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Susceptibility of common weeds and cultivated crops in major maize growing agroecological zones of Uganda to viruses causing maize lethal necrosis disease

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Abstract

Maize lethal necrosis (MLN) disease is caused when maize plants become co-infected with *Maize chlorotic mottle virus* (MCMV) and potyviruses notably *Sugarcane Mosaic Virus* (SCMV). Apart from maize, little is known about susceptibility of weed species and cultivated crop species usually growing in proximity with maize to MLN viruses in Uganda. The common weeds and crop plants were mechanically inoculated with combined sap from MCMV and SCMV infected maize plants. Samples were tested for MLN causing viruses by Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). The weeds that were susceptibility to MCMV were *Digitaria abyssinica*, *Eleusine africana* and *Roetboellia cochinchinensis*; while those susceptible to SCMV were *Pennisetum purpureum, Panicum maximum* and *Roetboellia cochinchinensis*. The cultivated crops were susceptible only to MCMV and included cassava (*Manihot esculenta*), groundnut (*Arachis hypogaea*) and bean (*Phaseolus vulgaris*). Common weeds and cultivated crops growing close to maize in Uganda have differential susceptibility to MLN causing viruses and can act as reservoirs of MLN causing viruses. It is critical to identify non MLN hosts in cultivated crops for crop rotation and early weeding to reduce on MLN virus inoculum in cropping systems.

Keywords: *Alternative host, Disease, Maize, Weeds, Inoculum*

1. Introduction

Maize lethal necrosis (MLN) disease of maize (*Zea mays*) caused by co-infection of maize plants with *Maize chlorotic mottle virus* (MCMV) and any cereal potyviruses notably *Sugarcane mosaic virus* (SCMV) has affected maize production of maize in different countries in East and Central African severely (Kitenge, 2012; Wangai *et al*., 2012; Asea, 2013; IPPC, 2014; Lukanda *et al*., 2014; and Adams *et al*., 2014). The sources of inoculum of

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MLN causing viruses have been documented to include; infected maize seed, insect vectors, infected maize plant residues, alternative host plants and more recently contaminated soils (Nault, 1978; Bockelman *et al*., 1982; Jensen *et al*., 1991; Scheets, 2004; and Mahuku *et al*., 2015). Strategies to control MLN in East and Central Africa have mainly concentrated management efforts on the maize crop (Mahuku *et al*., 2015), yet the occurrence of crops and weeds grown in association with maize is the main characteristic of farming systems in the region. Indeed according to Uyemoto (1983) when faced with a severe MLN epidemic, the rotation of the maize crop with soybean or sorghum reduces the incidence of MLN in the next season. However, several cultivated crops and weeds that grow within the vicinity of the maize crop could affect the dynamics of MLN through encouraging the survival of MLN causing viruses and perpetuation of the MLN disease. A clear determination of the mode of interaction of associated plants in the cropping system on pathogen survival and dynamics of disease are critical.

Alternative hosts have been documented to have a critical role they play in the propagation of various diseases in a variety of crop species (Tugume *et al*., 2008; González-Segnana *et al*., 2013; and Macharia *et al*., 2016). Indeed this is the basis of various studies established to determine how other species of plants (weeds and cultivated crops) in the ecosystem of maize interact with viruses. For instance studies on the host range of *Maize streak virus* (MSV) revealed over 45 new species in the Gramineae family that were capable of being hosts of MSV (Damsteegt, 1983). Earlier studies conducted elsewhere revealed that MCMV has a wide experimental host range of up to 19 grass species (Bockelman *et al*., 1982). Other studies have revealed that the host range for both SCMV and MCMV is restricted to members of the Gramineae family with maize and sugarcane being the natural hosts of MCMV and SCMV respectively (Scheets, 2004). A more recent study conducted in the East African region reported typical disease symptoms of MCMV in finger millet (*Eleusine indica*), foxtail millet (*Setaria italica*) and proso millet (*Panicum miliaceum*) after inoculation with MCMV (Mahuku *et al*., 2015). Indeed some species of plants do not show any symptoms of pathogens and may be considered not to be pathogen hosts (Schaad and Dianese, 1981; Gitaitis *et al*., 1998; and Fassihiani, 2000). For instance, sorghum (*Sorghum bicolor*) was reported to have high tolerance for MCMV infection due to the fact that the virus was detected in the plants after inoculation but the plants remained asymptomatic (Mahuku *et al*., 2015). Although there is information on the susceptibility of weed species to MLN causing viruses elsewhere, there is need to conduct studies in Uganda because of genetic differences in the weeds, cultivated crop varieties and probable variation in virus strains of MLN causing viruses. The current study hence set out to determine the relative susceptibility of prevalent weeds and common maize intercrops such as banana, cassava, groundnut, common bean, soya bean and sweet potato to host MCMV/SCMV and/or succumb to MLN in screen house conditions. These findings will be helpful in informing the management of MLN in Uganda and the East and Central African region.

2. Materials and methods

2.1. Experimental site

Screen house studies and virus serological tests were conducted at the National Agricultural Research Laboratories (NARL) at Kawanda, 13 km Bombo road, Wakiso district in central Uganda. NARL is located 32° 31'E, 0°252 N with an altitude of 1190 masl. The experimental site at NARL receives annual rainfall total of 1,250 mm with a daily average minimum temperature of 15 °C and maximum temperature of 29 °C, a mean relative humidity of 76% and is characterized by a bimodal rainfall pattern (Mukankusi *et al*., 2018). The screen house studies were repeated during three rainy seasons namely; 2014A, 2014B and 2015A, where "A" is the first rainy season (March-July) and "B" is the second rainy season (September-December).

2.2. Mechanical transmission studies

2.2.1. Collection and maintenance of virus isolates

Symptomatic maize samples were collected from MLN hotspot areas of Tororo, Mbale, Bulambuli and Sironko districts of Eastern Uganda based on earlier surveys (Mudde *et al*., 2018). The procedure for collection and maintenance of virus isolates was based on a protocol by Gowda *et al.* (2015) with slight modifications. Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) according to the general protocol by Clark and Adams (1977) was used to test for the presence of SCMV and MCMV in collected symptomatic maize leaf samples. Once confirmed for the presence of SCMV or MCMV by DAS-ELISA, both viruses were propagated on a popular susceptible variety Longe 5 in separate greenhouses. Two sealed separate screen houses were used for production of MCMV and SCMV inoculum. One gram of infected maize leaf samples collected from

the field in MLN hotspot areas was cut into small pieces using a sterile scalpel blade and placed in a mortar containing 5 ml of ELISA extraction buffer. Inoculum was then made by grinding the infected maize leaf in cold 0.1M Phosphate buffer at pH 7 following methods by Noordam (1973). The resultant extract of sap was centrifuged for two minutes at 12,000 rpm. The sap was decanted and carborundum added at the rate of 0.02 g/ml. The susceptible maize variety Longe 5 at the V2 stage (10 days after emergence) with two leaves was infected with MLN causing virus by rubbing extract of sap onto the maize leaves. Prior to inoculation, DAS-ELISA was done on the maize leaves from each separate MCMV and SCMV screen house to ascertain the purity of the inoculums at three weeks before inoculation.

2.2.2. Selection of test weed and cultivated crop species

The weed samples were obtained from five neighboring maize fields at NARL, Kawanda, Wakiso district, Uganda. Weed species similar to predominant weed species earlier identified from the surveys were included and grown in the disease indexing screen house. The assembled plants were identified to species level according to the taxonomic keys using reference herbarium collections available at the Department of Botany Herbarium, Makerere University and reference identification keys from Phillips *et al*. (2003). The 21 weed species tested were; *Imperata cylindrica Echinochloa pyramidalis, Sorghum aethiopicum, Eleusine indica, Panicum maximum, Brachiaria decumbens*, *Chloris gayana, Digitaria abyssinica, Cyperus rotundus, Paspalum notatum, Amaranthus dubious, Amaranthus spinosus, Axonopus flexousus, Bracharia brizantha, Cynodon dactylon, Eleusine africana, Paspalam sceobiculatum, Pennisetum purpureum, Rottboellia cochinchinensis, Sorghum aethiopicum* and *Sorghum arundinacium* (Table 1). A similar experiment was also established for the following crops commonly associated with maize stands from all agroecological zones in Uganda based on previous surveys in Uganda. The six most common cultivated crops used to represent cultivated crops based on data from previous surveys conducted in five major maize growing agroecological zones (Mudde *et al*., 2018) and included common bean (*Phaseolus vulgaris*), cassava (*Manihot esculenta*), groundnut (*Arachis hypogaea*), banana (*Musa sp*), soybean (*Glycine max*) and sweet potato (*Ipomoea batatas*) (Table 2).

Table 1: Weed species used in the experiment

Family	Species	Growth Habitat	Common Name
Amaranthaceae	*Amaranthus dubius*	Annual	Spleen amaranth
Poaceae	*Axonopus flexousus*	Perennial	Carpet grass
Poaceae	*Bracharia brizantha*	Perennial	Palisade grass
Poaceae	*Brachiaria decumbens*	Perennial	Signal grass
Poaceae	*Chloris gayana*	Perennial	Rhodes grass
Poaceae	*Commelina benghalensis*	Annual	Wandering jew
Poaceae	*Cynodon dactylon*	Perennial	Common star grass
Cyperaceae	*Cyperus rotundus*	Perennial	Nut grass
Poaceae	*Digitaria abyssinica*	Perennial	African couch grass
Poaceae	*Echinochloa pyramidalis*	Perennial	Antelope grass
Poaceae	*Eleusine africana*	Annual	Wild African finger millet
Poaceae	*Eleusine indica*	Annual	Wild finger millet
Poaceae	*Imperata cylindrica*	Perennial	Sword or spear grass
Poaceae	*Panicum maximum*	Perennial	Common guinea grass
Poaceae	*Paspalum notatum*	Perennial	Bahia grass
Poaceae	*Paspalum scrobiculatum*	Perennial	Kodo millet

Table 1 (Cont.)

Family	Species	Growth Habitat	Common Name
Poaceae	*Pennisetum purpureum*	Perennial	Elephant grass
Poaceae	*Rottboellia cochinchinensis*	Perennial	Itch grass
Poaceae	*Sorghum aethiopicum*	Perennial	Not defined
Poaceae	*Sorghum arundinaceum*	Perennial	Common wild sorghum
Poaceae	*Sorghum purpureosericeum*	Perennial	Silky sorghum

Table 2: Cultivated crop species used in the experiment

Family	Common Name	Botanical Name	Life Cycle
Convolvulaceae	Sweet potato	*Ipomoea batatas*	Annual
Euphorbiaceae.	Cassava	*Manihot esculenta*	Perennial
Fabaceae	Groundnuts	*Arachis hypogaea*	Annual
	Soybeans	*Glycine max*	Annual
	Common Beans	*Phaseolus vulgaris*	Annual
Musaceae	Bananas	*Musa sp*	Perennial

2.2.3. Soil preparation and potting

Black top soil from the forest, sand from the shores of Lake Victoria and fully decomposed cow manure from a nearby animal farm / kraal were transported to the Banana Resource Center at the NARL for reconstitution in a potted plant growth medium. They were mixed in the ratio of 3:1:1 and then steam sterilized in a metallic cylindrical drum for eight hours. To sterilize the soil, the bottom of the drum was fitted with a rack up to a height of 15 cm. The bottom was filled with water up to a height of 5 cm from the base. The top of the rack was lined with heat resistant micro-pore polyethylene mesh on which the mixed soil was rested. The soil was topped up to 1.2 m high and covered with same heat resistant micro-pore polyethylene mesh. Fire was set up below the bottom of the drum and boiled for eight hours after which it was deemed sterile. After five days, when the soil was cool, 0.25 kg was transferred into disposable plastic cups.

2.2.4. Raising of test weed and cultivated crop plants

Five pots of plants were used from each of the 21 weed species studied and replicated three times. A Ugandan maize susceptible cultivar, Longe five was included as a positive control. The weeds were transplanted from the field into two liter plastic bucket and allowed to establish for two weeks in the screen house. The cultivated crops were propagated from certified seed obtained from NASECO seed company and were planted as seed while the cassava and bananas were raised from cuttings and suckers respectively obtained from tissue culture generated disease free planting material at NARL. Pots were labeled according to weed species and randomly placed on a table in a screen house, one meter above the floor. They were watered once after every three days throughout the experimental period.

2.2.5. Mechanical inoculation of test weed and cultivated crop plants with MLN causing viruses

After establishment, the weed and crop plants were mechanically inoculated with combined sap obtained from MCMV and SCMV infected plants raised separately and in different screen houses.

Prior to inoculation, one gram of infected maize leaf samples collected from the screen house with MCMV infected maize plants at V2 stage was cut into small pieces using a sterile scalpel blade and placed in a mortar containing 5 ml of ELISA extraction buffer. Inoculum was then made by grinding the infected maize leaf in cold 0.1 M Phosphate buffer at pH 7 following methods by Noordam (1973). The resultant extract of sap was

centrifuged for two minutes at 12,000 rpm. The same procedure was followed for the SCMV infected maize plants that were raised in a separate green house. One gram of SCMV infected maize leaf samples collected from the screen house with SCMV infected maize plants at V2 stage was cut into small pieces using a sterile scalpel blade and placed in a mortar containing 5 ml of ELISA extraction buffer. Inoculum was then made by grinding the infected maize leaf in cold 0.1 M Phosphate buffer at pH 7 following methods by Noordam (1973). The resultant extract of sap was centrifuged for two minutes at 12,000 rpm. The two separate viral solutions in separate motors were poured into a Phillips® blender and mixed at 35,000 RPM for 10 minutes in a ratio of four parts of SCMV to one part MCMV. The sap was then decanted and carborundum added at the rate of 0.02 g / ml. Prior to inoculation, the leaves of the maize plant at the V2 stage were dusted with carborundum which facilitated creating of wounds on the leaves for entry of the viruses. The centrifuged supernatant mixture of SCMV and MCMV was rubbed onto leaves of three different pots with five plants of each selected weed species. The pots were replicated three times for each plant species and each experiment giving a total of 15 plants tested per species for each experiment. The screen house studies were repeated during three rainy seasons namely; 2015B, 2016A and 2016B, where "A" is the first rainy season (March-June) and "B" is the second rainy season (September-December). The experimental design was a randomized complete block (RCBD) with three replicates. The plants were inoculated three times and ELISA tests for MLN causing viruses (MCMV and SCMV) done 10 days after each inoculation. The plants were observed for any symptoms of these viruses. A virus free maize plant was included as a control check for both the viruses.

2.3. Virus identification

2.3.1. Serological detection of MLN causing viruses in weeds and cultivated crops

The presence of the presence of SCMV and MCMV in mechanically inoculated weed and crop leaf samples was determined using serological techniques. The procedure based on protocols by Clark and Adams (1977) was DAS-ELISA. The antisera used were procured from Agdia Inc. Elkhart, Indiana USA. The polyclonal antibodies utilized were anti-SCMV and anti-MCMV. In the test all the buffers were prepared according to the manufacturers specifications from Agdia Inc. Elkhart, Indiana USA. Plant leaf samples were crashed in a mortar at a ratio of 1:20 (w / v) in extraction buffer using pestle. In order to prepare the DAS-ELISA plates, 200 μl coating antibody for each specific MLN causing virus (MCMV and SCMV) was added into each well of microtitre pate (dilution 1:200 v / v of antibody: buffer) followed by two hours of incubation at 37 ^{o}C. The coated plates were then rinsed thrice in PBS-T (Phosphate Buffered Saline-Tween 20 pH 7.4). A total of 200 μl of the prepared plant test samples were added into each well in pairs followed by overnight incubation at 4 ^{o}C. Following incubation, the plates were rinsed thrice and 200 μl enzyme conjugate diluted in ECL buffer 1:200 (v / v) added to each well. Plates were incubated at 37 ^{o}C for three hours and rinsed thrice. A 200 μl freshly prepared substrate (1 mg / ml para-nitrophenyl-phosphate in substrate buffer) was added to each well and incubated at 37 ^{o}C for 60 minutes. Positive and negative control samples procured from Agdia Inc. Elkhart, Indiana USA, were included in the microtitre plates. Plates were then assessed through observation for a yellow color change and absorbance measured at 405 nm wavelength using a BIO-RAD® microtitre plate reader Model 680 (BIO-RAD Laboratories, Hercules, California, USA). All samples were assayed in pairs and the results confirmed to be positive if the there was a yellow color change and the absorbance was greater than or equal to twice the average reading of the negative (healthy) controls.

2.3.2. Molecular detection of MLN causing viruses in weeds and cultivated crops

Total RNA was extracted from leaves of weeds and cultivated crops with Trizol Reagent (Bioneer, South Korea) and used for cDNA synthesis using the *AccuPower* reverse transcription polymerase chain reaction (RT-PCR) PreMix kit (Bioneer Corporation, South Korea) following manufacturer's instructions. MCMV and SCMV primers which flank the coat protein gene of each virus and amplify a fragment of approximately 550 bp for MCMV and 900 bp for SCMV were used for RT-PCR (Wangai *et al*., 2012). Amplicons were resolved on 1.5% agarose gels in 1X TAE (45 mM Tris-acetate, 1 mm EDTA) for 45 minutes at 120 V. The gel was then stained in 0.5 μg / ml ethidium bromide for 25 min and the image captured using the Syngene G: BOX gel documentation system (Fredrick, MD, USA). A 100 bp DNA Ladder (Bioneer Corporation, South Korea) was used as the standard.

2.4. Data processing and statistical analysis

Data was collected on incidence which was calculated as a percentage of the number of plants ELISA positive for MCMV / SCMV per total number of plants inoculated. Data was also recorded on ELISA (A_{405nm}) absorbance readings recorded as Optical Density (OD) values and measured at 405 nm for virus tested plants after 60

minutes. The above data were then entered into Microsoft Excel Spreadsheet for cleaning and exported to GENSTAT 15 statistical software (Buysse *et al*., 2004) for further analysis. Data on MCMV/SCMV disease incidence and relative absorbance for ELISA were subjected to analysis of variance in a two-way (randomized blocks) with plant species and seasons as the factors and replicates as blocks. The means of the parameters including disease incidence and relative absorbance for ELISA were separated using Fishers' Protected Least Significant Difference (LSD) at 5% probability level (Gomez and Gomez, 1984).

3. Results

3.1. Susceptibility of weed species to Maize chlorotic mottle virus following mechanical Inoculation with MLN

Seven out of the 21 species of weeds that were screened became infected with MCMV after being mechanically inoculated (Table 2). Some infected weeds notably *Imperata cylindrica* produced characteristic MCMV symptoms with a chlorotic mosaic being the most common symptom (Figure 1). Some of the weeds that tested positive for MCMV using ELISA did not produce any symptoms.

Figure 1: Maize chlorotic mottle virus symptoms observed in *Imperata cylindrica* grass weed species following mechanical inoculation

Susceptibility to MCMV infection via mechanical inoculation significantly ($p < 0.05$) varied among weed species evaluated (Table 3). Overall, perennial weeds such as *Imperata cylindrica, Axonopus flexuous, Pennisetum purpureum, Digitaria abyssinica, Rottboellia cochinchinensis*, *Eleusine africana* and *Panicum maximum* were more susceptible to MCMV compared to other weeds evaluated as measured by the incidence of infection.

Digitaria abyssinica had the highest incidence (93.3%) among the species tested, followed by *Eleusine africana* (86.7%) and *Rottboellia cochinchinensis* (73.3%). Weeds with moderately high (40 to 70%) incidence of MCMV following mechanical inoculation included; *Pennisetum purpureum, Imperata cylindrica, Panicum maximum* and *Axonopus flexousus.*

Amaranthus dubius, Bracharia brizantha, Brachiaria decumbens, *Chloris gayana*, *Commelina benghalensis, Cynodon dactylon, Cyperus rotundus, Echinochloa pyramidalis, Eleusine indica, Paspalum notatum*, *Paspalum scrobiculatum, Sorghum aethiopicum, Sorghum arundinaceum* and *Sorghum purpureosericeum* were not susceptible to MCMV following mechanical inoculation.

Table 3: Susceptibility of weed species to MCMV after mechanical inoculation with MLN

Weed Species	Mean Incidence (%)	Mean ELISA (A_{405nm})[a]
Amaranthus dubius	**0.0a**	**0.279ab**
Axonopus flexousus	**66.7de**	**2.180h**
Bracharia brizantha	**0.0a**	**0.328bc**
Brachiaria decumbens	**0.0a**	**0.244a**
Chloris gayana	**0.0a**	**0.281ab**
Commelina benghalensis	**0.0a**	**0.270ab**
Cynodon dactylon	**0.0a**	**0.397c**
Cyperus rotundus	**0.0a**	**0.266ab**
Digitaria abyssinica	**93.3fg**	**1.503f**
Echinochloa pyramidalis	**0.0a**	**0.277ab**
Eleusine africana	**86.7f**	**2.332i**
Eleusine indica	**0.0a**	**0.295ab**
Imperata cylindrical	**53.3bc**	**1.593g**
Panicum maximum	**60.0cd**	**1.664g**
Paspalum notatum	**0.0a**	**0.270ab**
Paspalum scrobiculatum	**0.0a**	**0.249a**
Pennisetum purpureum	**46.7b**	**1.217e**
Rottboellia cochinchinensis	**73.3e**	**2.253h**
Sorghum aethiopicum	**0.0a**	**0.255ab**
Sorghum arundinaceum	**0.0a**	**0.258ab**
Sorghum purpureo-sericeum	**0.0a**	**0.271ab**
Infected maize (Positive control)	**100.0g**	**2.443i**
Healthy maize (Negative control)	**0.0a**	**0.573d**
Fisher's protected $LSD_{0.05}$	7.862	**0.07829**
CV (%)	**33.5**	**9.8**
***p*-Value**	**0.001**	**0.001**

Note: a – ELISA average is based on the mean absorbance for three experiments conducted. Readings taken after 60 minutes. Mean incidence and mean ELISA values that are followed by the same letter (a, b, c, d, e, f, g, h, i) within each column do not differ significantly to Fisher's protected least significant difference test at P< 0.05.

Incidence significantly ($p < 0.05$) varied among the weed species with the highest incidence of MCMV disease being recorded on *Digitaria abyssinica, Eleusine africana* and *Rottboellia cochinchinensis*. *Rottboellia cochinchinensis, Eleusine africana* and *Axonopus flexousus* had the highest virus titer recorded. The concentration of MCMV virus titer based on ELISA OD readings captured at A_{405nm} was significantly different ($p < 0.05$) in the different weed species following mechanical inoculation (Table 3). *Rottboellia cochinchinensis, Axonopus flexousus, Eleusine africana* that tested positive for MCMV, were from the Poaceae family and had high virus titer ranging

from OD readings of 2.180 to 2.333 which was comparable with the concentration of virus titer OD readings of 2.443 of susceptible Longe 5 maize variety used as control (Table 3).

3.2. Susceptibility of weeds to Sugarcane mosaic virus after mechanical inoculation with MLN

Six out of the 21 species of weeds screened became infected with SCMV after being mechanically inoculated with MLN (Table 4). Some infected weeds notably *Chloris gayana* produced characteristic SCMV symptoms with a chlorotic mosaic being the most common symptom (Figure 2).

Table 4: Susceptibility of weeds species to SCMV following mechanical inoculation

Weed Species	Mean Incidence (%)	Mean OD (A_{405nm})
Amaranthus dubius	0.0a	0.788d
Axonopus flexousus	0.0a	0.697d
Bracharia brizantha	53.3b	1.817g
Brachiaria decumbens	0.0a	0.368abc
Chloris gayana Kunth	0.0a	0.379bc
Commelina benghalensis	0.0a	0.487c
Cynodon dactylon	53.3b	1.715g
Cyperus rotundus	0.0a	0.745d
Digitaria abyssinica	0.0a	0.357ab
Echinochloa pyramidalis	0.0a	0.288ab
Eleusine africana	0.0a	1.258f
Eleusine indica	0.0a	0.353ab
Imperata cylindrica	53.3b	1.806g
Panicum maximum	86.7d	2.866j
Paspalum notatum	0.0a	0.972e
Paspalum scrobiculatum	0.0a	0.696d
Pennisetum purpureum	93.3e	2.882j
Rottboellia cochinchinensis	66.7c	2.146h
Sorghum aethiopicum	0.0a	0.287ab
Sorghum arundinaceum	0.0a	0.305ab
Sorghum purpureo-sericeum	0.0a	0.256a
Infected maize (positive control)	100.0f	2.647i
Healthy maize (negative control)	0.0a	0.676d
Fisher's protected $LSD_{0.05}$	3.998	0.12235
CV (%)	19.5	12.2
p-Value	<0.001	<0.001

Note: ELISA average is based on the mean absorbance for the three experiments conducted. Readings taken after 60 minutes. Means followed by the same letter in a column do not differ significantly to Fisher's protected least significant difference test at $p < 0.05$.

Figure 2: Chlorotic *Sugarcane mosaic virus* symptoms observed in *Cynodon dactylon* grass species following mechanical inoculation in a green house in Uganda

Some of the weeds that were positive in the ELISA test did not produce any symptoms. Susceptibility to SCMV infection via mechanical inoculation significantly ($p < 0.05$) varied among the weed species (Table 4). Overall, weed species such as *Imperata cylindrica, Bracharia brizantha, Panicum maximum, Cynodon dactylon, Rottboellia cochinchinensis* and *Pennisetum purpureum* were more susceptible to SCMV compared to other weeds evaluated as measured by the incidence of infection. *Pennisetum purpureum* had the highest incidence (93.3%) among the weed species tested, followed by *Panicum maximum* (86.7%). Weeds with moderately high (40 to 70%) incidence of SCMV following mechanical inoculation included; *Rottboellia cochinchinensis* (66.7%), *Bracharia brizantha* (53.3%), *Cynodon dactylon* (53.3%) and *Imperata cylindrica* (53.3%). The concentration of SCMV virus titer based on OD readings was significantly different ($p < 0.05$) in the different weed species following mechanical inoculation (Table 4). The virus titer concentration based on OD readings was highest in *Pennisetum purpureum* (2.882) followed by *Panicum maximum* (2.866) and *Roetboellia cochinchinensi* (2.146). These concentrations were comparable with the concentration of virus titre OD readings of 2.647 of susceptible Longe 5 maize variety which was used as a positive control (Table 4). No SCMV infections were observed on *Amaranthus dubius, Bracharia brizantha, Brachiaria decumbens*, *Chloris gayana, Commelina benghalensis, Cynodon dactylon, Cyperus rotundus, Echinochloa pyramidalis, Eleusine indica, Paspalum notatum*, *Paspalum scrobiculatum, Sorghum aethiopicum, Sorghum arundinaceum* and *Sorghum purpureo-sericeum* after mechanical inoculation.

3.3. Susceptibility of common cultivated crop species to Maize chlorotic mottle virus and Sugarcane mosaic virus following mechanical inoculation with MLN

The concentration of MCMV virus titer based on OD readings was significantly different ($p < 0.05$) in the different cultivated crop species following mechanical inoculation (Table 5). There was successful mechanical transmission of MCMV in the cassava (*Manihot esculenta*) (0.8813) followed by groundnut (*Arachis hypogaea*) (0.7557) and common bean (*Phaseolus vulgaris*) (0.6953) based on the virus tire concentrations. The MCMV virus titer concentration based on OD readings was highest in cassava (*Manihot esculenta*) (0.8813) followed by groundnut (*Arachis hypogaea*) (0.7557) and common bean (*Phaseolus vulgaris*) (0.6953). These concentrations were comparable with the concentration of virus titer OD readings of 1.3983 of susceptible Longe 5 maize variety used as positive control. However, subsequent PCR tests did not detect the MCMV in these cultivated crops and hence did not corroborate the ELISA findings. On the other had for SCMV, although the concentration of SCMV virus titer based on OD readings was significantly different ($p < 0.05$) in the different cultivated crop species following mechanical inoculation, there was no successful mechanical transmission of SCMV to the evaluated cultivated crops based on the titer concentrations. The SCMV titer concentration based on OD readings were all low and comparable to the negative control Longe 5 maize variety with an OD reading of 0.297.

Table 5: Susceptibility of common cultivated crop species to *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* based on ELISA test following mechanical inoculation with MLN

Cultivated Crop Species	Family	Mean OD (A_{405nm}) MCMV[a]	ELISA Result – MCMV	Mean OD (A_{405nm}) SCMV[a]	ELISA Result – SCMV
Musa sp.	Musaceae	0.398abc	–	0.251a	–
Manihot esculenta	Euphorbiaceae	0.881d	+	0.213a	–
Phaseolus vulgaris	Fabaceae	0.695bcd	+	0.219a	–
Arachis hypogae	Fabaceae	0.756cd	+	0.353a	–
Glycine max	Fabaceae	0.276a	–	0.231a	–
Ipomoea batatas	Convolvulaceae	0.320ab	–	0.467a	–
Zea mays (positive control)	Poaceae	1.398e	+	1.618b	+
Zea mays (negative control)	Poaceae	0.255a	–	0.297a	–
Fisher's protected $LSD_{0.05}$		0.386		0.272	
CV (%)		65.8		63.3	
p-Value		<0.001		<0.001	

Note: [a] – ELISA average is based on the mean absorbance for all three experiments conducted. Readings taken after 60 minutes. Means followed by the same letter in a column do not differ significantly to Fisher's protected least significant difference test at $p < 0.05$.

3.4. RT-PCR results for Maize chlorotic mottle virus and Sugarcane mosaic virus in mechanically inoculated weed species and cultivated crops with MLN

Most of the experimentally infected weed grass and few cultivated crop species showed very strong distinct bands as shown in representative gels for MCMV (Figure 3). The positively tested plants yielded RT-PCR

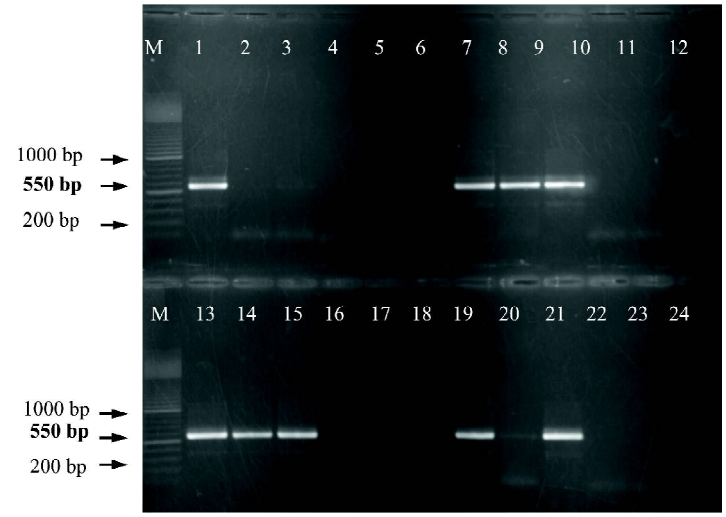

Figure 3: RT-PCR products of MCMV in weed samples tested after mechanical inoculation. Lane M: 100 bp DNA marker; lanes 1 = Positive control (maize), 2 = *Amaranthus dubius*, 3 = *Bracharia brizantha*, 4 = *Brachiaria decumbens*, 5 = *Chloris gayana*, 6 = *Commelina benghalensis*, 7 = *Digitaria abyssinica*, 8 = *Eleusine africana*, 9 = *Rottboellia cochinchinensis*, 10 = *Cynodon dactylon*, 11 = *Cyperus rotundus*, 12 = *Musa sp*, 13 = *Pennisetum purpureum*, 14 = *Arachis hypogaea*, 15 = *Panicum maximum*, 16 = *Echinochloa pyramidalis*, 17 = *Eleusine indica*, 18 = *Paspalum notatum*, 19 = *Manihot esculenta*, 20 = *Paspalum scrobiculatum*, 21 = *Phaseolus vulgaris*, 22 = negative control (maize), 23 = *Sorghum aethiopicum*, 24 = *Sorghum arundinaceum*

products of the expected size (550 bp), whereas no RT-PCR product was amplified from healthy maize plant as a negative control. SCMV was not detected using RT-PCR in both weeds and cultivated crops hence results are not presented.

4. Discussion

This study investigated whether commonly occurring weed species and cultivated crops in maize agro ecosystems would vary in their susceptibility to MLN causing viruses and therefore participate in the disease dynamics. The screening process revealed variation in susceptibility of weed and crop species to MLN causing viruses. Findings in this study revealed that most of the tested weeds species which were also from the Poaceae family were susceptible to MCMV. This study established *Panicum maximum, Axonopus flexuous, Digitaria abyssinica, Imperata cylindrica, Eleusine africana, Rottboellia cochinchinensis* and *Pennisetum purpureum* all from the Poacea family are susceptible to MCMV. This was in support of earlier work which indicated most of the weed hosts of MCMV in the Poaceae family are susceptible to MCMV (Uyemoto, 1980; Bockelman *et al*., 1982; Scheets, 2004; and Brunt *et al*., 2010). Susceptibility among wide variety of weed species was possibly attributed to the fact that the MCMV particle is very stable and can retain infectivity (Scheets, 2016). MCMV is readily mechanically transmissible to maize and other experimental hosts in laboratory settings. The MCMV genome also has the ability to encode for proteins that increase the accumulation of the virus in the plant (Scheets, 2016) and hence has higher chances of being detected during virus testing. As evident from studies conducted since 1984 (Uyemoto, 1980; Bockelman *et al*., 1982; Scheets, 2004; and Brunt *et al*., 2010), MCMV is known to have at least 19 different experimental alternative plant hosts. Therefore, this and earlier studies provide further evidence of the presence of MCMV in *Panicum maximum and Digitaria abyssinica*. All other MCMV alternative hosts were reported for the first time in the following species *Axonopus flexuous, Eleusine africana, Imperata cylindrica, Pennisetum purpureum* and *Rottboellia cochinchinensis.* The above findings suggest that the high incidence of this MCMV virus in endemic hotspots based on previous surveys by Mudde *et al.* (2018) may be partially associated with large numbers of MCMV sources in the major maize growing agroecological zones of Uganda.

Furthermore, this study revealed that the following perennial weeds *Panicum maximum, Bracharia brizantha, Imperata cylindrica, Cynodon dactylon, Rottboellia cochinchinensis* and *Pennisetum purpureum* are susceptible to SCMV and could potentially serve as reservoirs of the SCMV virus. The findings of this study are in agreement with earlier findings of Bhargava (1975) who reported *Pennisetum purpureum* as susceptible to SCMV; Kusia (2014) and Kusia (2015) who recorded *Cynodon dactylon* and *Bracharia brizantha* as susceptible hosts of SCMV and Louie (1980) who confirmed *Cynodon dactylon* as susceptible to SCMV. While it has been reported before that SCMV has at least 22 different alternative hosts mainly restricted to the Poaceae family (Teakle and Grylls, 1973; Tosic and Ford, 1972; Louie, 1980; Brunt *et al*. 1990; Bhargava, 1975; Louie, 1980; Kusia, 2014; and Kusia, 2015). This study also confirms *Pennisetum purpureum, Bracharia brizantha, Cynodon dactylon* and *Panicum maximum* as hosts of SCMV. Of all these species, only *Cynodon dactylon* exhibited characteristic systemic mosaic, yellow striping as described by Mahuku *et al*. (2015) while other SCMV infected weeds remained asymptomatic. This suggests that most of the common weeds growing in association with maize have the potential of surviving with the SCMV virus without any damage to the plant. This study has also established for the first time that *Imperata cylindrica* and *Rottboellia cochinchinensis* as experimental hosts of SCMV. Overall host range responses to MCMV and SCMV inoculations were similar in *Panicum maximum, Pennisetum purpureum, Imperata cylindrica* and *Rottboellia cochinchinensis,* all from Poaceae family and thus they could be candidates as over-seasoning hosts of both MCMV and SCMV and should be the target for MLN management. These ELISA based results were not confirmed in most of the weed species using PCR for SCMV but only for MCMV. These findings are in conformity to reports by Adams *et al*. (2014) and Mahuku *et al*. (2015) who reported low detection of SCMV using PCR despite positive results using ELISA. The probable reason for the low detection of SCMV could be due to the emergence of new strains with sequences in capsid protein that are different from the sequences used to design the primers. Indeed studies conducted by Adams *et al*. (2013) have confirmed that SCMV strains in the East African region are highly divergent.

As regards screening cultivated crops for susceptibility to MLN causing viruses, mechanical inoculation test results showed that common bean (*Phaseolus vulgaris*), cassava (*Manihot esculenta*) and groundnut (*Arachis hypogaea*) can habor MCMV despite the fact that they did not show symptoms. This contrasts the findings of Niblett and Claflin (1978) who reported that dicotyledonous species were not mechanically infected with MCMV. This result was thus unique and not expected for these dicotyledonous crops notably cassava, common

bean and groundnut apart from Banana monocot (Pursglove, 1972; and Pursglove, 1969). Prior to this, MCMV was only known to be found in Poaceae family (Scheets, 2004). However these results are in conformity with studies by Jiang *et al*. (1992) who showed that some dicotyledonous plants can be natural and artificial hosts of MCMV. Nonetheless the findings in this study suggest that these cultivated crops may carry the virus based on the virus titers that were comparable to the positive maize control. Hence this study could provide the first evidence of the potential role of cultivated dicot crops as hosts of MLN causing viruses. No information is available concerning the reaction of cultivated crops including common bean (*Phaseolus vulgaris*), cassava (*Manihot esculenta*), groundnut (*Arachis hypogaea*), banana (*Musa sp*), soybean (*Glycine max*) and sweet potato (*Ipomoea batatas*) to inoculation with MLN causing viruses and hence these findings provide the first report of the potential role these cultivated crops could play as reservoirs of MCMV potentially increasing the amount of virus inoculum within the field. The implications of these findings is crops like cassava (*Manihot esculenta*), commonly grown in these areas have some varieties that are late maturing and can hence provide a source of inoculum to the next season crop of maize. Furthermore, common bean and groundnut are usually grown as intercrops with maize and hence could also potentially provide a source of inoculum of MLN causing virus when grown with maize. However, the importance of the cultivated crops as alternate hosts needs further studies to determine if vectors that can survive on maize can also survive on these alternative hosts.

5. Conclusion

Potential alternative hosts of maize lethal necrosis causing viruses present in major maize growing agro ecological zones of Uganda differ in their susceptibility to MLN causing viruses. Most of the commonly occurring weeds and cultivated found in maize growing agro ecologies in Uganda are susceptible to MCMV. Continuous occurrence of MLN in the MLN hotspots of Uganda is linked to the continuous presence of these perennial weeds and cultivated crops grown in association with maize each season which can act as sources of inoculum for the main virus driving the MLN epidemic in Uganda. The prevalence of these perennial weeds in major maize growing agro ecological zones is likely to indicate a major source of the MLN viruses carried through the dry season when the maize crop has been harvested. In addition, the presence of cultivated crops and weeds plants as alternate hosts, presents the need to identify MLN non hosts in cultivated crops for crop rotation and early weeding to reduce on MLN virus inoculum in the agro ecosystem. Failure of farmers to adopt these management strategies may result in a constant increase in MLN disease inoculum in the MLN hotspot areas and contribute to early infection of most maize plants with resultant losses in yields that are severe.

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Conflicts of interest

There is no conflict of interests between the authors of this manuscript.

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