cucurbit aphid borne-yellows virus* (CABYV). In both crops, there was a higher incidence of virus-like diseases in central than in eastern region ($P = 0.000$). All the four viruses were detected with CMV as the most common, followed by WMV, ZYMV, and CABYV. Differences in virus incidences between the districts were not always significant. Single CMV, dual CMV+WMV and triple CMV+WMV+ZYMV were the most common single, and multiple infections in both crops. Watermelons contained more single virus infections than mixed infections ($P < 0.001$), but this difference was not significant in pumpkins ($P = 0.468$). In all, single virus infections were significantly higher in watermelons than pumpkins ($P < 0.001$). This is the first study to report incidence of viruses and virus-like diseases in watermelons and pumpkins in Uganda, and in eastern Africa as a region. The importance of these results with respect to crop production and next steps in virus disease management are discussed.

Key words: Cucurbits, DAS-ELISA, multiple virus infection, TAS-ELISA, virus incidence, Uganda.

INTRODUCTION

Plant diseases caused by viruses are major biotic constraints in crop production worldwide (Anderson et al., 2004, Alabi et al., 2011; Navas-Castillo et al., 2011;

Tzanetakis et al., 2013). However, the impact of plant virus diseases is exacerbated in the tropics because tropical conditions favour continuous presence of both

primary and secondary hosts and super-abundance of vectors that efficiently transmit the viruses (Fargette et al., 2006; Morales, 2007; Barult et al., 2010; Navas-Castillo et al., 2011; Geering and Randles, 2012; Fereres and Racciah, 2015). Moreover, virus diseases in non-priority crops at local or regional scale when ignored or left to 'fallow' may constitute none investigated pathosystems as sources of harmful viruses in surrounding cropping systems (Jones et al., 2010; Lebeda and Burdon, 2013). An example is the watermelon/pumpkin pathosystem in eastern Africa including Uganda that has not been studied, despite pumpkins being common intercrops of other well characterized pathosystems such as cassava (Legg et al., 2011; Ndunguru et al., 2015) and sweetpotato (Valverde, 2007; Clark et al., 2012).

Watermelon and pumpkins belong to the family Cucurbitaceae (cucurbits) which consists of approximately 118 genera, and over 820 species (Lebeda et al., 2006). There are several edible species such as melon (*Cucumis melo* L and *Citrullus lanatus* (Thunb) Matsum and Nakai), cucumber (*Cucumis sativus* L), pumpkin (*Cucurbita moschata* Duchesne) and (*Cucurbita maxima* Duchesne), zucchini and squash (*Cucurbita pepo* L.) (Lebeda et al., 2006). Cucurbits are produced in several parts of the world, of which China, USA, Europe and the Mediterranean countries together account for over 96% of the world's production (FAOSTAT, 2016). In Uganda and surrounding countries of eastern Africa, watermelons and pumpkins are the only main cucurbits of some economic importance. However, these crops have not been prioritized in Uganda. For example, only 7.0% and less than 1.0% of 3,630 households sampled in 6 rural districts of Uganda were involved in the production of pumpkins and watermelons, respectively in 2012 (Kabunga et al., 2014). Nonetheless, the food and high nutritional security benefits of fruit and vegetable production such as watermelon and pumpkin at household level can be improved if there were deliberate efforts to promote the intensification of smallholder production of these crops (Kabunga et al., 2014).

Worldwide, over 60 species of viruses in different taxonomic groups have been detected and/or characterized from cucurbits (Lecoq, 2003; Akhtar et al., 2012; King et al., 2012; Lecoq and Desbiez, 2012; Abrahamian and Abou-Jawdah, 2014; Romay et al., 2014). The Mediterranean region is implicated for the highest virus diversity in cucurbits from where at least 28 different viruses have been detected (Lecoq and Desbiez, 2012). This high virus diversity is attributed not

only to the genetic and ecological diversity of their hosts, but also to the huge diversity of agro-ecosystems, ranging from highly heated glass houses to traditional rain-fed cultivation providing favourable conditions for vector-mediated transmission of the viruses (Lecoq and Desbiez, 2012). Globally, *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family Bromoviridae) is the most widespread of the cucurbit-infecting viruses, followed by *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) (genus *Potyvirus*, family Potyviridae), and *Cucurbit aphid borne-yellow virus* (CABYV, genus *Polevirus*, family Luteoviridae) (Lecoq, 2003; Lecoq and Desbiez, 2012; Abrahamian and Abou-Jawdah, 2014; Romay et al., 2014).

However, this hierarchy is variable depending on time, host, and geography. Aphids of different species transmit CMV, WMV and ZYMV non-persistently, or CABYV persistently (Dodds et al., 1984; Kishaba et al., 1992; Lecoq and Desbiez, 2012), but seed-transmission of ZYMV (Simmons et al., 2011, 2013) and CMV (Zitter and Murphy, 2009; Jacquemond, 2012) are also known. The geographical and host range of plant viruses is delimited more by virus-vector than virus-host relations (Power and Flecker, 2003, Power, 2008), which in accordance with its broad vector range makes CMV cosmopolitan infecting over 1200 species in over 100 families of plants worldwide (Zitter and Murphy, 2009; Jacquemond, 2012; Lecoq and Desbiez, 2012).

In Africa, with an exception of a few studies in some northern, western and southern countries (Lecoq et al., 1994, 2001, 2016; Nono-Womdim, 2003; Yakoubi et al., 2008; Mnari-Hattab et al., 2009; Mohammed et al., 2014; Ibaba et al., 2015), studies of plant viruses infecting cucurbit crops are generally limited on the continent, and almost non-existent in eastern Africa including Uganda. Instead, a lot of effort has been invested in viruses of other crops (maize, cassava, sweetpotato, and banana), yet, pumpkins are often intercropped with these crops. In Uganda, watermelons are mainly grown as a single crop in relatively large fields, sometimes in the neighbourhood of other crops or the periphery of wetlands (Turyahabwe et al., 2013). In contrast, pumpkins are grown on a smaller scale usually integrated into the coffee-banana agro-forest production systems where coffee, cassava, sweetpotato, and banana are the main crops (Munyuli, 2011). The complex inter-cropping systems allow the direct exposure of pumpkins to many viruses that infect other crop plants into which they are intercropped in Uganda.

Between the year 2010 and 2012, we inadvertently

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#These authors contributed equally to this work.

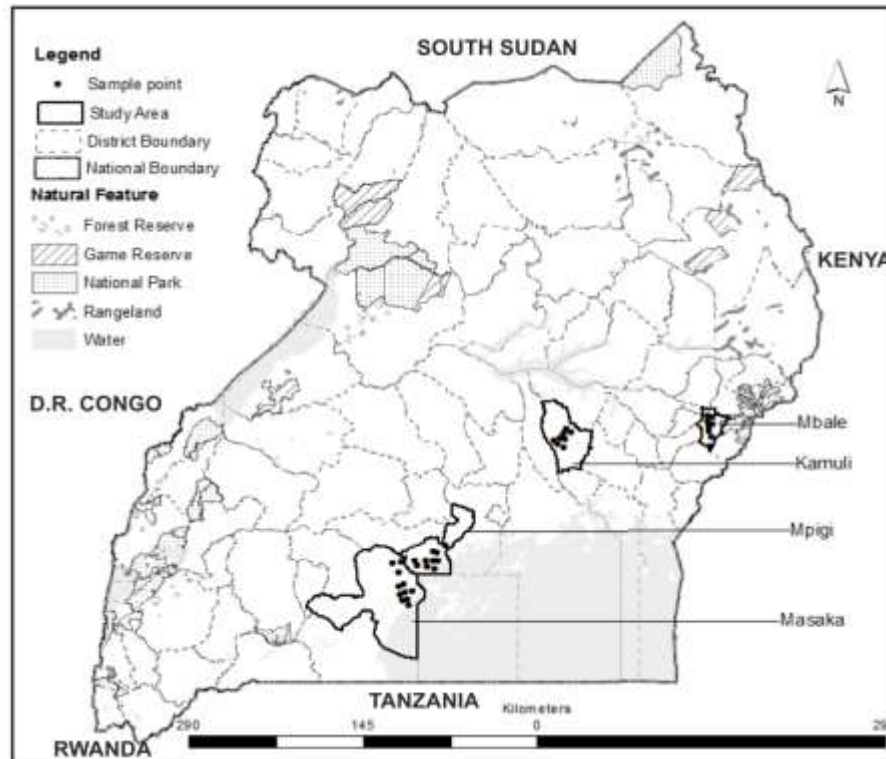


Figure 1. Map of Uganda showing the four districts (Mbale, Kamuli, Mpigi, and Masaka) that were surveyed for viruses and virus-like diseases in watermelons and pumpkins.

observed in various districts of Uganda typical virus disease symptoms (mosaics, yellowing, necrosis, stunting) on pumpkins and watermelons, similar to those described previously for cucurbits (Lecoq and Desbiez, 2012). Consequently, we followed up with systematic studies to assess symptoms and test for viruses in a few districts as a probe into this non-investigated pathosystem.

Therefore, the aims of this study were to determine the prevalence of virus-like diseases on watermelons and pumpkins in four districts located in different agro-ecological zones of Uganda, and to use serological methods for determining incidence of four viruses, namely, CMV, WMV, ZYMV and CABYV in the plants. The study was carried out in two surveys, during which 374 and 522 watermelon and pumpkin plants, respectively were assessed for the viruses and virus-like diseases.

MATERIALS AND METHODS

Watermelon and pumpkin fields surveyed

Two of the agro-climatic zones of Uganda, namely the central (Lake Victoria basin), and eastern zone that reflect different rainfall patterns and diversities of cropping systems (Mubiru et al., 2012; Dixon et al., 2014), were surveyed for pumpkins and watermelons and four viruses (CMV, WMV, ZYMV, and CABYV). The Lake Victoria basin (1174 to 1235 meters above sea level) experiences

two relatively dry periods (December to March and June to July), while peak rainfall (1250 to 1500 mm per annum) periods are in March to May and October to November, and a minimum temperature of 12°C (NEMA, 2009). Eastern zone has a mean altitude of 1075 meters above sea level) also receives high rainfall greater than 1200 mm twice a year during (April to June and July to September). The minimum temperature is 15°C (NEMA, 2009). The micro-climates, rainfall patterns and cropping regimes in these zone are traditionally influenced by Lakes Victoria and Kyoga (Huxley, 1965; Phillips and McIntyre, 2000; Herrman and Mohr, 2011).

From each agro-climatic zone, 2 districts that commonly grow pumpkins and watermelon (Mpigi and Masaka in the Lake Victoria basin; Kamuli and Mbale in Eastern zone) (Figure 1) were chosen and surveyed for plants of these crops displaying virus-like disease symptoms and those healthy-looking. The surveys were carried out in August to November 2013, and repeated on the same fields in January to March 2014. In each district, fields surveyed (located 20 to 40km apart) were chosen from areas known to most grow watermelons or pumpkins. Sampling of plants in field was conducted as described by Tugume et al. (2008). The plant samples were identified in fields (gardens) of watermelon, and pumpkins, or in fields where watermelon or pumpkins had been intercropped with cassava, banana, millet, maize, sorghum, or sweetpotato. The number of fields surveyed per district ranged from 3 to 4. Overall, 9 to 13 fields were surveyed throughout the study.

Sampling and virus disease assessment in watermelons and pumpkins

Leaf samples from 15 to 20 plants of watermelon, pumpkin or both, expressing virus-like symptoms were sampled from each field. In

addition, leaf samples were also collected from some symptomless plants. The leaf samples were picked by moving diagonally across the field from one individual plant to the next as described by Aamir et al. (2010). Because of the creeping growth habit of watermelons and pumpkins, care was taken not to sample the same plant more than once. Virus-like symptom severity was scored visually in the field on a scale of 0 to 5, based on extent of leaf damage; 0 = non-symptomatic; 1 = mild yellowing, mild rugosis or mild necrosis; 2 = yellowing symptoms, rugosis or necrosis; 3 = severe yellowing, severe rugosis and severe necrosis; 4 = severe yellowing, severe rugosis, severe necrosis and mild stunting; 5 = stunting, severe yellowing, severe necrosis as described previously by Coutts et al. (2011). The sampled leaves were placed in plastic polythene bags, placed in a cool box and transported to the laboratory at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) for serological detection of the viruses.

Serological detection of four viruses in watermelons and pumpkins

A total of 374 and 522 plants of watermelon and pumpkins, respectively, were each tested for four viruses; CMV, WMV, ZYMV, and CABYV. Leaf discs (3 to 5 discs, each 2-cm in diameter) were excised from leaves of a plant, combined, and tested for CMV, WMV, and ZYMV using Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA). In contrast, CABYV was tested using Triple Antibody Sandwich-Enzyme Linked Immunosorbent Assay (TAS-ELISA). DAS-ELISA and TAS-ELISA procedures were as described by Clark and Adams (1977), with minor modifications using polyclonal antibodies supplied by DMSZ Braunschweig (Germany) and monoclonal conjugate antibodies supplied by BIO-RAD (Hercules, California, United States). Each sample was tested in triplicate.

Briefly, DAS-ELISA plates were coated with WMV-, CMV- and ZYMV-specific polyclonal antisera (first antibody) in a coating buffer (pH 9.6) for 12-16 hours at 4°C. The excess unbound antibodies were washed off three times using 0.05 % Tween-20 in PBS buffer (Clark and Adams, 1977) after which sap of test plant samples (extracted using the sample extraction buffer) were added and plates incubated overnight at 4°C. After washing three times with 0.05 % Tween-20, a 3000-times diluted alkaline phosphatase conjugated monoclonal antibody (BIO-RAD) (the second antibody) was added, and the ELISA plates incubated for 4 h at 30°C. The plates were washed three times for the third time with 0.05% Tween-20.

In TAS-ELISA for testing CABYV, the addition of alkaline phosphatase conjugated monoclonal antibody (BIO-RAD) was preceded with a second CABYV-specific monoclonal antibody (DMSZ Braunschweig, Germany). These antisera were supplied with lyophilized leaf samples of pumpkins infected with CMV, WMV, CABYV, or zucchini infected with ZYMV, which were used as positive controls in the ELISA assays. The presence or absence of CMV, WMV, ZYMV, and/or CABYV was confirmed through color development following the addition of *p*-nitrophenyl phosphate (1 mg/mL) in substrate buffer after incubating at room temperature in the dark for 60 to 120 min. Absorbance values were measured at 405 nm using a microplate reader (BIO-RAD Laboratories, USA). Samples were considered to be positive only when their absorbance values were greater than three times that of the blank/negative control (Wang and Gonsalves, 1990).

Data analysis

Field virus-like disease incidence (here defined as the extent of disease infection of the field) was calculated by expressing the

number of infected plants as a percentage of the total number of plants in the field, estimated as percentage infection; in this case, 1 to 20%, 21 to 49%, 50 to 100% infection is considered as low, moderate, and high incidences, respectively (Nono-Womdim et al., 1996). Prevalence of disease in a given district was determined by expressing the total number of fields with plants showing virus-like symptoms as a percentage of all fields surveyed in that district. Data generated from DAS- and TAS-ELISA tests was recorded in binary form as 0 or 1 to imply absence or presence of a specific virus and analysed using StataCorp LP 4905 (Lakeway Drive College Station, Texas USA).

RESULTS

Fields of watermelon and pumpkin sampled

In all, 171 and 267 plant leaf samples of watermelon and pumpkins respectively were observed and collected for virus testing during August to November 2013. Testing was repeated on additional 176 and 255 leaf samples of watermelon and pumpkins respectively collected during January to March 2014. Thus, 347 plants of watermelon and 522 plants of pumpkins were assessed throughout the study. The plant samples were collected from fields where plants were found in varied cropping systems in close proximity (10 to 20 meters) to other crops or wild vegetation. For example, most watermelon samples were obtained from fields that had been planted as a monoculture crop (Figure 2) and in rare circumstances where watermelon was intercropped with maize or beans (*Data not shown*). In some cases, watermelon fields were neighbouring other crops such as banana, cassava, or sweetpotato (Figure 2A and B) while in other cases, isolated fields had been established far from other crop stands (Figure 2C). No watermelon samples were collected from Kamuli because watermelon fields were not found in the areas surveyed of this district in the two consecutive surveys. In contrast, pumpkin plants in all districts were either intercropped in maize, beans, sweetpotato, or banana (Figure 2D), although in some cases, the plants grew out of accidental seed drops on rubbish heaps near homesteads (Figure 2E and F). No field was found with watermelon and pumpkins intercropped with each other.

Three fields of watermelon in each district (except Kamuli; total of 9 fields) (Table 1) were observed and assessed in August to November 2013, and then repeated in January to March 2014 for incidence of virus-like diseases and plants tested for viruses. In contrast, a total of 13 pumpkin fields were assessed with 3 fields from each district, except Kamuli from where 4 fields were assessed (Table 1). On average, disease prevalence was lowest in fields from eastern Uganda than central Uganda (Table 1). For example in watermelons, lowest prevalence was recorded in Mbale (15.1 %) and highest in Mpigi for which almost two thirds of all plants in the fields showed virus-like diseases (57.8 %) (Table 1). This same trend was observed in pumpkins

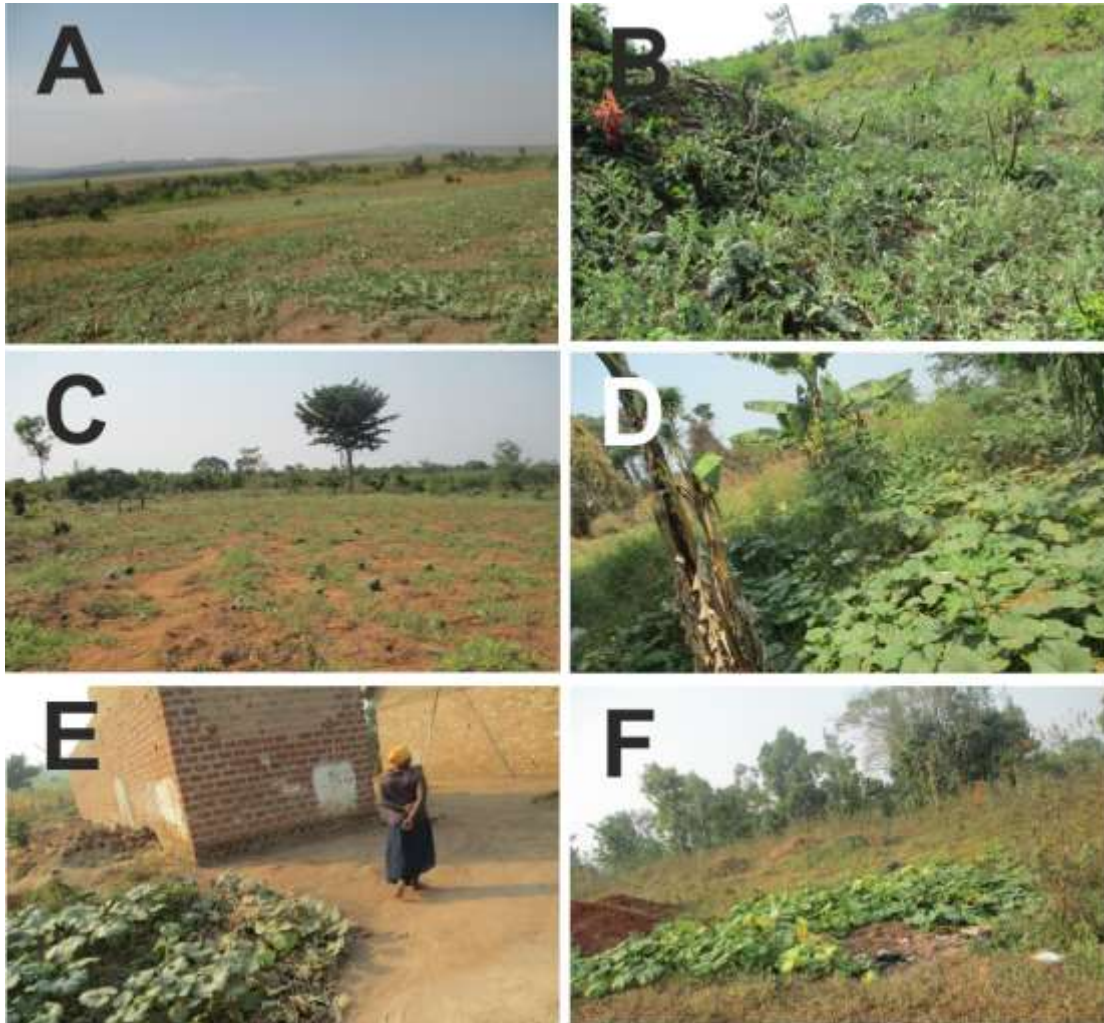


Figure 2. Examples of crop fields of watermelon (A, B and C) and pumpkins (D, E and F) in which the plants were observed for virus-like symptoms and collected for virus testing. Fields of watermelons near cassava and banana fields on the left in Masaka district (A); fields of watermelon planted in a newly cleared area growing next to sweetpotato in the background in Mbale district (B); watermelon field established in a new field not neighbouring any other crops in Masaka district (C). Watermelons were usually grown in relatively large monocultures (A, B and C) as compared to pumpkins which were commonly intercropped with other crops like banana, cassava and other crops (eg., D, taken in Masaka district) and near homesteads (for example, E, in Kamuli district). None of the watermelon plants were found in isolation from other watermelon plants, as opposed to some pumpkin plants found growing in isolation well detached from other crops (e.g., F, in Mpigi district).

with prevalence of 13.0 (Mbale) to 55.3% (Masaka).

Incidence of virus-like disease symptoms in watermelon

Of the 171 watermelon samples collected in the first survey (August to November 2013), 136 plants (79.5 %) were from symptomatic plants displaying various disease symptoms typical of virus infection in plants. In the second survey (January to March 2014), 143 of 176 samples (81.3 %) collected were from plants showing

virus-like symptoms. There was no statistically significant difference in severity of virus diseases symptoms between the two surveys ($\chi^2 = 8.9459$, $df = 5$, $P = 0.111$). In all, 68 plants (19.6 %) had no virus disease symptoms. Plants displayed various virus-like symptoms such as yellowing, rugosis, necrosis, blistering and stunting while some few showed a mixture of different symptoms (Figure 3). On average, virus-like disease incidence of 30.9, 45.7 and 52.9% was observed in Mbale, Mpigi, and Masaka, respectively. The most common virus-like disease symptoms observed in watermelon were yellowing and rugosis (24.8%), mild yellowing (22.5%),

Table 1. Prevalence of virus-like diseases in fields of watermelon and pumpkins from four districts of Uganda across the two surveys in 2013 and 2014. No watermelon fields were surveyed in Kamuli district because no watermelons were found grown there for the two consecutive surveys. Virus-like diseases incidence was 0.0% in watermelon in Mbale field 1 in 2013 and field 2 in 2014 because these fields were too young/newly planted (field 1 in 2013) or had just gotten harvested (field 2 in 2014).

District	Field	Incidence of virus –like diseases in the field					
		Watermelons			Pumpkins		
		Aug-Nov 2013	Jan-Mar 2014	Average	Aug-Nov 2013	Jan-Mar 2014	Average
Mbale	1	0.0	30.2	15.1	20.1	15.6	17.9
	2	60.7	0.0	30.4	10.6	15.4	13.0
	3	55.2	39.1	47.2	15.5	20.7	18.1
	Average	57.9	34.7	-	15.4	17.2	-
Kamuli	1	-	-	-	50.3	30.1	40.2
	2	-	-	-	40.2	20.1	30.2
	3	-	-	-	25.6	20.4	23.0
	4	-	-	-	30.2	25.8	28.0
	Average	-	-	-	36.6	24.1	-
Mpigi	1	60.0	55.6	57.8	70.9	30.1	50.5
	2	45.1	42.7	43.9	60.8	25.7	43.3
	3	50.2	20.3	35.3	40.7	60.0	50.4
	Average	51.8	39.5	-	57.5	38.6	-
Masaka	1	55.0	45.2	50.1	50.5	30.0	40.3
	2	60.0	50.3	55.2	70.6	25.4	48.0
	3	52.1	30.0	41.1	80.3	30.2	55.3
	Average	55.7	41.8	-	67.1	28.5	-



Figure 3. Some of the virus-like symptoms observed in watermelon plants in the field. Symptomless young (A) and old (B) plants which tested positive for CABYV and WMV, respectively. Rugosis in young (C) and leaf chlorosis in old plants (D). Many watermelon plants were found infested with aphids (E). Sometimes clarity of symptom description was hampered by the total destruction of watermelon shoot system by the virus-like diseases in which case only tinny leaf areas were left as is in (F, taken in Masaka district, central Uganda). In such cases, the diseases wiped out premature watermelon plants.

Table 2. Incidence of virus-like disease symptoms in watermelon and pumpkin plants in the districts of Mbale, Mpigi, Masaka, and Kamuli. Numbers in parentheses are the total number of samples showing a given virus-like disease symptom(s) while those outside the parentheses are their respective percentages in a given district. P values were deduced by comparison of virus-like disease severity between districts. Statistically significant values of P are indicated with an asterisk (*).

Symptom severity/District	Watermelon plants							Pumpkin plants							
	Mbale	Mpigi	Masaka	Total	Df	X ²	P	Mbale	Kamuli	Mpigi	Masaka	Total	Df	X ²	P
0-Symptomless	61.7 (42)	10.3 (7)	27.9 (19)	19.6 (68)	10	42.659	0.000*	37.3 (53)	26.8 (38)	13.4 (19)	22.5 (32)	27.2 (142)	15	45.976	0.000*
1-Mild yellowing	25.6 (20)	33.3 (26)	41.0 (32)	22.5 (78)	-	-	-	21.5 (37)	31.4 (54)	26.2 (45)	20.9 (36)	33.0 (172)	-	-	-
2-Yellowing, rugosis	29.1 (25)	38.4 (33)	32.6 (28)	24.8 (86)	-	-	-	12.1 (11)	39.6 (36)	24.2 (22)	24.2 (22)	17.4 (91)	-	-	-
3-Severe yellowing	30.5 (18)	49.2 (29)	20.3 (12)	17.0 (59)	-	-	-	12.2 (9)	24.3 (18)	27.0 (20)	36.5 (27)	14.2 (74)	-	-	-
4-Mild stunting	20.0 (10)	45.0 (18)	30.0 (12)	11.5 (40)	-	-	-	16.7 (6)	16.7 (6)	30.6 (11)	36.1 (13)	6.9 (36)	-	-	-
5-stunting	18.8 (3)	50.0 (8)	31.3 (5)	4.6 (16)	-	-	-	28.6 (2)	14.3 (1)	42.9 (3)	14.3 (1)	1.3	-	-	-

and severe yellowing (17.0%).

Most of the disease symptoms on watermelons were observed in Mpigi (94.2%) and Masaka (82.4%) as compared to Mbale (64.4%) (Table 2). Some of the symptoms in watermelon plants are shown in Figure 3. Most plants displaying severe yellowing and stunting were mostly encountered in samples from Mpigi (46.4%) and Masaka (30.4%) as compared to Mbale (23.2%). There was a higher incidence of virus symptoms in central Uganda districts (Mpigi and Masaka) than in the eastern district of Mbale (Table 2). These differences were statistically significant ($\chi^2 = 42.659$, $df = 10$, $P = 0.000$) (Table 2). In addition, virus-like diseases were present in all (100 %) of the twelve watermelon fields surveyed in central Uganda (Masaka and Mpigi), compared to 66.7% disease prevalence in watermelon fields in Mbale.

Incidence of virus-like disease symptoms in pumpkin

A total of 522 pumpkin plants (267 in the first, and 255 in the second survey) were assessed under this study in the four districts of Mbale, Kamuli, Mpigi and Masaka. Plants that displayed no

symptoms were 142 (19.6%) as compared to 380 plants (72.8%) which displayed various kinds of symptoms. Like for watermelons, the observed virus disease-like symptoms on pumpkin plants includes yellowing, rugosis, necrosis, blistering and stunting or a mixture of these symptoms (Table 2, Figure 4). The most common virus symptoms observed in pumpkins were mild to severe yellowing (33.0%), yellowing and rugosis (17.4%), and stunting (7.0%) (Table 2). More symptomatic pumpkin plants were observed in the central districts of Mpigi (32.6%), and Masaka (32.6%), as compared to the eastern districts of Mbale (18.6%) and Kamuli (16.3%), making the eastern districts with low disease incidences (Table 2). These differences were statistically significant ($\chi^2 = 45.976$, $df = 15$, $P = 0.000$) (Table 2). Prevalence of virus-like diseases was higher in central region (Masaka and Mpigi) where in each case 83.0% of the pumpkin fields had virus-like disease symptoms as compared to 50.0% in Mbale and 75.0% in Kamuli (eastern region). Considering watermelons and pumpkins together, higher disease incidence was recorded in the central than eastern districts surveyed, and watermelon showed a higher disease incidence (80.4 %) than pumpkins (72.8 %).

Incidence of four viruses in watermelons and pumpkins

In the 347 watermelon plants assayed, CMV was the most frequently detected virus, found in 74.1% of the plants. The other three viruses; WMV, ZYMV, and CABYV were detected in 61.4, 21.6 and 19.0% of watermelon plants respectively (Table 3). These viruses were detected in at least one watermelon plant from each of the three districts of Mbale, Mpigi, and Masaka. With the exception of WMV for which there was a significant difference in the incidence between districts ($\chi^2 = 24.896$, $df = 2$, $P = 0.000$), the other three viruses (CMV, ZYMV, and CABYV) showed no significant difference in incidence between the districts (Table 3). Most of the watermelon plants (36.2 %) infected with CMV were found in Mpigi (Table 3). Apart from CABYV, there were significant differences in the incidence of viruses between the two survey periods (Table 4).

Thus, CMV was more frequently detected in the first survey (August to November 2013) than in the second survey (January to March 2014) ($\chi^2 = 18.071$, $df = 1$, $P = 0.000$) (Table 4). Also, more WMV incidence was recorded in the first survey (57.3 %) than the second survey (42.7%) ($\chi^2 =$

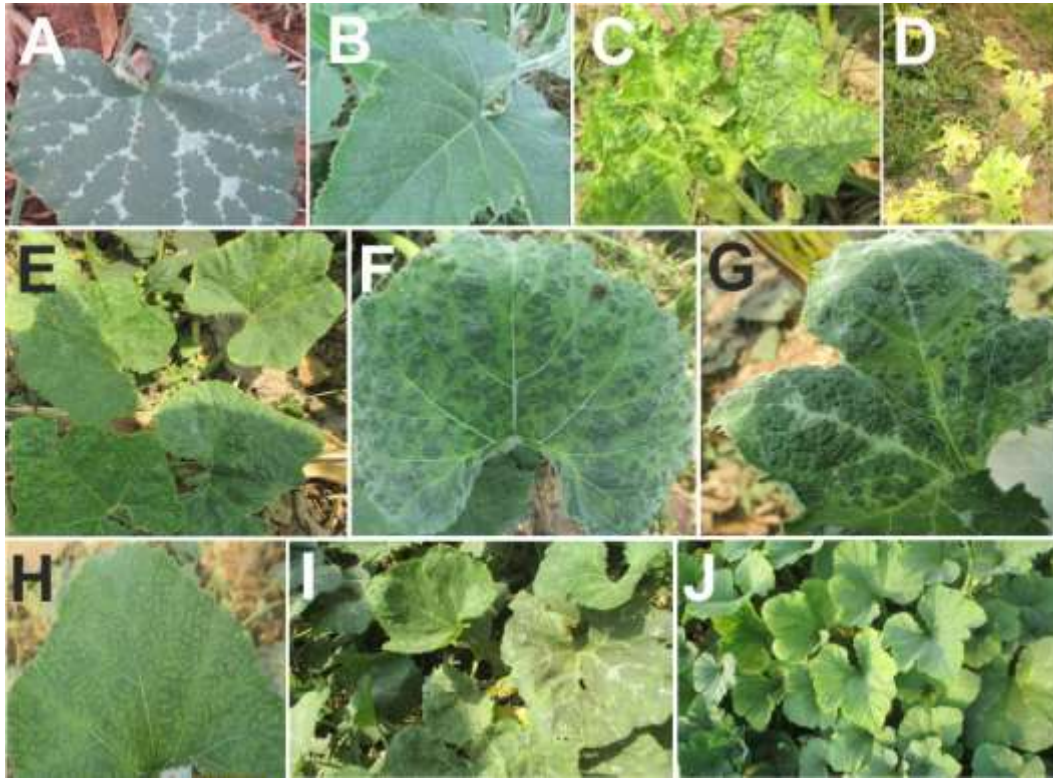


Figure 4. Some of the virus-like disease symptoms observed in on leaves of pumpkin plants in the field. Many symptomless leaves (for example, A) tested negative for all the viruses tested, while some other symptomless leaves tested positive for some viruses (e.g., B tested positive for CMV and WMV). Severe blistering of leaves (C), and blistering associated with severe chlorosis (D) in plants tested positive for all the four viruses tested. Mild rugosis as observed in leaves of whole plants (E) or when in advanced stages characterized by dark green islands (F) or severe in which case leaf starts to reduce in area (G). Mottling observed in plants testing positive for ZYMV (H). Plants with mild rugosis (I) or chlorosis and stunting symptoms (J) growing in close association with healthy looking plants; such a close association allows the ease of mechanical transmission of viruses in the field.

14.114, $df = 1$, $P = 0.000$) (Table 4). Furthermore, a higher number of more samples tested positive for ZYMV in the first survey (65.8 %) as compared to the second (34.2 %) ($\chi^2 = 10.612$, $df = 1$, $P = 0.001$). Five (5) samples (7.4%) from symptomless watermelon plants tested positive for at least one of the four viruses assayed. Contrastingly, 12 (4.3 %) samples from symptomatic watermelon plants were consistently negative for all the viruses tested under this study.

The order of occurrence of the four viruses in pumpkins followed similar pattern to that for watermelons. Thus, CMV was also the most frequently detected virus in 59.9% of 522 pumpkin plant samples tested, compared to 46.4, 20.1, and 16.9% of samples testing positive for WMV, ZYMV and CABYV, respectively (Table 3). Differences in the incidences of WMV and ZYMV between in the districts were not statistically significant ($P \geq 0.092$; Table 3). In contrast, incidences of CMV and CABYV in the districts were significantly different: CMV was highest in Masaka (30.7%) and lowest in Mbale (17.2%) while CABYV was highest in Kamuli (37.8%) and

lowest in Mbale (15.3%) ($P \leq 0.024$, Table 3). Incidence of CMV was significantly higher during the first survey as compared to the second survey ($\chi^2 = 8.594$, $df = 1$, $P = 0.003$) (Table 4). On the other hand, more samples tested positive for ZYMV and CABYV in the second survey than the first survey (Table 4). These differences were statistically significant ($\chi^2 = 10.352$, $df = 1$, $P = 0.001$ and $\chi^2 = 10.176$, $df = 1$, $P = 0.001$) respectively (Table 4). Eleven (11) symptomless pumpkin plants (7.7 %) tested positive for at least one virus compared to 23 symptomatic plants (6.1 %) that consistently tested negative for all the four viruses under this study.

Single and multiple virus infections

Of the 338 watermelon samples that tested positive for viruses, 233 samples (68.9 %) were singly infected with only one of the four viruses as compared to 105 samples (31.1 %) in which two or more (multiple) virus infections were detected (Figure 5). This difference was statistically

Table 3. Incidence of viruses infecting watermelons and pumpkins in the different districts. Numbers in parentheses are the total number of samples tested negative or positive while those outside the parentheses their respective percentages in a given district. P values were deduced by comparison of virus incidence in the different districts. Statistically significant values of P are indicated with an asterisk (*).

Virus	Incidence of viruses watermelon plants							Virus	Incidence of viruses watermelon plants						
	District	Negative	Positive	Total	Df	X2	p		District	Negative	Positive	Total	Df	X2	p
CMV	Mbale	42.2 (38)	31.1 (80)	34.0 (118)	2	3.681	0.159	CMV	Mbale	30.8 (64)	17.2 (54)	22.6 (118)	3	29.959	0.000*
	Mpigi	31.1 (28)	36.2 (93)	34.9 (121)	-	-	-		Mpigi	16.8 (35)	27.1 (85)	23.0 (120)	-	-	-
	Masaka	26.7 (24)	32.7 (84)	31.1(108)	-	-	-		Masaka	16.8 (35)	30.6 (96)	25.1 (131)	-	-	-
-	-	-	-	-	-	-	-	Kamuli	35.6 (74)	25.2 (79)	29.3 (153)	-	-	-	
WMV	Mbale	50.0 (67)	23.9 (51)	34.0 (118)	2	24.9	0.000*	WMV	Mbale	26.1 (73)	18.6 (45)	22.6 (118)	3	6.453	0.092
	Mpigi	26.1 (35)	40.0 (86)	34.9 (121)	-	-	-		Mpigi	21.8 (61)	24.4 (59)	22.9 (120)	-	-	-
	Masaka	23.9 (32)	35.7 (76)	31.1(108)	-	-	-		Masaka	21.8 (61)	28.9 (70)	25.1 (131)	-	-	-
ZYMV	Mbale	35.1 (95)	30.3 (22)	34.0 (118)	2	1.52	0.465	ZYMV	Kamuli	30.4 (85)	28.1 (68)	29.3 (153)	-	-	-
	Mpigi	35.4 (96)	32.9 (25)	34.8 (121)	-	-	-		Mbale	23.3 (97)	19.8 (21)	22.6 (118)	3	5.59	0.133
	Masaka	29.5 (80)	36.8 (28)	31.1 (108)	-	-	-		Mpigi	23.8 (99)	19.8 (21)	22.9 (120)	-	-	-
-	-	-	-	-	-	-	-	Masaka	22.8 (95)	33.9 (36)	25.1 (131)	-	-	-	
CABYV	Mbale	34.4 (100)	32.1 (18)	34.0 (18)	2	0.104	0.949	CABYV	Kamuli	30.1 (125)	26.4 (28)	29.3 (153)	-	-	-
	Mpigi	34.7 (101)	35.7 (20)	35.7 (20)	-	-	-		Mbale	24.1 (102)	15.3 (15)	22.5 (117)	3	9.453	0.024*
	Masaka	30.9 (90)	32.1 (28)	31.1 (108)	-	-	-		Mpigi	21.8 (92)	28.6 (28)	23.0 (120)	-	-	-
-	-	-	-	-	-	-	-	Masaka	26.7 (113)	18.4 (18)	25.1 (131)	-	-	-	
								Kamuli	27.4 (102)	37.8 (37)	29.4 (153)	-	-	-	

significant ($\chi^2 = 96.447$, $df = 1$, $P < 0.001$). In pumpkins, out of the 457 samples that tested positive for viruses, single virus infections were detected in 234 samples (51.2 %) while 223 plants (48.8 %) were found with two or more (multiple) virus infections (Figure 5). However, this difference was not statistically significant ($\chi^2 = 0.526$, $df = 1$, $P = 0.4683$). In both crops, CMV was the most frequently detected single virus infection, followed by WMV (Figure 5). While ZYMV and CABYV were the third and fourth most common single virus infections, respectively, in watermelon, this order was reversed in pumpkins (Figure 5). In both crops, the two most common

multiple infections were dual infections of CMV and WMV, and triple infections of CMV, WMV, and ZYMV (Figure 5). Overall, the difference in proportions of single virus infections was significantly higher in watermelon than pumpkins, whereas multiple infections were significantly more frequent in pumpkins than watermelon ($\chi^2 = 25.082$, $df = 1$, $P < 0.001$).

DISCUSSIONS

Several viruses and virus diseases infecting cucur-bitaceous crops including watermelons and

pumpkins have been reported in many parts of the world (Lecoq, 2003; King et al., 2012; Lecoq and Desbiez, 2012; Abrahamiam and Abou-Jawdah 2014; Romay et al., 2014). However, such reports are unavailable in Uganda or limited in eastern Africa as a region, with an exception of a recent report of CABYV, and *Pepo aphid-borne yellows virus* (PABYV, genus *Polerovirus*) in two watermelon samples from Tanzania (Desbiez et al., 2016). Therefore, this study becomes the first comprehensive report of viruses and virus-like diseases in watermelons and pumpkins in Uganda and the region. Our interest for the study was provoked by our frequent impromptu observations

Table 4. Variability in the incidence of the four viruses infecting watermelons and pumpkins across the two surveys conducted in August-November 2013 (survey 1) and January-March 2014 (survey 2) in Uganda. Numbers in parentheses are the total number of samples that tested negative or positive while those outside the parentheses are their respective percentages for each virus in a given district. P values were deduced by comparison of virus incidences between the two surveys. Statistically significant values of P are indicated with an asterisk (*).

Virus	Incidence of viruses infecting watermelons across the two surveys							Virus	Incidence of viruses infecting watermelons across the two surveys						
	Surveys	Negative	Positive	Total	Df	X2	P		Surveys	Negative	Positive	Total	Df	X2	P
CMV	1	30.0 (27)	56.0 (144)	49.3 (171)	1	18.071	0.000*	CMV	1	43.3 (90)	56.4 (177)	51.2 (267)	1	8.594	0.003*
	2	70.0 (63)	44.0 (133)	50.7 (176)	-	-	-		2	56.7 (118)	43.6 (137)	48.8 (255)	-	-	-
ZYMV	1	36.6 (49)	57.3 (122)	49.3 (171)	1	14.114	0.000*	WMV	1	52.1 (146)	50.0 (121)	51.2 (267)	1	0.238	0.625
	2	63.4 (85)	42.7 (91)	50.7 (176)	-	-	-		2	47.9 (134)	50.0 (121)	48.8 (255)	-	-	-
ZYMV	1	44.6 (121)	65.8 (50)	49.3 (171)	1	10.612	0.001*	ZYMV	1	47.6 (198)	65.1 (69)	51.2 (69)	1	10.352	0.001*
	2	55.4 (150)	34.2 (26)	50.7 (166)	-	-	-		2	52.4 (218)	34.4 (218)	48.8 (91)	-	-	-
CABYV	1	47.1 (137)	60.7 (34)	49.3 (171)	1	3.493	0.062	CABYV	1	54.6 (231)	36.7 (36)	51.2 (267)	1	10.176	0.001*
	2	52.9 (154)	39.3 (22)	50.7 (176)	-	-	-		2	45.4 (192)	63.3 (62)	48.8 (255)	-	-	-

made in some fields in Mpigi and Masaka (central Uganda) of the seemingly rampant virus-like diseases, first in pumpkins and later in watermelons between 2010 and 2012.

The study found higher severities and frequencies of virus disease symptoms in central than eastern region districts in both crops. Similarly, incidences of WMV (in watermelons) or CMV and CABYV (in pumpkins) were higher in central than eastern districts of Uganda. These results are consistent with previous reports on studies of viruses in different crops in Uganda. For example, virus infection inciting passion fruit woodiness disease showed a low incidence of up to 5% in eastern as compared to a high rate of 100% in central Uganda when 15 districts were surveyed (Ochwo-Ssemakula et al., 2012). Also, cultivated sweetpotato showed low incidences of viruses and virus diseases in eastern region compared to central region in multiple surveys of up to 35

districts of Uganda (Aritua et al., 1998, 2007; Mukasa et al., 2003). Cultivated sweetpotato cropping system in eastern Uganda is characterised by a break in two growing seasons as compared to central Uganda where the crop is continuously grown all year-round which explains low incidence in eastern versus high incidence in central (Mukasa et al., 2003). Whether such a scenario may also hold for watermelons and pumpkins is unknown. Geographical prevalence and distribution of plant viruses often have a strong bearing to virus-vector relations (Power and Flecker, 2003; Power, 2008) implying that availability vectors transmitting the viruses is a key determinant in high virus incidence (Ng and Falk, 2006; Bragard et al., 2013; Whitfield et al., 2015). All the viruses under the current study are known to be transmitted by aphids (Dodds et al., 1984; Kishaba et al., 1992; Lecoq and Desbiez, 2012), but CMV (Zitter and Murphy, 2009;

Jacquemond 2012; Sevik and Balkaya 2015) and ZYMV (Simmons et al., 2011, 2013) can also be seed-transmitted in different hosts. A relatively higher number of different aphid species were observed in central than eastern districts (*Our unpublished data*), and this may partly explain the observed low and high incidences in east and central respectively.

Besides involvement of vectors, differences in virus incidences may reflect regional differences in the abundance of susceptible vs. resistant host genotypes grown by farmers, and variabilities in environmental parameters influencing virus transmission and disease development (Fargette et al., 2006; Geering and Randles, 2012). Accordingly, virus infection and symptom development in plants is a function of variable factors of environment, virus strain, host plant species or genotype, age, and the host's physiological state (Cooper and Jones, 2006).

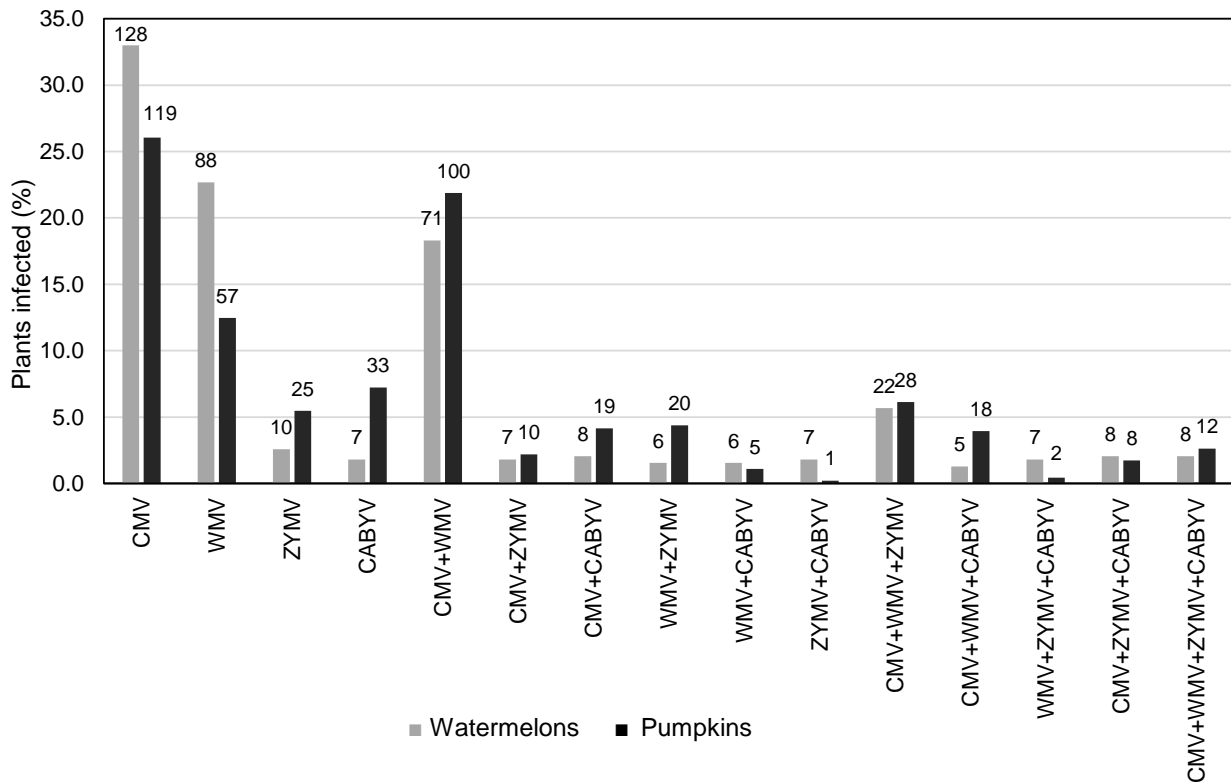


Figure 5. Natural occurrence of single and mixed virus infections in watermelon and pumpkin plants as a percentage of plants found infected with viruses. Number of plants detected with a virus or viruses is indicated above each bar.

Symptomatic plants testing negative for all the viruses tested implies the presence of other viruses not assessed. As many as 66 viruses have been reported in these crops in other parts of the world (Lecoq, 2003; King et al., 2012; Lecoq and Desbiez, 2012; Abrahamian and Abou-Jawdah 2014; Romay et al., 2014), which is much higher than the pre-selected virus species assessed under this study. For example, some strains or species of *Papaya ringspot virus* (PRSV), *Squash mosaic virus* (SqMV), *Watermelon chlorotic stunt virus* (WmCSV), *Pepo aphid-borne yellows virus* (PABYV) among others are known to infect these crops (Bananej and Vahdat 2008; Mohammed et al., 2012; Lecoq and Desbiez, 2012; Desbiez et al., 2016). Also, the methods used in the study were limited to serology using a highly stringent threshold of three times or more absorbance of the blank for a plant sample to be considered as virus infected, which might have affected the proportions of plants declared virus-infected with the viruses assayed. In contrast, symptomless virus infections as observed in this study are also common in many pathosystems implying cryptic virus infections (Roossink, 2010). In all, more extensive molecular diagnostic procedures that are independent of prior knowledge of symptoms such as siRNA deep sequencing (Kreuze et al., 2009; Boonham et al., 2014) must be employed on these crops to

broaden our understanding of virus infections. CMV was the most frequently detected virus both in single and mixed infections in watermelons and pumpkins, possibly because of a large number of aphid species (up to 60) known to transmit this virus to over 1200 plant host species (Lecoq and Desbiez, 2012). Some of the aphid species implicated in CMV transmission have been reported in Uganda (Orawu et al., 2015), and in watermelons and pumpkins (*Our unpublished data*). Similarly, many surveys in different vegetables susceptible to these viruses have reported high abundance of CMV as compared to the other viruses (Köklü and Yilmaz, 2006; Jossey and Babadoost, 2008; Quemada et al., 2008). Dual CMV+WMV and triple CMV+WMV+ZYMV were the most common mixed infections. It is expected that viruses transmitted by a common vector to the same host should depict higher frequencies of mixed infections than those transmitted by different vector agents (Opiyo et al., 2010; Salvaudon et al., 2013; Tugume et al., 2016; Tollenaere et al., 2016). Because all the viruses under this study are known to be aphid-borne (Lecoq and Desbiez, 2012), and their selection for assay was pre-determined, observed differences in single/mixed infections might be a reflection of variable transmission efficiencies, aphid populations, and/or host preferences by the aphid vectors.

In contrast, short cropping cycles of 3 to 4 months for watermelons compared to long cycles of 5 to 16 months including multiple and extended harvesting dates for pumpkins may result into high single virus infections in watermelons because the crops are exposed for a short inoculation time by viruliferous insect vectors in the field. Indeed, our study demonstrated that single virus infections were significantly higher in watermelon than pumpkins. Conversely, multiple infections were significantly more frequent in pumpkins than watermelon since pumpkins have long cropping cycles that expose them to repeated inoculations in the field.

Although watermelons and pumpkins are both currently widely grown in Uganda, cultivation of watermelons is a more recent activity in Uganda having started not earlier than 15 to 20 years ago (*Our personal observations*). In contrast, cultivation of pumpkins is known for over a century with several subsistence crop fields routinely intercropped with pumpkins and can be found in virtually all districts of Uganda. However, to-date, FAOSTAT database lacks production statistics for Uganda on watermelons and pumpkins (FAOSTAT, 2016) which might imply negligible production and associated economic benefits at a national level. Nonetheless, unlike pumpkins for which local non-improved seed is saved by subsistence farmers from a previous crop, most of watermelon cultivation in Uganda is conducted on relatively large farms for commercial purposes using commercially available (imported) improved or hybrid seed. Due to the nature of pumpkin cropping systems (semi-perennial, and mostly inter-cropped with other crops) in Uganda, it is likely that the viruses studied here might have long existed in pumpkins but remained unnoticed or a non-issue since the crop is not priority in Uganda (MAAIF, 2010; FAOSTAT, 2016).

With more recent arrival of fast-growing and short-lived watermelon – a host for the same viruses into the same cropping system, incidences of the same viruses on the pumpkins may get enhanced, even though these crops are mostly never intercropped with each other. Short-lived plant hosts of vectored plant viruses attract and amplify vector populations, and subsequent high virus incidence in long-lived hosts. A classic example is demonstrated under a natural ecosystem by introducing annual grass species that have overturned the dominance of perennial native bunchgrasses in California grasslands via increased infection with aphid-borne Barley/Cereal yellow dwarf viruses (Malmstrom et al., 2005; Borer et al., 2007; HilleRisLambers et al., 2010).

Also, feeding preferences or feeding behaviour of insect vectors of plant viruses can be altered after exposure to infected plants and acquisition of virus (Stafford et al., 2011; Ingwell et al., 2012; Moreno-Delafuente et al., 2013; Rajabaskar et al., 2014). In these contexts, a much higher abundance of aphids was observed on watermelons than on pumpkins in Uganda (*Our unpublished data*), and whether or not it has

implications with increased virus impact on pumpkins is an interesting subject of our future studies. Overall, the current study opens obvious opportunities for research at a number of fronts, considering the favourable tropical environments for virus disease development (Fargette et al., 2006; Thresh 2006; Jones 2009). Watermelons and pumpkins are important fruit and vegetable crops, respectively, in Uganda, but unfortunately, these are not prioritized for research (MAAIF, 2010; FAOSTAT, 2016), hence the several unknowns.

Firstly, how are the distributions of these and other viruses structured in broader geographical areas of Uganda and the region? – This requires diagnostic tools screening several samples but also considering many more virus species in broader areas than those covered under the current study because many more viruses probably infect these plants in Uganda. Secondary, what is the breadth and depth of genetic and genomic variability of the virus isolates, strains and species from watermelons and pumpkins in Uganda and the region? – This requires molecular genetic characterization of isolates from diverse geographical areas for their full or partial genomes and comparison with isolates/species from other parts of the world. It also allows testing of appropriate evolutionary hypotheses on the viruses. Thirdly, what is the breadth and depth biological variability of virus isolates from the different hosts? – This will involve biological characterizations of vector transmissibility between hosts, virus host ranges in common local weeds, virus-virus interactions and functional analysis of unique genomic segments that may exist. Fourthly, how is virus disease epidemiology in watermelons and pumpkins determined by factors of vector population dynamics across germplasm and geographic scales, cropping systems and husbandry practices? Assessing germplasm diversity across regions, the level yield losses due to virus diseases, spatio-temporal scales of disease prevalence will constitute the main activities under this research question. Importantly, East Africa as a region including Uganda is a cradleland and centre of diversification of dozens of important plant viruses decimating important crops there and elsewhere (Ndungruru et al., 2005, 2015; Tairo et al., 2005; Fargette et al., 2006; Tugume et al., 2010a,b, 2013; Mbanzibwa et al., 2011; Legg et al., 2011; Clark et al., 2012).

An in-depth assessment of viruses in watermelon and pumpkins as suggested earlier especially once conducted via regional alliances gives another chance to re-assess the significance of this region as a home of important plant viruses.

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Conflict of Interests

The authors have not declared any conflict of interests.

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