Journal of Plant Pathology (2021) 103:573–585
https://doi.org/10.1007/s42161-021-00811-7

ORIGINAL ARTICLE

Detection and distribution of viruses infecting hot pepper (*Capsicum* spp.) in Rwanda

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Received: 25 April 2020 / Accepted: 9 March 2021 / Published online: 15 March 2021

Abstract
Accurate diagnosis is a prerequisite to effective management of plant diseases especially those whose symptoms are not very specific. A survey was conducted from February to March 2018 to detect the presence and determine the distribution of six viruses infecting pepper in Rwanda. A total of 225 symptomatic samples were collected from high, mid and low-altitude zones, and analysed using serology assay with antibodies to cucumber mosaic virus (CMV), potato virus Y (PVY), pepper veinal mottle virus (PVMV), pepper mild mottle virus (PMMoV) and tobacco mosaic virus (TMV). Polymerase chain reaction was used to confirm the results from serology and to test for the presence of pepper vein yellows virus (PeVYV), which has no commercial antibodies. Amplified DNA fragments were sequenced and compared with other known pepper viruses available in the GenBank database. Seventy-three percent of the samples tested positive for at least one of the viruses. CMV, PVY, PVMV, TMV and PMMoV were detected in all zones but PeVYV was detected in the mid and low-altitude areas. CMV was the most widespread and was detected in 48% of the samples, followed by PVMV and PVY in 23.6% and 18.2%, respectively. There were both single (36%) and mixed (34.6%) infections of the viruses. Sequence and phylogenetic analysis confirmed the presence of CMV, PeVYV, PVMV and TMV in the country. This is the first report of TMV, PMMoV and PVY infecting pepper in Rwanda. Efforts towards the development of sustainable management for these viruses should be put in place to improve yields and quality of hot pepper.

Keywords Agro-ecological zones · *Capsicum* · Incidence · Mixed infection · Plant viruses

Introduction

Hot pepper (*Capsicum* spp.) is a spicy vegetable crop widely cultivated throughout the world. In Rwanda, production of hot pepper generates income for farmers and contributes to the development of the country's economy through the creation of employment and earning of foreign revenue (USAID 2018). For instance, in 2017 hot pepper contributed 4.5% of the foreign revenue generated from the sale of vegetables (NISR 2018). In addition to its economic importance, hot pepper is also known for its chemical and medicinal properties (Marin et al. 2004; Surh 2002). Although hot pepper production in Rwanda has increased over the past ten years, the productivity is low with yields currently estimated at 6.8 t/ha compared to the country's production potential of 15 t/ha (FAO 2017).

Viral diseases in pepper are the most destructive causing huge losses in different parts of the world in terms of quantity and quality of the produce (Olawale et al. 2012). According to Olawale et al. (2020) more than 45 viruses are associated with hot pepper in Africa. Among these viruses, 12 species have been reported in the eastern Africa region including *Potato virus* Y (PVY), *Pepper veinal mottle virus* (PVMV), *Chilli veinal mottle virus* (ChiVMV) and *Ethiopian pepper mottle virus* belonging to genus *Potyvirus*; *Cucumber mosaic virus* (CMV), genus *Cucumovirus*; *Pepper mild mottle virus* (PMMoV), *Tobacco mosaic virus* (TMV) and *Tomato mosaic virus,* genus *Tobamovirus*; *Pepper vein yellows virus* (PeVYV), genus *Polerovirus*; *Alfalfa mosaic virus,* genus *Alfamovirus*; *Tomato spotted wilt virus*, genus

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Tospovirus; and *Potato virus X*, genus *Potexvirus* (Dafalla, 2001; Haskias et al. 1999; IPM CRSP, 2008; Ndunguru and Kapooria 1999; Skelton et al. 2018). Information on viruses infecting pepper in Rwanda is scarce. So far, only three (CMV, PVMV and PeVYV) of these viruses have been detected in the country (Skelton et al. 2018).

Effective control of viral diseases requires a thorough understanding of the responsible pathogens and their distribution. Knowledge of the distribution of different pepper viruses in Rwanda is still limited. This information is essential in developing effective control strategies. There has been only one previous survey on hot pepper viruses carried out in 2016 (Skelton et al. 2018). However, the study did not cover the major production areas except for a few samples analysed for virus detection. Furthermore, regular surveys are recommended since viruses are diverse and new species/strains keep evolving over time. The present study aimed at detecting six viruses namely CMV, PVY, PVMV, PeVYV, PMMoV and TMV that have been previously reported to be prevalent in pepper in the eastern and other parts of Africa (Appiah et al. 2014; IPM CRSP, 2008; Olawale et al. 2020; Waweru et al. 2019), and determining their distribution in three agro-ecological zones (AEZs) in Rwanda.

Materials and methods

Study areas. A survey for hot pepper viruses was carried out in three (high, mid and low-altitude) AEZs of Rwanda from February to March 2018. Eight districts within the three AEZs were surveyed to cover areas where hot pepper is mainly grown (EU 2015). These districts were Rulindo and Gakenke (high-altitude areas), Huye and Nyanza (mid-altitude areas), Bugesera, Rwamagana, Nyagatare and Rusizi (low-altitude areas). The geographic locations of sampled sites are as shown in Fig. 1.

Assessment of incidence and severity of viral symptoms. A total of 92 hot pepper fields were assessed in the three AEZs. On a 10 by 10 m area, twenty plants were randomly selected along x-shaped transect stretching between

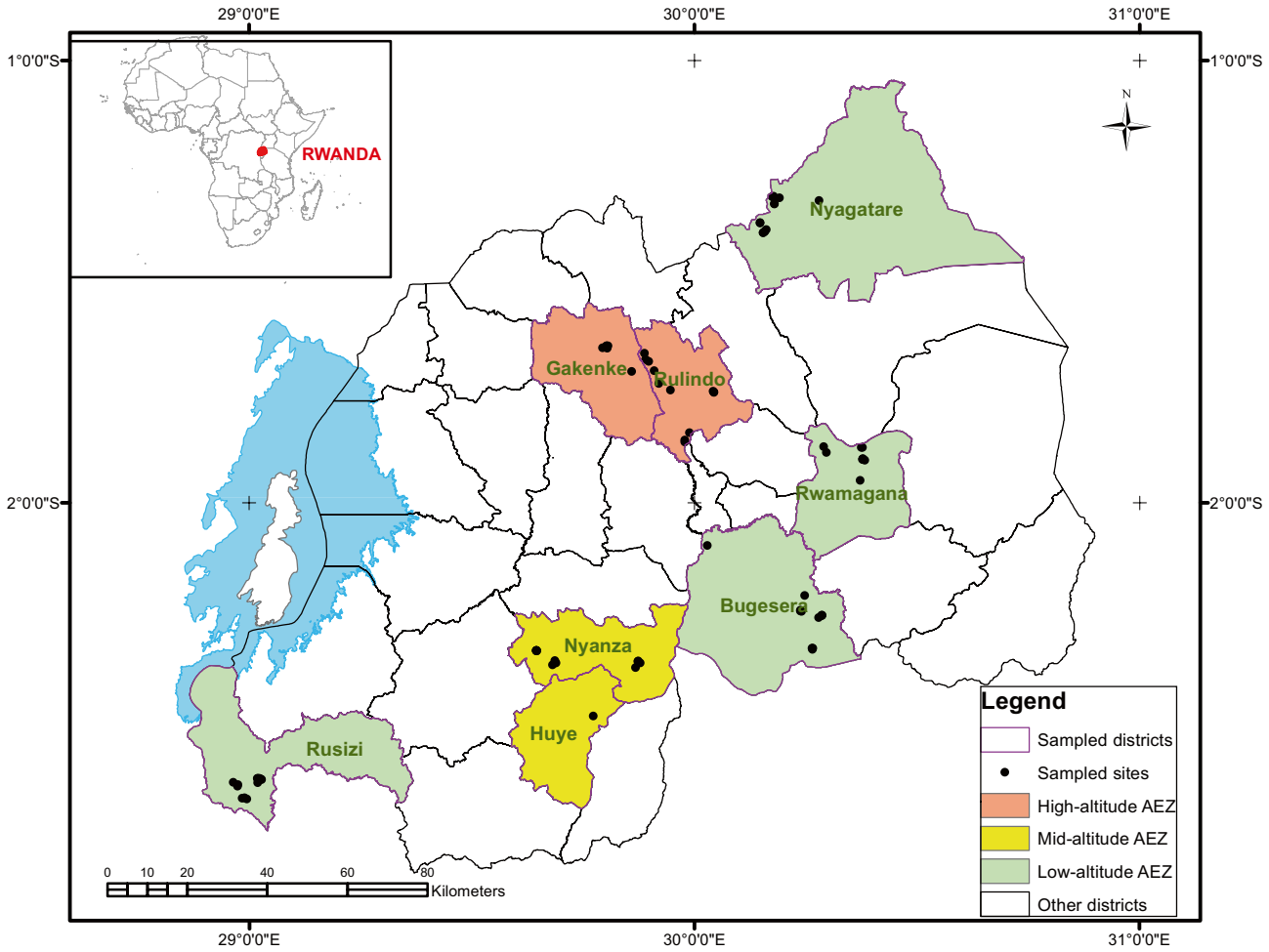

Fig. 1 Geographic location of the hot pepper fields sampled in three agro-ecological zones of Rwanda in 2018

opposite corners and assessed for virus symptoms incidence and severity. Disease incidence was expressed as a percentage based on the proportion of plants showing viral symptoms to the total number of plants observed per field, as described by Galanihe et al. (2004).

Viral symptom severity was determined using 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). For fields that exceeded 2 acres, one to five sub-sampling sites were assessed. The observations made from different sub-sites were summed up and the average incidence and severity calculated based on the total number of sub-sites observed. Prevalence of viral diseases was estimated as the percentage of hot pepper fields having virus-like symptoms to the total number of fields assessed per location (Shiferaw and Alemayehu, 2014).

Collection of diseased leaf samples. A total of 225 leaf samples were collected from suspected diseased plants showing virus-like symptoms from three AEZs. Each sample was collected from a different plant targeting five young leaves from different growing points of the plant. The samples were kept in envelopes containing silica gel and later transported to Phytopathology Laboratory of Rwanda Agriculture and Animal Resources Development Board (RAB) at Rubona station, Huye district and stored at room temperature until dry. After 4–5 days, they were ground in liquid nitrogen and powdered leaf samples were stored in 1.5 ml eppendorf tubes in duplicates at -40 °C (for ELISA test) and -80 °C (for RNA extraction) until analyzed.

Serological tests. Presence of five suspected pepper viruses reported in Africa namely; PVY, PVMV, PMMoV, CMV and TMV was tested in 225 samples using DAS-ELISA kits (LOEWE Biochemica GmbH company, Germany) following the manufacturer's protocol. The absorbance was measured at 405 nm using a microplate reader (BioTek ELX800, USA). A sample with ELISA reading of at least twice the average of the negative controls was considered as reacting positively for the target virus. All incubation plates were covered with sealing tape provided with the kits to maintain a uniform temperature. All buffers, negative and positive controls were provided with the kits.

RNA extraction and RT-PCR. Total ribonucleic acids (RNA) were extracted from 100 mg of frozen powdered hot pepper leaf tissue using cetyl-trimethyl ammonium bromide (CTAB) method as described by Allen et al. (2006) with slight modifications. In addition to phenol: chloroform: isoamyl alcohol (25:24:1), chloroform: isoamyl alcohol (24:1) was used to further remove traces of proteins and phenolic compounds. Virus-specific primers designed for this study based on the nucleotide sequence data of CMV-R1 (GenBank accession No. MG470800.1), PVMV-R1 (MG470801.1), PeVYV-R1 (MG470802.1) and TMV (AY360447.1) were used. Accessions MG470800.1, MG470801.1 and MG470802.1 are known sequences previously identified from hot pepper in Rwanda (Skelton et al. 2018) while AY360447.1 is a GenBank reference sequence for TMV. The CMV-F/R primers amplified a fragment of ~502 bp from the RNA3 segment, PeVYV-F/R a fragment of ~498 bp from RNA-dependent RNA polymerase, PVMV-F/R and TMV-F/R fragments size of ~502 bp and ~622 bp from the coat protein regions, respectively (Table 1). The targeted genes contain conserved regions among the viruses. The primers were designed using Primer3 software (http://primer3.ut.ee/) and synthesized by Inqaba Biotechnical

Table 1 Sequences of primers used for detection of CMV, PVMV, PeVYV and TMV in hot pepper samples collected in Rwanda

Primer	Sequence 5′ to 3’	Annealing Temperature (°C)	Fragment size (bp)	Region amplified
CMV_F	5′-GCTTCGCAATACGTTTTGACGG-3’	54	502	RNA3
CMV_R	5′-TACGACCAGCACTGGTTGATTC-3’	54	502	RNA3
PVMV_F	5′-AAGCCCTCATTGAAGGTCAACG-3’	54	502	Coat protein
PVMV_R	5′-ATCAACCATCACCCACATACCG-3’	54	502	Coat protein
PeVYV_F	5′-AGTACGTCTTCGAGACTACTGC-3’	54	498	RdRp
PeVYV_R	5′-TCTATAGTAGAGAGGTCGATCC-3’	54	498	RdRp
TMV_F	5′-TGATGATTCGGAGGCTACTGTC-3’	54	622	Coat protein
TMV_R*	5′-CCTTCGATTTAAGTGGAGGGAA-3’	54	622	Coat protein

RdRp RNA dependent RNA polymerase, *CMV* cucumber mosaic virus, *PVMV* pepper veinal mottle virus, *PeVYV* pepper vein yellows virus, *TMV* tobacco mosaic virus

*TMV reverse primer cross-react with PMMoV, however the sequence generated using both forward and reverse primers were specific to TMV

Industries (Pty) Ltd, South Africa. The specificity of the primer sequence was checked using Basic Local Alignment Search Tool provided online by the National Center for Biotechnology Information.

One-step RT-PCR was carried out to confirm/identify the viruses from DAS-ELISA positives and also to identify PeVYV for which a commercial antisera kit is not available. One Taq One-step RT-PCR Kit (Catalogue E531S5, New England Biolabs Inc.) was used, following the manufacturer's instructions. RT-PCR mixture comprised of 12.5 µl of 2X reaction mix, 1 µl of 25X enzyme mix, 1 µl of 10 µm forward primer, 1 µl of 10 µm reverse primer, 1 µl total of RNA and the reaction mix was made to 25 µl with PCR nuclease water. Thermal cycling conditions were: 48 °C at 15 min for RT; followed by 1 min at 94 °C for initial denaturation; 40 cycles of 94 °C at 15 s for denaturation, 54 °C at 30 s for annealing and 68 °C at 45 s for extension. The final extension was at 68 °C for 5 min. These conditions were the same for all the viruses. Optimization of the PCR conditions for the PVY and PMMoV primers was not successful and therefore the samples were not tested for the two viruses using RT-PCR.

DNA fragments 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. Purification of the amplified products was done using the QIAquick PCR Purification Kit (Qiagen, USA), following the manufacturer's protocol. Nine isolates (3-PeVYV, 3-CMV, 2-PVMV and 1-TMV) were selected based on different geographical regions where the samples were collected and the DNA fragments sent to Inqaba Biotechnical Industries (Pty) Ltd, South Africa for sequencing. A few isolates were sequenced due to limited resources.

Analysis of disease incidence and severity data. Data on virus disease incidence and severity in farmers' fields were subjected to one-way Analysis of variance (ANOVA) using statistical product and service solutions software (SPSS version 16). Comparison of means was done by Tukey test at 5% level of probability. Data on incidence of aphid-transmitted and seed-borne viruses were analysed separately. Chi-square was used to test for the differences in incidence among the viruses across the three AEZs.

Analysis of sequences and comparisons. The obtained Sanger sequences were trimmed using the CLC main work bench software and analysed with Basic Local Alignment Search Tool nucleotide (BLAST). Multiple sequence alignment of the obtained virus sequences with other known hot pepper viruses available in the GenBank database (Supplementary materials 1a-c) was done by ClustalW using MEGA X software (Kumar et al. 2018). The same size of the sequence fragments was used in the alignments and a phylogenetic tree constructed using unweighted pair group method averages (UPGMA). Tree branches were bootstrapped 1000 replications. Pairwise sequence comparisons were carried out on aligned sequences using Bioedit computer software. GenBank isolates used for phylogenetic analysis were selected based on host crop (pepper) and the targeted regions/genes. However, for TMV, only a few sequences from pepper are available in the GenBank therefore, isolates from other host crop were included. Where multiple isolates from the same origin/country exist, representative isolates were used. The sequences of nine virus isolates namely PeVYV-I4 (MT445648), PeVYV-R13 (MT445647), PeVYV-G12 (MT445649), PVMV-R12 (MT445645), PVMV-28 (MT445646), TMV-198 (MT445644), CMV-F1 (MW080679), CMV-G11 (MW080680) and CMV-R10 (MW080681) were deposited in the GenBank.

Results

Virus disease incidence and symptom severity in farmers' fields. A range of viral disease symptoms were observed in surveyed fields. These included dark green vein banding, reduced leaf size, leaf mosaic, bleaching, puckering, mottling, deformation, chlorotic veins and stunting (Fig. 2a-h). The low-altitude zone had the highest (53.4%) incidence of virus symptoms while the high and mid-altitude zone had 44.2% and 43.6%, respectively. Similarly, severity of symptoms was highest in the low-altitude zone (2.0) followed by high-altitude (1.9) and the lowest was mid-altitude zone (1.7). The incidence and severity of the viral symptoms did not differ across the AEZs (data not shown). Results from observed farmers' fields indicated prevalence of viral diseases was 100% in all zones.

Out of 225 leaf samples collected and analysed using five polyclonal antibodies, 56% (126 samples) reacted positively to the antibodies of one or more of the viruses tested. Using RT-PCR, a total of 76 samples selected from different geographical regions including 26 positive and 50 negative samples by serology were tested. A further 17.3% (39 of the negatives by serology) samples tested positive for the presence of one or more of the viruses. The 26 positive samples tested by serology were further supported by results from RT-PCR using PVMV, CMV and TMV primers. Overall, viruses were detected in 73.3% samples that were collected from the field. Slightly above a quarter (26.7%) of the samples tested negative. A summary of samples and the viruses detected using ELISA and RT-PCR are shown in Table 2.

Distribution of pepper viruses. Aphid transmitted viruses namely CMV, PVY and PVMV, and seed-borne viruses PMMoV and TMV were detected by serology. Among the aphid transmitted viruses, the most prevalent virus was CMV detected in 48% of the samples tested followed by PVMV in 23.6% and the least was PVY detected in 18.2% of the samples

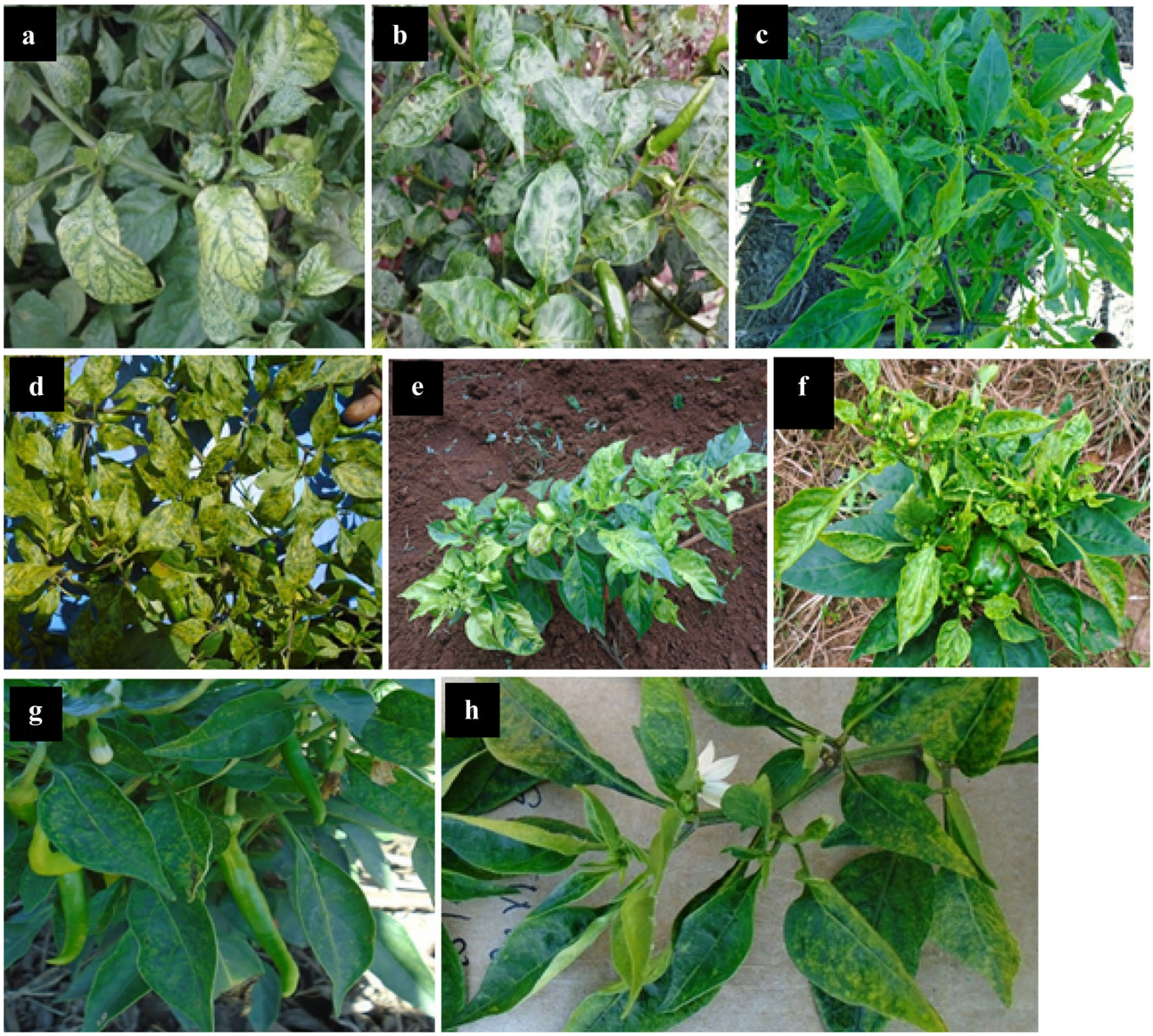

Fig. 2a-h Viral symptoms observed in farmers' fields on hot pepper plants, **a** dark green vein banding, **b** leaf puckering, **c** leaf distortion, **d** leaf mottling, **e** leaf bleaching, **f** stunting, **g** and **h** chlorotic veins of pepper leaves

(Table 3). Chi-square test revealed that CMV and PVMV incidence did not differ significantly across the zones. However, incidence of PVY differed significantly ($\chi 2 = 26.621$; $p < 0.001$), where it was higher in mid-altitude areas detected in 40% of the samples compared to high (13.3%) and low-altitude areas (8.6%). Proportions of infected samples with seed-borne viruses were about 16% for both PMMoV and TMV (Table 3). The viruses were distributed in all areas surveyed. Incidence of TMV was significantly ($\chi 2 = 8.146$; $p = 0.017$) higher in low-altitude zone and detected in 21.9% of the leaf samples, followed by 16.7% in the high-altitude and 5% in mid-altitude areas. PMMoV was present at 20%, 16.2% and 13.3% in high, low and mid-altitude areas, respectively. Incidence of seed-borne viruses across the zones differed significantly ($\chi 2 = 6.526$; $p = 0.038$) with highest prevalence in low-altitude areas (Table 3).

Types of virus infections. Among the samples tested using serology, the proportion of positive samples was 73.3%, consisting of both single (36%) and mixed (34.6%) infections. The most common single virus species infection was CMV detected in 33 samples while PVY was the least detected in 8 samples (Table 4). Among the dual infection, combinations of CMV + PVMV and CMV + PVY were the most prevalent detected in 19 and 18 samples, respectively. Other dual infections included CMV + PMMoV, CMV + TMV, PVY + TMV, PVY + PVMV and

Table 2 Summary of number of leaf samples tested and viruses detected by ELISA and RT-PCR in hot pepper from different sites and agro-ecological zones in Rwanda

Test	AEZ[3]	No. of samples tested	No. of positive samples	CMV*	PVY	PVMV	TMV	PMMoV	PeVYV
ELISA[1]	High altitude	60	27	19	7	7	9	11	nt[4]
	Mid altitude	60	45	31	24	12	3	8	nt
	Low altitude	105	54	30	9	13	16	16	nt
	Sub-total	225	126	80	40	32	28	35	
RT-PCR[2]	High altitude	15	13	11	nt	7	1	nt	0
	Mid altitude	20	20	15	nt	6	3	nt	4
	Low altitude	41	32	16	nt	11	8	nt	8
	Sub-total	76	65	42	0	24	12	0	12

[1]Enzyme linked immunosorbent assay;

[2]Reverse-transcription polymerase chain reaction

[3]Agro-ecological zone; [4]Not tested;

**CMV* cucumber mosaic virus, *PVY* potato virus Y, *PVMV* pepper veinal mottle virus, *TMV* tobacco mosaic virus, *PMMoV* pepper mild mottle virus, *PeVYV* pepper vein yellows virus

PVMV + PMMoV in order of decreasing importance. Triple infections occurred in 16 samples. A multiple infection involving four viruses (CMV + PVMV + TMV + PMMoV) was detected from two samples and five viruses (CMV + PVY + PVMV + TMV + PMMoV) were detected in 3 samples. Mixed infections were most prevalent in the low and mid-altitude areas (Table 4).

A selection of samples based on geographical locations and symptoms appearance were tested by RT-PCR for PeVYV. Of the 76 samples tested, 12 were positive. Seven collected from low AEZ had single infection while mixed infections of PeVYV + CMV and PeVYV + CMV + PVMV were detected in three and one sample, respectively collected from mid-altitude AEZ (data not shown). The combination of PeVYV + PVMV was detected from one sample collected from low-altitude AEZ.

Sequences and phylogenetic analysis. Using CMV-F/R primers, fragments with an expected size of 502 bp were amplified. DNA sequencing of 3 amplicons, isolate F1 (MW080679), G11 (MW080680) and R10 (MW080681) confirmed the presence of CMV. Phylogenetic reconstruction based on segment RNA3 nucleotide (nt) sequences (481 bp) suggested that the three isolates (MW080679, MW080680, MW080681) were CMV and formed a distinct group (clade A) with 100% bootstrap support together with previously isolated Rwandan strain (MG470800.1) and four isolates (MN422338.1, KP033526.1, MN422335.1 and KC527759.1) from South Korea (Fig. 3). Isolate AJ585522.1 from Australia and D12499.1 from Japan made an independent clade B while KT004544.1 from China was placed in an intermediate position between Japan and Rwanda-South Korea isolates. Clade C comprised of isolates from India and Italy, while clade D contained isolates KX525738.1 and MK440591.1 from Australia and USA, respectively. Pairwise nucleotides (nt) and deduced amino acids (aa) similarity among the Rwandan isolates ranged between 98.6–100% nt (99.3–99.7% aa). Identities of the Rwandan isolates to isolates from South Korea, China, Italy, India and Japan

Table 3 Proportion (%) of aphid-transmitted and seed-borne viruses detected in hot pepper leaf samples collected from three agro-ecological zones in Rwanda in 2018

Type	Virus	Low altitude	Mid altitude	High altitude	Total	χ2-test	P-value
Aphid-transmitted viruses	CMV*	42.9	56.7	45.0	48	2.587	0.274
	PVY	8.6	40	13.3	18.2	26.621	< 0.001
	PVMV	24.8	21.7	23.3	23.6	0.205	0.902
	Overall infected samples	61.9	71.7	56.7	61.8	3.48	0.176
Seed-borne viruses	TMV	21.9	5	16.7	16	8.146	0.017
	PMMoV	16.2	13.3	20	16.4	0.98	0.613
	Overall infected samples	34.3	16.7	23.3	26.7	6.526	0.038

CMV cucumber mosaic virus, *PVY* potato virus Y, *PVMV* pepper veinal mottle virus, *TMV* tobacco mosaic virus *PMMoV* pepper mild mottle virus

*Total number of samples tested is 225 (105 from low, 60 from both mid and high altitude areas)

Table 4 Frequency of single and mixed virus infections detected using serology in hot pepper leaf samples collected from three agro-ecological zones in Rwanda

Type of infection	Virus/combinations	Low altitude	Mid altitude	High altitude	Total
Single	CMV	15	10	8	33(14.7)*
	PVMV	9	1	6	16(7.1)
	PMMoV	9	4	0	13(5.8)
	TMV	7	1	3	11(4.9)
	PVY	2	5	1	8(3.5)
Total		42	21	18	81(36)
Double	CMV + PVMV	11	3	5	19(8.4)
	CMV + PVY	1	14	3	18(8.0)
	CMV + PMMoV	2	0	4	6(2.7)
	CMV + TMV	6	0	0	6(2.7)
	PVY + TMV	4	0	0	4(1.8)
	PVY + PVMV	1	2	0	3 (1.3)
	PVMV + PMMoV	0	1	0	1 (0.4)
	Sub-total	25	20	12	57 (25.3)
Triple	CMV + TMV + PMMoV	2	1	4	7 (3.1)
	CMV + PVY + PVMV	1	3	0	4 (1.8)
	CMV + PVMV + TMV	2	1	0	3 (1.3)
	CMV + PVMV + PMMoV	0	2	0	2 (0.9)
	Sub-total	5	7	4	16 (7.1)
Multiple	CMV + PVMV + TMV + PMMoV	2	0	0	2(0.9)
(4and 5)	CMV + PVY + PVMV + TMV + PMMoV	0	0	3	3(1.3)
	Sub-total	2	0	3	5(2.2)
Total (mixed infections)		32	27	19	78(34.6)

CMV cucumber mosaic virus, *PVY* potato virus Y, *PVMV* pepper veinal mottle virus, *TMV* tobacco mosaic virus, *PMMoV* pepper mild mottle virus.

*Total samples and values in brackets are proportion (%) of single and mixed virus infections detected to the total number of samples tested = 225

ranged from 77.6–98% nt (88.9–99.3% aa) which were above the cut off (65%) for species demarcation for *Cucumovirus* (data not shown). Alfalfa mosaic virus (AMV), accession MF990286.1 was used as outgroup.

For PeVYV, three amplicons G12 (MT445649), I4 (MT445648) and R13 (MT445647) with an expected size of ~ 498 bp obtained with primers PeVYV-F/R were sequenced. The three samples clustered together with the previously identified isolate MG470802.1 from Rwanda, and other isolates from Israel (HM439608.2), Spain (KY523072.1), Japan (LC126031.1, LC126045.1, AB594828.1), China (KP326573.1), Australia (KU999109.1) and Malaysia (MN337276.1) in Fig. 4. The sequence identities of the deduced amino acids (aa) sequences for partial RdRp were 97–100% aa (94.9–100% nt) between MT445649, MT445648, MT445647 and MG470802.1 from Rwanda. Comparison of the Rwandan isolates with those of Spain, Australia and Asian countries (Japan, Israel, China, Malaysia), revealed sequence identities ranging from 90.3–94.9% aa (90.5–96.8% nt) which were above the currently accepted < 90% threshold for genus *Polerovirus* dermacation (data not shown). Barley vein yellow dwarf virus (BYDV), accession EU332330.1 was used as outgroup.

Using PVMV-F/R, fragments with an expected band size of ~ 502 bp were amplified from the samples tested. From BLASTn analysis, isolates R12 (MT445645) and 28 (MT445646) sequences indicated the highest nucleotide identity of 98.5–98.9% to MG470801.1 previously isolated from hot pepper in Rwanda. Phylogenetic grouping of eighteen PVMV isolates based on nucleotide sequences (418 bp) corresponding to the partial coat protein gene was done (Fig. 5). The PVMV isolates separated into four groups. The first cluster comprised of isolates from Ghana (FM202327.1, NC011918.1), Japan (LC438542.1, LC438544.1, LC438545.1), China (KR002568.1, MN082715.1) and Taiwan (EU719646.1). The second clade B comprised of Mali isolates (GQ918276.1, GQ918276.1) while clade C comprised of Cameroon (AJ780967.1) and Ghana (AJ780968.1). Rwanda isolates (MT445645, MT445646, MG470801.1), Yemen (AJ780969.1) and Ethiopia (AJ780970.1) clustered together in clade D. Sequence identities between the Rwandan PVMV isolates ranged from 98–99% nt (99% aa) while to other isolates from

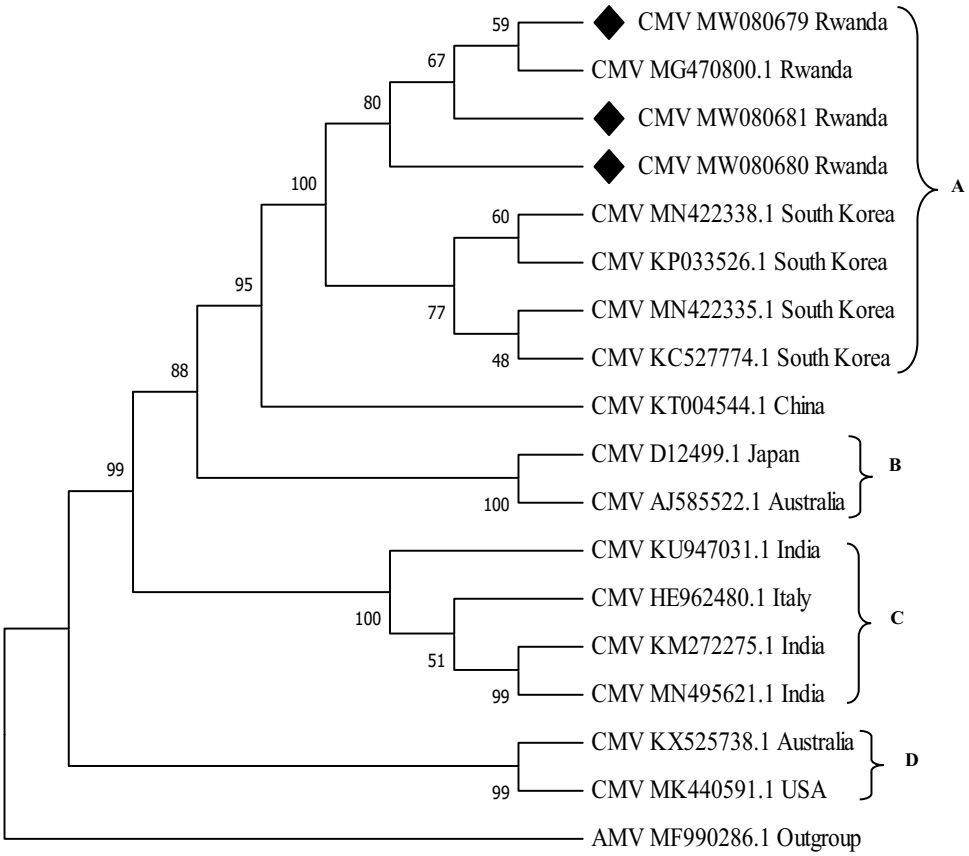

Fig. 3 Phylogenetic tree constructed with sequences of seventeen cucumber mosaic virus (CMV) isolates, genus *Cucumovirus*. The tree was based on alignments of 481 nucleotides of partial RNA3 segment and was rooted in the sequence of alfalfa mosaic virus (AMV), genus *Alfamovirus* (MF990286.1). Nodes bearing less than 50% bootstrap values support are collapsed. The accession numbers of the isolates and place of origin are indicated in the tree. Samples analysed in this study are indicated by the symbol ◆

Ethiopia, Senegal, Cameroon, Ghana, Japan, China and Taiwan were 76–79% nt (82–87% aa) which correspond the optimal species demarcation criterion (< 76% nt, < 82% aa) for the CP in genus *Potyvirus* species (data not shown). However, isolates from Rwanda (MT445645, MT445646) and Mali (GQ918274.1, GQ918275.1) seems to be separate species by their nt identities (74–75% nt) but not by their aa identities (82–83% aa). Squash vein yellowing virus (SqVYV), accession DQ812125.1 was used as outgroup.

For TMV, sequencing of one amplicon (622 bp) confirmed the identification of TMV. Phylogenetic analysis was done based on complete coat protein nucleotides sequences (Fig. 6). Isolate 198 (MT445644) from Rwanda, India (JQ895560.1), Africa (AY360447.1), China (AJ239099.1, JX993906.1, GU324660), United Kingdom (KY810785.1), Germany (AJ429081.1), South Korea (AB369275.1, AB354955.1), Thailand (AY633749.1) and Serbia (GQ340671.1) clustered together in one distinct clade A with 100% bootstrap value (Fig. 6). TMV isolates from pepper, tobacco, soya bean, eggplant, tomato and impatiens all clustered together in clade A. Rwandan isolate MT445644 showed 91.1–99.8% nt (92.7–99.8% aa) similarity to twelve isolates clustered together in clade A which is above the threshold (< 90%) for *Tobamovirus* species demarcation (data not shown). Tobacco rattle virus (TRV), accession JO4347.1) was used as outgroup.

Discussion

Viral diseases cause significant losses in the quantity and quality of pepper crop globally (Olawale et al. 2012). They are one of the most important constraints for pepper producers. The present study aimed at detecting six viruses namely CMV, PVMV, PVY, TMV, PMMoV and PeYV and determine their distribution in Rwanda. Results show that the viruses are widespread in the main areas where hot pepper is grown in the country. Of the six viruses included in this study, CMV, PVMV and PeVYV were previously reported infecting pepper in Rwanda (Skelton et al. 2018). To our knowledge, this is the first report of TMV, PMMoV and PVY in pepper crop in Rwanda. These findings therefore extend our knowledge on viruses infecting hot pepper in Rwanda.

Aphids-transmitted viruses namely CMV, PVMV and PVY were the most prevalent viruses infecting hot pepper in the surveyed AEZs in decreasing order of importance. The high prevalence of CMV, PVY and PVMV may be attributed to their broad host range and the fact that they are mainly transmitted by several species of aphids (Pernezny et al. 2003). Among the insect-vectors, aphids are the most prevalent in pepper fields in Rwanda (Waweru et al. 2020). The most common aphid species associated with peppers

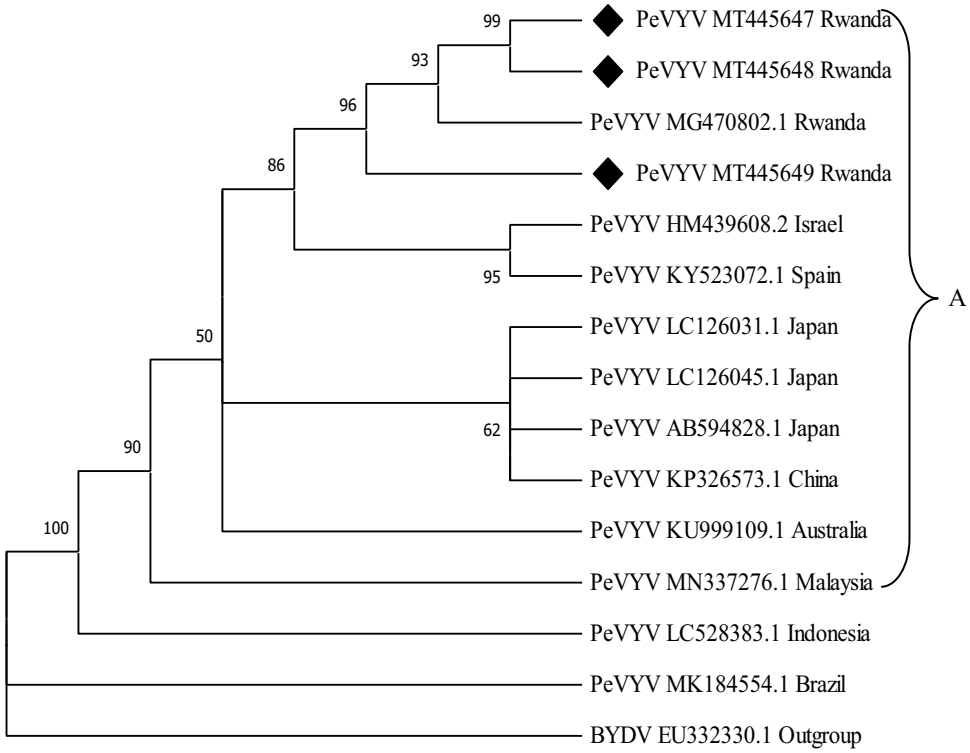

Fig. 4 Phylogenetic tree constructed with sequences of fourteen pepper vein yellows virus (PeVYV) isolates, genus *Polerovirus*. The tree was based on alignments of 475 nucleotides of partial RNA-directed RNA polymerase gene and was rooted in the sequence of barley vein yellow dwarf virus (BYDV), genus *Luteovirus* (EU332330.1). Nodes bearing less than 50% bootstrap values support are collapsed. The accession numbers of the isolates and place of origin are indicated in the tree. Samples analysed in this study are indicated by the symbol ◆

in the field are *Myzus persicae* and *Aphis gossypii* (Fajinmi et al. 2011). Both species are known to efficiently transmit CMV, PVY and PVMV in a non-persistent manner within a short period, among other aphid species (Mello et al. 2011; Palukaitis et al. 1992). It is likely that the same species of aphids are transmitting CMV, PVY and PVMV within the AEZs leading to an increase in prevalence of the viruses. CMV has been previously reported as a dominant virus in hot pepper, particularly in the tropical and semi-tropical regions (Choi et al. 2018; Myti et al. 2014; Olawale et al. 2012). Dafalla (2001) also reported that CMV and PVY were among the most common and damaging viruses infecting pepper in the Sub-Saharan region.

The non-significant differences in the mean prevalence of aphid-transmitted viruses amongst the three AEZ, shows the importance of the viral diseases in all the AEZ in the country. However, for PVY, the incidence of the disease was significantly different between zones with mid AEZ recording highest value. The variation may be due to several factors such as environment conditions, source of inoculum and insect-vectors (Njeru et al. 2008; Thresh et al. 2003). The widespread distribution of the viruses across all agro-ecological zones could also be attributed to inadequate farmers' knowledge on viral diseases and pest management methods, and poor agronomic practices such as continuous cropping, mono-cropping, use of uncertified seeds, free movement of planting materials from one location to another and field sanitation (Waweru et al. 2020).

The high incidence of the seed-borne viruses in the low altitude zone compared to the high and mid AEZs can be attributed to unchecked local and international exchange of planting materials. Majority of the hot pepper farmers from low AEZ obtain their seeds from exporting companies (Waweru et al. 2020). The level of PMMoV and TMV infections were generally low. This may suggest that the pathogens might have been recently introduced in pepper fields. Both viruses are seed-borne and could be spread unknowingly by farmers through infected seeds or as they work in the fields (Genda et al. 2005). Besides, some of the farmers normally recycle planting materials or use uncertified seeds and these practices may provide a means to perpetuate the diseases (HCA 2012). Therefore, the government should emphasize on testing of seed prior to planting and educate the farmers not to recycle seeds but rather use certified seed only. Farmers' awareness of the viruses should be raised so that they can be cautious and conscious while working in the fields.

In this study, the presence of PeVYV was confirmed from a few samples analysed, its distribution is yet to be confirmed. This is because there are no commercial antisera that would allow processing bulk leaf samples. However, from the few samples analysed, the virus was detected in low and mid-altitude AEZs. PeVYV was first isolated in Israel in 2010 (Dombrovsky et al. 2010). Since then it has been detected in African countries which include Sudan, Benin, Tunisia and Mali (Afouda et al. 2013; Alfaro-Fernández

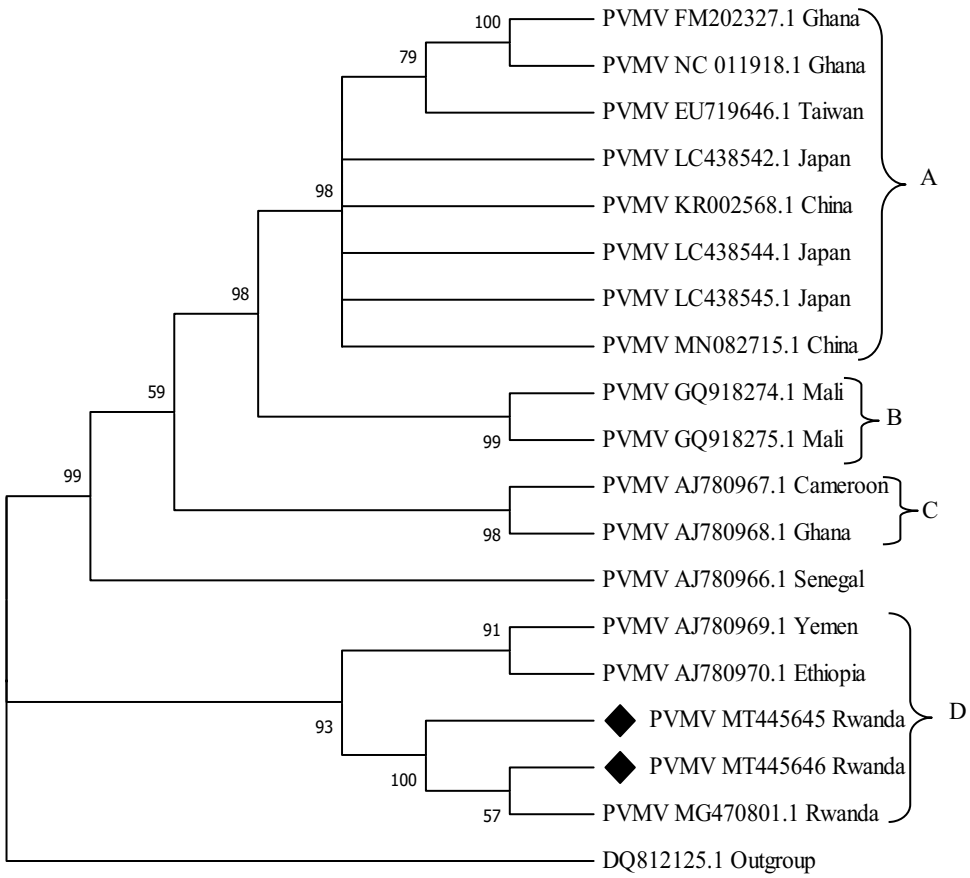

Fig. 5 Phylogenetic tree constructed with sequences of eighteen pepper veinal mottle virus (PVMV) isolates, genus *Potyvirus*. The tree was based on alignments of 418 nucleotides of partial coat protein gene and was rooted in the sequence of squash vein yellowing virus (SqVYV), genus *Ipomovirus* (DQ812125.1). The accession numbers of the isolates and place of origin are indicated in the tree. Samples analysed in this study are indicated by the symbol ◆

et al. 2014; Buzkan et al. 2013; Knierim et al. 2013). More recently PeVYV was detected in Rwanda by Skelton et al. (2018) in samples collected from the high-altitude AEZ (Rulindo district) and low-altitude AEZ (Kirehe and Kayonza districts). Considering the present finding and previous results by Skelton et al. (2018), it is evident that the virus is present in the low, mid and high-altitude AEZs. The results also suggest that PeVYV can co-infect with other viruses. For example, it was present as a double infection with CMV or PVMV and a triple infection as PeVYV + CMV + PVMV. Therefore, further research need to be carried out to understand the virus, its epidemiology and economic significance.

Mixed infections in pepper increase the intensity of disease symptoms, leading to significant losses in yield (Olawale et al. 2012). The occurrence of double, triple and multiple infections among the viruses detected was observed in the three AEZs, which could have serious consequences in their management and the resultant yield obtained by farmers. Double infection of CMV + PVY and CMV + PVMV were the most common. The co-infection of CMV with *Potyvirus* is common and has been reported in other countries like Ivory Coast and Nigeria, based on serological analysis of diseased pepper leaf samples (Olawale et al. 2015; Sorho et al. 2014). Conversely, mixed infections of CMV with *Tobamovirus* (TMV or PMMoV) and *Potyvirus* with *Tobamovirus* as was revealed in this study have also been documented in Ghana (Appiah et al. 2014). Mixed infections are quite common in nature not only on pepper but also in other solanaceous crops, and are associated with serious virus problems in pepper production (Afouda et al. 2013). The mixed infections cause synergistic or antagonistic interactions (Syller 2012).

The presence of mixed virus infections from several genera in farmers' hot pepper field is likely to cause varying levels of losses in quantity and quality leading to a significant reduction in yield. There are no specific studies done in Rwanda, however, yield losses of 10.84 to 50.51%, 54.5 to 64.3%, 20–70%, 75–95% and up to 90% have been reported elsewhere due to CMV, PVMV, PVY, PMMoV and TMV, respectively (Avilla et al. 1997; Chitra et al. 2002; Fajinmi et al. 2012; Guldur and Caglar, 2006; Rahman et al. 2016). Like in other countries, it is expected that these viruses will cause varying degrees of damage and yield losses, and thus threaten pepper production in Rwanda. This is a cause for concern in economic terms and hence, the need for diseases management strategies that target these viruses. About 26.7% of the apparently diseased samples were negative, an indication of possible presence of other viruses infecting the crop. Considering the symptoms (dark green vein banding, reduced leaf size, leaf mosaic, bleaching, puckering,

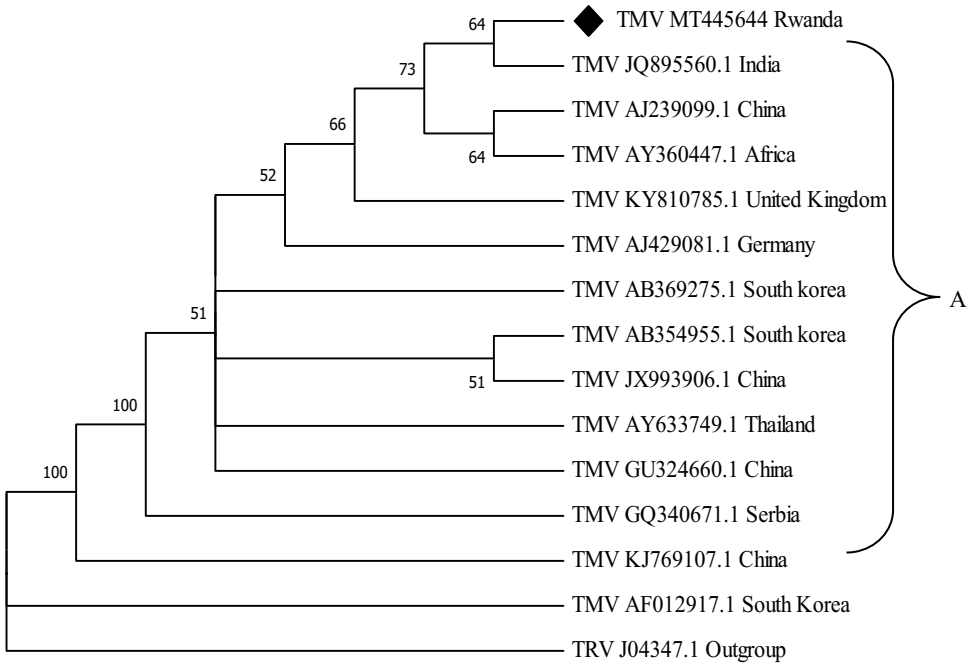

Fig. 6 Phylogenetic tree constructed with sequences of fourteen tobacco mosaic virus (TMV) isolates, genus *Tobamovirus*. The tree was based on alignments of 622 nucleotides of coat protein gene and was rooted in the sequence of tobacco rattle virus (TRV), genus *Tobravirus* (JO4347.1). Nodes bearing less than 50% bootstrap values support are collapsed. Bootstrap values (1,000 replications) are shown as percentages at the branch points. The accession numbers of the isolates and place of origin are indicated in the tree. The sample analysed in this study is indicated by the symbol ◆

mottling, deformation, chlorotic veins leaf mosaic, mottle and dark green vein-banding) observed in the samples collected, more assays targeting other viruses is therefore necessary. Pepper viruses such as pepper mottle virus, tobacco etch virus and ChiVMV which exhibits similar symptoms in infected pepper and have been reported in Africa might be present in the country and would be important to test (Njukeng et al. 2013; Olawale et al. 2015).

The sequence and phylogenetic analysis of the Rwandan CMV, PVMV, TMV and PeVYV isolates confirmed the identity of the viruses. Sequence identities between the Rwandan isolates ranged from 97–100%, suggesting low genetic variability. Phylogenetic analysis of TMV resulted in a tree with one main part. TMV isolates from different tobacco, soya bean, eggplant, tomato and impatiens clustered together in a distinct branch. There was no evidence of branching pattern based on differences in plant hosts as observed in previous research by Alishiri et al. (2013). Considering the observed low diversity, it is possible that host species do not contribute to differentiation of the virus population. According to the criteria established by the International Committee on Taxonomy of Viruses (ICTV), demarcation thresholds recommended for members of the genus *Polerovirus* is < 90% aa sequence identity of any gene, *Tobamovirus* < 90% nt identity, *Potyvirus* < 76% nt (< 82% aa) of coat protein gene and *Cucumovirus* < 65% nt sequence identity (Adams et al. 2005, 2012; Domier 2012; Wylie et al. 2017). Based on the ICTV criteria, the degree of similarities between the Rwandan isolates to other isolates of either PeVYV, TMV, or CMV reported in the GenBank were well above the thresholds suggesting that the isolates are not new virus species. Overall, the Rwandan pepper isolates of CMV, PVMV and PeVYV clustered together indicating that, their geographical origin and phylogenetic relatedness could be correlated. However, complete genomes sequences will be needed to fully characterize the viruses. In addition, further characterization of PVMV isolates would be useful to clarify their taxonomic status as their amino acid and nucleotide identities were just above the demarcation threshold for differentiation between species in the genus *Potyvirus*.

Conclusion

This study detected and identified six virus species namely cucumber mosaic virus, pepper veinal mottle virus, potato virus Y, pepper mild mottle virus, tobacco mosaic virus and pepper vein yellows virus that are widely distributed in all major hot pepper growing areas in Rwanda. The viruses exist either as single or mixed infections. This is the first time that TMV, PMMoV and PVY are being reported in pepper fields in Rwanda. It will be important for the government to strengthen extension services to educate farmers on diseases and pests management and strengthen the hot pepper seed certification system to prevent the spread of viral diseases that might threaten income security to smallholder farmers. Further assays targeting viruses other than those tested in this study are recommended. In addition, further studies should be carried out to determine the effect of mixed infections on severity and yield losses. Resistance breeding and other control strategies focusing on viruses are urgently needed.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42161-021-00811-7.

Authors contributions All authors have materially participated in the research.

Funding This work was funded by the United States Agency for International Development, as part of the Feed the Future initiative, under the CGIAR Fund, award number BFS-G-11–00002, and the predecessor fund the Food Security and Crisis Mitigation II grant, award number EEM-G-00–04-00013; with additional support from Rwanda Agriculture and Animal Resources Development Board (RAB).

Availability of data and material All data is available if and when needed.

Declarations

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate Not applicable as the research did not involve human participants.

Consent for publication Not applicable as the research did not involve human participants.

Conflicts of interest The authors declare that they have no conflict of interest.

References

Adams MJ, Heinze C, Jackson AO, Kreuze JF, Macfarlane SA, Torran L (2012) Family *Virgaviridae*. In: King AMO, Lefkowitz E, Adams MJ, Carstens EB (eds) Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London, United Kingdom, pp 1139–1162

Adams MJ, Antoniw JF, Fauquet CM (2005) Molecular criteria for genus and species discrimination within the family *Potyviridae*. Arch Virol 150(3):459–479

Afouda LAC, Kotchofa R, Sare R, Zinsou V, Winter S (2013) Occurrence and distribution of viruses infecting tomato and pepper in Alibori in northern Benin. Phytoparasitica 41:271–276

Alfaro-Fernández A, El Shafie EE, Ali MA, El Bashir OOA, Córdoba-Sellés MC, Font San Ambrosio MI (2014) First report of *pepper vein yellows virus* infecting hot pepper in Sudan. Plant Disease 98(10):1446. https://doi.org/10.1094/PDIS-03-14-0251-PDN

Alishiri A, Rakhshandehroo F, Zamanizadeh H, Palukaitis P (2013) Prevalence of tobacco mosaic virus in Iran and evolutionary analyses of the coat protein gene. Plant Pathol J 29(3):260–273

Allen GC, Flores-Vergara MA, Krasynanski S, Kumar S, Thompson WF (2006) A modified protocol for rapid DNA isolation from plant tissues using cetyl-trimethyl ammonium bromide. Nat Protoc 1(5):2320–2325

Appiah AS, Quartey EK, Amoatey HM, Nunekpeku W, Owusu-Ansah M, Ofori S (2014) Response of nine cultivars of pepper (*Capsicum* spp.) to infection by four viruses under natural field conditions in the coastal savanna zone of Ghana. Res J Appl Sci Eng Tech 7(5):903–907

Avilla C, Collar JL, Duque M, Fereres A (1997) Yield of bell pepper (*Capsicum annuum*) inoculated with CMV and/or PVY at different time intervals. J Plant Dis Protect 104(1):1–8

Buzkan N, Arpaci BB, Simon V, Fakhfakh H, Moury B (2013) High prevalence of *Poleroviruses* in field-grown pepper in Turkey and Tunisia. Arch Virol 158(4):881–885. https://doi.org/10.1007/s00705-012-1553-y

Chitra TR, Prakash HS, Albrechtsen SE, Shetty HS, Mathur SB (2002) Indexing of leaf and seed samples of tomato and bell pepper for Tobamoviruses. Indian Phytopathol 55:84–86

Choi S, Lee JH, Kang WH, Kim J, Huy HN, Park SW, Son EH, Kwon JK, Kang BC (2018) Identification of *Cucumber mosaic* resistance 2 (cmr2) that confers resistance to a new *Cucumber mosaic virus* Isolate P1 (CMV-P1) in pepper (*Capsicum* spp.). Front Plant Sci 9:1106

Dafalla GA (2001) Situation of tomato and pepper viruses in Africa. In: d'A Hughes, J.BO Odu (eds.) Proceedings of a Conference on "Plant Virology in Sub Saharan Africa". International Institute of Tropical Agriculture, June 4–8, Ibadan, Nigeria, pp 18–24

Domier LL (2012) Family *Luteoviridae*. In: Andrew MQK, Elliot L, Michael JA, Carstens EB (eds) Virus Taxonomy: ninth report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London, United Kingdom, pp 1045–1053

Dombrovsky A, Glanz E, Pearlsman M, Lachman O, Antignus Y (2010) Characterization of *Pepper yellow leaf curl virus*, a tentative new *Polerovirus* species causing a yellowing disease of pepper. Phytoparasitica 38:477–486

Fajinmi AA, Popoola AR, Fajinmi OB (2012) Incidence and epidemiology of *Pepper veinal mottle virus*, a Potyvirus disease of pepper. Arch Phytopathol Plant Protect. https://doi.org/10.1080/03235408.2012.694250

Fajinmi AA, Odebode CA, Fajinmi OB (2011) The effect of agro-ecological zones on the incidence and distribution of aphid vectors of pepper veinal mottle virus on cultivated pepper (*Capsicum annuum* L.) in Nigeria. J Cent Eur Agric 12 (3):528–542

FAO (2017) FAO Statistics Division 2017. www.Faostat.org

Galanihe LD, Priyantha MGDL, Yapa DR, Bandara HMS, Ranasinghe JADAR (2004) Insect pest and diseases incidences of exotic hybrid chilli pepper varieties grown in the low country dry zone of Sri Lanka. Ann of Sri Lanka 6:99–106

Genda Y, Sato K, Nunomura O, Hirabayashi T, Ohnishi J, Tsuda S (2005) Immunolocalization of pepper mild mottle virus in *Capsicum annuum* seeds. J Gen Plant Pathol 71:238–242

Guldur ME, Caglar BK (2006) Outbreaks of pepper mild mottle virus in greenhouses in Sanliurfa. Turkey J Plant Pathol 88(3):339–342

Hiskias Y, Lesemann DE, Vetten HJ (1999) Occurrence, distribution and relative importance of viruses infecting hot pepper and tomato in the major growing areas of Ethiopia. J Phtopathol 147(1):5–11

Horticultural Council for Africa (HCA) (2012) Integrating the horticultural council for Africa in the Nile basin initiative's analysis and sustainability of cross border trade in fruits and vegetables along the selected corridor. Final report November, 2012, pp36

Crsp IPM (2008) Annual report. USAID, Virginia Tech, Integrated Pest Management Collaborative Research Support Program, p 190

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547–1549

Knierim D, Tsai W, Kenyon L (2013) Analysis of sequences from field samples reveals the presence of the recently described *Pepper vein yellows virus* (genus *Polerovirus*) in six additional countries. Arch Virol 158(6):1337–1341

Marin A, Ferreres F, Tomas-Barberan FA, Gil M (2004) Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). J Agric Food Chem 52:3861–3869

Mello AFS, Olarte RA, Gray SM, Perry KL (2011) Transmission efficiency of *Potato virus Y* strains PVY^{O} and $PVY^{N\text{-}Wi}$ by five aphid species. Plant Dis 95:1279–1283

Myti S, Khandaker SA, Akhter S, Uddin A, Kamruzzaman M, Faruq O, Biswas GC (2014) Identification of the most prevalent and

spatially disperse virus on chilli at Northern and Eastern part of Bangladesh. Int J Biosci 5(7):40–49
National Institute of Statistics of Rwanda (NISR) (2018). Rwanda statistical year, December 2018, pp 193
Ndunguru J, Kapooria RG (1999) Identification and incidence of virus diseases of *Capsicum annuum* in the Lusaka Province of Zambia. EPPO Bull 29(1–2):183–189
Njeru RW, Bagabe MC, Nkezabahizi D, Kayiranga D, Kajuga J, Butare L, Ndirigwe J (2008) Viruses infecting sweet potato in Rwanda: occurrence and distribution. Ann Appl Biol 153:215–221
Njukeng PA, Ngwakum WN, Mbong GA (2013) Relative prevalence, incidence and severity of two viruses infecting cultivated pepper in the western highlands of Cameroon. Agric Sci Res J 3(8):261–266
Olawale A, Ajose T, Osijo I, Onyeanusi H, Matthew J, Aliyu TH (2020) Management of viruses and viral diseases of pepper (Capsicum spp.) in Africa. https://doi.org/10.5772/intechopen.92266
Olawale A, Balogun OS, Akinyemi SOS, Kumar PL (2015) Surveys of virus diseases on pepper (*Capsicum spp.)* in South-west Nigeria. Afr J Biotechnol 14(48):3198–3205
Olawale A, Olusegun SB, Kehinde TK (2012) Occurrence and distribution of *Pepper veinal mottle virus* and *Cucumber mosaic virus* in pepper in Ibadan. Nigeria Virol J 9:79
Palukaitis P, Roossinck MJ, Dietzgen RG, Francki RI (1992) Cucumber mosaic virus. Adv Virus Res 41:281–348
Pernezny K, Robert PD, Murphy JF, Goldberg NP (2003) Compendium of pepper diseases. The American Phytopatholgical society 1:24–25. Plant Dis 68:597–599. https://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1984Articles/PlantDisease68n07_597.1984 Accessed on 18 Nov 2017
Rahman MS, Akanda AM, Mian IH, Bhuiyan MKA, Hossain MM (2016) New Sources of resistance to *Cucumber mosaic virus* in *Capsicum annuum*. J Crop Sci Biotechnol 19(3):249–258
Shiferaw M, Alemayehu C (2014) Assessment of hot pepper (*Capsicum* species) diseases in southern Ethiopia. Int J Sci Res, pp 91–95
Skelton A, Uzayisenga B, Fowkes A, Adams I, Buxton-Kirk A, Harju V, Forde S, Ward R, Ritchie B, Rutherford M, Offord L, Umulisa C, Waweru B, Kagiraneza B, Karangwa P, Fox A (2018) First report of *Pepper veinal mottle virus*, *Pepper vein yellows virus* and a novel *Enamovirus* in hot pepper (*Capsicum* sp.) in Rwanda. New Dis Rep 37: 5
Sorho F, Dembele D, Cherif M, Bolou BA, Kassi F, N'guessan AC, Kone D, (2014) Identification and distribution of some viral diseases of solanaceous in Ivory Coast. Int J Sci 3(9):55–62
Surh YJ (2002) Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food Chem Toxicol 40:1091–1097
Syller J (2012) Facilitative and antagonistic interactions between plant viruses in mixed infections. Mol Plant Pathol 13(2):204–216. https://doi.org/10.1111/J.1364-3703.2011.00734.X
Thresh JM, Fargette D, Jeger MJ (2003) Epidemiology of tropical plant viruses. In: Loebenstein G, Thottapill G (eds) Viruses and virus-like diseases of major crops in developing countries. Kluwer Academic Publishers, Dordrecht, pp 55–77
EU (2015) Baseline Report on the Rwanda Horticulture Organization Survey Final Report. European Union External Cooperation Program for Rwanda. March 2014
USAID (2018) Postharvest loss assessment of green chillies in Rwanda, April 30, 2018, Pp 76
Waweru BW, Kilalo DC, Kimenju JW, Rukundo P., Miano DW (2020) Farmers' knowledge and perceptions of viral diseases of hot pepper (Capsicum sp.) and their management in Rwanda. Fundam Appli Agric 5(3):319–329
Waweru BW, Kilalo DC, Miano DW, Kimenju JW, Rukundo P (2019) Diversity and economic importance of viral diseases of pepper (*Capsicum* spp.) in Eastern Africa. J Appl Hortic 21(1):70–76
Wylie SJ, Adams M, Chalam C, Kreue J, Lopez-Moya L, Ohshima K, Praveen S, Rabenstein F, Stenger D, Wand A, Zerbini FM (2017) ICTV report consortium. ICTV virus taxonomy profile: Potyviridae. J Gen Virol 99 (3): 352–354