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Evaluation of hot pepper (*Capsicum* spp.) genotypes for resistance to viruses and aphids in Rwanda

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Hot pepper is an important crop in Rwanda but viral diseases and pests are major constraints to its production. Field experiments were conducted to evaluate the resistance of 18 hot pepper genotypes (4 commercials, 5 introduced and 9 local) to natural infection by viruses and aphid infestation, in two agro-ecological zones of Rwanda. Fourteen genotypes were further evaluated for resistance to *Cucumber mosaic virus* (CMV) under screenhouse conditions. Disease incidence and severity were recorded in all experiments while population of aphids was assessed in the field. Diseased leaf samples from each genotype in the field were analysed using polymerase chain reaction to detect the presence of viruses, while samples from the screenhouse were analysed using serological assay. Results showed significant ($p<0.05$) differences in disease incidence and severity among genotypes. Three genotypes namely PBC 462, 00767PPR and 0802PPR were rated as resistant to viral diseases while genotype HP 0117, PP9852-170 and PP9950-5197 were moderately resistant. All commercial and most of the local genotypes were susceptible compared to the introduced lines. There was no difference in genotype infestation by the aphids. The genotypes that are resistant to viruses are recommended for use by growers and in breeding programs.

1. Introduction

Hot pepper (*Capsicum* spp.) is an important vegetable crop grown throughout the world. In Rwanda, it is produced for both local consumption and export to the European market (NAEB, 2015). The crop plays an important role in poverty alleviation through income generation and creation of employment to both farmers and the hot pepper value chain actors. Hot pepper production has increased in recent years in Rwanda but, the average yield is still low at around 6.8 t ha^{-1} which is lower than

50% of the country's potential yield of 15 t ha^{-1} (RDB, 2010; FAO, 2017). The low and poor quality produce have been attributed to abiotic and biotic factors, of which diseases caused by viruses play a significant role (Skelton *et al*., 2018).

Pepper is attacked by more than 68 viruses globally, of which about 15 have been identified in Africa (Njukeng *et al.,* 2013; Aliyu, 2014; Kenyon *et al.*, 2014). *Cucumber mosaic virus* (CMV), *Pepper veinal mottle virus* (PVMV), *Tobacco mosaic virus* (TMV) and *Potato virus Y* (PVY) have been reported as the most prevalent in Sub-Saharan Africa (Dafalla, 2001). In Rwanda, *Pepper vein yellows virus* (PeVYV), PVMV and CMV have been detected in hot pepper (Skelton *et al*., 2018). The CMV is ranked among the most economically important viruses of hot pepper not only in Rwanda but also in some other countries such as India where yield losses ranging from 10 to 50% are documented (Rahman *et al*., 2016). On the other hand, the crop is attacked by more than 21 insects which include aphids, whiteflies, thrips among others (Niranjanadevi *et al*., 2018). Aphids, whiteflies, and thrips are vectors of various viruses infecting hot pepper. These insect pests especially the aphids, are serious threat to hot pepper production not only due to losses caused through direct damage but also they are vectors of devastating viruses (Kenyon *et al*., 2014).

Various management options have been proposed to reduce virus diseases of hot pepper in the field. These measures include the use of virus-free planting materials, resistant varieties, borders crops, pesticides and roguing (Wang *et al.*, 2006; Degri and Ayuba, 2016). Farmers in Rwanda mainly rely on insecticides to control insect-vectors. Unfortunately, the insecticides do not achieve 100% control of the vectors. Hence, the insect-vectors develop resistance against the active ingredient after repeated application of the insecticides within a short time and more so the insecticides negatively affect the environment (Kenyon *et al*., 2014). The use of resistant cultivars offers the most economical, effective and durable solution in mitigating the negative effects due to diseases and aphids in hot pepper production (Visalakshi and Pandiyan, 2018). Resistant varieties are highly preferred because they not only reduce the pest population and the virus inoculum in the farming system but they are also compliant with other methods (Frantz *et al*., 2004). Several studies have evaluated the resistance of wild and cultivated hot pepper genotypes to virus infection and aphids' infestation leading to the release of virus-resistant lines in different parts of the world (Frantz *et al*., 2004; Appiah *et al*., 2014; Choi *et al*., 2018). However, information on hot pepper genotypes that are resistant to virus infection and vector infestation is not documented in Rwanda. The current study focused on local, commercial and introduced genotypes that have not been evaluated before for the resistance to viruses or aphids in Rwanda.

It is important to assess the genotypes in different environments in the field to identify the relative host resistance, as some genotypes found resistance at one location turns out to be susceptible to another place. Screenhouse assessment using artificial inoculation techniques is important for validation of resistance. In this study, both field and screenhouse experiments were conducted to (1) evaluate the reaction of different hot pepper genotypes (local, commercial and introduced) to natural virus infections and aphids' infestations in two agro-ecological zones of Rwanda, and (2) evaluate the reaction of selected hot pepper genotypes to infection by *Cucumber mosaic virus* under screenhouse conditions.

2. Materials and Methods

Reaction of hot pepper genotypes to virus infection and aphid infestation under field conditions

Source of seeds

A total of 18 hot pepper genotypes that included nine local collections obtained from Rwanda National Genbank, five introduced lines provided by World Vegetable Center, Eastern and Southern Africa-Tanzania and four commercial varieties from seed companies were evaluated (Table 1). Previous studies indicate that the introduced genotype PP9950-5197 is resistant to CMV, PVY and *Chilli veinal mottle virus* while genotype ICPN 18-7 is resistant to PVY (Gniffke *et al*., 2013). Similar studies by Reddy *et al*. (2014) showed the introduced genotype PP9852-170 as resistant against CMV. California wonder (sweet pepper) variety which has been used as a susceptible control for viruses in previous studies (Murphy and Bowen, 2006) was also included in the present study.

Study areas

The study was conducted at Rubona and Gashora research fields belonging to the Rwanda Agriculture and Animal Resources Development Board (RAB) during two successive growing seasons; long rains (end

March-July, 2018) and short rains (end October, 18-March, 2019). Rubona station is located at an altitude of 1692.9m, latitude S 2°28'59.59" and longitude E29°46'22.46", in midland AEZ. Gashora is located at an altitude of 1331.1m, latitude S 2°15'22.11" and longitude E30°17'12.43", in lowland AEZ. The characteristics of the two AEZs are shown (Table 2).

Raising of the seedlings

Seeds of the different genotypes were sown and raised in trays containing sterilized sandy loam soil (1:2) in the screenhouse. At 2-3 leaf stage, the seedlings were transplanted into plastic potting bags (5 × 9 × 4 cm) containing steam-sterilized sandy loam soil and maintained for six weeks in the screenhouse. Before transplanting to the field, the seedlings were confirmed to be free from *Pepper mild mottle virus* (PMMoV), PVMV, TMV, PVY and CMV using DAS-ELISA. The kits were obtained from Loewe Biochemica GmbH company, Germany) and used according to instructions from the manufacturer.

Establishment of the field experiments

The experiments were carried out in the open field and depended on natural virus infections and aphid infestation from the uncultivated fields. The experiments were laid out in a randomized complete block design (RCBD) with three replications. The blocking was done according to soil fertility gradient and nearness to the uncultivated land, such that each treatment had an equal chance of vector infestation. Each replicate had eighteen experimental plots, measuring 2.5 m by 3 m each, with a 1 m wide path between the plots. An experimental plot contained 24 seedlings planted on 4 rows, at a spacing of 60 cm by 45 cm. At planting, approximately 500 g of organic manure was used per plant and 15 g of NPK (17:17:17) fertilizer was applied one week later. A month after transplanting, 3.5 g of urea (46:0:0) per

Table 1 - List of genotypes evaluated for reaction to infection by viral diseases and aphids under field conditions in Rwanda

Genotype	Species	Type	Source
00765PPR [1]	*C. annum*	Local	RNGB
00767PPR	*C. baccatum*	Local	RNGB
00774PPR	*C. annum*	Local	RNGB
00775PPR	*C. chinense*	Local	RNGB
00786PPR	*C. annum*	Local	RNGB
00791PPR	*C. chinense*	Local	RNGB
00792PPR	*C. frutescens*	Local	RNGB
00802PPR	- [2]	Local	RNGB
00795PPR	*C. chinense*	Local	RNGB
PBC 462	*C. annum*	Introduced	WVC
PP9950-5197	*C. annum*	Introduced	WVC
HP 0117	*C. annum*	Introduced	WVC
PP9852-170	*C. annum*	Introduced	WVC
ICPN 18-7	*C. annum*	Introduced	WVC
Long Red Cayenne	*C. annum*	Commercial	Simlaw seed company
Bird-eye hybrid (Oiseau pili pili)	*C. frutescens*	Commercial	Technisem Company
Red Scotch bonnet	*C. chinense*	Commercial	Exporter
California Wonder	*C. annum*	Commercial	Kenya seed company

[1] Code of the local genotypes as found in the database of Rwanda National GenBank;
[2] Unknown species;
RNGB= Rwanda National GenBank;
WVC= World Vegetable Center.

Table 2 - Characteristics of the two agro-ecological zones of Rwanda where the study was conducted

Site/District	AEZ*	Relief	Elevation (m)	Rainfall (mm)	Temperature (°C)
Rubona/ Huye	Midlands	Dissected plateaus	1600-1900	1100-1400	17-20
Gashora/Bugesera	Lowlands	Pediplains	900-1600	850-1100	20-21

AEZ= Agro-ecological zone. Source: Verdoodt and Van Roanst, 2003.

plant was applied. Both preventive and curative fungicidal sprays were applied at regular intervals to control fungal diseases. The spray regime was dependent on symptom appearance and prevailing weather conditions. Weeding was done two times in a month. Insecticides were not sprayed at all.

Data collection

During plant growth, data was collected at a 14-days interval starting from two to ten weeks after planting (WAP) in the field. Ten plants were randomly selected from the middle rows of each plot and tagged. These plants were used for the assessment of viral disease incidence and symptom severity during the experimental period.

Determination of disease incidence and symptom severity. Virus disease incidence was expressed as a percentage based on the proportion of infected/diseased plants to the total number of plants observed per plot, as described by Galanihe *et al.* (2004).

Symptom severity was scored for the ten tagged plants in a plot based on a scale of 1-5 as described by Olawale *et al.* (2015) with slight modifications, where: 1 = no symptoms; 2 = mild symptoms of mosaic/mottling/yellowing on few leaves (<25% of the plant affected); 3 = moderate symptoms of mosaic/puckering/mottling/vein clearing/yellowing on many leaves (26-50% of the plant affected); 4 = severe symptoms of mosaic/puckering/mottling/vein clearing/yellowing/stunting (51-75% of the plant affected) and 5 = severe symptoms of mosaic/puckering/mottling/vein clearing/yellowing/ stunting/ necrosis (>75% of the plant). Percentage severity was calculated as the sum of all disease rating per genotype expressed as a percentage of the total number of observations multiplied by maximum disease scoring scale (5).

Determination of the area under disease progress curve. The area under the disease progress curve (AUDPC) was estimated to compare different responses of the tested hot pepper genotypes. Estimated percentages of symptom severity recorded at different times during the experimental period were used to calculate AUDPC using the following equation as described by Campbell and Madden (1990).

$$AUDPC\ \Sigma^{n-1} = (Y_i + Y_{i+1})/2\ (t_{i+1} - t_i)$$

where Σ = summation; n = number of successive readings, Y_i = disease severity at time t_i and Y_{i+1}= disease severity at time t_{i+1}.

Detection of the viruses. Detection of the suspected viruses in the experimental plots was carried out using RT-PCR. At 10 WAP, approximately five young leaves from diseased plants of the eighteen genotypes were collected and placed in envelopes containing silica gel. The samples were later transported to the Phytopathology Laboratory of RAB at Rubona and stored at room temperature for 4-5 days to dry. Later, the samples were grounded in liquid nitrogen to fine powders that were stored at -80°C until analyzed. In total, 68 symptomatic leaf samples were collected from Rubona and Gashora's experimental sites.

Total ribonucleic acid was extracted from 100 mg of frozen powdered hot pepper leaf tissues using acetyl trimethyl ammonium bromide (CTAB) method as described by Allen *et al*. (2006) with modifications. Amplification of CMV, PVMV, PeVYV was done using One Taq One-step RT-PCR Kit (Catalogue E531S5, New England Biolabs Inc.), following the manufacturer's instructions. Amplified products were generated using virus-specific primers that were designed during this study based on the nucleotide sequence data of CMV-R1 (GenBank accession no. MG470800.1), PVMV-R1(MG470801.1), PeVYV-R1 (MG470802.1). The CMV primers amplified a fragment of ~502 bp, PVMV a fragment of ~502 bp and PeVYV a fragment of ~498 bp (Table 3).

Thermal cycling conditions were: 48°C at 15 min

Table 3 - Primers used for detection of Cucumber mosaic virus, Pepper veinal mottle virus, and Pepper yellows virus

Virus	Primer *	Sequence	Amplification size (bp)
CMV	MG470800_1F	5'-GCTTCGCAATACGTTTTGACGG-3'	502
CMV	MG470800_1R	5'-TACGACCAGCACTGGTTGATTC-3'	502
PVMV	MG470801_1F	5'-AAGCCCTCATTGAAGGTCAACG-3'	502
PVMV	MG470801_1R	5'-ATCAACCATCACCCACATACCG-3'	502
PeVYV	MG470802_1F	5'-AGTACGTCTTCGAGACTACTGC-3'	498
PeVYV	MG470802_1R	5'-TCTATAGTAGAGAGGTCGATCC-3'	498

* Primers developed during this study.

for RT; followed by 1 min at 94°C for initial denaturation; 40 cycles of 94°C for 15 s, 54°C for 30 s and 68°C for 45 s for denaturation, annealing, and extension, respectively. The final extension was at 68°C for 5 min. These conditions were similar for the three viruses. The PCR products were separated by electrophoresis in 1.2% agarose gel stained with ethidium bromide at 100 V for 40 min in 1 × Tris-Acetate-EDTA (TAE) buffer. Gels were visualized under UV light.

Assessment of aphid population. Monitoring of aphid populations was done at a 14-days interval starting from 2nd to 12th WAP. Un-winged aphids were monitored on four plants that were randomly selected from the center of each plot. Observations were carried out on six leaves (2 upper, 2 middle and 2 lower leaves) per plant. A small camel-brush was used to dislodge and collect aphids present into small-plastic bottles containing 70% ethanol and transported to the Phytopathology Laboratory of RAB at Rubona for identification and counting. Winged aphids were captured using yellow water traps (YWT) made from yellow plastic containers that were placed in the middle of each plot and filled with 1.5 litre of tap water (Blackman and Eastop, 2000). Five millilitre of formaldehyde (10%) was added per trap to preserve the insect. The collected aphids were counted and identified to species level using existing entomological keys and stereomicroscope based on their morphological features as described by Martin (1983) and Blackman and Eastop (2000).

Reaction of hot pepper genotypes to Cucumber mosaic virus under controlled conditions

Genotypes tested

Fourteen hot pepper genotypes selected from the field trials were evaluated for resistance to CMV in the screenhouse. The experiment was carried out to validate the genotypes resistance to virus infection under controlled conditions. The genotypes tested included; seven local collections (00765PPR, 00767PPR, 00774PPR, 00786PPR, 000792PPR, 00795PPR and 00802PPR), five introduced lines (PBC 462, PP9950-5197, HP0117, PP9852-170 and ICPN 18-7) and two commercial varieties (Long Red Cayenne and Red Scotch Bonnet) as indicated in Table 1.

Inoculation of CMV

Fifty seedlings of each genotype were raised in the screenhouse. Before inoculation, the seedlings were confirmed to be free of viruses using DAS-ELISA as described in Materials and Methods. At the 5-6 leaf stage, the plants were mechanically inoculated with a local isolate of CMV. The virus was propagated and maintained in a hot pepper cultivar Scotch bonnet in the screenhouse. Infected leaves were harvested and homogenized (1: 10 w/v) in 0.1 M phosphate buffer (pH7.0) containing 0.01% of sodium sulfite. The sap was sieved to remove plant debris and 0.06% of silicon carbide was added to enhance injury and increase points of entry of the virus. Two leaves per test plant were rub-inoculated with sap extract as described by Noordam (1973). After 5 mins the inoculated plants were rinsed with distilled water to remove the excess of the inoculum. Inoculated plants were maintained in an insect free screenhouse (average 27.8°C temp, 70.8% relative humidity). In total, forty-eight plants of each genotype were mechanically inoculated with CMV. Ten healthy plants of each genotype inoculated with phosphate buffer alone (with no inoculum) were maintained as control. The symptoms development on inoculated plants were recorded up to 3 weeks' post-inoculation. At this time all the plants from the susceptible local check showed typical symptoms of CMV. The incidence and symptoms severity of CMV were evaluated on all plants as described in Materials and Methods.

Detection of CMV

The confirmation of CMV infection was performed using DAS-ELISA following the procedures described by Clark and Adam (1977) on representative samples from each hot pepper genotypes. The kits were obtained from Deutsche Sammlung Von Mikroorganismen und Zellkulturen (DSMZ, Germany) and used according to instructions from the manufacturer. A healthy sample and extraction buffer were used as negative controls. A positive control was provided with the kit. Absorbance values were read at 405 nm using a microplate reader (BioTek ELX800, USA). Due to a large number of plants, only representative samples (7) were collected from each genotype and analysed. A sample was considered positive when the absorbance value at 405 nm (A405) exceeded the mean of negative controls by a factor of two.

Classification of the hot pepper genotypes for resistance to viral diseases

The rating of the genotypes was done as described by Rahman *et al*. (2016). Based on disease incidence and severity indices for both field and

screenhouse experiments, each genotype was allocated a score of 1 to 4. Scoring for virus incidence was: <20% =1, 21-30%=2, 31-50%=3 and >51%=4. Whereas, disease severity: <1=1, 1.1-2.0=2, 2.1-3.0=3 and >3.0=4. Based on cumulative scores i.e. incidence and severity indices, the genotypes were categorized into four groups: <3= resistant (R), 4-6 = moderately resistant (MR) and 7-8 = susceptible (S). The scores for the field experiments were made on pooled data obtained from the two sites and both cropping seasons than data of individual site or season.

Data analysis

Data on disease incidence (%) and symptom severity were square-root transformed, and aphids' populations were log-transformed before subjecting to the analysis of variance (ANOVA). Means of values regarding AUDPC were worked out using the Microsoft excel program. The AUDPC values were directly subject to ANOVA. Data were analyzed using SAS statistical software program and the means were separated using the least significant difference (LSD) test at p=0.05.

3. Results

Reaction of the hot pepper genotypes to virus infection and aphid infestation under field conditions
Weather conditions at the experimental sites

The average monthly rainfall, minimum and maximum air temperature during the two cropping seasons (March-July, 2018 and October 2018-March, 2019) at Gashora and Rubona experimental sites are shown in figure 1. In Rubona, temperatures ranged from 14.4 -24.2°C in season one and 15.2-24.9°C in season two. At Gashora, there were wide variations between the minimum and maximum temperatures from 12.9-27.7°C in season one and 13.7-28.2°C in season two.

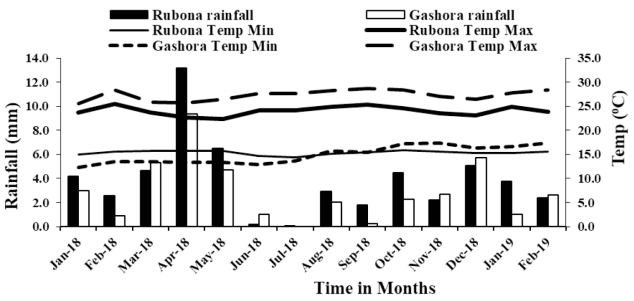

Fig. 1 - The microclimate of the experimental sites during the two cropping seasons. Temp- Temperature, Min-Minimum, Max-Maximum.

Incidence of virus diseases

There were significant differences in disease incidence between sites (p= <.0001), seasons (p= <.0001) and among genotypes (p = <.0001). The differences were observed from 4th WAP and increased with time, ranging from 3% to 100% at 10 WAP in both seasons and locations (Tables 4 and 5). At Rubona, higher disease incidence was recorded from both commercial and local genotypes, except for 00767PPR and 00802PPR compared to introduced genotypes in all sampling periods (Table 4). In season one, genotype 00767PPR was the least infected with the viruses as the disease incidence (DI) level was only 3%, followed by 00802PPR with 10%, PP9950-5197 with 20% and PBC 462 with 30% at 10 WAP (Table 4). The remaining genotypes had DI greater than 60%. In season two, disease incidence levels were generally lower in all genotypes compared to season one. The least infected genotype was PBC 462 with DI of 3% followed by 00802PPR and PP9852-170 both having 10%. This was followed by six genotypes (PP9950-5197, PBC 462, ICPN 18-7, 00767PPR, 00786PPR, 00765PPR) with incidence levels of ≤ 35% compared to the remaining genotypes that showed DI of ≥55% at 10 WAP (Table 4).

On the other hand, a similar trend was observed at Gashora, where higher disease incidence levels were recorded in season one compared to two (Table 5). Genotype PBC 462 was the least infected with DI of 13%, followed by ICPN 18-7 with 30%, PP9950-5197 with 47% and the remaining genotypes had incidence levels greater than 70% in season one (Table 5). In season two, 5 genotypes (00767PPR, PP9950-5197, PBC 462, PP9852-170 and ICPN 18-7) showed DI levels of ≤ 50%. In both sites, the highest spread of viral diseases was recorded on both commercial and local except for the genotype 00767PPR and 00802PPR. At 10 WAP, all the genotypes had developed symptoms of viral diseases but, high variability existed between genotypes. The interactions of site and season (p = <.0001), site and genotype (p = <.0001), season and genotype (p = 0.0025) were also highly significant. These results indicated that the incidence of viral diseases was dependent on the site and season the experiments were conducted.

Area under disease progress curve

The total amount of disease that occurred in the experiments was estimated and presented as the area under the disease progress curve (AUDPC). The mean AUDPC values differed significantly within sites

Table 4 - Incidence (%) of viral diseases recorded on eighteen hot pepper genotypes grown under field conditions during two seasons at Rubona Station, Huye District in Rwanda

Genotype	Type	Season one (March to June, 18)					Season two (Mid-Oct. 18 to March, 19)				
		2WAP	4WAP	6WAP	8WAP	10WAP	2WAP	4WAP	6WAP	8WAP	10WAP
00765PPR	Local [1]	0	0	43 a	97 a	100 a	0	3 b	10 bc	23 abd	33 bcdef
00767PPR	Local	0	0	0 e	3 f	3 f	0	0 b	3 c	10 bcd	17 def
00774PPR	Local	0	3	33 abc	90 ab	97 ab	0	0 b	17 ab	36 a	60 abcd
00775PPR	Local	0	10	33 abc	70 bc	87 abc	0	0 b	7 bc	20 abcd	77 ab
00786PPR	Local	0	10	37 ab	77 abc	90 ab	0	3 b	7 bc	20 abcd	30 cdef
00791PPR	Local	0	3	33 abc	80 abc	93 ab	0	0 b	0 c	23 abcd	97 a
00792PPR	Local	0	7	30 abcd	87 ab	97 ab	0	0 b	0 c	33 ab	70 abc
00802PPR	Local	0	0	0 e	3 f	10 f	0	0 b	3 c	7 bcd	10 f
00795PPR	Local	0	0	30 abcd	83 ab	97 ab	0	0 b	0 c	20 abcd	83 a
PBC 462	Introduced [2]	0	0	0 e	13 de	30 e	0	0 b	0 c	3 cd	3 f
PP9950-5197	Introduced	0	0	7 de	10 f	20 ef	0	3 b	3 c	3 cd	13 ef
HP 0117	Introduced	0	0	10 cde	37 de	63 d	0	0 b	0 c	7 bcd	13 ef
PP9852-170	Introduced	0	0	7 de	37 de	63 d	0	0 b	0 c	3 cd	10 f
ICPN 18-7	Introduced	0	0	13 bcde	40 d	63 d	0	0 b	0 c	0 d	14 f
Long red cayenne	Commercial [3]	0	0	23 abcde	77 abc	90 ab	0	3 b	10 bc	27 abcd	57 abcde
Bird eye hybrid	Commercial	0	0	7 de	40 d	70 cd	0	0 b	10 bc	33 ab	73 abc
Red Scotch bonnet	Commercial	0	3	33 abc	57 cd	80 bcd	0	0 b	10 bc	23 abd	70 abc
California Wonder	Commercial	0	0	37 ab	83 ab	100 a	0	13 a	23 a	30 abc	83 a
LSD (0.05)			10	24	26	19		5	11	28	46
P-Value			0.3699	0.0027	<.0001	<.0001		0.0002	0.0167	<.0001	<.0001

The values represent means of un-transformed data. Means comparison done by least significant difference (LSD) test on transformed data. Data transformed by square root (X+1).
Means with the same letters within a column are not significantly different (P<0.05). n= 10 replicated thrice.
WAP= Weeks after planting;
[1] Genotypes collected from farmers' field and conserved in Rwanda National GenBank;
[2] Genotypes from World Vegetable Center;
[3] Varieties obtained from seed companies and are grown for commercial purposes in Rwanda.

and thus, data were not pooled together. In Rubona, the total amount of disease was significantly ($p< 0.0001$) higher in season one with mean AUDPC values of 230.9 compared to season two that recorded 117.1. In both seasons, all commercial and local genotypes (except 00767PPR and 00802PPR) recorded high levels of disease compared to introduced genotypes (Table 6). Genotypes 00767PPR, 00802PPR, PBC 462 and PP9950-5197 consistently recorded lower AUDPC values of less than 100 in both seasons. Besides, genotypes 00786PPR, PP9950-5197, PP9852-170 and ICPN 18-7 recorded values of less than 100 but only in season two.

In Gashora, the AUDPC values were higher in both seasons compared to Rubona. However, a similar trend was observed where the amount of diseases was more in season one with values of 243.9 than season two 198.9 (Table 6). Introduced and the two local genotypes (00767PPR and 00802PPR) had low AUDPC values compared to the rest of the genotypes. Genotypes PBC 462 and PP9950-5197 recorded values of less than 100 in both seasons, while genotypes 00767PPR, PP9852-170 and ICPN 18-7 had AUDPC values of less than 100 in season two only. On the other hand, genotypes 00792PPR, 00795PPR and the four commercial genotypes were the most infected with the viral diseases in both sites during the two seasons.

Detection of the viruses in hot pepper genotypes

Three viruses were detected in the samples analyzed namely CMV, PeVYV and PVMV. *Cucumber mosaic virus* infection was the most abundant in both sites, detected in 53.1% of the samples in Rubona and 75% in Gashora, followed by PeVYV with 31.3% and 2.8%, respectively (Fig. 2). The PVMV infections were the least abundant and only detected in 21.9% of the samples in Rubona. Double infections of CMV+PeVYV were common and detected in both sites, 12.5% in Rubona and 2.7% in Gashora, followed

Table 5 - Incidence (%) of viral diseases recorded on eighteen hot pepper genotypes grown under field conditions during two seasons at Gashora Station, Bugesera District in Rwanda

Genotype	Type	Season one (March to June, 18)					Season two (Mid-Oct. 18 to March, 19)				
		2WAP	4WAP	6WAP	8WAP	10WAP	2WAP	4WAP	6WAP	8WAP	10WAP
00765PPR	Local [1]	0	0	0 d	90 a	97 ab	0	0	3	33becd	70 abc
00767PPR	Local	0	0	17 cd	40 bc	87 abc	0	0	0	0 e	10 f
00774PPR	Local	0	3	17 cd	90 a	100 a	0	0	10	63 ab	83 ab
00775PPR	Local	0	0	13 cd	77 ab	97 ab	0	0	3	13 de	53 bcde
00786PPR	Local	0	3	13 cd	100 a	100 a	0	0	0	57 bc	73 a
00791PPR	Local	0	0	17 cd	97 a	100 a	0	0	0	17 cde	83 ab
00792PPR	Local	0	7	23 abc	100 a	100 a	0	0	10	60 ab	87 ab
00802PPR	Local	0	0	0 d	43 b	63 cd	0	0	0	40 bcde	60 bcd
00795PPR	Local	0	3	17 cd	97 a	100 a	0	0	0	27 bcde	80 ab
PBC 462	Introduced [2]	0	0	0 d	0 d	13 f	0	0	0	7 efg	20 ef
PP9950-5197	Introduced	0	0	0 d	10 d	47 de	0	0	0	3 de	13 f
HP 0117	Introduced	0	0	0 d	17 bcd	70 cd	0	0	7	33 bcde	53 bcde
PP9852-170	Introduced	0	0	0 d	17 bcd	73 bc	0	0	3	23 bcde	33 cdef
ICPN 18-7	Introduced	0	0	7 cd	14 cd	30 ef	0	0	0	7 de	23 def
Long red cayenne	Commercial [3]	0	3	13 cd	87 a	100 a	0	0	10	100 a	100 a
Bird eye hybrid	Commercial	0	7	37 ab	100 a	100 a	0	0	10	43 bcd	60 bcd
Red Scotch bonnet	Commercial	0	0	20 bc	90 a	100 a	0	0	3	33 bcde	70 abc
California Wonder	Commercial	0	13	40 a	93 a	100 a	0	0	20	100 a	100 a
LSD (0.05)			9	18	29	24			13	40	39
P-Value			0.2336	0.0006	<.0001	<.0001			0.1196	<.0001	<.0001

The values represent means of un-transformed data. Means comparison done by least significant difference (LSD) test on transformed data. Data transformed by square root (X+1);
Means with the same letters within a column are not significantly different (P<0.05). n= 10 replicated thrice;
WAP= Weeks after planting;
[1] Genotypes collected from farmers' field and conserved in Rwanda National GenBank;
[2] Genotypes from World Vegetable Center;
[3] Varieties obtained from seed companies and are grown for commercial purposes in Rwanda.

Table 6 - Means of the area under disease progress curve (AUDPC) of viral diseases recorded on eighteen genotypes of hot pepper during two seasons in Rubona and Gashora sites

Genotype	AUDPC in Rubona site		AUDPC in Gashora site		Pooled
	Season one	Season two	Season one	Season two	
00765PPR	240 cdef	86 bcd	264 c	203 cdefg	197cd
00767PPR	9 h	33 cd	146 d	22 h	52 e
00774PPR	294 abc	178 ab	298 bc	334 abc	279 abc
00775PPR	281 bcd	191 ab	315 bc	129 efgh	233 bc
00786PPR	364 ab	95 bcd	311 bc	272 cde	261 abc
00791PPR	320 abc	207 ab	316 abc	191 cdefg	259 abc
00792PPR	398 a	199 ab	388 a	300 bcd	321 ab
00802PPR	21 h	34 cd	149 d	169 defgh	93 e
00795PPR	273 bcde	179 ab	333 abc	240 cdef	257 abc
PBC 462	63 gh	15 d	78 d	57 gh	55 e
PP9950-5197	62 gh	52 cd	83 d	30 h	56 e
HP 0117	165 efg	46 cd	128 d	131 efgh	117 de
PP9852-170	148 fg	27 cd	137 d	97 fgh	102 de
ICPN 18-7	179 def	16 d	93.6 d	63 gh	89 e
Long red cayenne	308 abc	146 abc	344 ab	448 a	316 ab
Bird-eye	312 abc	179 ab	346 ab	251 cde	276 abc
Red Scotch bonnet	331 abc	177 ab	350 ab	198 cdefg	264 abc
California Wonder	379 ab	248 a	312 bc	445 ab	346 a
LSD (0.05)	109.2	123.7	72.9	149.5	99.9
P-value	< 0.0001	0.0011	< 0.0001	< 0.0001	< 0.0001

The values represent means of three replicates. Means with the same letters within a column are not significantly different (P<0.05). Means comparison done by Least significant difference (LSD) test.

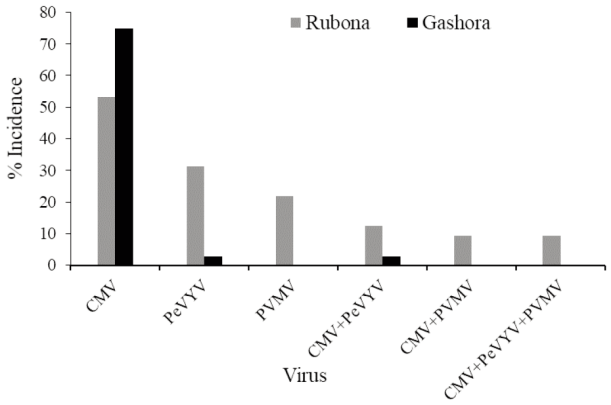

Fig. 2 - Overall incidence of viruses detected in leaf samples of hot pepper genotypes from Rubona and Gashora in Rwanda.

by CMV+PVMV detected in 9.4% of the samples tested in Rubona. Triple infection of CMV+PeVYV+PVMV was detected in 9.4% of the samples from Rubona. All hot pepper genotypes were infected by CMV.

Assessment of aphids

Aphid populations differed significantly between sites (p<0.0001), season (p=0.0075) and thus, data were analysed separately. In both seasons, the aphid population was significantly (p<.0001) higher in Rubona compared to Gashora (Table 7). Three species of aphids were observed. These were *Aphis gosypii* Glover, *Macrosiphum euphorbiae* Thomas and *Acyrthosiphon pisum* (Harris). The *A. gosypii* and *M. euphorbiae* were the most abundant in both sites, while *A. pisum* was observed in Gashora only.

All genotypes were infested by aphids but the difference in numbers were not significant (p = 0.0923) among the genotypes (Table 8). The mean number of aphids per plant ranged from 4 to 108 in Rubona and 4 to 19 in Gashora, while the mean number of aphids per leaf ranged from 0.8 to 18 and 0.6 to 3.2, respec-

Table 7 - Mean number of aphids captured in hot pepper fields during two cropping seasons in Rubona and Gashora experimental sites

Season	Rubona site			Gashora site			
	A. gosypii	*M. euphorbiae*	Total aphids	*A. gosypii*	*M. euphorbiae*	*A. Pisum*	Total aphids
Feb-June 2018	167 ± 30 a	10 ± 1 b	177 ± 30 a	32 ± 6 a	0 ± 0 b	11 ± 1 a	43 ± 5 a
Oct. 2018 -March 2019	48 ± 4 b	80 ± 11 a	128 ± 10 a	26 ± 5 a	28 ± 2 a	0 ± 0 b	54 ± 6 a
LSD (0.05)	59	21	62	16	5	2	16
P-value	0.0017	<0.0001	NS	NS	< 0.0001	<0.0001	NS

The values represent means and standard errors of three replicates.
NS= Not significant at 0.05 level.

Table 8 - Number of aphids associated with different hot pepper genotypes in Rubona and Gashora's experimental sites

Genotype	Rubona site			Gashora site			Mean aphids/plant
	Total aphids	Aphids/plant	Aphids/leaf	Total aphids	Aphids/plant	Aphids/leaf	
00765PPR	178.3	32.8	5.5	53.3	11.7	1.9	22.3
00767PPR	64.8	5.5	0.9	29	5.2	0.8	5.3
00774PPR	178.5	31.5	5.3	37.3	6.2	1	18.8
00775PPR	125.2	19.2	3.2	56	11.3	1.9	15.3
00786PPR	258.3	54.8	9	56.3	9.5	1.6	32.2
00791PPR	117.2	20.3	3.4	66.3	11.8	2	16.1
00792PPR	125.2	18.3	3	45	9.5	1.6	13.9
00802PPR	113	21.3	3.6	36.3	6.7	1.1	14
00795PPR	100.8	10.1	1.7	65.5	14	2.3	12.1
PBC 462	120	19.5	3.2	38	5.7	0.9	12.6
PP9950-5197	117.5	19.3	3.2	62.2	11	1.8	15.1
HP 0117	289.5	108	18	29.7	5.5	0.9	56.8
PP9852-170	205.8	42.2	7	94.5	19.3	3.2	30.8
ICPN 18-7	136.2	25.3	4.2	35	6.2	1	15.8
Long Red Cayenne	171	33.8	5.6	59.8	11	1.8	22.4
Bird-eye	72.2	4.8	0.8	26	4	0.6	4.4
Scotch Bonnet	83.7	10.2	1.7	50.7	9.7	1.6	9.92
California Wonder	287.8	79.2	13	40.5	7.8	1.3	43.5
P-value (0.05)	NS	NS	NS	NS	NS	NS	NS

The values represent means of untransformed data.
NS= Not significant at 0.05 level.

tively. Except HP 0117 and California wonder at Rubona site, the rest of the genotypes showed low numbers of aphids which did not exceed recommended chemical control action thresholds of 10 aphids per leaf. The population exhibited a negative correlation with minimum (r = -0.04, -0.22) and maximum temperature (0.50, -0.73) while, the correlation was positive with average rainfall (0.37, 0.68) in Rubona and Gashora, respectively. These correlations were not significant at the 5 percent level (data not shown).

Classification of hot pepper genotypes for resistance to viral diseases under field conditions

Commercial genotypes were more susceptible to virus infections than the new lines from World Vegetable Center. Various degrees of symptoms were observed on most genotypes during the evaluation period. These included leaf mosaic, crinkling, chlorosis, vein banding, and leaf deformation. Based on incidence and severity indices from both locations and seasons only five genotypes rated resistant to viral diseases i.e. 00767PPR, 00802PPR, PBC 462, PP9950-5197 and ICPN 18-7 with total scores between 2-3; three moderately resistant 00765PPR, HP 0117 and PP9852-170 with scores between 4-6; and nine susceptible 00775PPR, 00786PPR, 00774PPR, 00786PPR, 00792PPR, Long Red Cayenne, Bird Eye Hybrid, Red Scotch Bonnet, and California Wonder with scores between 7-9 (Table 9). Two local genotypes (00767PPR, and 00802PPR) and three introduced genotypes (PBC 462, PP9950-5197 and ICPN 18-7) showed resistance to viral diseases in both locations.

Reaction of hot pepper genotypes to CMV under artificial inoculation conditions

Disease incidence

A significant difference ($p<0.05$) in disease incidence and symptoms severity was observed between the genotypes tested (Table 10). Infected plants showed systemic symptoms of CMV infection including leaf mosaic, mottle, crinkling, small and deformed leaves, and stunting with varying degrees of severity (Fig. 3). Six genotypes (Red Scotch Bonnet, 00795PPR, 00792PPR, 00786PPR, 00774PPR, and Long red cayenne) developed symptoms thirteen days' post-inoculation (dpi) and the first three

Table 9 - Classification of the hot pepper genotypes based on incidence (%) and severity indices of virus-induced diseases under field conditions

Genotype	Incidence (%)				Severity indices				Cumulative rating	Host reaction
	Season one	Season two	Pooled	Rating	Season one	Season two	Pooled	Rating		
00765PPR	97	51.5	74.3 ab	4	2.4	1.6	2 d	2	6	MR
00767PPR	45	11.5	28.3 e	2	1	0.3	0.7 ef	1	3	R
00774PPR	96.5	71.5	84 a	4	2.8	2.7	2.8 abc	3	7	S
00775PPR	83.5	65	74.3 ab	4	2.6	2.2	2.4 cd	3	7	S
00786PPR	88.5	51.5	70 abc	4	2.9	2.1	2.5 bcd	3	7	S
00791PPR	90	90	90 a	4	2.9	2.7	2.8 abc	3	7	S
00792PPR	93.5	78.5	86 a	4	3.5	3	3.3 a	4	8	S
00802PPR	33	24	28.5 e	2	0.8	1.1	1 ef	1	3	R
00795PPR	91.5	81.5	86.5 a	4	2.8	2.9	2.9 abc	3	7	S
PBC 462	13	11.5	12.3 e	1	0.2	0.4	0.3 f	1	2	R
PP9950-5197	28.5	13	20.6 e	1	0.7	0.5	0.6 ef	1	2	R
HP 0117	53.5	33	43.3 cde	3	1.2	0.9	1.1 e	2	5	MR
PP9852-170	55	21.5	38.3 cde	3	1.2	0.7	1 ef	1	4	MR
ICPN 18-7	35	15	25 e	2	0.6	0.5	0.6 ef	1	3	R
Long Red Cayenne	88.5	78.5	83.5 a	4	3	3.3	3.2 ab	4	8	S
Bird-eye	76.5	55	65.8 abcd	4	3	2.5	2.8 abc	3	7	S
Scotch bonnet	80	71.5	75.6 ab	4	3	2.3	2.7 abcd	3	7	S
California Wonder	91.5	68.5	75.6 a	4	2.9	2.9	2.9 abc	3	7	S
LSD			33.6				0.7			
P-value			0.0004				<.0001			

The values represent means of un-transformed data. Means comparison done by Least significant difference (LSD) test on transformed data. Means with the same letters within a column are not significantly different ($P<0.05$). Incidence scores; 20% =1, 21-30%=2, 31-50%=3 and >51%=4. Severity scores; <1=1, 1.1-2.0=2, 2.1-3.0=3 and >3.0=4. Cumulative scores i.e. incidence + severity indices; < 3= resistant (R), 4-6 = moderately resistant (MR) and 7-8 = susceptible (S).

Table 10 - Reaction of hot pepper genotypes against Cucumber mosaic virus under screenhouse conditions

Genotype	Incidence (%)					Severity indices					Cumulative rating	Host reaction
	13dpi	15dpi	17dpi	19dpi	Rating	13dpi	15dpi	17dpi	19dpi	Rating		
00765PPR	0.0 d	0.0 d	60.4 bc	77.1 b	4	1.0 c	1.0 d	1.8 d	2.7 c	3	7	S
00767PPR	0.0 d	0.0 d	0.0 g	2.1 h	1	1.0 c	1.0 d	1.0 e	1.0 f	1	2	R
00774PPR	10.4 cd	41.7 c	41.7 cd	50.0 de	3	1.1 c	1.4 cd	2.1 d	3.1 c	4	7	S
00786PPR	4.2 cd	8.3 d	22.9 def	25 fg	2	1.1 c	1.1 cd	1.3 e	1.4 ef	2	4	MR
00792PPR	75.0 b	75 bc	75.0 ab	75 b	4	2.3 ab	2.8 b	3.2 bc	4.0 b	4	8	S
00802PPR	0.0 d	0.0 d	0.0 g	18.8 fgh	1	1.0 c	1.0 d	1.0 e	1.2 f	2	3	R
00795PPR	75.0 b	75.0 b	81.3 ab	100.0 a	4	2 b	2.7 b	3.7 ab	5.0 a	4	8	S
PBC 462	0.0 d	0.0 d	4.2 efg	12.5 gh	1	1.0 c	1.0 d	1.0 e	1.2 f	2	3	R
PP9950-5197	0.0 d	0.0 d	8.3 efg	22.9 fg	2	1.0 c	1.0 d	1.0 e	1.3 f	2	4	MR
HP 0117	0.0 d	0.0 d	2.1 fg	35.4 ef	3	1.0 c	1.0 d	1.0 e	1.5 def	2	5	MR
PP9852-170	0.0 d	0.0 d	2.1 fg	14.6 gh	1	1.0 c	1.0 d	1.0 e	1.2 f	2	3	R
ICPN 18-7	0.0 d	0.0 d	25.0 de	56.3 cd	4	1.0 c	1.0 d	1.0 e	2.0 de	2	6	MR
Long red cayenne	16.7 c	29.2 c	89.6 a	97.9 a	4	1.2 c	1.5 c	2.7 c	4.5 ab	4	8	S
Red Scotch bonnet	91.7 a	93.8 a	93.8 a	100.0 a	4	2.4 a	3.3 a	4.1 a	4.8 a	4	8	S
LSD (0.05)	12.6	15.3	21.4	19.8		0.3	0.5	0.6	0.7			
P value	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001			

The values represent means of un-transformed data. Means comparison done by Least significant difference (LSD) test on transformed data. Data transformed by square root (X + 1). Means with the same letters within a column are not significantly different (P<0.05).
Incidence scores: 20% =1, 21-30%=2, 31-50%=3 and >51%=4.
Severity scores: <1=1, 1.1-2.0=2, 2.1-3.0=3 and >3.0=4.
Cumulative scores i.e. incidence + severity indices: < 3= resistant (R), 4-6 = moderately resistant (MR) and 7-8 = susceptible (S).
n = 16 replicated three times.
Dpi= Days post-inoculations.

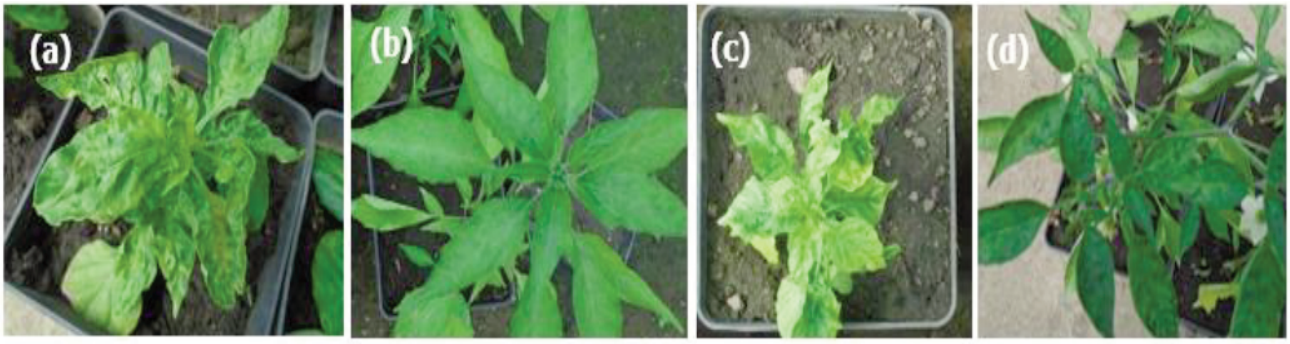

Fig. 3 - Symptoms of *Cucumber mosaic virus* on different hot pepper genotypes. (a) leaf mosaic, crinkling and distortion in commercial genotype Scotch Bonnet, (b) mottling in local genotype 00774PPR, (c) leaf distortion and stunting in local genotype 00795PPR, (d) leaf mosaic in introduced genotype ICPN18-7.

showed high levels of CMV infection (Table 10). Introduced genotypes PBC462, PP9950-5197, HP 0117, PP9852-170, ICPN18-7, and local genotype 00765PPR displayed symptoms at seventeen dpi while the remaining two local genotypes 00767PPR and 0802PPR showed symptoms at nineteen dpi. A total of six genotypes namely 00765PPR, 00792PPR, 00795PPR, 00774PPR, Long red cayenne and Red scotch bonnet had disease incidence between 50-100%, severity 2.7-5 at nineteen dpi and thus rated as susceptible to CMV (Table 10). Genotypes PP9950-5197, 00786PPR, HP 0117 and ICPN 18-7 had moderate levels of infection displaying 22-35.4% disease incidence at nineteen dpi and thus classified as moderately resistant to CMV. Among the 14 hot pepper genotypes tested, only four including two local (00767PPR, 0802PPR) and two introduced (PBC 462 and PP9852-170) showed resistant reaction against CMV with disease incidence ranging from 2-18.8% and severity 1.0-1.2 at nineteen dpi. A positive reaction to CMV was revealed by ELISA for the tested samples from all genotypes.

4. Discussion and Conclusions

Host plant resistance is an important factor in the integrated management of pests. The present study was undertaken to identify genotypes that can be used in production or as sources of resistance to viruses and aphids in hot pepper breeding programs. All the genotypes tested in this study were infected by the viruses observed either in the field or screenhouse however, there were some resistant genotypes found based on incidence and symptoms severity of the viral diseases.

In the field, five genotypes 00802PPR, *C. baccatum* 00767PPR, *C. annuum* PBC 462, PP9950-5197 and ICPN 18-7 were resistant to the viral diseases while *C. annuum* 00765PPR, HP 0117 and PP9852-170 were moderately resistant. The rest of the genotypes were susceptible to the viral diseases. These variations among the genotypes might be due to various factors that include genetic make-up, the strain of the virus and their combinations, time of infection and prevailing environmental conditions (Visalakshi and Pandiyan, 2018). Such variations reveal the diversity present within the genotypes that needs to be exploited. In previous studies under field conditions, various genotypes from *C. baccatum*, *C. annuum* and *C. frutescens* species have displayed variable resistance to some viruses such as PVMV, TMV, CMV, *Pepper mild mottle virus, Chili veinal mottle virus* and *Leaf curl virus* (Appiah *et al*., 2014; Rahman *et al*., 2016; Bandla and Beena, 2018; Fajinmi *et al*., 2018). For instance, C. *baccatum* PI 439381-1-3 was reported as resistant to CMV and PMMoV under field conditions (Suzuki *et al*., 2003). Our result, reports some additional sources of resistance from *C. baccatum* and *C. annuum* species that could be valuable in hot pepper breeding programs as well as cultivation if preferred by farmers.

The CMV, PVMV and PeVYV were detected in leaf samples collected from fields and CMV was the most abundant. These three viruses have also been reported to infect pepper previously in Rwanda (Skelton *et al*., 2018). The high incidence of CMV in the field could be attributed to several factors including wide host range, climatic conditions and efficiency of vector transmission as reported by Shah *et al*. (2009). Appiah *et al*. (2014) in their study also observed a high incidence of CMV in the range of 75% to 83.3% on pepper cultivars. All genotypes evaluated were infected with CMV and almost half of them showed multiple (double or triple) infections of CMV with either PVMV or PeVYV or both, which could have serious consequences in their management. Mixed infections of CMV and PVMV in pepper have been reported in previous studies (Aliyu, 2014; Appiah *et al*. 2014). Mixed infections in pepper plants increase the severity of disease symptoms leading to significant yield losses (Arogundade *et al*., 2012). Thus, understanding the interactions of these viruses is crucial for the development of efficient and sustainable management strategies such as resistant varieties (Syller, 2012).

Results from screenhouse showed that all genotypes developed symptoms to CMV infection albeit at different levels of severity and time, confirming the virulence of the local CMV isolate used. The plants developed systemic symptoms including mosaic, mottle, leaf crinkling and distortion and stunting which were similar to symptoms described by Rahman *et al*. (2016). Two local genotypes 0802PPR and *C. baccatum* 00767PPR, and two introduced genotypes *C. annuum* PBC 462 and PP9852-170 were found resistant to CMV while one local *C. annuum* genotypes 00786PPR and three introduced genotypes PP9950-5197, HP 0117 and ICPN 18-7 were moderately resistant. The previously published resistant genotypes (PP9852-170 and PP9950-5197) to CMV in screenhouse conditions were also resistant in our study (Gniffke, *et al*., 2013; Reddy, *et al*., 2014). However, genotype ICPN 18-7 that was previously reported as susceptible to CMV was moderately resistant in the current study (Gniffke, *et al*., 2013). The reason for these differences could be attributed to the use of different strain of CMV. Various sources of resistance to CMV in pepper have been identified in *C. annuum*, *C. baccatum* and *C. frutescens* species (Grube *et al*., 2000; Chaim *et al*., 2001; Caranta *et al*., 2002; Suzuki *et al*., 2003; Rahman *et al*., 2016). The present findings prove that natural resistance or tolerance exists in tested *C. annuum* and *C. baccatum* genotypes. As different strains of CMV exist, it is desirable to test the identified pepper genotypes against multiple strains of CMV to validate their resistance.

In both field and screenhouse experiments, genotype 00767PPR, 0802PPR and PBC 462 were consistently resistant to viral diseases while genotype HP 0117 was moderately resistant, providing evidence that the reactions of these genotypes to the virus might be due to genetic factors. However, unlike under field conditions where genotypes PP9950-5197 and ICPN 18-7 were categorized as resistant to viral

diseases, they reacted differently when subjected to the artificial inoculation with CMV and grouped as resistant. This might be due to disease escape in the field. Similar observations were made by Ashfaq *et al*. (2014), where two chili genotypes C-7 and C-8 showed a different reaction to CMV under controlled and uncontrolled conditions. On the other hand, genotype PP9852-170 was resistant to CMV under controlled conditions while in the field, it was grouped as moderately resistant to viral diseases. This may be due to the complex nature of the viruses' infection in the field where more than two viruses occur in combination. As was evident in this study where single and mixed infections of CMV, PVMV, and PeVYV were observed and their presence might have contributed towards variations in the reaction of the host in the field. These variations in the observation may also be due to variations in inoculum load and environmental conditions that might have interfered with plant behaviour.

The categorization of genotypes into resistant, moderately resistant and susceptible was based on the incidence and severity of the viral diseases on the host. However, it is noteworthy that the genotypes 00802PPR, 00767PPR, PBC 462, PP9950-5197 and ICPN 18-7 classified as resistant to viral diseases had the lowest AUDPC values of less than 100 in the field while the highest AUDPC value was recorded in susceptible check California wonder 346. Lower AUDPC values indicate a lower disease development rate. These genotypes had low AUDPC values which implies that the plant defence mechanism against the viruses could be mediated by resistance (R) genes which are observed as complete resistance or extreme resistance (ER) and that the virus replication could have been hindered or gone undetectable among the infected cells (Ingvardsen *et al*., 2010). The reaction of pepper cultivar to the viral diseases is governed by the resistance genes which can be brought by a single gene or multiple genes (Kang *et al*., 2010; Kim *et al*., 2017). However, genes responsible for their resistance in particular for the two local accessions are unknown and mechanisms that underlie their resistance are yet to be understood. This is important information that could help to determine useful markers to support breeding processes.

Aphid species are important agricultural pests because they have a broad host range and transmit many important plant viruses. In this study, three species of aphids were recorded in the pepper fields and the most abundant in both sites were *A. gosypii* and *M. euphorbiae*. These findings agree with previous studies by Meena *et al*. (2013) and Rajput *et al*. (2017) who reported the infestation of hot pepper fields with *A. gosypii* in India. Similar results on *M. euphorbiae* were reported by Djieto-Lordon *et al*. (2014) in Cameroon. The presence of *A. pisum* in Gashora was understandable since there was a pigeon peas field near the experimental plots. These polyphagous insects belong to the *Hemiptera* order and they are important pests because of the ability to transmit several viruses in pepper. According to Fajinmi *et al.* (2011); Dombrovsky *et al.* (2010) and Zitter and Murphy, (2009) *A. gosypii* efficiently transmits CMV, PeVYV, and PVMV which were detected in this study.

There was no difference in genotypes infestation by the aphids. Besides, complete genotype resistance to aphids' infestation was not recorded in any of the genotypes tested. Bird-eye hybrid was the less preferred by the aphids (4.4 aphids/plant) followed by 00767PPR (5.3 aphids/plant) and Red scotch bonnet (9.9 aphids/plant) while the most preferred was genotype HP 0117 (56.8 aphids/plant) followed by California wonder (43.5 aphids/plant) and 00786PPR (32.2 aphids/plant). Unlike other plant species such as soybean where a lot has been done on resistance to aphids (Hill *et al*., 2004), only a few studies have been conducted on pepper (Frantz *et al*., 2004; Sun *et al*., 2018). Sun *et al*. (2018), in their studies, identified *C. baccatum* accession PB2013071 as highly resistant, while the accessions PB2013062 and PB2012022 as intermediate resistant to *M. persicae* under screen house conditions. Recently, quantitative trait loci (QTLs) conferring resistance to *M. persicae* in pepper was detected (Sun *et al*., 2019). In the present study, prevailing weather conditions, especially at the Gashora site, negatively affected the population of aphids leading to low infestation on pepper plants. Thus, further efforts are needed to identify and validate the resistance of these genotypes to aphids under controlled and uncontrolled conditions.

Most of the varieties grown in the country including the commonly grown commercial varieties were found to be susceptible to viral diseases. A relatively higher number of resistant lines from introduced material indicates that the World Vegetable Center germplasm collection has a wider genetic base than local material. Since viruses cause serious diseases of hot pepper around the world, the results of this study may be promising and could be used in the for-

mulation of integrated control strategies for the management of these destructive 1diseases. The use of resistant pepper genotypes to manage the viral diseases can potentially replace or minimize the application of harmful pesticides and could be used as an important component of integrated pest management (IPM) which is a promising approach to sustainable agriculture.

In the present study, three genotypes 00767PPR, 00802PPR and PBC 462 consistently rated as resistant to viral diseases while genotype HP 0117, PP9852-170, PP9950-5197 and ICPN 18-7 were moderately resistant under field and screenhouse conditions. As revealed from the study, most of the local genotypes and all of the commercially grown pepper genotypes tested were susceptible. Therefore, the identified genotypes especially the ones from World Vegetable Center are recommended for adoption by growers. The two local collections 00767PPR and 00802PPR are not preferred cultivars for commercial production and thus, they can be utilized in breeding programs as potential sources for virus resistance. Farmers should be encouraged to use hot pepper varieties that are resistant to viruses as part of a management program to maximize yields. Further studies are needed to identify and validate resistance of the tested genotypes to aphids under controlled and uncontrolled conditions.

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