PREVALENCE AND MANAGEMENT OF VIRAL DISEASES AND ASSOCIATED VECTORS IN HOT PEPPER (*Capsicum spp*.) PRODUCTION IN RWANDA

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This thesis is my original work and has not been submitted for award of a degree in any other University.

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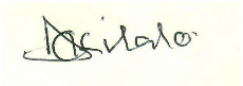
Dr. Dora C. Kilalo  Date: 23/08/2021

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To my parents Mr Charles Waweru and Mrs Jemimah Njoki, and husband Dr Edouard Rurangwa who have motivated me to learn and continue learning. Your support in many ways made me come this far.

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LIST OF ABBREVIATIONS

AEZ	Agro-ecological zone
ANOVA	Analysis of variance
AUDPC	Area under the disease progress curve
BLASTn	Basic Local Alignment Search Tool
bp	Base pair
cm	Centimetre
CP	Coat protein
CTAB	Cetyl-trimethyl ammonium bromide
°C	Degree celsius
DAS-ELISA	Double antibody sandwich-enzyme linked immunosorbent assay
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immunosorbent assay
g	Gram
ha	Hectare
IPM	Integrated pest management
JKUAT	Jomo Kenyatta University of Agriculture and Technology
L	Litre
LSD	Least significant difference
M	Molar
m	Metre
MEGA	Molecular evolutionary genetic analysis
mg	Microgram
min	Minute(s)
ml	Millilitre
mM	Millimolar
mm	Millimetre
NaCl	Sodium chloride
NPVs	Non-persistent viruses
nt	Nucleotide(s)
PCR	Polymerase chain reaction

RAB	Rwanda Agriculture and Animal Resources Development Board
RCBD	Random complete block design
RdRp	RNA-dependent RNA polymerase
RNA	Ribonucleic acid
RNGB	Rwanda National Genbank
rpm	Revolutions per minute
RT	Room temperature
RT-PCR	Reverse-transcription polymerase chain reaction
sec	Second (s)
spp	Species
TAE	Tris-Acetate-EDTA buffer
Tons	Tonnes
µl	Microlitre
µm	Micrometre
Var	Variety
w/v	Weight/volume
WAP	Weeks after planting
WVC	World Vegetable Center
YWT	Yellow water trap

LIST OF ACRONYMS

CABMV	*Cowpea aphid borne mosaic virus*
CMV	*Cucumber mosaic virus*
PepMoV	*Pepper mottle virus*
PeVYV	*Pepper vein yellows virus*
PMMoV	*Pepper mild mottle virus*
PVMV	*Pepper veinal mottle virus*
PVY	*Potato virus Y*
TEV	*Tobacco etch virus*
TMGV	*Tobacco mild green mosaic virus*
TMV	*Tobacco mosaic virus*
ToMV	*Tomato mosaic virus*
TSWV	*Tomato spotted wilt virus*
TYLCV	*Tomato yellow leaf curl virus*

ABSTRACT

Hot pepper (*Capsicum* spp.) is a source of income, employment and foreign exchange in Rwanda. However, production of the crop is constrained by diseases and pests, mainly viruses and their vectors. Reports on presence of viral diseases in Rwanda are available but there is limited information on the pathogens responsible and their management. This study was carried out to establish farmers' perceptions and knowledge of viral diseases, to determine the prevalence of viruses associated with hot pepper, to screen hot pepper genotypes for reaction to viruses and aphids, and to evaluate the effect of companion crops on aphids and associated-viral diseases.

To establish farmers' perceptions and knowledge of viral diseases, a survey was carried out in major hot pepper growing areas from February to March 2018 covering low, mid and high-altitude agro-ecological zones (AEZs). A total of 101 randomly selected farmers were interviewed, and results revealed that pests and diseases were the main production constraints as indicated by 86.1% of the farmers. Awareness of viral diseases varied significantly ($\chi 2$ = 20.116; $p < 0.001$) among farmers across the AEZs as well as their knowledge on causes and management of the viral diseases ($\chi 2 = 26.896$; $p = 0.003$).

During the survey mentioned above, 225 symptomatic leaf samples of pepper were collected and analysed to detect six viruses using serology assay and reverse-transcription polymerase chain reaction (RT-PCR). Incidence of viruses transmitted by aphids {*Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY), *Pepper veinal mottle virus* (PVMV)} did not differ significantly ($\chi 2 = 3.48$; $p = 0.176$) across AEZs while for seed-borne viruses {*Tobacco mosaic virus* (TMV) and *Pepper mild mottle virus* (PMMoV)} it differed significantly ($\chi 2 = 6.526$; $p = 0.038$). The CMV was the most prevalent identified in 48% of the samples. Single and mixed virus infections were present in 36 and 34.6% of the samples, respectively.

Sequence as well as phylogenetic analysis confirmed the identity of the Rwandan CMV, PVMV, TMV and PeVYV virus isolates.

The reaction of 18 hot pepper genotypes (9 local accessions from Rwanda National Genbank, 5 introduced lines from World Vegetable Center and 4 commercial varieties from seed companies) to viral infection and aphid infestation in the field was evaluated in mid and low AEZs. Fourteen (14) of the best field performing genotypes were further assessed for their reaction to artificially inoculated CMV in the screenhouse. Incidence of viral diseases and severity varied significantly ($p < 0.05$) among genotypes in the field and screenhouse. Genotype 00767PPR, 0802PPR and PBC 462 were consistently resistant while genotype HP 0117, PP9852170 and PP99505197 were moderately resistant under field and screenhouse conditions. The population of aphids on the genotypes did not differ significantly ($p > 0.05$) in genotypes.

The potential of companion crops to reduce aphids and associated viral diseases in hot pepper was evaluated for two seasons. The CMV, PVMV and PVY were the viruses detected in the plots while for aphid species, it was M. euphorbiae and A. gossypii. The border crops (maize, sorghum, sunflower), significantly reduced ($p < 0.05$) the incidence of viral diseases in hot pepper by 31.9, 46.5 and 54.8%, respectively compared to the control. Intercropping was also effective where reductions in virus incidence were 35.3, 41.2 and 51.6%, respectively. Aphids population was not ($p > 0.05$) affected by treatments both experiments.

These findings enhance the existing knowledge of causal agents of hot pepper viral diseases and their distribution in Rwanda. Genotypes found to be resistant maybe useful for breeding programs and hot pepper growers. Farmers can adopt the utilization of sorghum, sunflower and maize as border or inter-crops to control the spread of non-persistently aphid-transmitted viruses within hot pepper fields. This study provides important information for designing of long-term strategies for management aphids and virus diseases.

CHAPTER ONE:

INTRODUCTION

1.1. Background information

Hot pepper (*Capsicum* spp.) is mainly produced by small-scale growers, globally (Olawale *et al.,* 2015). The crop originated from Mexico, but is presently widely cultivated throughout the tropical, sub-tropical and temperate zones (Olatunji and Afolayan, 2018). On a worldwide scale, production of green hot peppers ranked 7th after tomatoes, onions, cucumbers, cabbages, eggplants and carrots, among other vegetable crops in 2018 (FAOSTAT, 2019). Sixty-seven percent of the crop is produced in Asia with China being the lead producer (FAOSTAT 2019). Estimated world annual production of green hot pepper was 36,771,482 tons with Africa accounting for 3,478,095 tons, while the eastern Africa region produced 100,896 tons in 2018 (FAOSTAT, 2019). The main producers in Africa include Nigeria (747,367), Egypt (713,752) and Algeria (651,045) tons.

In East Africa, Rwanda is one of the leading producers of green hot pepper ranking 3rd after Ethiopia and Tanzania in Africa with 5,009 tons' annual production on average (FAOSTAT, 2019). The main hot pepper production areas, where it is a cash crop, are Nyagatare, Kayonza, Rwamagana and Bugesera in eastern; Rulindo and Gakenke in northern; and Nyanza and Ruhango districts in southern Province of Rwanda, (EU, 2015). The crop is a source of income, employment, foreign exchange earnings and raw material for the processing industries thus, playing a role in Rwandan economy (USAID, 2018).

Despite the increase in area planted with hot pepper and the total production in Rwanda, the average yield of 6.8 t ha^{-1} is low and 50% lower than that of Egypt and Algeria the leading producers in Africa (FAOSTAT, 2019). Production of this crop is constrained by many abiotic and biotic factors. These include high cost of seeds, lack of proper and adequate inputs, drought, low soil fertility, lack of storage facilities, shortage of improved varieties,

fluctuations of prices, non-availability of credit, lack of technical knowledge, and high infestations of diseases and insect pests (Geetha and Selvarani, 2017). Biotic factors, particularly, diseases caused by fungi, bacteria and viruses are reported as the most harmful in hot pepper production (Dagnoko *et al.,* 2013).

Viral diseases cause complex symptoms such as chlorosis, puckering, vein banding, deformation, mosaic, mottle, reduced leaf size and stunting, resulting to serious losses in plant vigor and yield (Nono-Womdim, 2001; Olawale *et al.,* 2012). At least 68 viruses are reported infecting pepper globally (Pernezny *et al.,* 2003). Among these viruses, 15 are documented in Africa (Njukeng *et al.,* 2013; Aliyu, 2014), out of which four are the most prevalent in the sub-Saharan Africa namely *Tobacco mosaic virus* (TMV), PVMV, CMV and PVY (Dafalla, 2001). This study was carried out to identify selected viruses limiting hot pepper production and assess potential sustainable management methods for the enhanced productivity.

1.2. Statement of the problem of the study

Low and poor quality hot pepper produce is usually obtained, although the crop is of economic importance in Rwanda as a source of income, employment and foreign exchange earner. The average yield of 6.8 t ha^{-1} currently is below 15 t ha^{-1} yield potential earlier reported in the country (RDB, 2010; FAOSTAT, 2019). Viral diseases are limiting production and expansion of the hot pepper sector. Yield losses of 40 to 100% due to viruses in hot pepper have been reported (Olawale *et al.,* 2012). Therefore, management of viral diseases sustainably is important to improve yields.

The indigenous knowledge of farmers has a significant role in managing viral diseases and the associated vectors sustainably. Therefore, being cognisant of the perceptions and management practices of viral diseases by farmers, prior to engaging in any research is

important. However, there is scarce or no documentation of the farmers' knowledge and perceptions of hot pepper virus diseases, causes and their management in Rwanda. On the other hand, to control viral diseases effectively needs correct identification and an understanding of the pathogens involved. There is inadequate information concerning identity of pathogens causing viral diseases and the distribution within Rwanda.

Most of the viruses associated with hot pepper are known to be transmitted by insect vectors namely aphids, whiteflies or thrips and as such farmers mainly rely on insecticides to manage them (Schreinemachers *et al.,* 2015). Unfortunately, the aphids and whiteflies have developed resistance against some of the active ingredients (such as organophosphates, pyrethroids, carbamates) after repeated application of the insecticides (Houndété *et al.*, 2010; Kenyon *et al*., 2014; Naveen *et al.,* 2017). This, coupled with the increasing concerns over environmental and health considerations, calls for less application of pesticides to manage the vectors. Therefore, there is need for alternative effective strategies that are locally available, economically viable in resource-challenged production systems and safe to the environment. Use of resistant varieties and cultural practices are among the most promising alternatives. 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 compatible with other methods (Frantz *et al*., 2004). However, information on hot pepper genotypes that can resist or tolerate to virus infection and vector infestation in the country is scarce. The potential of cultural practices such as companion cropping as a component of integrated pest management is yet to be exploited.

1.3. Justification of the study

Identifying the causal viral pathogens is a basic step to the development of strategies for management that are appropriate and effective to improve the yield of hot pepper. Farmers' perceived knowledge of the diseases and current practices for their management would provide useful information to incorporate into scientific knowledge while developing effective management strategies. In addition, information generated from screening hot pepper genotypes for virus and aphids' resistance is useful for breeding programs intended to improve the available varieties and also develop new ones. Integrating cultural techniques for the management of virus-induced diseases and associated vectors in hot pepper is important. The cultural practices are locally available, ecologically and economically friendly measures. Given that most of the stakeholders involved in hot pepper production in Rwanda are small-scale farmers, information generated provides a sustainable approach to pest management.

1.4. Objectives of the study

The overall objective was to increase productivity and farmers' income in Rwanda through sustainable management of the viruses that attack hot pepper.

1.4.1. Specific objectives of the study

The specific objectives were:

i. To establish farmers' perceptions and knowledge of viral diseases and their management in Rwanda.
ii. To identify selected viral diseases infecting hot pepper and their distribution in different agro-ecological zones.
iii. To evaluate the reaction of different hot pepper genotypes to virus infection and aphid infestation.

iv. To determine the efficacy of selected companion crops as border or inter-crops in the management of aphids and associated viruses in hot pepper.

1.5. Hypothesis

i. Farmers' perceptions and knowledge of viral diseases and their management do not differ irrespective of hot pepper production areas in Rwanda.

ii. Distribution of selected viruses infecting hot pepper is not influenced by agro-ecological zones.

iii. There is no variability in the reaction of different hot pepper genotypes to infection by viruses and aphid infestation.

iv. Border and intercropping as systems of companion-cropping do not affect the population of aphids and associated viruses in hot pepper.

CHAPTER TWO:

LITERATURE REVIEW

2.1. Origin and description of hot pepper

Pepper (hot, bell and sweet) belongs to the genus *Capsicum*, in the family *Solanaceae*. The genus contains 5 domesticated (*C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens*) and 22 wild species (Bosland and Votava, 2000; Dagnoko *et al.*, 2013). *Capsicum annuum* originated in Mexico while *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens* originated in South America (OECD, 2006). The center of diversity of the genus *Capsicum* is in south-central South America. In 1493, Columbus and other early explorers, introduced *Capsicum* to Europe and its cultivation has since spread throughout the world (OECD, 2006). Today, Capsicum species are cultivated in the tropical, sub-tropical and temperate regions (FAOSTAT, 2017). *Capsicum annuum*, *C. frutescens* and *C. chinense* are the commonly grown varieties in Rwanda.

Globally, *C. annuum* is the most important because of its economic benefits, nutritional and medicinal value of its fruits (Al-Snafi, 2015; Saleh *et al.*, 2018). *Capsicum annuum* is a small herb with an average height of about 1 m. The size of the leaf is 4 to 13 cm by 1.5 to 4 cm including the entire margin while the shape is either ovate, oblong-ovate or ovate-lanceolate (Al-Snafi, 2015). The flowers are small, white or purplish in color. *Capsicum* fruits may be red, green, orange and yellow in color (Li, 2000). Species of *C. annuum* require well-drained loamy soil that is rich in organic matter (Li, 2000). Temperature between 25 to 30°C is required for seed germination while for optimal growth, they prefer between 18 to 30°C. *Capsicum annuum* grows from lowland to 2,000 m above sea level.

2.2. Nutritional value and medicinal properties of hot pepper

Hot pepper fruits are well known for its high nutritional value and provide essential micronutrients such as vitamin A, B5, C and E (Tocopherols), and macronutrients e.g. protein, carboyhdrates, fats and dietary fiber that are of great importance for human health and growth (Tripathi and Mishra, 2009; Yuni *et al*., 2013; Saleh *et al.,* 2018). Mature fruits of hot pepper are rich in phtytochemicals including phenolics, vitamins (C and E), flavonoids and carotenoids that are essential anti-oxidants (Howard *et al.,* 2000). Hot pepper is also high in minerals such as potassium, phosphorous, calcium, magnesium and iron (Pawar *et al.,* 2011). Nutritional and anti-oxidants level vary depending on species, varieties and consumption forms. The hotness and antioxidant level are proportional to the amount of capsaicin present in the hot pepper (Chu *et al.*, 2003).

Besides the nutritional benefits, hot pepper is used for different therapeutic purposes due to their capsaicin content. Capsaicin is known to give relief from cold symptoms, disorders in the digestive system as well as reducing the risk of cardiometabolic diseases (Chu *et al.,* 2003; Olatunji and Afolayan, 2018). The activation of TRPV1 (transient receptor potential vanilloid subtype 1) in different target organs or tissues by capsaicin play a role in cardiometabolic protection (Geppetti and Trevisani, 2004; Sun *et al.,* 2016; Saleh *et al.,* 2018). Capsaicin increases the permeability and absorption capacity of the intestinal wall surface and thus enhances the uptake of micronutrients (Olatunji and Afolayan, 2018).

Dietary antioxidants found in pepper are important in protecting body cells as well as fighting off free radicals and thus, protect the body against various diseases e.g. diabetics, anemia among others (Lee *et al.*, 2010; Olatunji and Afolayan, 2018). Hot pepper has antimicrobial and anticancer properties. Previous studies by Ito *et al.* (2004) showed that the growth of leukemic cells can be directly suppressed by capsaicin.

2.3. Constraints to production of hot pepper

Hot peppers are affected by several abiotic and biotic (insects and diseases) factors causing considerable economic losses. Insect pests, which cause significant losses in pepper include aphids (*Myzus persicae*, *Aphis ssp*, and *Macrosiphum euphorbiae*), thrips (*Frankliniella sp*), whitefly (*Bemisia tabaci*), mediterranean fruit fly (*Ceratitis capitate*), red spider mites (*Tetranychus spp*) and fruit borers (*Lepidopterae spp*) (Dagnoko *et al.,* 2013). Yield losses due to damage by insect pests on chilli can reach up to 100%, depending on several interacting factors (Messiaen *et al.,* 1991). Apart from direct feeding on plants, some of these insects are vectors of destructive viruses (Kenyon *et al*., 2014).

On the other hand, diseases also have significant effects on hot pepper production (Dagnoko *et al.,* 2013). Bacterial wilt (*Ralstonia solanacearum*), soft rot (*Erwinia carotovora*), phytophthora root rot, anthracnose (*Colletotrichum capsici*); and virus-induced diseases are the most important in hot pepper production (Mekonen and Chala, 2014; Asare-Bediako *et al*., 2015). Losses in yields of up to 90% are documented on hot pepper production due to these diseases (Grube *et al.,* 2000).

In addition, abiotic factors such as low soil fertility, drought, excessive rainfall and salinity have also affected hot pepper production significantly. Bosland and Votava (2000) reported more than 50% yield reductions in pepper due to water shortage and salinity. In addition, lack of or an excess of moisture in the soil has led to the fall of plants' organs in hot pepper (Black *et al.,* 2010). Nutrient deficiency also affects the quality and yield of hot pepper, for instance, calcium deficiency leads to blossom-end rot, a physiological disorder (Hochmuth and Hochmuth, 2009). With the current situation of climate change and increased population, adverse effects due to abiotic constraints are expected especially in vulnerable regions. It is anticipated that the adverse effects of climate change will lead to more serious pest and disease attacks on the crop (Zayan, 2019; Nwaerema, 2020; Skendžic *et al.,* 2021).

2.4. Viruses associated with hot pepper

Viral diseases are key limiting factors of hot pepper production worldwide (Abdalla *et al.*, 1991; Shah *et al.*, 2008). Atleast 68 viruses are detected in peppers in different parts of the world where they are grown (Pernezny *et al.*, 2003). Out of these, 15 viruses in seven genera (*Alfamovirus*, *Begomovirus*, *Cucumovirus*, *Polerovirus*, *Potexvirus*, *Potyvirus*, and *Tobamovirus*) are recorded in Africa (Waweru *et al*., 2019). The viruses include CMV, PeVYV, PVMV, PMMoV, PVY, TMV, *Alfalfa mosaic virus*, *Blackeye cowpea mosaic virus*, *Cowpea aphid borne mosaic virus*, *Potato virus X*, *Pepper mottle virus*, *Tomato yellow leaf curl virus*, *Tobacco etch virus*, *Tomato mosaic virus* and *Tobacco mild green mosaic virus* (Sidaros *et al.*, 2009; Dombrovsky *et al.*, 2010; Njukeng *et al.*, 2013; Aliyu, 2014; Kenyon *et al.*, 2014; Leke *et al.*, 2015; Olawale *et al.*, 2012, 2015). The distribution, transmission, host range and symptoms expression of some of the most important viruses is discussed below.

2.4.1. *Pepper veinal mottle virus*

Pepper veinal mottle virus (genus *Potyvirus*, family *Potyviridae*), was originally described as *PVY* group, however, later it was recognized as a member of potyvirus group (Harrison *et al.*, 1971). In Africa, PVMV was first reported in *C. annum* and *C. frutescens* from Ghana (Brunt and Kenten, 1971) and since then, it has spread to other countries especially in the sub-saharan region (Huguenot *et al.*, 1996; Gorsane *et al.*, 1999; Tsai *et al.*, 2010; Olawale *et al.*, 2012; Njukeng *et al.*, 2013). In East Africa, PVMV is reported in Ethiopia, Kenya, Tanzania, Uganda and Rwanda (Dafalla, 2001; IPM CRSP, 2008; Skelton *et al*., 2018). Although it is mainly reported in Africa, PVMV is also identified in India (Nagaraju and Reddy, 1980), Afghanistan (Lal and Singh, 1988), South Korea (Ha *et al.*, 2008), Taiwan (Cheng *et al.*, 2009) and recently in China (Zhang *et al.*, 2016).

Yield losses of 54.5-64.3% and disease incidence of as high as 100% due to PVMV were recorded in hot pepper in Nigeria (Alegbejo and Abo, 2002; Fajinmi *et al.,* 2012). At least eight species of aphid spread PVMV in a non-persistent way, of which *M. persicae*, *A. craccivora*, *A. spiraecola* and *A. gossypii* are the most efficient vectors in nature (Fajinmi *et al.,* 2011). Mechanical transmission of PVMV has been documented by Moury *et al.* (2004). Several symptoms are associated with PVMV on pepper; mottle, mosaic, curling, vein banding, yellowing, blistering, deformation, ring spots and severe stunting (Fajinmi *et al*.,1998; Tsai *et al*., 2010).

2.4.2. *Potato virus Y*

Potato virus Y (genus *Potyvirus*, family *Potyviridae*), is among the five most economically damaging viruses occurring worldwide. In East Africa, PVY is associated with hot pepper in Zimbabwe, Zambia, Kenya, Tanzania, Malawi, Madagascar and Ethiopia (Haskias *et al*., 1999; Ndunguru and Kapooria, 1999; Dafalla, 2001; Karavina *et al*., 2016). In spite of this, PVY as a virus infecting hot pepper has not received much attention in the region and other parts of Africa. A lot of the research done has focused on PVY strains infecting potato. Therefore, more work is needed on strains of the virus that attack hot pepper. According to Singh *et al*. (2008), strains of PVY from pepper will not infect potato and vice versa. Since the virus is causing substantial losses on hot pepper in the Eastern Africa region (Dafalla, 2001), in-depth studies to identify the pathotypes present, incidence, losses associated with PVY and management within pepper fields are necessary.

According to Avilla *et al*. (1997) and Olawale *et al.* (2020), yield losses resulting from PVY infection on hot pepper ranged between 20 to 70%. The virus is spread non-persistently by several species of aphids and also by mechanical means as demonstrated by Schramn *et al*. (2011). The PVY infects many hosts which include potato, tobacco, pepper, tomato and

several species of weeds (Kerlan and Moury, 2008). Symptoms associated with PVY include mosaic, mottling, dark green vein-banding, vein-clearing, stunting, and the fruits are small and deformed (AVRDC, 2004a).

2.4.3. *Pepper mottle virus*

Pepper mottle virus (genus *Potyvirus*, family *Potyviridae*) has been isolated in several countries namely California (Abdalla *et al.*, 1991), Japan (Ogawa *et al.*, 2003), Taiwan (Cheng *et al.*, 2011), Cuba (Quiñones *et al.*, 2011), India (Kaur *et al.*, 2014) and Cameroon (Njukeng *et al.*, 2013). Disease incidence in the fields can reach up to 100% infection-causing the abandonment of the fields (Green and Kim,1991).

The green peach aphid (*M. persicae*), cowpea aphid (*A. craccivora*) and cotton melon aphid (*A. gosspii*) spread PepMoV in a non-persistent way. The virus is spread more efficiently by the green peach aphid and it is sap-transmissible. *Pepper mottle virus* causes mottle diseases of *capsicum* species and other solanaceous crops. Several crops are host of PepMoV namely bell pepper, tomato, tobacco, ground cherry and night shade. Symptoms caused by PepMov are vein banding, mottling, puckering/crinkled leaves, mild chlorosis, stunting, mottled and deformed fruit. Symptoms are more severe on the foliage and fruit (Quiñones *et al.*, 2011).

2.4.4. *Tobacco etch virus*

Tobacco etch virus (genus *Potyvirus*, family *Potyviridae*) has been identified in Europe, Asia, America and Africa (Buzkan *et al.*, 2015; Olawale *et al.*, 2015). It is spread by >10 aphids' species in non-persistent manner (AVRDC, 2004b). *Myzus persicae* transmit the virus more efficiently compared to other species. *Tobacco etch virus* (TEV) transmission is easy by mechanical means and there is no evidence of spread by seed. The virus infects many species of *Solanaceae* including pepper, tobacco and tomato. It also infects many perennial weeds

including *Solanum nigrum*, *S. aculeatissimum*, *Chenopodium album*, *Datura stramonium*, *Linaria canadensis*, and *Physalis spp.* (https://www.plantwise.org). Typical symptoms on either leaves or fruits include vein clearing, mottling and necrotic lines or etching (AVRDC, 2004b).

2.4.5. *Pepper mild mottle virus*

Pepper mild mottle virus (genus *Tobamovirus*, family *Virgaviridae*) was first described in the USA in 1952 and has since become an important pathogen in other countries where hot pepper is grown (McKinney, 1952). The virus is present in America, Asia and Europe (CABI/EPPO, 2009; Ahmad *et al.*, 2015; Ali and Ali, 2015). The PMMoV is also associated with pepper in some East and West Africa countries (Ndunguru and Kapooria, 1999; Dafalla, 2001; IPM CRSP, 2008; Appiah *et al*., 2014). The PMMoV causes significant damage on the quality of fruits leading to considerable yield losses. For instance, Martínez-Ochoa (2003) observed that the disease incidence of 20 to 80% on hot pepper plants, results in yield losses of 50 to 100%. Similar studies by Guldur and Caglar (2006) reported that the disease incidence of 60 to 95% caused 75 to 95% yield loss.

The spread of PMMoV is through mechanical transimission, and infected seed, debris and soil (Ikegashira *et al*., 2004; Genda *et al*., 2005). *Pepper mild mottle virus* is not transmitted by insects. The major host of this virus is *Capsicum spp*. however, it infects up to 24 species belonging to *Solanaceae* family and other species in *Chenopodiaceae*, *Cucurbitaceae*, *Labiatae* and *Plantaginaceae* through experiments (Wetter, 1984). Various symptoms are associated with PMMoV disease including leaf mottling, puckering, malformations, small and deformed fruits marked by off-coloured sunken areas, and stunted growth (Guldur and Caglar, 2006; Nikolay, 2014). The symptoms are far more pronounced on younger infected plants compared to old infected plants (Sevik, 2011).

2.4.6. *Tobacco mosaic virus*

Tobacco mosaic virus (genus *Tobamovirus*, family *Virgaviridae*) is the first-ever plant virus to be identified globally (Scholthof, 2008). The virus is reported to infect pepper in South America, Asia and African countries and therefore it is spread worldwide (Abdalla *et al.*, 1991; Alishiri *et al.*, 2013; Olawale *et al.*, 2015). In Eastern Africa, the virus is present in Uganda, Tanzania, Zimbabwe, Sudan and Zambia (Ndunguru and Kapooria, 1999; Dafalla, 2001; IPM CRSP, 2008). Heavy yield losses due to this virus have been reported in tobacco, tomato and pepper. According to Chitra *et al*. (2002), the yield losses resulting from TMV infection on bell pepper can reach up to 90%.

Like other tobamoviruses, TMV survives for a long time on infected plant debris (Moury and Verdin, 2012). It is spread mechanically by contact between plants and seed but it is not spread by insect-vectors. *Tobacco mosaic virus* infects over 125 crop species such as tomato and pepper among others (Kumar *et al*., 2011). Leaf mosaic, leaf curling, deformation and stunted growth are symptoms observed on pepper plants (Kumar *et al*., 2011; Pazarlar *et al*., 2013).

2.4.7. *Cucumber mosaic virus*

Cucumber mosaic virus (genus *Cucumovirus*, family *Bromoviridae*) occurs globally in temperate, tropical and sub-tropical parts of the world (Olawale *et al.*, 2012). The CMV is among the most widespread viruses in Africa (Ndunguru and Kapooria, 1999; Dafalla, 2001; IPM CRSP, 2008; Appiah *et al*., 2014; Skelton *et al*., 2018). Rahman *et al*. (2016) observed 10 to 37% yield losses due to CMV infections in hot pepper plants.

The virus is transmitted non-persistently by >80 aphid species; *A. gossypii*, and *M. persicae* being the most efficient vectors (Palukaitis *et al*., 1992). According to Ali and Kobayashi (2010), CMV is also seed-transmitted in hot pepper. The CMV infects over 1200 plant

species from including monocotyledons and dicotyledons (Zitter and Murphy, 2009). Symptoms of CMV on hot pepper plants vary widely but the most common are leaf mosaic, mottling, shoe-string and fern-like leaves, vein banding and clearing, stunting and small fruit (Zitikaite and Samuitien, 2009; Kapoor, *et al*., 2018).

2.4.8. *Pepper vein yellows virus*

Pepper vein yellows virus (genus *Polerovirus*, family *Luteoviridae*) was identified first in Israel by Dombrovsky *et al.* (2010). It is now present in five more African countries i.e. Tunisia (Buzkan *et al*., 2013), Mali (Knierim *et al.,* 2013), Sudan (Alfaro-Fernandez *et al.,* 2014), Ivory Coast (Bolou *et al.,* 2015) and Rwanda (Skelton *et al*., 2018). The PeVYV is also reported in Europe (Villanueva *et al.,* 2013), North America (Alabi *et al.,* 2015) and Asia (Liu *et al.,* 2016). Infection rates of up to 100% are reported in pepper due to PeVYV (Tomassoli *et al.,* 2016).

The spread of PeVYV is through aphids (*A. gossypii* and *M. persicae*) in a circulative and non-propagative manner (Dombrovsky *et al*., 2010; Murakami and Kawano, 2017). Host plants include *Capsicum spp.* and *Solanum nigrum* (Knierim *et al*., 2013; Alabi *et al*., 2015). Vein clearing, curling, deformation, reduced size, puckering, interveinal chlorosis or yellow patches are among the commonly observed symptoms on pepper leaves (Murakami and Kawano, 2017).

2.4.9. *Tomato yellow leaf curl virus*

Tomato yellow leaf curl virus (TYLCV) is mostly common in Africa especially in the West and Central regions, and has been reported to attack hot pepper (Reina *et al.,* 1999; Leke *et al.,* 2015; Adel, 2016). It is spread naturally by whitefly, *Bemisia tabaci* (Rojas *et al.,* 2005). Mechanical transmission of some begomoviruses have been reported, although most of them require Agrobacterium-mediated transfer (agro-inoculation) as reported by Rojas *et al.* (2005). The TYLCV infect mainly dicotyledonous and wild plants. General symptoms associated with the virus are leaf crumple, curl, foliar mosaic, mottle and growth stunting of plants among others (Akhtar *et al.,* 2007).

From the review, a lot has been done on the identification of hot pepper viruses in other countries. However, knowledge of the identity and distribution of hot pepper viruses in Rwanda is not documented. The impact of these viral pathogens on hot pepper quality and yield has not been assessed.

2.5. Methods of detecting viruses associated with hot pepper

Identification of viral diseases by visual observations of the common symptoms is not easy as plants can display the same features when responding to unfavourable environmental conditions, nutritional deficiencies and infection by insect pests (van der Want and Dijkstra, 2006). Hence, various methods geared toward identifying hot pepper viruses have been developed. Indicator plants are commonly used to confirm or detect virus infection especially for sap-transmitted viruses (Eman, 2006; Fajinmi, 2010; Alwabli *et al.,* 2017). However, this method does not identify the actual causative agent of the disease and therefore, serological tests are preferred.

Several serological-based methods have been used in the identification of hot pepper viruses. The enzyme-linked immunosorbent assay (ELISA) is the most frequently applied method to

detect virus infection within plant material, seeds and insect vectors (Naidu and Hughes, 2001; Webster *et al.,* 2004). Specific antiserum against several hot pepper viruses have been developed and used in diverse studies (Fajinmi, 2010; Olawale *et al.,* 2015; Almudena *et al.,* 2016). The advantages of using ELISA include; the process can be semi-automated, a large number of samples can be analysed at once and only little amount of antibody is required. In addition, it is somehow safe and eco-friendly as radioactive substances are not required (Naidu and Hughes, 2001). However, ELISA tests are labor-intensive, the cost of producing antibody is high, require large volume of sample, and it takes a long time to complete ELISAs.

Tissue blot immunoassay (TBIA) method was reported for identification of PMMoV and PepMoV (Eman, 2006; Han *et al.,* 2007). Unlike conventional ELISA, extraction of the samples in TBIA is not required and thus, tubes/containers to store extracts are not needed. Furthermore, loading of the sample to the membrane is precise and does not require other loading devices (Rocha-Pena and Lee, 1991; Lin *et al.,* 2000). Immunodiffusion method have been used to identify TMV, ToMV and PMMoV (Wetter, 1984). Though this method is simple and economical, it is relatively insensitive and mainly used for identification of viral antibodies in persistent viral diseases as the antigens are constantly present. Thus, samples showing positive reaction are recommended for re-testing to validate or confirm the results (Kibenge *et al.,* 2016).

Dot blot immunoassay have been used for the detection of PepMoV (Eman, 2006). The advantage of this method over other complex blotting is that procedures for the gel are not required and therefore saves on time. The weakness is that it does indicate the size of the targeted protein. Immunofluorescence have also been used for the detection PMMoV (Genda *et al.,* 2005) while direct immunostaining assay have been used for detecting TMV, PMMoV, TMGV and ToMV in seeds.

Molecular based methods are commonly used due to their high accuracy, sensitivity and reliability compared to serological methods. Identification of hot pepper viruses by conventional PCR and RT-PCR have been achieved at different taxonomic levels depending on the specificity of the primers used (Fajinmi, 2010; Cheng *et al.*, 2011; Almudena *et al.*, 2016; Alwabli *et al.*, 2017). On the other hand, multiplex PCR has been applied to identify ToMV and TMV in pepper (Vinayarani *et al.,* 2011) and in differentiating PVY strains in mixed infections (Lorenzen *et al.,* 2006). Recently, RT-loop-mediated isothermal amplification assay was applied to identify PeVYV. Other methods include immuno-capture polymerase chain reaction used to identify PMMoV, dot-blot hybridization to detect PepMoV (Eman, 2006), and Bougatef *et al.* (2005) used nested PCR to improve detection of PVY in pepper. The PCR methods are sensitive and highly accurate but limited in identification of virus species and strains from cross-reacting viruses and thus, the need for sequencing to reveal the genome of the specific virus or strain. In the present study, some of these techniques were used in combination to confirm and identify viruses associated with hot pepper in Rwanda. These include ELISA and RT-PCR used to screen samples collected from farmers and experimental fields followed by sequencing.

2.6. Insect-vectors of viruses associated with hot pepper

Most of the plant viruses are dependent on vectors for their transmission and survival (Fereres and Raccah, 2015). Previously reported vectors of hot pepper viruses include aphids, whiteflies and thrips belonging to the orders *Hemiptera* and *Thysanoptera* and that possess pierce-sucking mouthparts. The most economically important virus vectors are Hemipterans that are known to transmit more than 70% of the insect-borne viruses (Fereres and Raccah, 2015). Transmission of viruses by insects is classified into two categories depending on; the time the vector remains viruliferous or the route of the virus within its vector (Brault *et al.*,

2010). First category includes persistent, semi-persistent or non-persistent transmitted viruses while the second category comprise of non-circulative or circulative transmitted viruses.

Aphids, whitefly and thrips are insects belonging to the family *Aphididae*, *Aleyrodidae* and *Thripidae*, respectively. These vectors mainly occur in warm climates where they are pests of herbaceous plants. In temperate climate they are usually pests in protected environments such as greenhouses. Aphids typically feed on young leaves and shoots while whiteflies and thrips feed on leaves (Dagnoko *et al.*, 2013). The structure of their mouthparts, searching behaviour for host plants, the wide host range and high reproductive rates contribute to the efficiency of the insects as virus carriers.

Aphids are the most insect-vectors associated with hot pepper viruses belonging to the genera *Potyvirus*, *Alfamovirus*, *Cucumovirus*, and *Polerovirus*. Majority of viruses attacking pepper in Africa including PVMV, PVY, PepMoV, TEV, CABMV, CMV and AMV are transmitted non-persistently by aphids while PeVYV is persistently transmitted (Dombrovsky *et al.*, 2010; Aliyu, 2014). Conversely, whitefly-transmitted pepper virus reported in Africa include TYLCV, genus *Begomovirus* while thrip-transmitted-pepper virus include *Tomato spotted wilt virus* (TSWV), genus *Tospovirus* (Orosz, 2012; Leke *et al.*, 2015).

Aphids are the main pathway for plant virus spread and account for the transmission of two-third of insect-vectored plant viruses (Brault *et al*., 2010). The species of aphid are in the genera *Aphis*, *Myzus* and *Macrosiphum*. However, the majority of the aphid species mainly associated with virus transmission in hot pepper are found in the genus *Aphis* (e.g. *A. craccivora, A. gossypii, A. fabae, A. spiraecola*) and *Myzus* (*M. persicae*) (Pernezny *et al.*, 2003; Dombrovsky *et al.*, 2010; Fajinmi *et al.*, 2011; Murakami and Kawano, 2017). The efficiency of virus transmission depends on aphid species, biotypes within a species, aphid life stages, virus strains and isolates as well as host plants (Mello *et al*., 2011). Conversely, the population of aphids, presence of inoculum and the period over which host plants are

exposed to insect-vectors might also influence plant infection by the viruses (Difonzo *et al.*, 1996).

From the reviewed literature, it is evident that majority (>50%) of the hot pepper viruses encountered in Africa are transmitted by aphids and therefore the present study mainly focused on aphids as vectors of hot pepper viruses. There is limited information on aphid species associated with hot pepper fields and their management in Rwanda.

2.7. Management of viral diseases in hot pepper

Virus induced-diseases cause significant losses in hot pepper especially in warm regions (tropical and semi-tropical) which provide favorable conditions for the reproduction of vectors and spread viruses. Several strategies have been applied to control viral diseases and minimize their losses. However, host plant resistance and control of vector populations are the two main methods used in the management of plant viruses (Brault *et al.,* 2010).

2.7.1. Management of vectors associated with hot pepper

Both cultural practices and insecticides have been used for the management of vectors. Cultural methods used include crop sanitation, the use of reflective mulches, the use of barrier crops and manipulation of planting density. Degri and Ayuba (2016) intercropped pepper with maize and millet leading to a significant reduction in aphids' infestation, improved growth and enhanced yields of hot pepper. In addition, Karungi *et al.* (2013) demonstrated that the use of transparent plastic mulch led to 43% reductions in occurrence of whiteflies while close plant spacing reduced by 36% in hot pepper. Similar studies have also been demonstrated outside Africa for control of CMV and PVY in hot pepper (Fereres, 2000). The use of mulches, particularly reflective mulches intended to reduce landing rates of

flying insects such as aphids and to delay the incidence of viruses has been extensively evaluated (Kumar and Poehling, 2006).

Other practices are field sanitation, rouging of infected crops, using disease-free planting materials, weeding, early planting and integrated pest control measures (Alegbejo, 2002). Virus-free seed are used as an effective way of controlling the effects of seed transmitted viruses (Wang, 2006). Integrating cultural-control techniques has the potential to reduce insect pests and resulting transmission of viruses. For example, the use of barrier crops with other pest-control methods.

Application of insecticides to control vectors in peppers has considerably increased over recent years in many countries in an attempt to control viral diseases associated with these vectors (Kenyon *et al.,* 2014). Several studies on the use of insecticides to control insect vectors have been conducted. For example, Faniqliulo *et al.* (2014) reported that a combination of Acibenzolar-S-Methyl and Cyantraniliprole allowed the best control of TSWV transmission by thrips. The bio-pesticides also have a role to play in the control of the vectors (Pandey *et al.,* 2010). For instance, neem seed kernel extract was used to manage whitefly populations resulting in reduced incidences of leaf curl disease in hot pepper (Pandey *et al.,* 2010).

2.7.2. Host plant resistance

Use of resistant varieties is one of the best means for controlling pests and diseases (Byoung-Cheorl *et al.,* 2005; Duveiller *et al.,* 2007). Many virus-resistant hot pepper genotypes have been developed and released. For instance, thirteen parental and nine cross lines of hot pepper from India were found to be resistant to PepMoV, TEV, PVY and CMV (Shashikumar and Madhavi, 2017). Soler *et al.* (2015) found resistance to TSWV in one accession from *C. baccatum*. Three varieties of hot pepper 'GKC29', 'BS-35' and 'Bhut Jolokia' were reported to have resistance to *Pepper leaf curl virus* (Kumar *et al.,* 2006). The resistance of hot pepper lines against *Chilli veinal mottle virus* was demonstrated in Pakistan where six exotic lines were found resistant (Shah *et al.,* 2011). Ashfaq *et al.* (2014) reported nine lines of hot pepper as highly resistant to CMV. The use of resistant varieties is the most effective approach of controlling vectored plant viruses however, there is limited information on hot pepper genotypes resistance to virus or aphids in Rwanda which may be of great importance to the management of the diseases and pests in the field.

CHAPTER THREE:

FARMERS' KNOWLEDGE AND PERCEPTIONS OF VIRAL DISEASES AFFECTING HOT PEPPER AND THEIR MANAGEMENT

Abstract

Increased hot pepper productivity can only be achieved after addressing factors that challenge its production. The objective of this study was to reveal the farmers' knowledge and perceptions of virus diseases and their management in Rwanda. A survey was conducted in major hot pepper growing areas between February and March 2018 covering low, mid and high-altitude agro-ecological zones (AEZs). A structured questionnaire was used to collect data from 101 respondents and analysed by descriptive statistics. Pests and diseases were the main challenges to hot pepper production as indicated by majority of farmers (86.1%) of which key among them are viral diseases, aphids and whiteflies. Farmers' awareness of viral diseases varied significantly ($\chi 2 = 20.116$; $p < 0.001$) across the AEZs as well as knowledge on causes of the viral diseases ($\chi 2 = 26.896$; $p = 0.003$). Majority of the farmers from the mid-altitude AEZ were not aware of the viral diseases. Only 17.8 and 25.7% of the farmers correctly linked the cause of the viral diseases to insect vectors and the use of infected seeds, respectively. Training among farmers on pepper production also differed significantly ($\chi 2 = 12.671$; $p = 0.002$) among the AEZs with low-altitude having the highest number of farmers who did not receive any training. Generally, knowledge of viral diseases and their management was lacking in two-thirds of the farmers across all AEZs. Training ($\chi^2 = 29.205$; $p < 0.001$) and age ($\chi^2 = 10.421$; $p = 0.005$) strongly influenced farmers' awareness of viral diseases. Awareness creation on viral diseases and integrated disease management through farm-level training is needed. Our findings provide fundamental information for designing long-term management options for virus-induced diseases of hot pepper in Rwanda.

3.1. Introduction

Hot pepper is one of the most promising horticultural commodities in Rwanda and is among the crops prioritized by the government for export diversification (MINAGRI, 2014). The crop is mainly cultivated for local consumption, income generation, export and processing industries. Rwanda is the third producer in the East African region and is ranked 19th in Africa producing 5,009 tons of green pepper in 2018 (FAOSTAT, 2019). In 2017/2018, the contribution of hot pepper to the total revenue from export of vegetables in Rwanda was estimated at 4.5% (NISR, 2018).

Over the years, the production of hot pepper has increased in Rwanda from 2,600 tons in 2008 to 5,009 tons in 2018 (FAOSTAT, 2019). Despite the increase in production, farmers have been recording low yield compared to other leading Africa countries such as Egypt which produced 713, 752 tons in 2018 (FAOSTAT, 2019). The average yield of 10 t ha^{-1} in the last five years, is below the country's potential of 15 t ha^{-1} (RDB, 2010; FAOSTAT, 2019). Consequently, local production fails to meet the domestic market demand. This gap in yield might be due to several biotic and abiotic constraints. According to Melesse *et al*. (2014), identification of sources of risks and their management plays a crucial role in achieving sound and sustainable production of vegetables.

There is strong evidence that diseases and pests are becoming increasingly important as limitations to the production of hot pepper in Rwanda (Olawale *et al*., 2012). Aphids, whiteflies, thrips, mealybugs, fruit borers among others are the significant insect pests, attacking hot pepper at stages of growth (Bugti *et al*., 2014; Djieto-Lordon *et al*., 2014). Among the diseases, bacterial wilt, soft rot, phytophthora root rot, anthracnose and virus-induced diseases are the most challenging in hot pepper production (Mekonen and Chala, 2014; Asare-Bediako *et al*., 2015). The wide range of diseases and pests documented on hot pepper raises concerns and calls for the development of sustainable pest management

strategies. Farmers' indigenous knowledge can play a major role in attaining adequate interventions and sustainable management.

Several studies on farmers' knowledge and perceptions of diseases and pests, and their control in vegetables have been done in Cameroon, India and Japan (Nagaraju *et al*., 2002; Oo *et al.*, 2012; Abang *et al*., 2014). Previous study by Skelton *et al*. (2018) identified some viruses affecting the production of hot pepper in Rwanda. However, there is limited information on perceived constraints, farmers' perception and knowledge of hot pepper pests and diseases. Building capacity among farmers is one of vital strategy for management of viral diseases, with understanding of the current status of farmers' knowledge being the first step. This study aimed at assessing farmers' knowledge and perceptions of virus diseases, causes and applied control practices in hot pepper. This information will be important in developing an effective management strategy for hot pepper viral diseases.

3.2. Materials and methods

Study sites: The survey was conducted in high, mid and low-altitude AEZs covering seven main hot pepper-producing areas in Rwanda (EU, 2015), from February to March 2018 during the long rain season. The geographical location of the surveyed areas in Rwanda is shown in Fig 3.1.

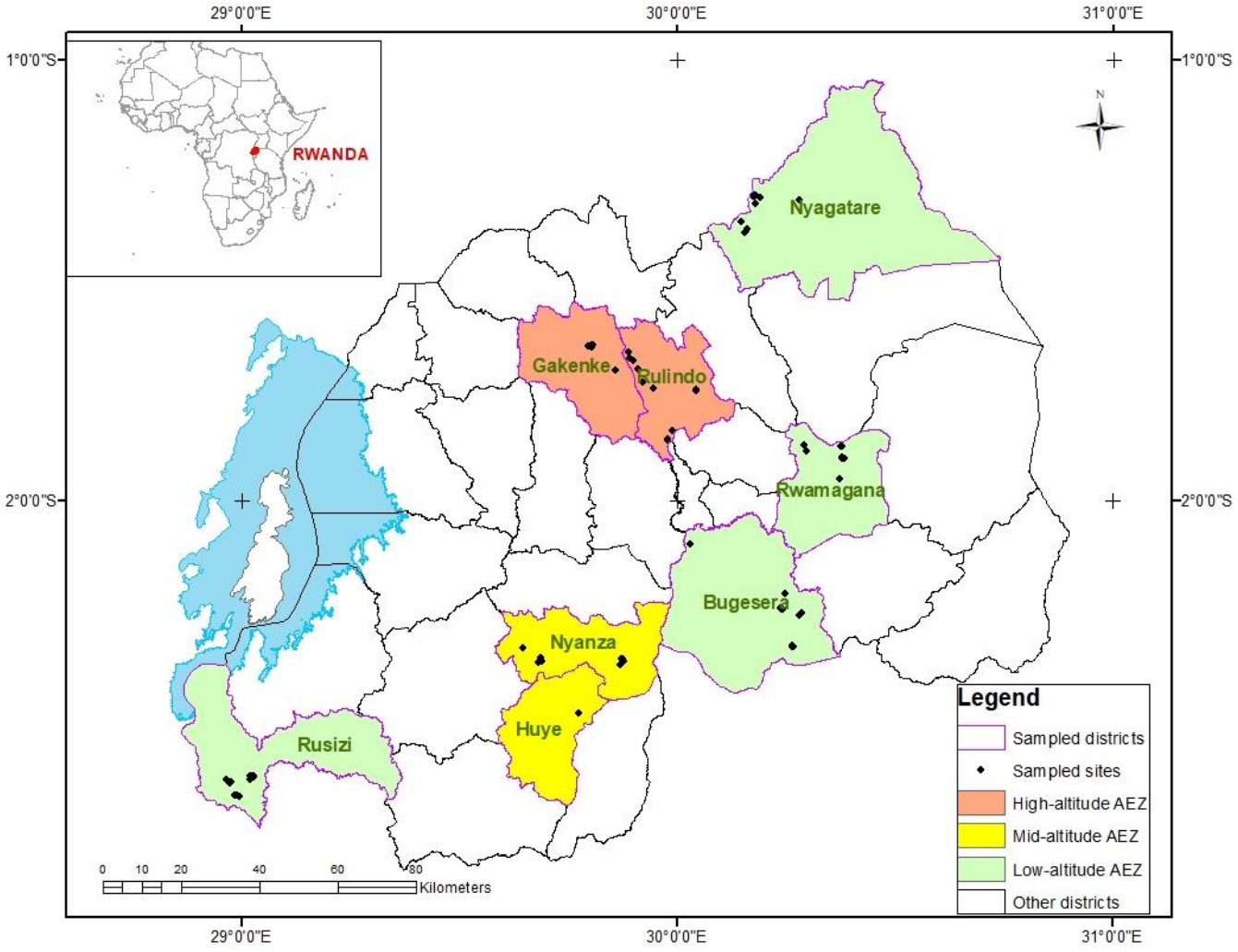

Figure 3.1. A map of Rwanda showing the geographic location of the areas where the survey was carried out in three agro-ecological zones.

Sampling procedure and interviews: A multiple-stage sampling technique was applied to choose the farmers for the survey with the AEZs as the strata. In the first stage, the districts to be surveyed within the AEZs were chosen based on the intensity of hot pepper production. In the second stage, at least two sectors were purposively selected based on the number of farmers involved in the production of hot pepper from each district. A sector is an administrative entity that is made up of several villages. Simple random sampling was used in the last stage to select 10% of the total farmers (230 in high, 140 in mid and 640 farmers in low-altitude zone) involved in hot pepper production in each of the selected sector

(Mohammed, 2016). The selection was done in consultation with the sector agronomists. In total, 101 hot pepper farmers were interviewed and distributed as 23 in high, 14 in the mid and 64 in low-altitude AEZ, depending on the proportion of farmers involved in hot pepper production across the regions. The low-altitude AEZ has the highest number of farmers involved in pepper production. Face to face interviews with the individual farmer were carried out by three enumerators in local language.

Information on demographic characteristics of households including gender, age, the experience in hot pepper farming and training; farm characteristics and production systems including land owned, the area under hot pepper production, varieties grown, input usage, source of planting materials and type of cropping systems; constraints encountered by farmers' in hot pepper production; and farmers' perception and knowledge of viral diseases, causes and management practices were collected using a questionnaire that contained open-ended and closed questions (Appendix 1). The questionnaire was pretested with ten farmers and revised. Printed colour photographs of virus-infected plants and other major diseases of hot pepper were shown to farmers to assist in the identification.

Data analysis: The data recorded in the questionnaire were coded and entered into an excel spreadsheet and later transferred to Statistical Package for the Social Sciences (SPSS version 16) program for descriptive and correlation analysis. Cross tabulations were used to determine the relationships among variables in the three AEZs. Pearson chi-square was used to test for differences in variables across the three AEZs. Correlations and relationships between farmers' knowledge of viral diseases and the independent variables (gender, age, farming experience, the area under hot pepper production and training) were conducted using Cramer's V test.

3.3. Results

3.3.1. Demographic characteristics of the interviewed hot pepper farmers

The majority of the farmers who were interviewed were males at an average of 80.2% (Table 3.1). A quarter of the farmers were 35 years and below, 72% between 36-65 years while 3% were 66 years and above with an overall mean of 44.7 years. The years of experience in hot pepper farming did not significantly vary ($\chi 2 = 7.775$; $p = 0.255$) across the AEZs (Table 3.1). Majority of the farmers had between 1.1 to 5 years' experience in hot pepper farming. Coverage of training on pepper production also differed significantly ($\chi 2 = 12.671$; $p = 0.002$) across the AEZs with highest number of farmers who did not receive any training found in low-altitude zone. Only 19% of the farmers from all AEZs had been trained or had access to extension information regarding agronomic practices and management of diseases and pests, mainly from extension officers, exporting companies and school/colleges.

Table 3.1. Percentage of hot pepper farmers interviewed in three agro-ecological zones in Rwanda and their demographic characteristics in February to March 2018

Variable	High altitude	Mid altitude	Low altitude	Overall mean	Chi-square test	P-value
Gender						
Male	87	78.6	78.1	80.2	0.858	0.651
Female	13	21.4	21.9	19.8		
Age of the respondents						
≤ 35	34.8	25	20.7	24.7	2.339	0.674
36-65	60.9	75	75.9	72		
≥ 66	4.3	0	3.4	3.2		
Years of experience						
≤ 1 year	50.0	58.3	23.3	37.0	7.775	0.255
1.1-5 year	41.7	33.3	66.7	53.7		
5.1-10	0.0	8.3	6.7	5.6		
≥ 10.1	8.3	0.0	3.3	3.7		
Training on pepper production						
Yes	43.5	23.1	9.5	19.2	12.671	0.002
No	56.5	76.9	90.5	80.8		

3.3.2. Constraints experienced by farmers in the production of hot pepper

Across all the AEZs, majority (86.1%) of the farmers ranked diseases and insect pests as their number-one problem in hot pepper production followed by lack of technical knowledge (38.6%), unfavourable weather conditions (37.6%), unstable markets (30.7%), lack of credit facilities (16.8%) and high cost of inputs (13.9%) among others (Table 3.2). However, the importance of these constraints varied across the AEZs (Table 3.2). In the high-altitude zone, the top five constraints were the diseases and insect pests (73.9%), unpredictable weather conditions (30.4%), inadequate technical knowledge (26.1%), unstable markets (17.3%) and inadequate capital or lack of credit facilities (13%). In the mid-altitude AEZ, all (100%) farmer respondents reported that pests and diseases were the major constraint followed by the high cost of inputs (42.9%), unstable markets (42.9%), inadequate technical knowledge (35.7%) and lack of quality seeds (28.6%). On the other hand, diseases and insect pests (84.8%), unpredictable weather conditions (45.5%), inadequate technical knowledge (42.4%), unstable market (31.8%) and inadequate capital or lack of credit facilities (19.7%) were the leading constraints mentioned by farmers from the low-altitude areas.

3.3.3. Farmers' perceptions of viral diseases associated with hot pepper

Awareness of viral diseases was at 33% among the hot pepper farmers (Table 3.3). However, farmers' awareness of viral diseases varied significantly ($\chi 2 = 20.116$; $p = <0.001$) across the AEZs. The majority of the farmers from the mid-altitude AEZ were aware of the viral diseases. Viral diseases were regarded as the most serious across the three AEZs by 71.9% of the farmers followed by fungal diseases as reported by 22.8% of the farmer respondents and lastly bacterial diseases by 5.3% (Table 3.3). Concerning the stage of growth at which farmers observed viral symptoms, about 40% reported flowering and fruiting stage,

respectively, followed by vegetative stage (16.5%) and the least was at the seedling stage (3%). These farmer proportions differed ($\chi 2 = 18.833$; $p = <0.016$) across the AEZs.

Table 3.2. Percentage of farmers in three agro-ecological zones in Rwanda who stated various constraints to production of hot pepper in February to March 2018

Constraints*	High altitude	Mid altitude	Low altitude	Overall mean
Pests and diseases	73.9	100	84.8	86.1
Inadequate technical knowledge	26.1	35.7	42.4	38.6
Unpredictable weather conditions	30.4	7.1	45.5	37.6
Unstable market	17.3	42.9	31.8	30.7
Inadequate capital/lack of credit facilities	13	7.1	19.7	16.8
High cost of inputs	8.7	42.9	9.1	13.9
Price fluctuations	0	14.2	16.7	12.9
Lack of quality seeds	8.7	28.6	4.5	8.9
Lack of postharvest facilities	0	0	12.1	7.9
Shortage of land	4.3	0	9.1	6.9
Delayed payment by exporting companies	0	7.1	9.1	6.9
Expensive irrigation facilities	4.3	0	7.6	5.9
Low yields of local varieties	4.3	7.1	1.5	3.0
Poor soil conditions	0	7.1	0	1.0
Lack of extension services	0	7.1	0	1.0
Difficulties in irrigation due to land topography	0	0	1.5	1.0

*Multiple responses

Table 3.3. Percentage of farmers in three agro-ecological zones in Rwanda and their perception of diseases affecting production of hot pepper in February to March 2018

Variable	High altitude	Mid altitude	Low altitude	Overall mean	Chi-square test	P value
Farmers awareness of viral diseases						
Yes	43.5	83.3	19.4	33	20.116	<0.001
No	56.5	16.7	80.6	67		
Observed diseases by farmers*						
Fungal diseases	21.7	21.4	28.1	22.8	1.694	0.792
Bacterial diseases	13	7.1	3.1	5.3		
Viral diseases	78.3	85.7	81.3	71.9		
Growth stage at which symptoms of viral disease are seen						
Seedling	5.9	0	2.2	3	18.833	0.016
Vegetative	11.8	80	11.1	16.5		
Flowering	52.9	20	37.8	40.3		
Fruiting	29.4	0	48.9	40.3		

*Multiple responses

3.3.4. Farmers perception about sources or causes of viral diseases associated with hot pepper

The farmers' perception about sources or causes of the viral diseases varied significantly among the AEZs ($\chi 2 = 26.896$; $p = 0.003$). About a quarter (25.7%) and slightly below a fifth (17.8%) of the respondents were able to correctly link the viral diseases to infected seed and insect-vectors, respectively (Table 3.4). In contrast, about a third of the farmers thought that the viral diseases were caused by bad weather and/or poor soils, respectively while (23.8%) did not know the cause at all.

Table 3.4. Percentage of farmers in three agro-ecological zones in Rwanda and their knowledge about sources or causes of virus infections of hot pepper in February to March 2018

Sources or causes of infection*	High altitude	Mid altitude	Low altitude	Overall mean	Chi-square test	P value
Infected seed	30.4	42.9	19.7	25.7	26.896	0.003
Insect vectors	30.4	7.1	15.2	17.8		
Bad weather	30.4	7.1	42.4	35.6		
Poor soils	21.7	50.0	33.3	33.7		
Do not know	30.4	0.0	25.8	23.8		

*Multiple responses

3.3.5. Farmers' knowledge of insect pests infesting hot pepper

Among the arthropod pests infesting hot pepper, the aphids were the most serious insect across the AEZs reported by slightly above half (51.4%) of the farmers (Table 3.5). The whiteflies followed and were reported by 12.9% respondents while the mites (2%) and thrips (2%) were ranked third. Forty-per cent of the farmers did not know that insects infest hot pepper. Farmers' perceptions of insect pests infesting hot pepper did not vary ($\chi 2 = 13.641$; $p = 0.190$) across the AEZs but the management of insect pests differed significantly ($\chi 2 = 16.913$; $p = <0.001$) across the AEZs (Table 3.5). All the farmers from the mid-altitude AEZ engaged in the management of the insects followed by high-altitude AEZ (65.2%) and the least were farmers from low-altitude AEZ (40.3%). The main method used to control insects by the majority of the farmers (82.5%) was insecticides namely cypermethrin, endosulfan and profenofos 40% + cypermethrin 4%. A few (8.8%) of the farmers used cultural practices and traditional products, respectively to control insect pests. Cultural practices included crop rotation, mulching and the use of border crops such as tobacco and sunflower to control insects from hot pepper plants.

Table 3.5. Percentage of farmers in three agro-ecological zones in Rwanda who stated various insect pests associated with hot pepper and their management in February to March 2018

Variable	High altitude	Mid altitude	Low altitude	Overall mean	Chi-square test	P value
Insects observed in fields*						
Aphids	56.5	78.6	43.8	51.4	13.641	0.190
Whiteflies	13	35.7	7.8	12.9		
Broad mites	4.3	7.1	0	2		
Thrips	0	7.1	1.6	2		
Do not know	34.8	7.1	50	40.6		
Do you control insects						
Yes	65.2	100	40.3	54.1	16.913	<0.001
No	34.8	0	63.3	45.9		
Type of control used for insects						
Insecticides	60.9	92.9	32.8	82.5	6.488	0.166
Cultural practices	4.3	0	4.7	8.8		
Traditional products	4.3	0	6.3	8.8		

*Multiple responses

3.3.6. Farmers' perceived yield losses in hot pepper due to viral diseases

Most of the farmers (95.3%) were aware that viral diseases could cause yield losses. The farmer perceptions of yield losses across the three AEZs did not vary significantly ($\chi 2$ = 4.406; p = 0.110). About one-fifth of the farmers estimated yield losses of less than 25% while 39.2, 17.7 and 22.8% of the farmers estimated 25-50, 50-75 and more than 75% yield losses, respectively (Table 3.6).

Table 3.6. Percentage of farmers in three agro-ecological zones in Rwanda stating the expected yield losses due to virus-induced diseases in February to March 2018

Variable	High altitude	Mid altitude	Low altitude	Overall mean	Chi-square test	P value
Do you expect to lose yields due to viral diseases						
Yes	94.4	84.6	98.2	95.3	4.406	0.110
No	45.6	15.4	1.8	4.7		
Expected yield losses by farmers						
< 25 %	12.5	36.4	19.2	20.3	11.846	0.065
25-50 %	43.8	9.1	44.2	39.2		
50-75 %	31.2	36.4	9.6	17.7		
>75 %	12.5	18.2	26.9	22.8		

3.3.7. Farmers options for managing viral diseases on hot pepper

The management options used by farmers varied widely ($\chi 2 = 35.135$; $p < 0.001$) across the AEZs. The farmers from the three AEZs relied mainly on synthetic pesticides to control viral diseases (Table 3.7). Application of pesticides was markedly higher in the mid-altitude AEZ compared to other zones while rouging of virus-infected plants was mainly practised in the low-altitude areas. Overall, the most common option used to manage viral diseases was spraying pesticides (fungicides and insecticides) reported by 36.6% of the farmers. The commonly used fungicides were Copper oxychloride 50% WP, and metalaxyl-M 4% w/w and mancozeb 64% w/w that were used erroneously to target viral diseases. Other methods included cultural control practices such as rouging of diseased plants used by 24.8% of the farmers, field sanitation by 8.9%, crop rotation by 2%, the use of quality seeds by 2% and the least was planting of different varieties of hot pepper by 1% of the farmers.

Table 3.7. Percentage of farmers in three agro-ecological zones in Rwanda stating various options they used to manage viral diseases on hot pepper in February to March 2018

Control strategy[1]	High altitude	Mid altitude	Low altitude	Overall mean	Chi-square test	P value
Spraying pesticides[2]	47.8	92.9	20.3	36.6	35.135	<0.001
Rouging of infected plants	0	21.4	34.4	24.8		
Crop rotation	0	0	3.1	2		
Field sanitation	4.3	7.1	10.9	8.9		
Use of quality seeds	0	7.1	1.6	2		
Use of different varieties	0	7.1	0	1		
Did nothing	47.8	7.1	48.4	42.6		

[1]Multiple responses; [2]Some of the pesticides used by farmers were not appropriate e.g. fungicides

3.3.8. Farm characteristics and production systems

Hot pepper farming is dominated (96%) by small-scale farmers who owned 0.405 to 2 ha of land under pepper production while a few (4%) owned 2.1 to 5 ha (Table 3.8). The cropping systems ($\chi 2 = 20.235$; $p < 0.001$) and source of planting materials ($\chi 2 = 20.032$; $p = 0.010$) varied across the AEZs. Intercropping was practised by 55% while mono-cropping was done by 45% of the farmers. The main crops intercropped with hot pepper included banana (*Musa* spp.), coffee (*Coffea arabica*) and arrowroots (*Colocasia esculenta*). Commonly grown varieties of hot pepper included hybrids of the Bird-eye (62%), Scotch bonnet (36%) and Long cayenne (2%) (Fig. 3.2). Slightly over a half (56%) of the farmers obtained their seeds from export companies that contracted them and about a third (34%) got seeds from their neighbours (34%) (Table 3.8). A small percentage of the farmers sourced seeds from their farms (6%) and agro-dealers (4%).

Table 3.8. Percentage of farmers in three agro-ecological zones in Rwanda and characteristics of their hot pepper farms in February to March 2018

Variable	High altitude	Mid altitude	Low altitude	Overall Mean	Chi-square test	P value
Area under hot pepper (ha)						
0.405-2	100	85.7	96.9	96	4.99	0.820
2.1-5	0	14.3	3.1	4		
Cropping systems						
Mono-cropping	34.8	100	35.9	44.6	20.235	<0.001
Intercropping	65.2	0	64.1	55.4		
Source of seeds						
Own field	18.2	0.0	3.1	6	20.032	0.010
Neighbour	36.4	57.1	28.1	34		
Agro-dealer	9.1	7.1	1.6	4.0		
Export companies	36.4	57.1	67.2	56		

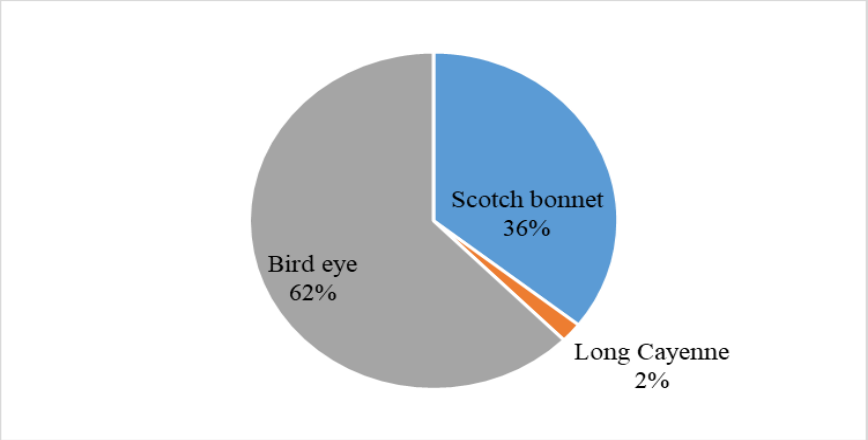

Figure 3.2. Percentage of farmers in three agro-ecological zones in Rwanda stating the commonly grown hot pepper varieties in February to March 2018

3.3.9. Factors influencing farmers' knowledge of virus diseases in hot pepper

Five factors which are gender, age of the farmer, the area under hot pepper production, the experience of the farmer, and training were evaluated. Training (χ^2 = 29.205; p <0.001) and age of the farmer (χ^2 = 10.421; p = 0.005) significantly influenced farmers' awareness of viral diseases. Cramer's V test showed a strong positive association (0.552) between training and farmers awareness of viral diseases in hot pepper. Conversely, the other three factors namely gender (χ2 = 1.159; Cramer's V = 0.109), the area under hot pepper production (χ2 = 3.331; Cramer's V = 0.185) and the farmer experience (χ2 = 0.982; Cramer's V = 0.136) correlated positively with farmers' awareness of viral diseases. However, the relationships were not significant.

Table 3.9. Factors influencing farmers' knowledge and perceptions of viral diseases in hot pepper in Rwanda during a survey carried out in February to March 2018

Variable	χ2*	P value	Cramer V test
Age of farmer	10.421	0.005	0.340
Gender	1.159	0.282	0.109
Area under hot pepper	3.331	0.068	0.185
Training	29.205	< 0.001	0.552
Farmer experience	0.982	0.806	0.136

*Pearson chi-square

3.4. Discussion

The survey results revealed that the diseases and pests are the major challenges faced by hot pepper producers in the country. One of the reasons for the increased disease and pest pressure could be climate change (Nwaerema, 2020). Moist and warm climates favour the development of most pests and diseases (Abang *et al*., 2014). Another reason is the poor pest management due to inadequate farmers' technical knowhow and the high cost of inputs. Poor understanding and management of pests leads to increased incidences of diseases and pests. Indeed, inadequate technical know-how and high cost of inputs were among the top five major constraints mentioned by the farmers. A similar survey reported by Musebe *et al.* (2017) revealed that insect diseases and pests, coupled with lack of high-quality seeds and the high cost of inputs were the main challenges that led to low and unstable yields in the production of vegetables in Rwanda. Diseases and pests remain a major challenge in hot pepper production not only in Rwanda but also in other producing countries such as Nigeria and Ghana (Mohammed, 2016; Orobiyi *et al*., 2013). The diseases and pests cause economic problems to the farmers and therefore, there is a necessity to develop sustainable management strategies.

Among the diseases, virus-induced diseases are serious hindrances to hot pepper farming as perceived by farmers. Two-fifths of the farmers used uncertified planting materials from own fields, neighbours and local markets. Locally, the exchange of planting materials is uncontrolled and the seed system is informal. These might have contributed towards the spread of the virus-induced diseases (RADA, 2002; HCA, 2012). Besides, aphids, whiteflies and thrips were the most recurrent insect pests across the three AEZs. These insect pests are vectors of devastating viruses (Meyer, 2003; Niranjanadevi *et al*., 2018;) and therefore, contribute to wide spread of most of the viral diseases. A previous study by Schreinemachers *et al.* (2015) reported virus diseases as the major constraint to pepper production in Tamil

Nadu, India. The perceived yield losses due to viral diseases estimated to range from 25 to >75% by the interviewed farmers is important and reveal the necessity to implement effective viral diseases management program in hot pepper fields of Rwanda.

Even though the farmers could identify virus symptoms based on leaf crinkling and curling, only a minority correctly linked insect vectors and infected seed in the transimission of hot pepper viruses. This might be attributed to the inaccessibility of accurate information. As reported in this study and the work of Abang *et al*. (2014), the majority of the farmers relied mainly on farmer-to- farmer interactions for information. Besides, four-fifths of the farmers had not received formal agricultural training leading to limited farmers' knowledge of pathogens involved, spread and management of the diseases across the AEZs. This concurs with Schreinemachers *et al*. (2015) findings that knowledge of the cause, spread and management of virus diseases was limited among farmers. For instance, only 8 and 18% of the interviewed farmers could identify the cause of virus diseases symptoms in chilli from Thailand and Vietnam, respectively (Schreinemachers *et al*., 2015). Also, most of the farmers had less than 5 years' of experience in hot pepper farming. According to Nagaraju *et al.* (2002), farmers can also get informed through vast experience in farming. The farmers from the mid-altitude region generally had more knowledge of plant viruses than those from the high and the low-altitude zones, depending on how extension services and the export companies contracting the farmers had paid attention to this issue.

Two-fifths of the farmers relied on pesticides for management of viral diseases. They mixed various pesticides including fungicides and insecticides in single sprays. Fungicides were used erroneously to target viral diseases, which indicated inadequate farmers' knowledge of plant viruses and the need for training. The findings are similar to Schreinemachers *et al*. (2015) who found that majority of the chilli farmers applied fungicides for viral diseases control. Use of pesticide in the low-altitude zone was markedly lower compared to the mid

and the high-altitude zones. This was driven by the international market demands, as the majority of farmers from the low-altitude AEZ had been restricted from using pesticides by the export companies. The second commonly used management option was roguing of infected plants and burying especially in the low-altitude areas. Hoque *et al*. (2003) demonstrated effectiveness of roguing in the management of Jute leaf mosaic disease. Other cultural options used included field sanitation by regularly weeding, the use of quality seeds, crop rotation with unrelated crops and planting of different varieties in order of importance. These cultural practices are effective in reducing the initial level of inoculum and the rate of spread of the diseases and therefore, farmers should be encouraged to make use of them in combination with other management options (Dale and Ogle, 1997; Thresh, 2003a).

The management of insect pests was mainly by the use of insecticides. However, continuous use of the insecticides leads to the development of resistance by the insects in addition to health and environment risks (Kenyon *et al*., 2014). This calls for a need to develop alternative methods that are sustainable and environmentally safe, given the threats posed by pesticide residue to the environment and human health. Utilization of synthetic pesticides by farmers as one of the main technique for pest management in vegetables is also reported in West Africa (Abang *et al*., 2014). Apart from insecticides application, farmers also used cultural practices including crop rotation, mulching and the use of border crops e.g. tobacco and sunflower to control insects. Some of these practices are documented, for example, the use of crop borders in potato field was effective in the control of aphid infestation (Olubayo *et al*., 2009). Bearing in mind the risks related to the use of insecticides, farmers should be encouraged to integrate these cultural practices with other safe pest-suppression methods to sustainably manage insect pests.

Slightly above half of the hot pepper farmers interviewed practised intercropping with the aim of maximizing land use. Besides, the majority of farmers especially from the low-altitude areas intercropped with perennial crops such as banana and coffee to provide shade for hot pepper crop during the dry season. Apart from maximising land use, intercropping has other benefits including improving soil fertility and control of diseases and pests (Rämert, 2002). Intercropping is effective in the management of non-persistent viruses and associated vectors in several crops (Damicone *et al*., 2007; Fajinmi and Fajinmi, 2010). For example, incidence of *Pepper veinal mottle virus* in hot pepper was reduced by 76.2, 88.1 and 80.2% when intercropped with maize, cassava and plantain, respectively (Fajinmi and Fajinmi, 2010). The findings from the studies imply that intercropping could be used as a tool for pest and disease suppression in hot pepper production. However, farmers require training since the majority do not understand the principle behind using intercropping as a practice for diseases and pests' management.

Hot pepper farming is dominated by small-scale farmers of which the majority are men. This probably is because hot pepper is more of a cash crop than food security crop and like in many of the African countries, men dominate in the production of cash crops (World Bank, 2009). Also, due to the fact that in most African cultures, where men are available, they come forward and volunteer to provide information. Slightly above two-thirds of the farmers were in their active age and thus, can participate actively in the farming activities and at the same time are expected to adopt innovations more readily than older farmers (Asare-Bediako *et al*., 2015). Most of the respondents had less than 5 years' of experience an indication that most of them ventured in farming after hot pepper was set as a priority crop for export diversification by the government in 2014 (MINAGRI, 2014). Through the sensitization from the government, more farmers engaged in the production of hot pepper.

The findings revealed that diseases and pests are key factors in limiting production of hot pepper in Rwanda. Farmers lack accurate information on the cause, spread and control of the diseases. Majority of the farmers get information through farmer-to-farmer interactions. Thus, strategies such as farm-level training need to be put in place to avail this information to farmers and increase their knowledge of viral diseases and pests' management. The presence of viral symptoms in three agro-ecological zones calls for a need to identify the pathogens responsible for the diseases and the mode of spread. This will help in the development of efficient and sustainable control strategies.

CHAPTER FOUR:

DETECTION AND DISTRIBUTION OF SELECTED VIRUSES INFECTING HOT PEPPER

Abstract

Accurate diagnosis is a prerequisite to effective management of plant diseases especially those whose symptoms are not very specific. A survey was carried out in February to March 2018 to determine the prevalence of six hot pepper viruses in Rwanda. A total of 225 symptomatic samples were collected from 92 fields in high, mid and low-altitude agro-ecological zones (AEZs), and analysed using ELISA with antibodies to CMV, PVY, PVMV, PMMoV and TMV. The RT-PCR was used to confirm the results from ELISA and to test for the presence of PeVYV, which has no commercial antibodies. Amplified RT-PCR fragments were sequenced and compared with other known hot pepper viruses available in the GenBank database. Seventy-three (73) per cent of the samples tested positive for at least one of the viruses. The CMV, PVY, PVMV, TMV and PMMoV were detected in samples from the three AEZs but PeVYV was detected only in the mid and low-altitude AEZs. The CMV was the most prevalent and was detected in 48% of the samples, followed by PVMV and PVY detected in 23.6 and 18.2% of the samples tested, respectively. Incidence of aphid-transmitted viruses (CMV, PVMV, PVY) did not differ significantly ($\chi 2 = 3.48$; $p=0.176$) across AEZs. However, the incidence of seed-borne viruses across the AEZs differed significantly ($\chi 2 = 6.526$; $p = 0.038$) with highest prevalence in low AEZ. Generally, proportions of infected samples with seed-borne viruses were about 16% for both PMMoV and TMV, respectively. There were both single (36%) and mixed (34.6%) infections of these six viruses. The combinations of CMV with PVY or PVMV were the most common. 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. Efforts towards the development of sustainable management for these viruses should be put in place to improve yields and quality of hot pepper.

4.1. Introduction

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). Despite this economic importance, cultivation of this crop is constrained by several biotic and abiotic factors that lead to low yields (Bosland and Votava, 2000; Dagnoko *et al.,* 2013).

Among the biotic factors, viral diseases are the most destructive, causing enormous losses in hot pepper all over the world (Olawale *et al*., 2012). According to Olawale *et al.* (2020) more than 45 viruses have been reported to infect hot pepper in Africa. Among these viruses, 12 species are reported in the eastern Africa region namely PVY, PVMV, ChiVMV and EPMV belonging to genus *Potyvirus*; CMV, genus *Cucumovirus*; PMMoV, TMV and ToMV, genus *Tobamovirus*; PeVYV, genus *Polerovirus*; AMV, genus *Alfamovirus*; TSWV, genus *Tospovirus*; and PVX, genus *Potexvirus* (Dafalla, 2001; Haskias *et al*., 1999; IPM CRSP, 2008; Ndunguru and Kapooria, 1999). Information on viruses infecting hot pepper in Rwanda is scarce. So far, only three of these viruses namely CMV, PVMV and PeVYV are reported in the country (Skelton *et al*., 2018).

Effective control of virus-induced diseases requires a thorough understanding of the responsible pathogens and their distribution. Knowledge of the distribution of different hot 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 main 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. The present study aimed at detecting six viruses namely CMV, PVY, PVMV, PeVYV, PMMoV and TMV and determining their distribution in three AEZs of Rwanda.

4.2. Materials and methods

Study areas: A survey for hot pepper viruses was carried out in 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). The districts were Rulindo and Gakenke (high), Huye and Nyanza (mid), Bugesera, Rwamagana, Nyagatare and Rusizi (low-altitude AEZ). The geographic locations of sampled sites are shown in Fig. 3.1 section 3.2 of chapter three. Characteristics of the AEZs are as shown in Table 4.1.

Table 4.1. Characteristics of the three agro-ecological zones in Rwanda where the study was conducted

AEZ*	Area surveyed	Relief	Elevation (m)	Rainfall (mm)	Temperature (°C)
High altitude	Rulindo, Gakenke	Mountainous	>1900	1400-2000	15-17
Mid altitude	Nyanza	Dissected Plateaus	1600-1900	1100-1400	17-20
Low altitude	Bugesera, Nyagatare, Rwamagana, Rusizi	Pediplains	900-1600	850-1100	20-21

*AEZ: Agro-ecological zone, Source: Verdoodt, 2003

Assessment of virus disease incidence and severity in hot pepper farmers' fields: 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 opposite corners and assessed for virus disease incidence and severity.

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

Severity of viral diseases was determined using a scale of 1-5 as described by Olawale *et al.* (2015) with slight modifications, where: 1 = healthy plant; 2 = mild symptoms on few leaves of mosaic/mottling/yellowing (< 25% of the plant affected); 3 = moderate symptoms on many leaves of mosaic/puckering/mottling/vein clearing/yellowing (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 on the plant of mosaic/puckering/mottling/vein clearing/yellowing/ stunting/ necrosis (>75% of the plant). For fields that exceeded 2 acres, one to five sampling sites were assessed. The observations made from different sites were summed up and the average incidence and severity calculated based on the total number of 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 district (Shiferaw and Alemayehu, 2014).

Collection of diseased hot pepper leaf samples: A total of 225 symptomatic leaf samples were collected from the three AEZs. The samples were collected from suspected diseased plants showing virus-like symptoms. A sample was collected from an individual plant targeting five young leaves from different growing points of the plant. Contamination of the samples was avoided by disinfecting hands with 70% ethanol and changing of hand gloves after collecting each sample. 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. The samples were stored at room temperature (RT) ±25°C until dry, and after 4-5 days they were ground in liquid nitrogen. The powdered leaf tissues 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 test of collected diseased hot pepper leaf samples: The presence of five suspected hot pepper viruses reported in eastern and other parts of Africa namely PVY, PVMV, PMMoV, CMV and TMV was tested in the samples using DAS-ELISA (Clark and Adams, 1977; IPM CRSP 2008; Appiah *et al.,* 2014; Waweru *et al.,* 2019; Olawale *et al*., 2020). The kits were obtained from LOEWE Biochemica GmbH company (Germany) and used following the instructions from the manufacturer. Powdered leaf tissues of each sample were removed from a freezer -40°C and left to thaw. Two hundred (200) µl of specific coating antibody (IgG) of each virus, diluted (1:200) in coating buffer was dispensed into each well of the microtiter plate and incubated at 37°C for 4 hrs. The plates were washed with wash buffer four times after the incubation period.

Two hundred (200) µl of the test sample diluted 1:20 (w/v) in sample buffer was added into duplicate wells and then incubated overnight at 4°C. This was followed by washing the plates four times with wash buffer. Two hundred (200) µl of antibody-AP-conjugate diluted (1:200) in conjugate buffer was added into wells and incubated at 37°C for 4 hrs and later washed four times with wash buffer. Two hundred (200) µl of the freshly prepared substrate (1 mg/ml para- nitrophenyl- phosphate in substrate buffer) was added to each well and incubated at 37°C for 60 mins. The absorbance was measured at 405nm 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. Incubation plates were

covered with sealing tape provided with the kits to avoid edge effects and to maintain a uniform temperature. All buffers, negative and positive controls were provided with the kits.

Extraction of total ribonucleic acids from hot pepper leaf samples: Total ribonucleic acid (RNA) was extracted from hot pepper leaf tissue by cetyl-trimethyl ammonium bromide (CTAB) method as described by Allen *et al.* (2006) with slight modifications. Initially, a stock solution of the CTAB extraction buffer (500 ml) was prepared: 100 ml of 2% CTAB, 50 ml of 100 mM Tris-HCl, 20 ml of 20 mM EDTA, 200 ml of 2 M NaCl and 130 ml of sterile water. The buffer was sterilized at 121°C for 15 minutes and later stored in a cabinet at RT. Before any extraction, 3% Mercapto-ethanol was added to the working solution of CTAB buffer i.e. 30µl per 1ml of CTAB buffer used.

Approximately 100 mg of frozen tissue powder was transferred into 1.5 ml eppendorf tube and 900 µl of CTAB buffer containing Mercapto-ethanol preheated at 65°C for 15 mins was added. After vortexing for 30 secs, the samples were incubated at 65°C for 40 mins in a water bath and every 10 mins, the tubes were inverted to allow mixing and later kept at RT for 10 mins. The samples were centrifuged at 12000rpm for 5 mins at RT to remove non-soluble debris. The upper supernatant (~700 µl) was transferred into a new sterilized 1.5 ml eppendorf tubes containing an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). The solution was mixed gently by inverting the tubes for 10 mins at RT, followed by centrifuging at 12000 rpm for 10 mins at RT to separate the phases. The upper aqueous phase (~500 µl) was carefully transferred into a new clean 1.5 ml eppendorf tubes containing 500 µl of chloroform: isoamyl alcohol (24:1). The mixture was mixed gently by inverting the tubes for 10 mins at RT and later on centrifuged at 12000 rpm for 10 mins at RT. The nucleic acids were precipitated by transferring the upper aqueous phase (~500 µl) into a new sterilized 1.5 ml eppendorf tubes containing 350 µl of cold isopropanol (stored at -20°C). The

mixture was vortexed for 30 secs and the tubes incubated at -20°C for 2 hrs to increase the yield of the RNA.

The RNA was recovered by centrifuging the mixture at 13000rpm for 10 mins and discarding the supernatant leaving a white pellet at the bottom of the tube. This was followed by addition of 500 µl of cold 70% ethanol (stored at -20°C) and the pellet was washed by tapping the tube with the fingers. The contents in the tube were centrifuged at 13000rpm for 5 mins at RT and the supernatant was discarded. The pellet was left to dry for 40 mins at RT and later dissolved in 50 µl of PCR-treated water. The yield and quality of total RNA were checked using a spectrophotometer and 1.8% agarose gel electrophoresis stained with ethidium bromide. The total RNA obtained was stored at -80°C until analyzed.

Reverse transcription-polymerase chain reaction: One-step RT-PCR was carried out to confirm/identify the viruses from DAS-ELISA positives and also to identify PeVYV for which commercial antisera kit is not available. The amplification of CMV, PVMV, PeVYV and TMV was done using One Taq One-step RT-PCR Kit (Catalogue E531S5, New England Biolabs Inc.), following the manufacturer's instructions. The DNA products were generated using virus-specific primers that were 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). Accession 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 gene, PVMV-F/R fragment of ~502 bp from the polyprotein gene and TMV-F/R fragment of ~622bp from the coat protein region (Table 4.2). 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 Industries (Pty) Ltd, South Africa.

The RT-PCR mixture comprised of 12.5 µl of 2X reaction mix, 1 µl of 25X enzyme mix, 1µl of 10 µm forward primer, 1µl of 10 µm reverse primer, 1µl total of RNA and the reaction mix was made to 25 µl with PCR nuclease water. Thermal cycling conditions were: 48°C at 15 mins for reverse-transcription; followed by 1 min at 94°C for initial denaturation; 40 cycles of 94°C at 15 secs for denaturation, 54°C at 30 secs for annealing and 68°C at 45 secs for the extension. The final extension was 68°C at 5 mins. These conditions were same for all the viruses tested. 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.

Table 4.2. Sequences of the primers used for detection of CMV, PVMV, PeVYV and TMV in hot pepper samples collected in Rwanda

Primer	Sequence 5' to 3'	Fragment size (bp)	Region amplified
CMV_F	5' - GCTTCGCAATACGTTTTGACGG -3'	502	RNA3
CMV_R	5' - TACGACCAGCACTGGTTGATTC -3'	502	RNA3
PVMV_F	5'- AAGCCCTCATTGAAGGTCAACG -3'	502	Polyprotein
PVMV_R	5'- ATCAACCATCACCCACATACCG -3'	502	Polyprotein
PeVYV_F	5' - AGTACGTCTTCGAGACTACTGC -3'	498	RdRp[1]
PeVYV	5' - TCTATAGTAGAGAGGTCGATCC - 3'	498	RdRp
TMV_F	5' – TGATGATTCGGAGGCTACTGTC - 3'	622	CP[2]
TMV_R*	5' – CCTTCGATTTAAGTGGAGGGAA - 3'	622	CP

[1]RNA-dependent RNA polymerase; [2]Coat protein; CMV-*Cucumber mosaic virus*; PVMV-*Pepper veinal mottle virus*; PeVYV-*Pepper vein yellows virus*; TMV-*Tobacco mosaic virus*. *TMV reverse primer cross-react with *Pepper mild mottle virus* however, the sequence generated using both forward and reverse primers were specific to TMV.

Gel electrophoresis of amplified products from hot pepper diseased leaf samples: One point two (1.2) per cent of standard agarose was dissolved in 100 ml of 1×Tris-Acetate-EDTA buffer (TAE) (0.04 M Tris-Acetate, 0.001 M EDTA, pH 8.0) by heating in a microwave for 3 mins until the solution was clear. The mixture was allowed to cool to touch and 2 µl of ethidium bromide was added and mixed gently by shaking. The agarose was poured into the gel tray that had been pre-fitted with the comb and left for 1 hr at RT to solidify. The gel was later immersed in an electrophoresis tank filled with TAE buffer and the comb was removed carefully to expose the wells. The DNA containing samples were mixed with 6× loading dye at a ratio of (5:6) and carefully loaded on each well. A standard DNA molecular marker (1 kilobyte DNA ladder) was loaded in one well to estimate the sizes of the RT-PCR products being analysed. The gel was run at 100 volts for 40 mins. The nucleic acids were visualized under ultraviolet transilluminator and photographs taken.

Purification and sequencing of amplified products from hot pepper diseased leaf samples: The QIAquick PCR Purification Kit (Qiagen, USA) was used to purify amplified products following the manufacturer's instructions. After elution, 50 µl of purified products were saved in eppendorf tubes, the concentration of DNA was estimated using a spectrophotometer (Nanodrop) and later the products were preserved in -80°C. Nine isolates (3-PeVYV, 3-CMV, 2-PVMV and 1-TMV) were selected based on different geographical regions where the samples were collected and purified DNA fragments were sequenced at Inqaba Biotechnical Industries (Pty) Ltd, South Africa. Nine isolates were sequenced due to limited resources.

Analysis of data on disease incidence and severity: Data on virus disease incidence and severity in farmers' fields were subjected to one-way Analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS version 16). Comparison of means was done by least significant difference test at 5% level of probability. Data on incidence of aphids-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.

Sequence analysis and comparisons: The obtained Sanger sequences were trimmed using the CLC main workbench software and analysed with Basic Local Alignment Search Tool nucleotide (BLASTn). Multiple sequence alignment of the obtained virus sequences with other known hot pepper viruses available in the GenBank database (Appendix 2a, b & 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. The evolutionary distances were computed using the maximum composite likelihood method (Tamura and Kumar, 2004). 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 full sequences of the targeted regions/genes. However, for TMV, only a small number of isolates 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 dermarcation criteria for viruses and virus species identified in the study was done following recommended thresholds for members of the genus Polerovirus, Tobamovirus, Cucumovirus and Potyvirus established by the International Committee on Taxonomy of Viruses (ICTV) (Adams *et al.*, 2012, 2005; Domier, 2012; Wylie *et al.*, 2017).

4.3. Results

Incidence and severity of virus diseases on hot pepper in farmers' fields: A range of viral disease symptoms were observed in the fields. These included dark green vein banding, reduced leaf size, leaf mosaic, mottling, bleaching, puckering, deformation, chlorotic veins and stunting (Fig. 4.1a-h).

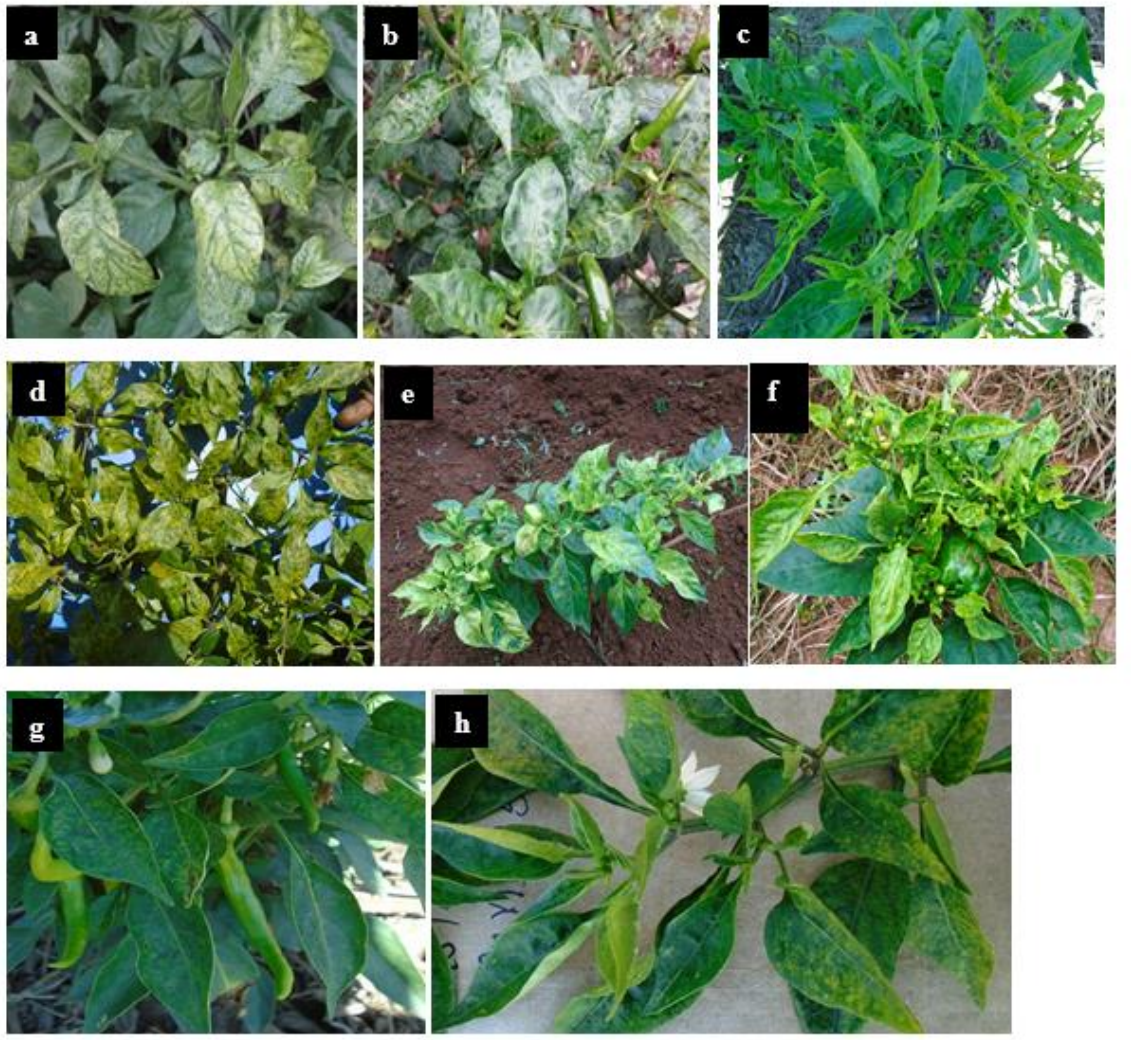

Figure 4.1. Virus-induced symptoms observed on hot pepper plants in surveyed farmers' fields in February to March 2018: (a) = dark green vein banding; (b) = leaf puckering; (c) = leaf distortion; (d) = leaf mottling; (e) = leaf bleaching; (f) = stunting; (g & h) = chlorotic veins of leaves.

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 viral diseases 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 ($p>0.542$) and severity ($p>0.353$) of the viral diseases 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 4.3.

Table 4.3. Summary of number of leaf samples tested and viruses detected by ELISA and RT-PCR in hot pepper in samples collected from three agro-ecological zones of Rwanda in February to March 2018

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
	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[4]	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*

Distribution of hot pepper viruses in surveyed agro-ecological zones: 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 (Table 4.4). Chi-square test revealed that CMV and PVMV incidence did not differ significantly across AEZs. 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%) in Table 4.4. Proportions of infected samples with seed-borne viruses were about 16% for both PMMoV and TMV, respectively (Table 4.5). The viruses were distributed in all AEZ 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% of samples from high, low and mid-altitude areas, respectively. Incidence of seed-borne viruses across the AEZs differed significantly ($\chi 2$ =6.526; p = 0.038) with highest prevalence in low AEZ (Table 4.5).

Table 4.4. Proportion of aphid-transmitted viruses detected in hot pepper leaf samples collected from three agro-ecological zones of Rwanda in February to March 2018

Agro-ecological zone	No. of samples tested	*CMV	PVY	PVMV	Overall infected samples
Low altitude	60	42.9	8.6	24.8	61.9
Mid altitude	60	56.7	40	21.7	71.7
High altitude	105	45.0	13.3	23.3	56.7
Total	225	48	18.2	23.6	61.8
$\chi 2$-test		2.587	26.621	0.205	3.48
P-value		0.274	<0.001	0.902	0.176

*****CMV-*Cucumber mosaic virus*; PVY-*Potato virus Y*; PVMV-*Pepper veinal mottle virus*

Table 4.5. Proportion of seed-borne viruses detected in hot pepper leaf samples collected from three agro-ecological zones of Rwanda in February to March 2018

Agro-ecological zone	No. of samples tested	*TMV	PMMoV	Overall infected samples
Low altitude	60	21.9	16.2	34.3
Mid altitude	60	5	13.3	16.7
High altitude	105	16.7	20	23.3
Total	225	16	16.4	26.7
χ2-test		8.146	0.98	6.526
P-value		0.017	0.613	0.038

*TMV-*Tobacco mosaic virus*; PMMoV-*Pepper mild mottle virus*

Types of virus infections found in hot pepper diseased leaf samples: Among the samples tested, the proportion of positive samples was 73.3%, consisting of both single (36%) and mixed (34.6%) infections (Table 4.6). The single virus species infections were 14.7%-CMV, 7.1%-PVMV, 5.8%-PMMoV, 4.9%-TMV and 3.5%-PVY. Among the mixed infections, double infections were detected in 25.3% of the samples. The combination of CMV+PVMV was the most prevalent and was detected in 8.4% of the positive samples. The other dual infections were CMV+PVY (8%), CMV+PMMoV (2.7%), CMV+TMV (2.7%), PVY+TMV (1.8%), PVY+PVMV (1.3%) and PVMV+PMMoV (0.4%) in Table 4.7. The proportion of triple infection was 7.1% while multiple infection was 2.2%. Mixed infections were most prevalent in the mid and low-altitude areas.

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.

Table 4.6. Frequency of single and mixed virus infections detected using serology in hot pepper leaf samples collected from three agro-ecological zones of Rwanda in February to March 2018

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 (4and 5)	CMV + PVMV + TMV + PMMoV	2	0	0	2(0.9)
	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)

*Total and values in brackets are proportion (%) of single and mixed virus infections detected to the total number of samples tested = 225. CMV-*Cucumber mosaic virus*; PVY-Potato virus Y; PVMV-*Pepper veinal mottle virus*; TMV-*Tobacco mosaic virus*; PMMoV-*Pepper mild mottle virus*

Sequences and Phylogenetic analysis: The obtained sequences of nine virus isolates namely PeVYV-I4 (accession MT445648), PeVYV-R13 (acc. MT445647), PeVYV-G12 (acc. MT445649), PVMV-R12 (acc. MT445645), PVMV-28 (acc. MT445646), TMV-198 (acc. MT445644), CMV-F1(acc. MW080679), CMV-G11 (acc. MW080680) and CMV-R10 (acc. MW080681) were deposited in the GenBank.

Using CMV-F/R primers, fragments with an expected size of 502 bp were amplified (Fig. 4.2). 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 sequences (481 bp) suggested that the three isolates were CMV and form 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. 4.3). Isolate AJ585522.1 (Australia) and D12499.1 (Japan) made an independent clade B while KT004544.1 (China) was placed in an intermediate position between Japan and Rwanda-South Korea isolates. Clade C comprised of isolates from India and Italy. Pairwise nucleotides (nt) and deduced amino acids (aa) similarities 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 ranged from 77.6-98% nt (88.9-99.3% aa) which were above the 65% cut off point for species demarcation for *Cucumovirus* (Appendix 3). *Alfalfa mosaic virus*, MF990286.1 was used as outgroup.

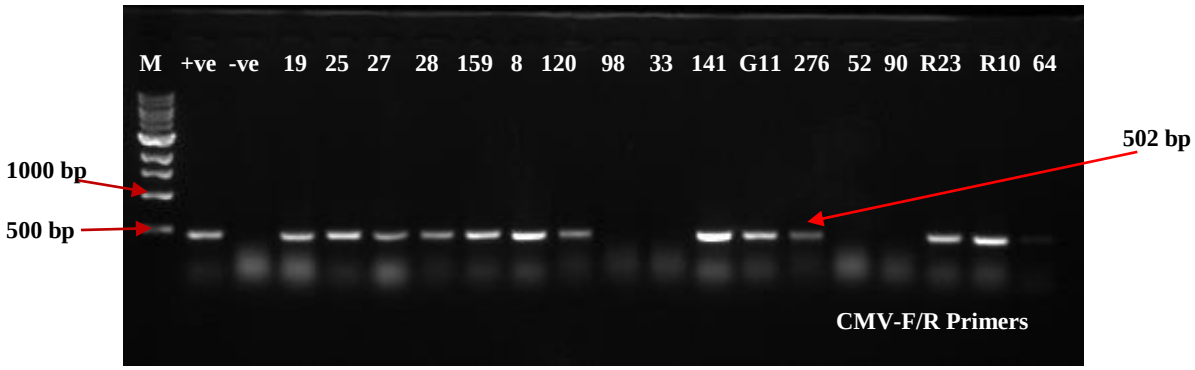

Figure 4.2. Detection of *Cucumber mosaic virus* from diseased leaf samples collected from farmers' fields by RT-PCR using CMV-F/R primers. Amplified products analysed on 1.2% agarose gel at 100 volts for 40 minutes. Lane M - 1 Kb DNA ladder, lane +ve - positive, lane –ve - negative control, lanes 19-159 samples collected from the high-altitude areas, lanes 8-276 samples from the low-altitude and lanes 52-64 samples from the mid-altitude areas. The primers amplified a ~502 bp product from infected leaf samples.

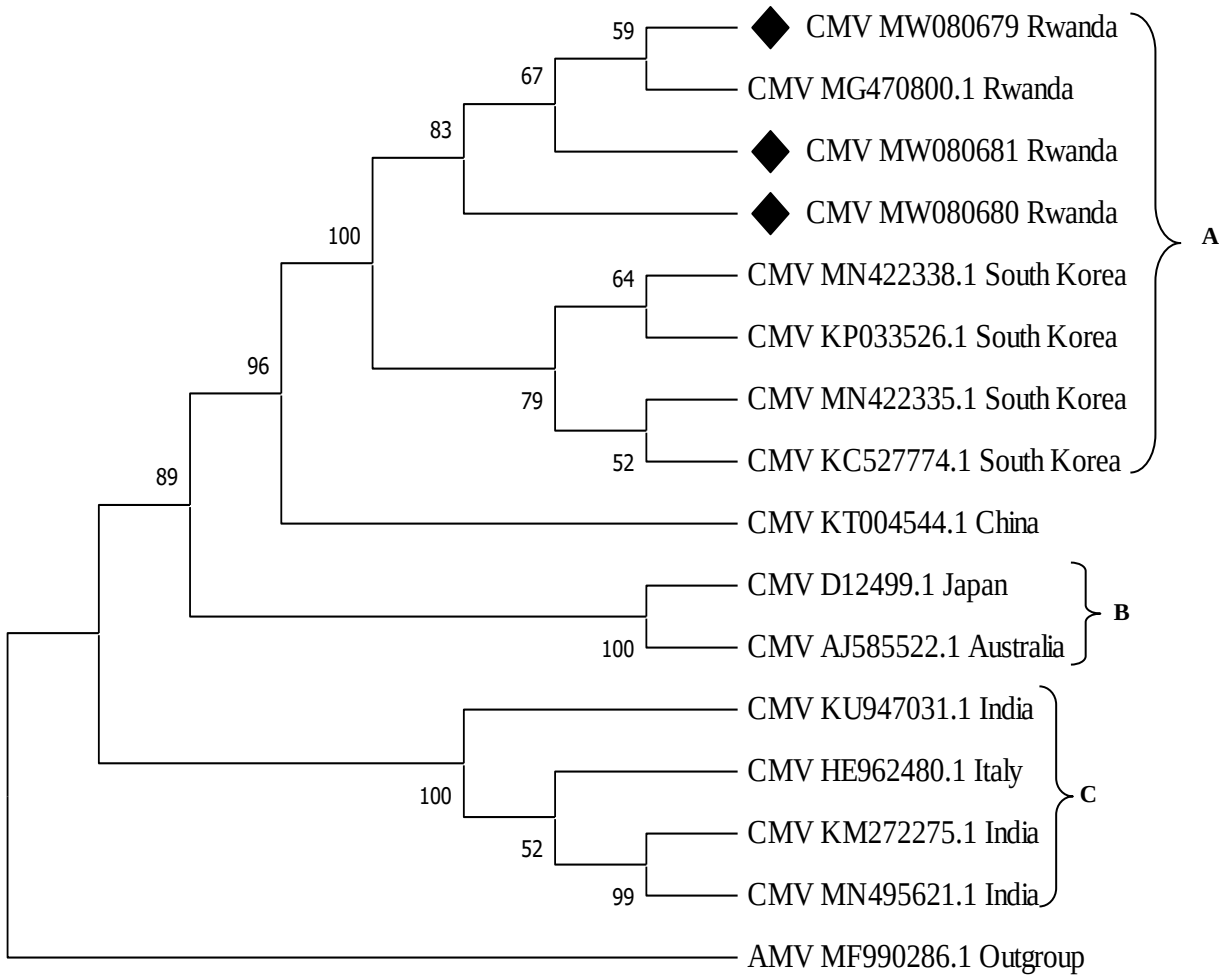

Fig. 4.3. Phylogenetic tree constructed with sequences of eighteen *Cucumber mosaic virus* (CMV) isolates, genus *Cucumovirus*. The tree was based on alignments of 481 nucleotides of partial RNA3 segment and was rooted in the sequence of *Alfalfa mosaic virus* (AMV), genus *Alfamovirus* (MF990286.1). 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 ◆

Three amplicons, G12 (accession number MT445649), I4 (MT445648) and R13 (MT445647) for PeVYV with an expected size of ~498 bp obtained with primers PeVYV-F/R were sequenced (Fig 4.4). 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. 4.5. 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 demarcation threshold 90% aa for genus *Polerovirus* (Appendix 4). *Barley vein yellow dwarf virus*, EU332330.1 was used as outgroup.

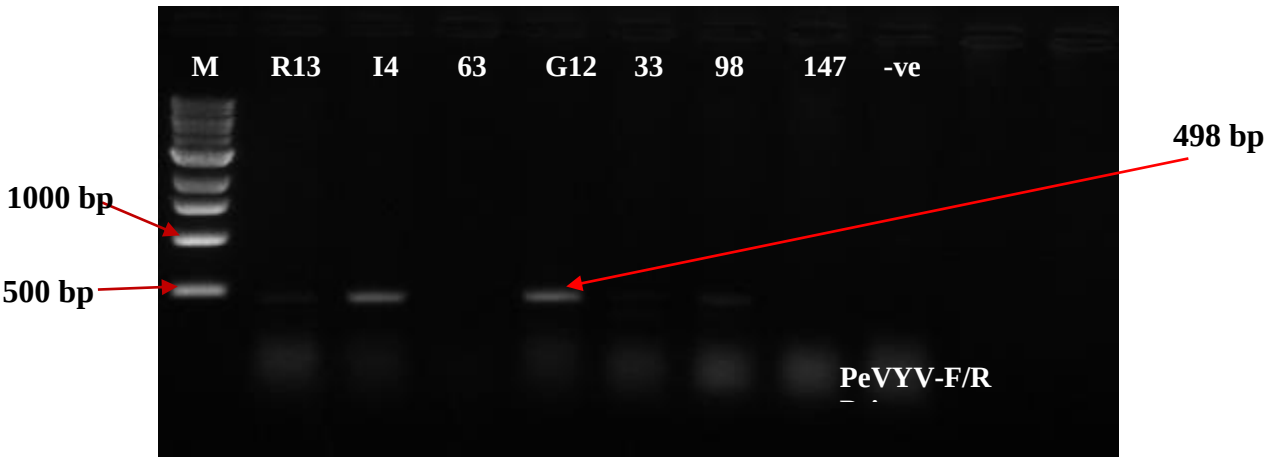

Figure 4.4. Detection of *Pepper vein yellows virus* from diseased leaf samples collected from farmers' fields by RT-PCR using PeVYV-F/R primers. Amplified products analysed on 1.2% agarose gel at 100 volts for 40 minutes. Lane M - 1 Kb DNA ladder, lanes R13-63 samples collected from the mid-altitude areas, lanes G12-147 samples from the low-altitude areas and lane –ve - negative control. The primers amplified a ~498 bp product from infected leaf samples.

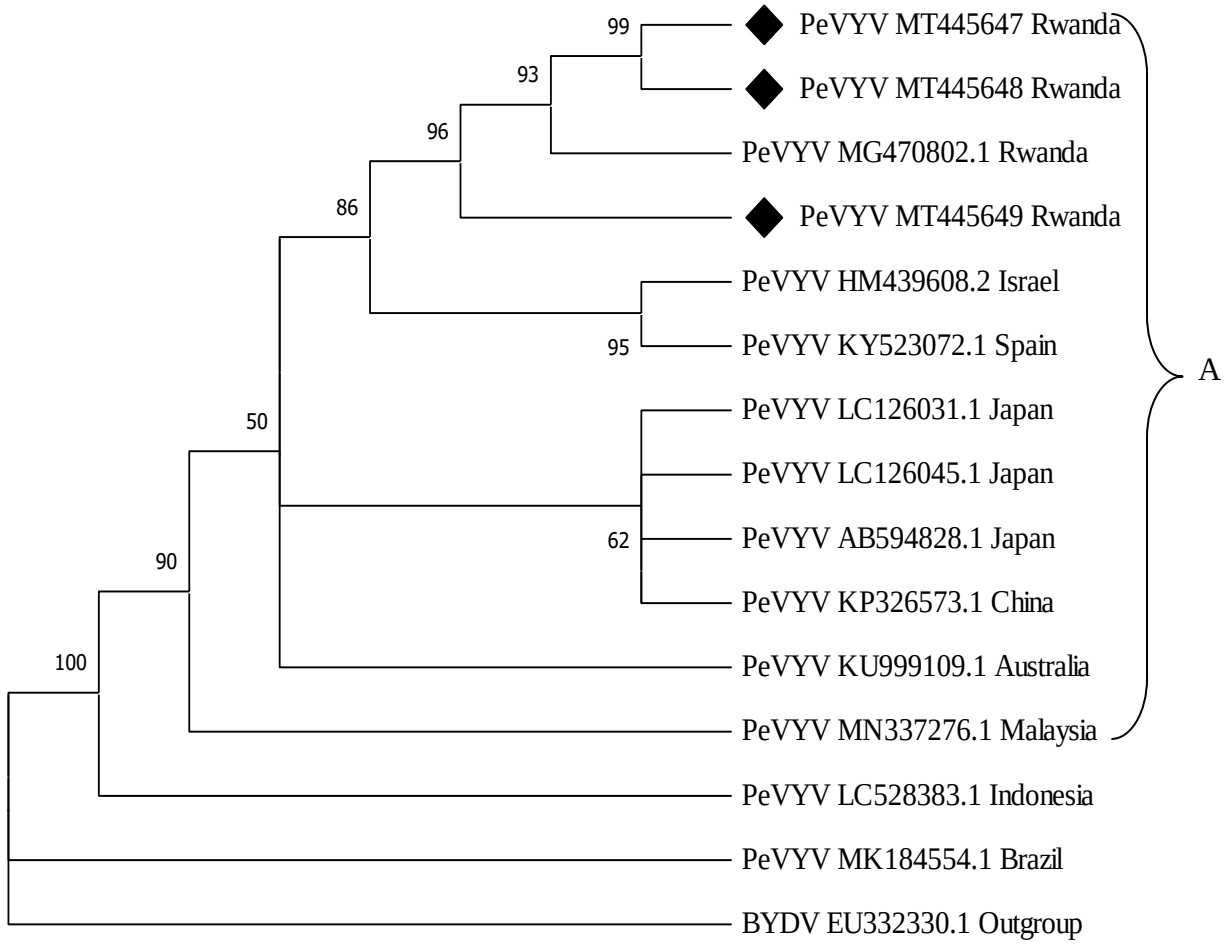

Figure 4.5. Phylogenetic tree constructed with sequences of fourteen *Pepper vein yellows virus* (PeVYV) isolates, genus *Polerovirus*. The tree was based on alignments of 475 nucleotides of partial RNA-directed RNA polymerase gene and was rooted in the sequence of Barley vein yellow dwarf virus (BYDV), 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 ◆.

Using PVMV-F/R, fragments with an expected band size of ~502 bp were amplified from the samples tested (Fig 4.6). Phylogenetic analysis of isolate R12 (MT445645), 28 (MT445646) and other PVMV isolates was done based on partial (418 bp) coat protein (CP) gene (Fig. 4.7). 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 consisted 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 isolates were 98-99% nt (99% aa) while to other isolates from Ethiopia, Senegal, Cameroon, Ghana, Japan, China and Taiwan were 76-79% nt (82-87% aa) which correspond to the optimal species demarcation criterion (>76% nt, >82% aa) for the CP in genus *Potyvirus* (Appendix 5). 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*, DQ812125.1 was used as outgroup.

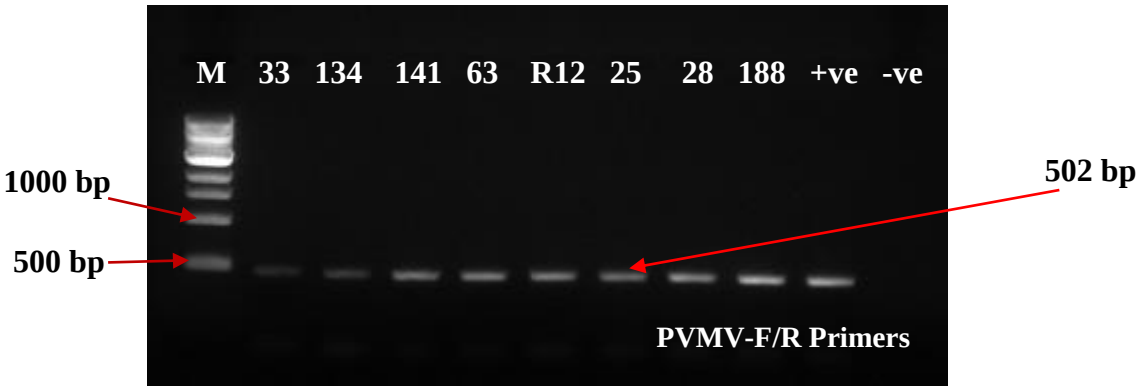

Figure 4.6. Detection of *Pepper veinal mottle virus* from diseased leaf samples collected from farmers' fields by RT-PCR using PVMV-F/R primers. Amplified products analysed on 1.2% agarose gel at 100 volts for 40 minutes. Lane M - 1 Kb DNA ladder, lanes 33-141 samples collected from the low-altitude areas, lanes 63-R12 samples from the mid-altitude, lanes 25-188 samples from the high-altitude areas, lane +ve - positive and lane –ve - negative control. The primers amplified a ~502 bp product from infected leaf samples.

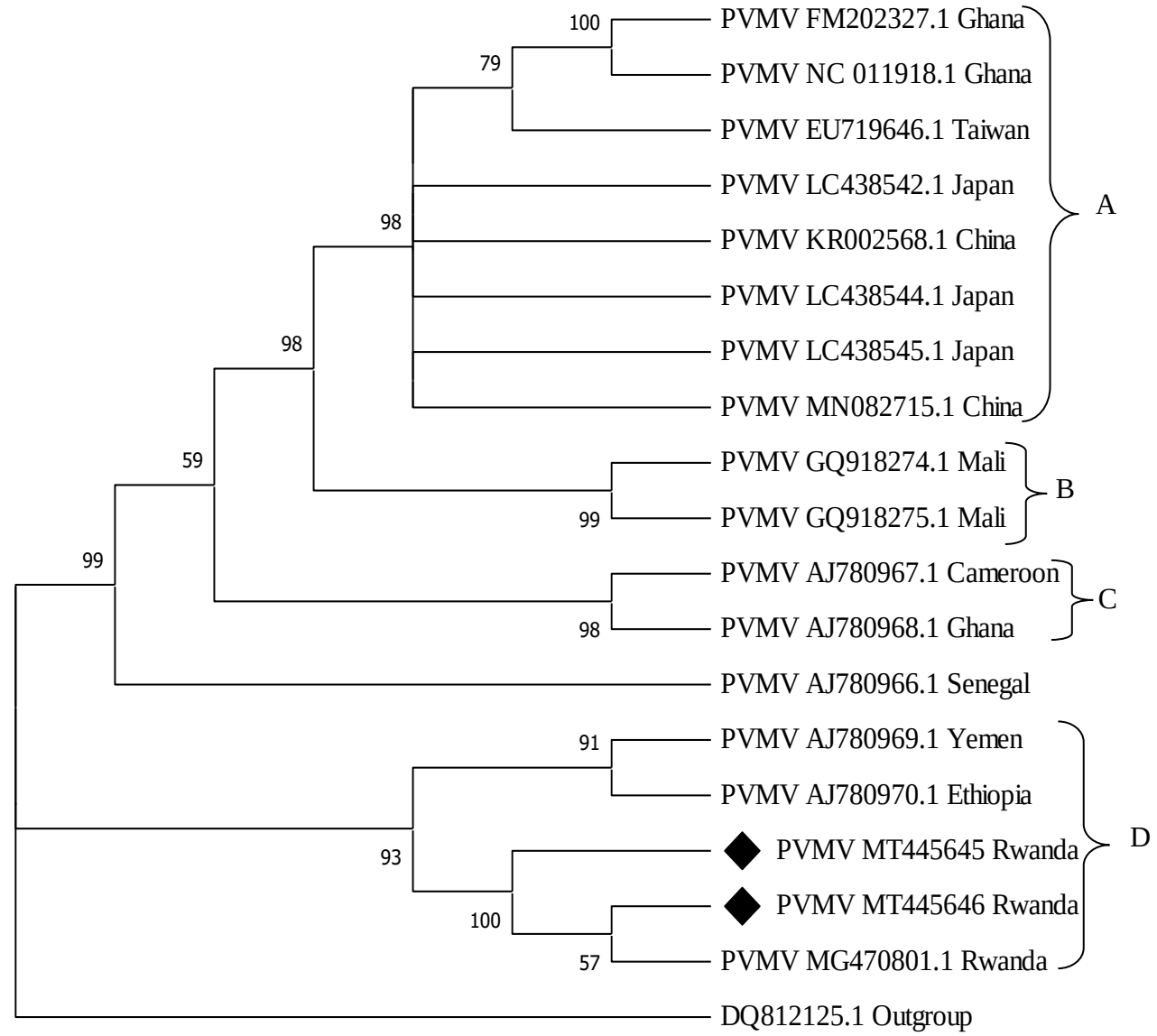

Figure 4.7. Phylogenetic tree constructed with sequences of eleven *Pepper veinal mottle virus* (PVMV) isolates, genus *Potyvirus*. The tree was based on alignments of 418 nucleotides of partial coat protein gene and was rooted in the sequence of *Squash vein yellowing virus* (SqVYV), genus *Ipomovirus* (DQ812125.1). 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 ◆.

For TMV, fragments with an expected band size of ~622 bp were obtained using primers TMV-F/R (Fig 4.8). Sequencing of one amplicon confirmed the identification of TMV. Phylogenetic analysis was done based on complete coat protein nucleotides sequences (Fig. 4.9). 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. 4.9). 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 (Appendix 6). *Tobacco rattle virus* (TRV), accession JO4347.1) was used as outgroup.

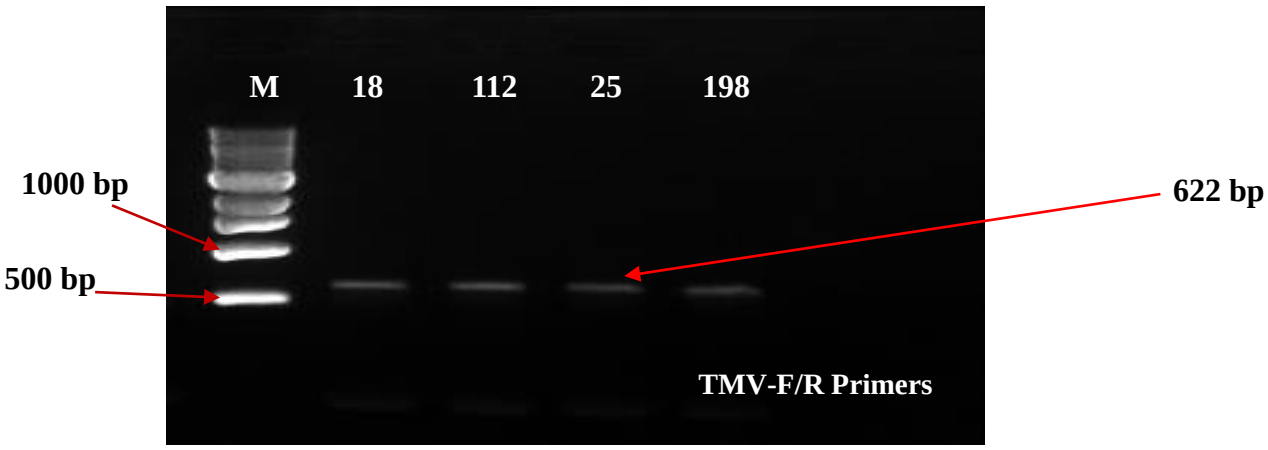

Figure 4.8. Detection of *Tobacco mosaic virus* from diseased leaf samples collected from farmers' fields by RT-PCR using TMV-F/R primers. Amplified products analysed on 1.2% agarose gel at 100 volts for 40 minutes. Lane M - 1 Kb DNA ladder, lanes 18-112 samples collected from the low-altitude areas and lanes 25-198 samples from the high-altitude areas. The primers amplified a ~622 bp product from infected leaf samples.

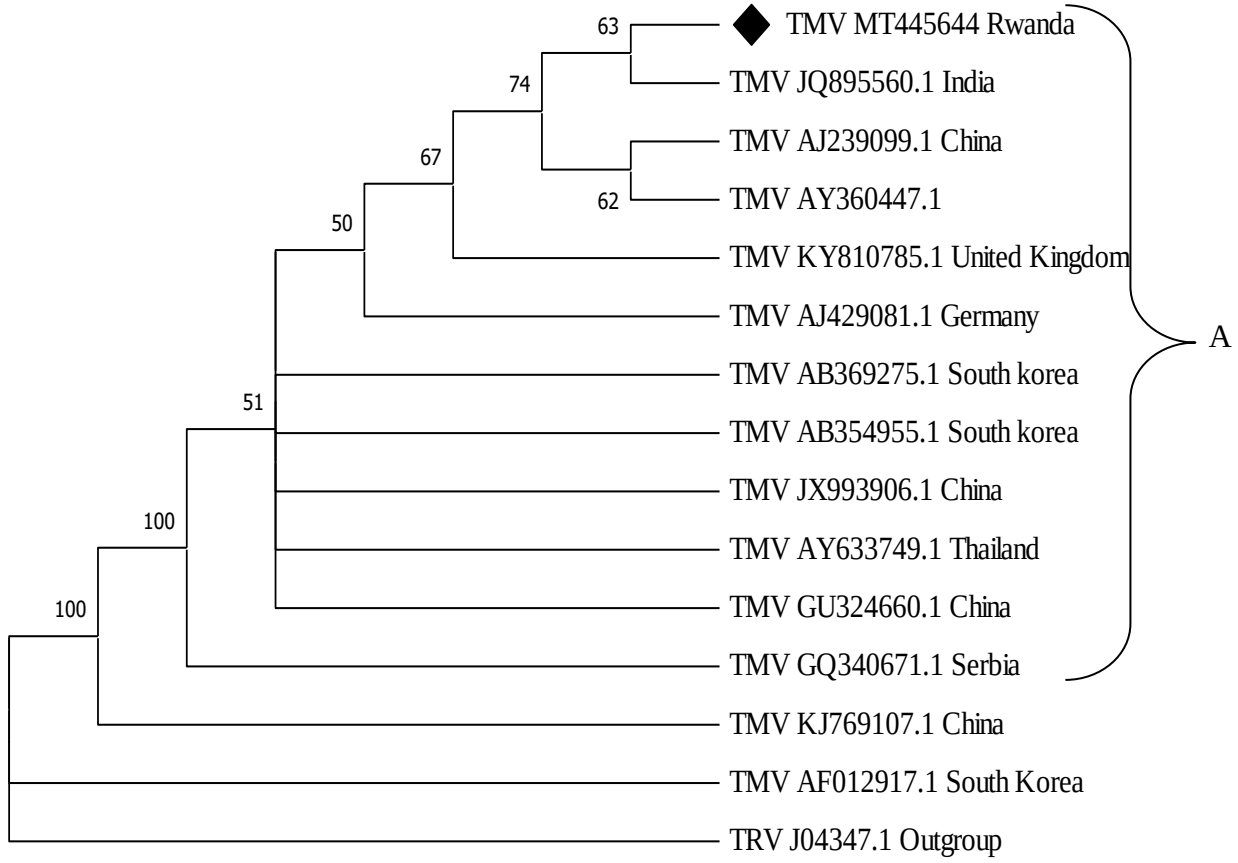

Figure 4.9. Phylogenetic tree constructed with sequences of fifteen *Tobacco mosaic virus* (TMV) isolates, genus *Tobamovirus*. The tree was based on alignments of 622 nucleotides of coat protein gene and was rooted in the sequence of *Tobacco rattle virus* (TRV), genus *Tobravirus* (JO4347.1). Nodes 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 ◆.

4.4. Discussion

The study revealed that six viruses namely CMV, PVMV, PVY, TMV, PMMoV and PeVYV were prevalent in the surveyed hot pepper production areas. Of the six viruses included in the present study, CMV, PVMV and PeVYV were previously reported infecting hot pepper in Rwanda (Skelton *et al.,* 2018). To our knowledge, this is the first report of TMV, PMMoV and PVY in hot pepper fields in Rwanda. These findings, therefore, are significant contribution to the current knowledge of 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 transmitted by several species of aphids in a non-persistent manner within a short period (Pernezny *et al*., 2003). Among the insect-vectors, aphids are the most prevalent in pepper fields in Rwanda (Waweru *et al*., 2020a). The most common aphid species associated with peppers 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 aphids' species (Palukaitis *et al*., 1992; Mello *et al*., 2011). 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 (Olawale *et al*., 2012; Myti *et al*., 2014; Choi *et al*., 2018). Dafalla (2001) also reported that CMV and PVY are among the most common and damaging viruses infecting pepper in the Sub-Saharan region.

The non-significant differences in the mean prevalence of aphids-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 (Thresh *et al*., 2003b; Njeru *et al*., 2008). 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 poor sanitation (Waweru *et al*., 2020a).

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 exchange of planting materials. Majority of the hot pepper farmers from low AEZ obtain their seeds from exporting companies (Waweru *et al*., 2020a). 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 production of seeds under appropriate conditions to ensure that the seeds are not infected 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 the low and mid-altitude AEZs. The 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; 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 areas (Rulindo district) and low-altitude areas (Kirehe and Kayonza districts). Considering the present finding and previous results by Skelton *et al*. (2018), it is evident that the virus is widespread in all AEZs where hot pepper is produced. The results also suggest that PeVYV can co-infect with other viruses. For example, it was present in dual infection with CMV and triple infections with CMV + PVMV. Therefore, further research need to be conducted to understand the virus, its epidemiology and economic significance.

Mixed infections in hot pepper increase the severity of disease symptoms, leading to significant yield losses (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 was the most common followed by CMV + PVMV. 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 (Sorho *et al*., 2014; Olawale *et al*., 2015). On the other hand, mixed infections of CMV with *Tobamovirus* (TMV or PMMoV) and *Potyvirus* with *Tobamovirus* as revealed in this study have also been documented in Ghana (Appiah *et al*., 2014). Mixed infections are quite common in nature not only on hot pepper but also in other solanaceous crops and are associated with serious virus problems in hot pepper production (Afouda *et al*., 2013). The mixed infections cause synergistic or antagonistic interactions (Syller, 2012).

The presence of mixed infections of viruses from several genera in high incidence in farmers' hot pepper field is expected to cause varying levels of damage leading to considerable yield reduction in quantity and quality. 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% are reported elsewhere due to CMV, PVMV, PVY, PMMoV and TMV, respectively (Avilla *et al*., 1997; Chitra *et al*., 2002; Guldur and Caglar, 2006; Fajinmi *et al*., 2012; 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 hot 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. No virus was detected in about 26.7% of the apparently diseased samples, an indication of possible presence of other viruses infecting the crop. More assays targeting viruses other than those tested in this study is therefore necessary.

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 of any gene (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, PVMV 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 the present study, six virus species were identified 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 prevalent in all major hot pepper growing areas. This is the first time that TMV, PMMoV and PVY are reported in hot pepper fields in Rwanda. Resistance breeding and other control strategies focusing on these viruses are urgently needed.

CHAPTER FIVE:

EVALUATION OF HOT PEPPER (*Capsicum* spp.) GENOTYPES FOR RESISTANCE TO VIRUSES AND APHIDS

Abstract

Production of hot pepper in Rwanda is mainly constrained by virus-induced diseases and pests. Eighteen (18) hot pepper genotypes (9 local, 5 introduced, 4 commercial) were evaluated for resistance to viruses' infection and infestation by aphids in two agro-ecological zones of Rwanda. Best performing genotypes (14) in the field were evaluated further for their reaction to *Cucumber mosaic virus* (CMV) through artificial inoculation. The most predominant virus attacking hot pepper in Rwanda. Data on incidence and severity of viral diseases were recorded at 7 and 14-days interval for both screenhouse and field experiments, respectively. The population of aphids in the field was assessed at 14-days interval. Polymerase chain reaction (PCR) was used to analyse diseased leaf samples from the field to detect the viruses present while serological assay was used for screenhouse samples. Incidence and severity of viral diseases significantly ($p \leq 0.05$) differed among genotypes in field and screenhouse experiments. Five genotypes namely 00767PPR, 00802PPR, PBC 462, PP9950-5197 and ICPN 18-7 were grouped as resistant to the viral diseases while genotype 00765PPR, HP 0117 and PP9852-170 were moderately resistant. Four genotypes 00767PPR, 0802PPR, PBC 462 and PP9852-170 were resistant to CMV while genotypes 00786PPR and PP9950-5197 were moderately resistant in the screenhouse. Three genotypes namely PBC 462, 00767PPR and 0802PPR were consistently rated as resistant to viral diseases while genotype HP 0117, PP9852-170 and PP9950-5197 were moderately resistant under field and screenhouse conditions. Genotype reaction to aphids' infestation did not vary significantly ($p \geq 0.05$). The five introduce genotypes that showed resistance to viral diseases are recommended for adoption by growers as they have high market demand while, the two local

genotypes 00767PPR and 00802PPR are recommended for breeding programs as potential sources for virus resistance.

5.1. Introduction

Globally, the most important diseases affecting production of hot pepper are caused by viruses (Kenyon *et al.*, 2014). Currently, pepper is attacked by more than 68 viruses globally, of which have been 15 are identified in Africa (Aliyu, 2014; Kenyon *et al.*, 2014). The CMV, TMV, PVMV, and PVY are the most predominant in Sub-Saharan Africa (Dafalla, 2001). In Rwanda, three viruses namely PeVYV, PVMV and CMV have so far been associated with hot pepper (Skelton *et al*., 2018). The CMV is among the most economically important viruses of hot pepper not only in Rwanda but also in 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 infested by more than 21 insects which include aphids, whiteflies, and thrips, among others (Niranjanadevi *et al*., 2018). Yield losses of up to 100% in chilli due to damage by insect pests can be achieved depending on a number of interacting factors (Messiaen *et al*., 1991). In addition to losses caused by direct feeding on plants, some of these insects including whiteflies, aphids and thrips are vectors of devastating viral diseases (Kenyon *et al*., 2014).

Various management options have been proposed to reduce virus infections in hot pepper. The measures include the use of virus-free planting materials, resistant varieties, habitat modification such as border crops, pesticides and roguing (Wang, 2006; Degri and Ayuba, 2016). Farmers in Rwanda mainly rely on insecticides to control insect vectors. Unfortunately, insecticides do not achieve 100% kill of the vectors. Hence, the insect vectors develop resistance against the active ingredient after repeated application of insecticides within a short time and more so, insecticides negatively affect the environment (Kenyon *et*

al., 2014). Utilization of cultivars that are resistant offers the most effective and durable solution in mitigating the negative effects of the diseases and pests in hot pepper production (Visalakshi and Pandiyan, 2018).

Resistant varieties not only reduce the pest population and the virus inoculum in the farming system but they are also compatible with other methods (Frantz *et al*., 2004). Previous studies have assessed the resistance of wild and cultivated pepper genotypes to viruses and aphids leading to the release of virus-resistant lines in some of countries (Frantz *et al*., 2004; Choi *et al*., 2018). However, there is no information on the reaction of hot pepper genotypes grown in Rwanda to infection by viral diseases and infestation by aphids.

It is important to assess the genotypes in different environments in the field to identify the relative host resistance, as resit may vary depending on the location. Screenhouse assessment using artificial inoculation techniques is important for validation of resistance. In this study, both field and screenhouse trials were carried out to; evaluate the reaction of different genotypes of hot pepper (local, commercial and introduced) to natural virus infections and infestation by aphids, in two agro-ecological zones of Rwanda, and evaluate the reaction of hot pepper genotypes to infection by CMV under screenhouse conditions.

5.2. Materials and methods

5.2.1. Evaluation of hot pepper genotypes reaction to virus infections and aphid infestations under field conditions

Experimental sites: The study was conducted at Rubona and Gashora research fields belonging to RAB during long (end March-July, 2018) and short rain (end October, 18-March, 2019) seasons. Rubona station is located at an altitude of 1692.9 m, latitude S 2°28′59.59″ and longitude E29°46′22.46″, in the mid-altitude AEZ. Gashora is located at an

altitude of 1331.1 m, latitude S 2º15′22.11″ and longitude E30°17′12.43″, in the low-altitude AEZ. The characteristics of the two AEZs are shown in Table 4.1, section 4.2 of chapter four**.**

Source of seeds: A total of 18 hot pepper genotypes including 9 local collections obtained from Rwanda National Genbank, 5 introduced lines provided by World Vegetable Center, Eastern and Southern Africa-Tanzania and 4 commercial varieties from seed companies were evaluated (Table 5.1). Previous studies reported the introduced genotype PP9950-5197 is resistant to CMV, PVY and ChiVMV while ICPN 18-7 is resistant to PVY (Gniffke *et al.*, 2013). Similar studies by Reddy *et al*. (2014) showed the introduced genotype PP9852-170 is resistant to CMV. California wonder (sweet pepper) variety was included as a susceptible control to viruses (Murphy and Bowen, 2006).

Table 5.1. List of hot pepper genotypes that were evaluated for reaction to viral diseases and aphids under field conditions in Rwanda

Genotype	Species	Type	Source
00765PPR[1]	*C. annum*	Local	RNGB[3]
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[2]
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 RNGB; [2]Unknown species; [3]Rwanda National Genbank; [4]World Vegetable Center.

Raising of hot pepper seedlings: The seeds of the different genotypes were sown in trays containing sterilized sandy loam soil (1:2) and raised under screenhouse conditions. At 2-3 leaf stage, the seedlings were transplanted into plastic potting bags (5 × 9 × 4 cm) containing the same media used in trays and maintained for six weeks in the screenhouse. Before transplanting to the field, seedlings were confirmed to be free from PMMoV, PVMV, TMV, PVY and CMV using DAS-ELISA. The kits were obtained from Loewe Biochemica GmbH company, Germany and used following instructions from the manufacturers. Details of the protocol is provided in section 4.2 of chapter four.

Establishment of field experiments: Randomized complete block design (RCBD) was used with three replications. The experiments depended on natural virus infections and aphid infestation from the surrounding uncultivated fields. The blocking was done according to soil fertility gradient and proximity 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 60 cm between rows and 45 cm between plants. At planting, approximately 500 g of organic manure was used per plant and a week later, 15 g/plant of NPK (17:17:17) fertilizer was applied. A month after planting, 3.5 g of urea (46:0:0) per plant was applied. Depending on symptom appearance and prevailing weather conditions, preventive and curative fungicidal sprays were applied at regular intervals. Weeds were removed regularly (2 times/month). Insecticides were not sprayed at all.

Assessment of incidence and severity of viral diseases: During plant growth, data was collected at a 14-days interval starting from two to ten weeks after planting (WAP). From each plot, ten plants from the middle rows were randomly selected and tagged. Incidence and severity of viral diseases were assessed following procedures described in section 4.2 of chapter four. 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). The estimated percentage severity was used to calculate area under disease progress curve (AUDPC) 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}.

Identification of viruses in the experimental plots: RT-PCR was used to identify suspected viruses present in the experimental plots. At 10 WAP, five young leaves from diseased plants of the eighteen genotypes were collected and kept in envelopes containing silica gel. The samples were later transported to the Phytopathology Laboratory of RAB at Rubona and stored at room temperature (±25°C) for 4-5 days to dry. Later, using liquid nitrogen the samples were ground in to fine powder and stored at -80°C until analyzed. In total, 68 symptomatic leaf samples were collected from Rubona and Gashora's experimental sites.

Acetyl-trimethyl ammonium bromide (CTAB) method with slight modifications was used to extract total ribonucleic acid from a 100 mg of frozen powdered tissues of hot pepper (Allen *et al*., 2006). One Taq One-step RT-PCR Kit (Catalogue E531S5, New England Biolabs Inc.) was used for amplification of CMV, PVMV and PeVYV following the manufacturer's instructions. Amplified products were generated using virus-specific primers for CMV, PVMV, PeVYV. Details of the primers used are provided in Table 4.2, section 4.2 of chapter four.

The RT-PCR conditions were: 15 mins for RT at 48°C; followed by 1 min for initial denaturation at 94°C; 40 cycles for denaturation at 94°C for 15 secs, 30 secs for annealing at 54°C, and 45 secs for the extension at 68°C. The final extension was at 68°C for 5 mins. The three viruses were amplified using same conditions. Amplified products were separated by electrophoresis on 1.2% agarose gel stained with ethidium bromide at 100 volts for 40 mins in 1 × Tris-Acetate-EDTA (TAE) buffer. Gels were visualized under UV light.

Assessment of aphid population: Observation of aphids was carried out at a 14-days interval starting from 2nd to 12th WAP. Un-winged aphids were observed on four randomly selected plants from the centre of each plot. The observations were carried out on six leaves (2 upper, 2 middle and 2 lower parts) 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. Yellow water traps (YWTs) made from yellow plastic containers were used for monitoring winged aphids. These traps were filled with 1.5 litres of tap water and placed at the middle of each plot immediately after planting hot pepper (Blackman and Eastop, 2000). Five millilitres of formaldehyde (10%) was added per trap to preserve the insect. The collected aphids were counted and identified to species level using stereomicroscope and the existing entomological keys based on their morphological features (Table 5.2) as described by Blackman and Eastop (2000) and Martin (1983).

Table 5.2. Morphological features that were used to identify aphids' species

Species	Body colour	Antennal tubercles	Siphunculi	Dorsal abdominal pigmentation
Myzus persicae	Green or olive	Well developed with inner sides converging	Clavate	Always bears a dorsal black patch
Macrosiphum euphorbiae	Green or olive, yellow/orange	Well developed with inner margin diverging distally	Cylindrical or tapering	No pigment completely green
Aphis gossypii	Black or green	Less developed or absent	-	Black tranverse bars on abdominal side
Aphis fabae	Black	Less developed	Short and same length with caudal	No abdominal marking all dark
Rophalosiphum maidis	Blue-green or grey	Less developed	-	Dark strip in the middle

Source; Blackman and Eastop (2000) and Martin (1983).

5.2.2. Evaluation of hot pepper genotypes reaction 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 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 5.1 section 5.2.1 of this chapter.

Inoculation with *Cucumber mosaic virus*: Fifty seedlings of each genotype were raised in the screenhouse. Before inoculation, the seedlings were confirmed to be free from PMMoV, PVMV, TMV, PVY and CMV using DAS-ELISA as described in section 5.2.1. At 5-6 leaf stage, the plants were mechanically inoculated with a local isolate of CMV. *Cucumber mosaic virus* was used as it was found to be most prevalent virus infecting hot pepper during the survey carried in chapter four. The virus was propagated and maintained in hot pepper cultivar Scotch bonnet in a 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 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 the sap extract as described by Noordam (1973). After 5 mins, the plants were rinsed with distilled water to remove the excess of the inoculum and maintained in an insect free screenhouse (average 27.8°C temperature, 70.8% relative humidity). Forty-eight plants of each genotype were inoculated with CMV. Ten healthy plants per genotype inoculated with phosphate buffer alone (with no inoculum) were maintained as control. Inoculated plants were observed for symptoms

development up to 3 weeks' post-inoculation. At this time all the plants from the susceptible control showed typical symptoms of CMV.

Assessment of incidence of *Cucumber mosaic virus* and severity*:* The incidence of CMV and severity in inoculated plants were assessed following procedures described in section 4.2 of chapter four.

Identification of *Cucumber mosaic virus:* DAS-ELISA was used to confirm CMV infection on representative samples from the genotypes (Clark and Adam, 1977). The kits were obtained from Deutsche Sammlung Von Mikroorrganismen und Zellkulturen (DSMZ, Germany) and used according to instructions from the manufacturer. Negative controls A included a healthy sample and extraction buffer while positive control was provided with the kit. A microplate reader (BioTek ELX800, USA) was used to determine the absorbance values at 405 nm (A405). Due to the large number of plants, only representative samples (7) were collected from each genotype and analysed. A sample with an absorbance values at 405 nm exceeding the mean of negative controls by a factor of two was considered positive.

5.2.3. Classification of genotypes for resistance to viral diseases

The rating of the genotypes was carried out as described by Rahman *et al*. (2016). Based on incidence and severity indices of viral diseases for field and screenhouse experiments, a score of 1 to 4 was allocated per genotype. Scoring for virus incidence was: <20% =1, 21-30%=2, 31-50%=3 and >51%=4 while for disease severity was: <1=1, 1.1-2.0=2, 2.1-3.0=3 and >3.0=4. Based on cumulative scores i.e. the incidence and severity indices, the genotypes were categorized into 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.

5.2.4. Data analysis

Data on incidence (%) and severity of viral diseases were square-root transformed, and aphids' population was log-transformed before subjecting to analysis of variance (ANOVA). Trends of aphid population trends across sites and seasons was also analyzed. Means of values regarding AUDPC were worked out using the Microsoft Excel program. The AUDPC values were directly subject to ANOVA. Fisher's protected least significant difference (LSD) test at 5% level of probability was used for means separation using Statistical Analyzing System (SAS Institute, Cary, NC) program.

5.3. Results

5.3.1. Evaluation of hot pepper genotypes reaction to virus infections and aphid infestations under field conditions in Rubona and Gashora Research station

Incidence of viral diseases: Significant variation in disease incidence among sites ($p < .0001$), seasons ($p < .0001$) and among genotypes ($p < .0001$) were observed. 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 5.3 and 5.4).

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 5.3). During long rains, genotype 00767PPR was the least infected with the viruses with disease incidence (DI) of 3%, followed by 00802PPR, PP9950-5197, PBC 462 with DI level of 10, 20 and 30% at 10 WAP, respectively (Table 5.3). The remaining genotypes had DI level greater than 60%. During the short rains, incidence levels of the viral diseases were generally lower in all genotypes compared to long rains season. The least infected genotype was PBC 462 with DI level of 3% while genotype 00802PPR and

PP9852-170 had DI level of 10%, respectively. This was followed by six genotypes (PP9950-5197, PBC 462, ICPN 18-7, 00767PPR, 00786PPR, 00765PPR) with the incidence levels of ≤ 35% while the remaining genotypes had DI of ≥55% at 10 WAP (Table 5.3).

On the other hand, a similar trend was observed at Gashora, where higher disease incidence levels were recorded in long rains compared to short rains (Table 5.4). Genotype PBC 462 was the least infected with DI of 13%, followed by ICPN 18-7 and PP9950-5197 with DI of 30 and 47%, respectively. The remaining genotypes had incidence levels of greater than 70% in long rains season (Table 5.4). During short rains, 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 genotypes except for 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. The results revealed that virus incidence was dependent on the site and season the experiments were conducted.

Table 5.3. Percentage incidence of viral diseases in eighteen hot pepper genotypes grown under field conditions during two seasons at Rubona Research Station, Huye District in Rwanda

		Long rains (March to June,18)					Short rains (Mid-Oct. 18 to March,19)				
Genotype	Type	2WAP[1]	4WAP	6WAP	8WAP	10WAP	2WAP	4WAP	6WAP	8WAP	10WAP
00765PPR	Local	0	0	43^{a}	97^{a}	100^{a}	0	3^{b}	10^{bc}	23^{bda}	33^{ebdfc}
00767PPR	Local	0	0	0^{e}	3^{f}	3^{f}	0	0^{b}	3^{c}	10^{bdc}	17^{edf}
00774PPR	Local	0	3	33^{bac}	90^{ba}	97^{ba}	0	0^{b}	17^{ba}	36^{a}	60^{bdac}
00775PPR	Local	0	10	33^{bac}	70^{bc}	87^{bac}	0	0^{b}	7^{bc}	20^{bdac}	77^{ba}
00786PPR	Local	0	10	37^{ba}	77^{bac}	90^{ba}	0	3^{b}	7^{bc}	20^{bdac}	30^{edfc}
00791PPR	Local	0	3	33^{bac}	80^{bac}	93^{ba}	0	0^{b}	0^{c}	23^{bdac}	97^{a}
00792PPR	Local	0	7	30^{bdac}	87^{ba}	97^{ba}	0	0^{b}	0^{c}	33^{ba}	70^{bac}
00802PPR	Local	0	0	0^{e}	3^{f}	10^{f}	0	0^{b}	3^{c}	7^{bdc}	10^{f}
00795PPR	Local	0	0	30^{bdac}	83^{ba}	97^{ba}	0	0^{b}	0^{c}	20^{bdac}	83^{a}
PBC 462	Introduced	0	0	0^{e}	13^{ed}	30^{e}	0	0^{b}	0^{c}	3^{dc}	3^{f}
PP9950-5197	Introduced	0	0	7^{ed}	10^{f}	20^{fe}	0	3^{b}	3^{c}	3^{dc}	13^{ef}
HP 0117	Introduced	0	0	10^{edc}	37^{ed}	63^{d}	0	0^{b}	0^{c}	7^{bdc}	13^{ef}
PP9852-170	Introduced	0	0	7^{ed}	37^{ed}	63^{d}	0	0^{b}	0^{c}	3^{dc}	10^{f}
ICPN 18-7	Introduced	0	0	13^{ebdc}	40^{d}	63^{d}	0	0^{b}	0^{c}	0^{d}	14^{f}
Long red cayenne	Commercial	0	0	23^{ebdac}	77^{bac}	90^{ba}	0	3^{b}	10^{bc}	27^{bdac}	57^{ebdac}
Bird eye hybrid	Commercial	0	0	7^{ed}	40^{d}	70^{dc}	0	0^{b}	10^{bc}	33^{ba}	73^{bac}
Red Scotch bonnet	Commercial	0	3	33^{bac}	57^{dc}	80^{bdc}	0	0^{b}	10^{bc}	23^{bda}	70^{bac}
California Wonder	Commercial	0	0	37^{ba}	83^{ba}	100^{a}	0	13^{a}	23^{a}	30^{bac}	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

Value represent the 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 superscript letters within a column are not significantly different ($P<0.05$); [1]Weeks after planting; n= 10 replicated thrice.

Table 5.4. Percentage incidence of viral diseases in eighteen hot pepper genotypes grown under field conditions during two seasons at Gashora Research Station, Bugesera District in Rwanda

		Long rains (March to June,18)					Short rains (Mid-Oct. 18 to March,19)				
Genotype	Type	2WAP[1]	4WAP	6WAP	8WAP	10WAP	2WAP	4WAP	6WAP	8WAP	10WAP
00765PPR	Local	0	0	0^{d}	90^{a}	97^{ba}	0	0	3	$33b^{ecd}$	70^{bac}
00767PPR	Local	0	0	17^{dc}	40^{cb}	87^{bac}	0	0	0	0^{e}	10^{f}
00774PPR	Local	0	3	17^{dc}	90a	100^{a}	0	0	10	63^{ba}	83^{ba}
00775PPR	Local	0	0	13^{dc}	77^{ba}	97^{ba}	0	0	3	13^{ed}	53^{bedc}
00786PPR	Local	0	3	13^{dc}	100a	100^{a}	0	0	0	57^{bc}	73^{a}
00791PPR	Local	0	0	17^{dc}	97a	100^{a}	0	0	0	17^{ecd}	83^{ba}
00792PPR	Local	0	7	23^{bac}	100^{a}	100^{a}	0	0	10	60ba	87^{ba}
00802PPR	Local	0	0	0^{d}	43^{b}	63^{dc}	0	0	0	40^{becd}	60^{bdc}
00795PPR	Local	0	3	17^{dc}	97^{a}	100^{a}	0	0	0	27^{becd}	80^{ba}
PBC 462	Introduced	0	0	0^{d}	0^{d}	13^{f}	0	0	0	7^{fge}	20^{fe}
PP9950-5197	Introduced	0	0	0^{d}	10^{d}	47^{ed}	0	0	0	3^{ed}	13^{f}
HP 0117	Introduced	0	0	0^{d}	17^{cbd}	70^{dc}	0	0	7	33^{becd}	53^{bedc}
PP9852-170	Introduced	0	0	0^{d}	17^{cbd}	73^{bc}	0	0	3	23^{becd}	33^{fedc}
ICPN 18-7	Introduced	0	0	7^{dc}	14^{cd}	30^{ef}	0	0	0	7^{ed}	23^{fed}
Long red cayenne	Commercial	0	3	13^{dc}	87^{a}	100^{a}	0	0	10	100^{a}	100^{a}
Bird eye hybrid	Commercial	0	7	37^{ba}	100^{a}	100^{a}	0	0	10	43^{bcd}	60^{bdc}
Red Scotch bonnet	Commercial	0	0	20^{bc}	90^{a}	100^{a}	0	0	3	33^{becd}	70^{bac}
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

Values represent the 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 same superscript letters within a column are not significantly different ($P<0.05$); n= 10 replicated thrice; [1]Weeks after planting.

Area under disease progress curve: The total amount of disease that occurred in the experiments was estimated and presented as the AUDPC. The mean AUDPC values differed significantly within sites thus, data not pooled together. In Rubona, the total amount of disease significantly ($p < 0.0001$) differed between season one and two (Table 5.5). In both seasons, all commercial genotypes and local collections (except 00767PPR and 00802PPR) recorded high levels of disease compared to introduced genotypes (Table 5.5). 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 in season two.

In Gashora, the AUDPC values were higher in both seasons compared to Rubona (Table 5.5). The introduced genotypes and two local collections (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. 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 viruses in hot pepper genotypes in the experimental plots: From the samples collected from the field, three viruses (PeVYV, PVMV, CMV) were detected. The CMV was the most abundant in both sites, detected in 53.1% of the samples in Rubona and 75% in Gashora, followed by PeVYV detected in 31.3 and 2.8% of the samples, respectively (Fig. 5.1). The PVMV was detected in 21.9% of the samples in Rubona. Double infection of CMV+PeVYV were common and detected in 12.5 and 2.7% in Rubona and Gashora, respectively. Triple infection of CMV+PeVYV+PVMV was detected in 9.4% of the samples from Rubona. All genotypes were infected by CMV.

Table 5.5. Means of the area under disease progress curve (AUDPC) of viral diseases in eighteen hot pepper genotypes during two seasons in Rubona and Gashora Research Station in Rwanda

	AUDPC in Rubona		AUDPC in Gashora	
Genotype	Long rains	Short rains	Long rains	Short rains
00765PPR	240^{fedc}	86^{bdc}	264^{c}	203^{gefdc}
00767PPR	9^{h}	33^{dc}	146^{d}	22^{h}
00774PPR	294^{bac}	178^{ba}	298^{bc}	334bac
00775PPR	281^{bdc}	191^{ba}	315^{bc}	129^{gefh}
00786PPR	364^{ba}	95^{bdc}	311^{bc}	272^{edc}
00791PPR	$320b^{ac}$	207^{ba}	316^{bac}	$191g^{efdc}$
00792PPR	398^{a}	199^{ba}	388^{a}	300^{bdc}
00802PPR	21^{h}	34^{dc}	149^{d}	169^{gefdh}
00795PPR	273^{bedc}	179^{ba}	333^{bac}	240^{efdc}
PBC 462	63^{hg}	15^{d}	78^{d}	57^{gh}
PP9950-5197	62^{hg}	52^{dc}	83^{d}	30^{h}
HP 0117	165^{feg}	46^{dc}	128^{d}	131^{gefh}
PP9852-170	148^{fg}	27^{dc}	137^{d}	97^{gfh}
ICPN 18-7	179^{fed}	16^{d}	93.6^{d}	63^{gh}
Long red cayenne	308^{bac}	146^{bac}	344^{ba}	448^{a}
Bird-eye	312^{bac}	179^{ba}	346^{ba}	251^{edc}
Red Scotch bonnet	331^{bac}	177^{ba}	350^{ba}	198^{gefdc}
California Wonder	379^{ba}	248^{a}	312^{bc}	445^{ba}
LSD $_{(0.05)}$	109.2	123.7	72.9	149.5
P-value	< 0.0001	0.0011	< 0.0001	< 0.0001

Values represent the 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.

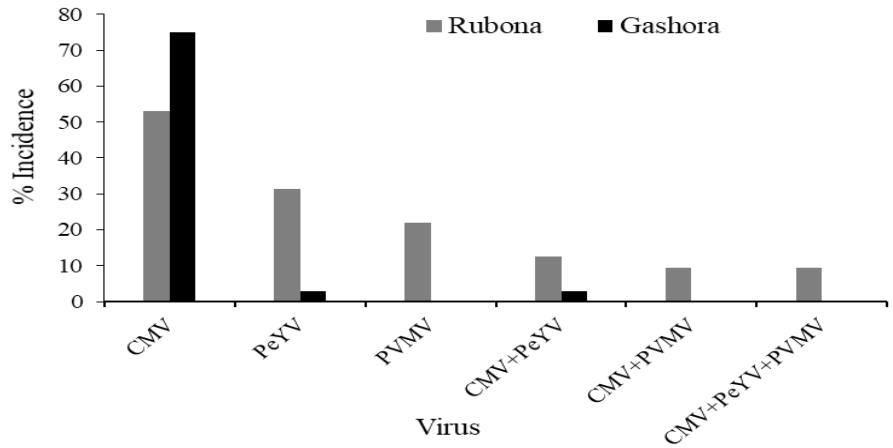

Figure 5.1. Overall incidence of viruses detected in leaf samples collected from different hot pepper genotypes in Rubona and Gashora experimental sites, Rwanda.

Assessment of aphids' population on hot pepper in the experimental plots: Aphid populations differed significantly between sites ($p < 0.05$) and season ($p < 0.05$), and thus data were analysed separately. However, genotype reaction to aphids' infestation did not vary significantly ($p = 0.0923$). In both seasons, the aphid population was significantly (p $p < 0.05$) higher in Rubona compared to Gashora (Table 5.6). Three species of aphids were observed. These were *Aphis gossypii* Glover, *Macrosiphum euphorbiae* Thomas and *Acyrthosiphon pisum* (Harris). *A. gossypii* and *M. euphorbiae* were the most abundant in both sites, while *A. pisum* was observed in Gashora site.

Table 5.6. Mean number of aphids captured in hot pepper fields during two cropping seasons in Rubona and Gashora experimental sites

	Rubona site			Gashora site			
Season	*A. gossypii*	*M. euphorbiae*	Total aphids	*A. gossypii*	*M. euphorbiae*	*A. Pisum*	Total aphids
Feb-June18	167 ± 30^a	10 ± 1^b	177 ± 30^a	32 ± 6^a	0 ± 0^b	11 ± 1^a	43 ± 5^a
Oct.18-Mar19	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[1]	ns	< 0.0001	<0.0001	ns

Values represent the means and standard errors of three replicates; [1]Not significant at 0.05 level.

All genotypes were infested by aphids but the difference in numbers was not significant among the genotypes (Table 5.7). The average number of aphids per plant varied from 4 to 108 in Rubona and 4 to 19 in Gashora, while the number of aphids per leaf ranged from 0.8 to 18 and 0.6 to 3.2, respectively. Except HP 0117 and California wonder at Rubona site, the rest of the genotypes showed low levels of aphids' infestation which did not exceed recommended chemical control action thresholds of 10 aphids per leaf.

Table 5.7. Number of aphids associated with different hot pepper genotypes in Rubona and Gashora experimental sites in Rwanda

	Rubona site			Gashora site			Mean aphids/plant
Genotype	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
LR Cayenne[1]	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 W[2]	287.8	79.2	13	40.5	7.8	1.3	43.5
P-value $_{(0.05)}$	ns [3]	ns	ns	ns	ns	ns	ns

Values represent the means of untransformed data; [1]Long red cayenne; [2]California wonder; [3]Not significant at 0.05 level.

The trends of aphid population trends varied significantly ($p < 0.05$) between sites during the two cropping seasons. However, population of aphids among the genotypes did not differ significantly ($p > 0.05$) therefore, data was pooled together. Population of aphid at Rubona peaked in the fourth week of May and continued into the second week of June followed by a sharp drop in the fourth week of June in long rains (Fig.5.2). On the contrary, the population of aphids at Gashora site remained consistently low throughout the sampling periods. In short rains, the population peaked from the third week in November to the third week in December at Rubona, followed by a sudden drop in the first week of January 2019 (Fig.5.3). On the other hand, two peaks were observed in Gashora i.e. in the third week of December 2018 and January 2019, respectively.

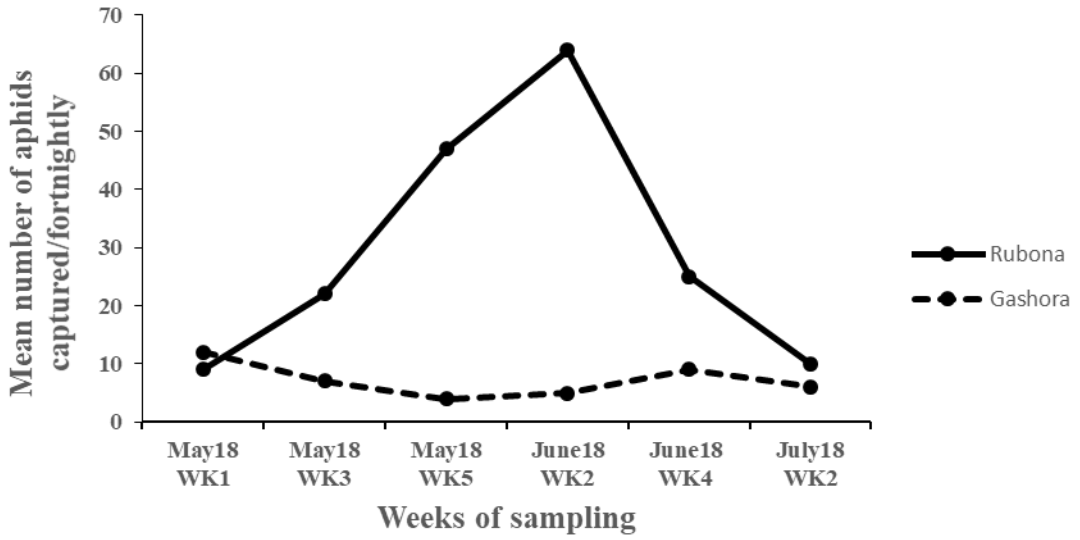

Figure 5.2. Aphids population dynamics in Rubona and Gashora sites in the short rains season between May and June 2018 in Rwanda.

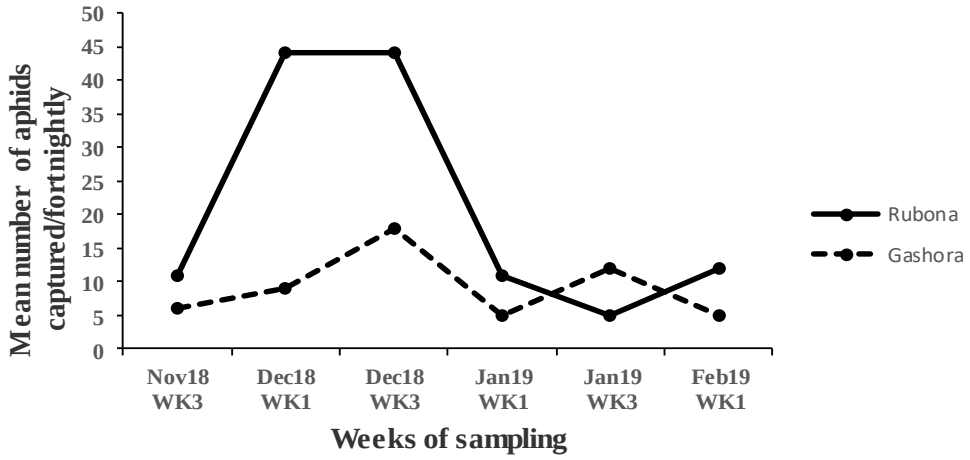

Figure 5.3. Aphids population dynamics in Rubona and Gashora sites in the long rains season between November 2018 and February 2019 in Rwanda.

Classification of genotypes for resistance to viral diseases in the field: Commercial genotypes were more susceptible to virus infection than new lines from the World Vegetable Center. Various types symptoms were observed on most genotypes during the evaluation period. These included leaf mosaic, crinkling, chlorosis, vein banding, and leaf deformation. Based on cumulative scores of the incidence and severity from both locations and seasons, only five genotypes were 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 5.8). 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.

Table 5.8. Classification of the hot pepper genotypes based on the incidence and severity scores of virus-induced diseases under field conditions in Rwanda

Genotype	Incidence (%)				Severity scores				Total rating	Host reaction
	Long rains	Short rains	Pooled	Rating	Long rains	Short rains	Pooled	Rating		
00765PPR	97	51.5	74.3^{ba}	4	2.4	1.6	2^{d}	2	6	MR
00767PPR	45	11.5	28.3^{e}	2	1	0.3	0.7^{fe}	1	3	R
00774PPR	96.5	71.5	84^{a}	4	2.8	2.7	2.8^{bac}	3	7	S
00775PPR	83.5	65	74.3^{ba}	4	2.6	2.2	2.4^{dc}	3	7	S
00786PPR	88.5	51.5	70^{bac}	4	2.9	2.1	2.5^{bdc}	3	7	S
00791PPR	90	90	90^{a}	4	2.9	2.7	2.8^{bac}	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^{fe}	1	3	R
00795PPR	91.5	81.5	86.5^{a}	4	2.8	2.9	2.9^{bac}	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^{fe}	1	2	R
HP 0117	53.5	33	43.3^{dec}	3	1.2	0.9	1.1^{e}	2	5	MR
PP9852-170	55	21.5	38.3^{dec}	3	1.2	0.7	1^{fe}	1	4	MR
ICPN 18-7	35	15	25^{e}	2	0.6	0.5	0.6^{fe}	1	3	R
LR Cayenne[1]	88.5	78.5	83.5^{a}	4	3	3.3	3.2^{ba}	4	8	S
Bird-eye	76.5	55	65.8^{bdac}	4	3	2.5	2.8^{bac}	3	7	S
Scotch bonnet	80	71.5	75.6^{ba}	4	3	2.3	2.7^{bdac}	3	7	S
California W[2]	91.5	68.5	75.6^{a}	4	2.9	2.9	2.9^{bac}	3	7	S
LSD $_{(0.05)}$			33.6				0.7			
P value			0.0004				<.0001			

Values represent the means of untransformed data; Means with the same letters within a column are not significantly different (P<0.05); Means comparison done by Least significant difference (LSD) test on transformed data; 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; Total rating i.e. incidence + severity indices: < 3= resistant (R), 4-6 = moderately resistant (MR) and 7-8 = susceptible (S); n = 16 replicated three times; *days post-inoculation; [1]Long red cayenne; [2]California wonder.

5.3.2. Evaluation of hot pepper genotypes reaction to artificial inoculation with *Cucumber mosaic virus* under screenhouse conditions

Incidence of CMV in hot pepper: The reaction to CMV varied significantly ($p<0.05$) between the genotypes evaluated (Table 5.9). Systemic symptoms including mosaic, mottle, leaf crinkling, small and deformed leaves, and stunting were observed (Fig 5.4). Six genotypes (Red scotch bonnet, 00795PPR, 00792PPR, 00786PPR, 00774PPR, and Long red cayenne) developed symptoms thirteen days' post-inoculation (dpi) (Table 5.9). Genotypes PBC462, PP9950-5197, HP 0117, PP9852-170, ICPN18-7, and 00765PPR displayed symptoms at seventeen dpi while 00767PPR and 0802PPR at nineteen dpi. A total of six genotypes namely 00765PPR, 00792PPR, 00795PPR, 00774PPR, Long red cayenne and Red scotch bonnet had disease incidence of 50-100%, severity of 2.7-5 at nineteen dpi and thus rated as susceptible to CMV (Table 5.9). Genotypes PP9950-5197, 00786PPR, HP 0117 and ICPN 18-7 had moderate levels of infection showing 22-35.4% CMV incidence at nineteen dpi and thus classified as moderately resistant. Among the 14 genotypes tested, only four (00767PPR, 0802PPR, PBC 462 and PP9852-170) showed resistant reaction against CMV with the incidence of 2-18.8% and severity of 1.0-1.2 at nineteen dpi. A positive reaction to CMV was revealed by ELISA for tested samples from all genotypes.

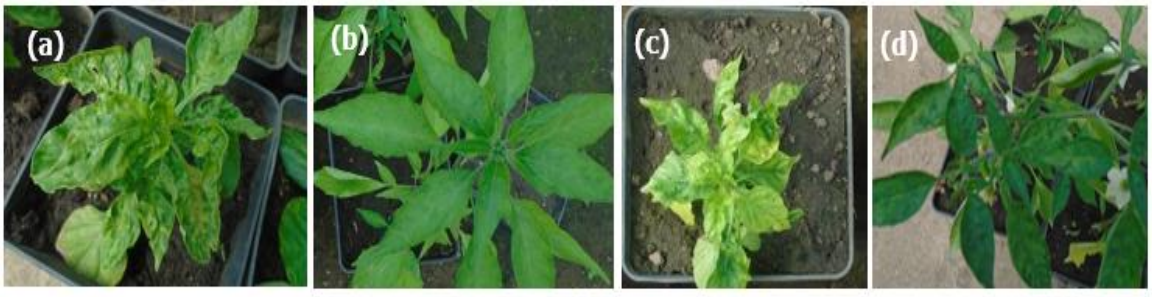

Figure 5.4. Symptoms of *Cucumber mosaic virus* on hot pepper genotypes: (a) mosaic, leaf crinkling and distortion in commercial genotype scotch bonnet; (b) mottling in local genotype 00774PPR; (c) leaf distortion and stunting in local genotype 00795PPR; (d) leaf mosaic in introduced genotype ICPN18-7.

Table 5.9. Reaction of hot pepper genotypes against *Cucumber mosaic virus* under screenhouse conditions

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

Values represent the 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 superscript letters within a column are not significantly different ($P<0.05$); n = 16 replicated three times; dpi*= days post-inoculation.

5.4. Discussion

The hot pepper genotypes tested in this study displayed variability in severity of virus induced 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. annuum, C. baccatum* and *C. frutescens* species have displayed variable resistance to some viruses such as PVMV, TMV, CMV, PMMoV and ChiVMV (Fajinmi *et al*., 2013; Appiah *et al*., 2014; Rahman *et al*., 2016). For instance, C. *baccatum* PI 439381-1-3 showed some resistance to PMMoV and CMV in field trials (Suzuki *et al*., 2003). Our result, reports some additional sources of resistance from *C. annuum* and *C. baccatum* species that could be valuable in hot pepper breeding programs as well as cultivation if preferred by farmers.

The CMV, PeVYV and PVMV were identified in leaf samples collected from fields and the former was the most abundant. A previous study in Rwanda by Skelton *et al*. (2018) also associated the three viruses with hot pepper. Several factors including wide host range, climatic conditions and efficiency of vector transmission could be attributed to the high incidence of CMV (Shah *et al*., 2009). Appiah *et al*. (2014) also observed a high incidence of CMV ranging from 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 might have serious implications in their management. Mixed infection of CMV and PVMV in hot pepper is reported in previous studies (Aliyu, 2014; Appiah *et al*. 2014). Mixed infections increase the severity of disease symptoms leading to significant yield losses (Olawale *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).

In the screenhouse, two local genotypes 0802PPR and *C. baccatum* 00767PPR, and two introduced genotypes *C. annuum* PBC 462 and PP9852-170 were categorized as 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 difference might be attributed to the strain of CMV used which was different. Various sources of resistance to CMV in pepper have been identified in *C. annuum, C. baccatum* and *C. frutescens* species (Grube *et al.,* 2000; 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 was 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 moderately resistant. This might be due to disease escape in the field. Similar observations were made by Ashfaq *et al*. (2014), where two chilli 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 observations may also be due to variations in inoculum load, virus combinations 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 of the viral diseases and severity 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 while susceptible check California wonder had the highest value of 346 in the field. Lower AUDPC values indicate a lower disease development rate. This implies that the plant defense mechanism against the viruses could have been mediated by resistance (R) genes (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 (Kim *et al*., 2017; Kang *et al*., 2010). However, genes responsible for the resistance in particular for the two local accessions are unknown and mechanisms that underlie their resistance are yet to be understood. This information is important and 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, *A. gossypii* and *M. euphorbiae* species were the most abundant in in the hot pepper fields both sites. These findings agree with previous study by Rajput *et al*. (2017) who reported the infestation of hot pepper fields with *A. gossypii* 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 Dombrovsky *et al.* (2010), Fajinmi *et al.* (2011), and Zitter and Murphy (2009), *A. gossypii* efficiently transmits CMV, PeVYV, and PVMV which were detected in this study.

Results obtained in the field showed no significant variations in genotypes infestation by aphid. The 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 (43.5aphids/plant) and 00786PPR (32.2 aphids/plant). Unlike other plant species such as soybean where a lot has been done on resistance to aphids only a few studies on pepper have been conducted (Frantz *et al*., 2004; Hill *et al*., 2004; Sun *et al*., 2018). Accession PB2013071 (*C. baccatum*) was identified as highly resistance to *M. persicae* under screenhouse conditions, while accessions PB2013062 and PB2012022 were moderately resistance (Sun *et al*., 2018). Recently, quantitative trait loci (QTLs) conferring resistance to *M. persicae* in pepper was detected (Sun *et al*., 2019). There is need for further studies to identify and validate the resistance of these genotypes to aphids under controlled and uncontrolled conditions.

The study has shown that most of the varieties grown in the country including the commonly grown commercial varieties are susceptible to viral diseases. A relatively higher number of resistant lines from introduced genotypes 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 formulation of integrated strategies for the control of these destructive diseases. There is also a need for evaluating other options that can be combined in integrated management strategy which is a promising approach to sustainable agriculture.

CHAPTER SIX:

EFFECT OF BORDER CROPS AND INTERCROPPING ON APHID INFESTATION AND THE ASSOCIATED VIRAL DISEASES IN HOT PEPPER

Abstract

Aphids are associated with loss in yield and quality of pepper crop due to damage they cause and the transmission of viruses in a non-persistent manner. The aim of the study was to determine the potential of border crops and intercrops in the management of aphids and the associated viruses in hot pepper production. Field experiments were carried out for two seasons in 2018/2019 at Rubona Research station, Huye District, Rwanda. Maize (*Zea Mays* L.), sorghum (*Sorghum bicolor* L.) and sunflower (*Helianthus annuus*) were tested as companion crops associated with hot pepper either as border crops or as intercrops. Randomized complete block design was used with four treatments and three replications per experiment. Data on aphid population, the virus disease incidence, the area under the disease progress curve (AUDPC), growth and yield of hot pepper were recorded. Diseased leaf samples were collected and analysed serologically to detect the viruses present in the experimental plots. Aphid-transmitted viruses; *Cucumber mosaic virus*, *Potato virus Y* and *Pepper veinal mottle virus*, and aphid species, namely *Aphis gossypii* and *Macrosiphum euphorbiae* were identified in the experimental plots. The use of maize, sorghum or sunflower as border crops or as intercrops significantly reduced ($p<0.05$) the incidence of viral diseases in hot pepper compared with control at later stages of growth. At 12 weeks after transplanting (WAP), use of maize, sunflower or sorghum as border crops significantly reduced ($p<0.05$) the incidence of viral diseases by 24, 31 and 38% compared with the control in season one and by 32, 54 and 46% in season two. Similarly, intercropping with

maize, sunflower or sorghum was also effective in reducing incidence of viral diseases in hot pepper by 24, 35 and 27% in season one, and by 39, 39 and 32% in season two, respectively. The AUDPC differed significantly ($p<0.05$) while the population of aphids did not differ ($p>0.05$) among treatments for both experiments. Results revealed that maize, sorghum and sunflower can be utilized either as border crops or intercrops for the control of viral diseases in pepper production. This technology is environmentally friendly, easily adaptable and appropriate since the production of pepper is mainly done by resource-poor farmers.

6.1. Introduction

Hot pepper (*Capsicum* spp.), is susceptible to several viral diseases which significantly affect its production. Non-persistent viruses (NPVs), for instance, CMV, PVMV and PVY vectored by several aphid species are considered as the economically most important viruses attacking hot pepper in Sub-Saharan Africa (Dafalla, 2001). Other important viruses of pepper transmitted by aphids in Africa include; *Pepper mottle virus* (PepMov) and TEV (Njukeng *et al*., 2013; Olawale *et al*., 2015). Among these NPVs, CMV and PVMV are reported to attack hot pepper crop in Rwanda (Skelton *et al*., 2018). The viruses cause mosaic, mottling, yellowing, vein banding, distortion, plant stunting and distortion of the fruits that renders them unmarketable (Waweru *et al*., 2019).

One of the main insect pests of crops especially in the tropical regions are the aphids that cause major economic losses. Among the aphids, the *A. gossypii* Glover, *M. euphorbiae* and *Myzus persicae* Sulz are the most efficient transmitters of viruses in pepper (Weintraub, 2007). Farmers rely mostly on applications of insecticides to control aphids, aimed at decreasing the populations before they damage the crop (Hooks and Fereres, 2006). However, this method has been found not effective in reducing the spread of the NPVs

because the virus is transmitted before the insecticides act to kill the aphids (Fereres, 2000). Also, many aphid species have developed resistance to various chemicals in use (Li and Han, 2004). Integrating cultural-control techniques such as companion-cropping with other pest-suppression methods are likely to reduce insect pest numbers and the resulting transmission of viruses.

"Companion cropping" is the practice of establishing two or more crop species together within the same field for cultural benefits such as pest management and increased yield (Kuepper and Dodson, 2001). Different spatial arrangements of the companion crops have been studied as a cultural control strategy for insect pests and associated viral diseases. One such arrangement is the planting of companion crops on the perimeter of the field containing the primary crop forming borders. These borders visually or physically obstruct movement or migration of insect pests into the primary crop, and can be helpful to reduce the spread and transmission of insect-vectored viruses (Hooks and Fereres, 2006). Companion crops can also be inter-planted with the primary crop either in alternating rows or within the same row as intercrops. Intercropping has several benefits such as suppression of insect-pest population, interfering with the insects' search for the host crop for feeding or breeding, and cleaning the mouthparts of the insect-vectors and therefore preventing or reducing the spread of pathogens. In Rwanda, the practice of growing two or more crops simultaneously on the same field is common and this is due to scarcity of land.

To date, the use of companion crops as crop borders has provided protection against several insect-vectors and associated NPVs on several plant species. For instance, use of sorghum, maize, wheat (*Triticum aestivum* L.), common bean (*Phaseolus vulgaris* L.) and garden pea (*Pisum sativum* L.) as crop borders resulted in reduced incidence of PVY and aphid infestation in potato *(Solanum tuberosum* L.) (Olubayo *et al*., 2009). Kibaru (2004) observed

reduced aphid infestation in potato plots bordered by wheat, sorghum and maize compared with unprotected potato plots. Similarly, significant reductions (13%) in CMV was achieved in pepper plots surrounded by sorghum (Fereres, 2000), while more than 50% reductions in the incidence of PVY on pepper was achieved with borders of sunflower (Simmons, 1957).

Intercropping has also been evaluated for effects on NPVs and insect-vectors. Intercropping pepper with millet, maize and sorghum resulted in a significant reduction in aphid colonization by 17.5, 51.3 and 52.6%, respectively (Degri and Ayuba, 2016). Intercropping with maize, cassava (*Manihot sp*.) and plantain (*Musa sp*.) reduced incidence of PVMV in pepper by 76.2, 88.1 and 80.2%, respectively (Fajinmi and Fajinmi, 2010). Similarly, Damicone *et al*. (2007) observed 43-96% reductions in *Watermelon mosaic virus* and *Papaya ringspot virus* incidence in pumpkin plants intercropped with sorghum.

In this present study, maize, sorghum and sunflower were used as companion crops associated with hot pepper either as border crops or as intercrops. These crops were selected based on their height difference with the main crop and also because they are common crops cultivated by farmers in Rwanda. According to Dhanju *et al*. (1995) the use of companion plants taller than the primary crop can impede the movement or migration of pests into the cropping system. There is a likelihood of these companion crops to intercept aphids in flight. The study focused on; to assess the potential of border crops or intercrops to control aphid infestation in hot pepper, and to limit the transmission of non-persistent viruses.

6.2. Materials and methods

Experimental site: The field experiments were carried out at Rubona, RAB Research station in the Huye District during two successive growing seasons 2019A (December, 2018-April, 2019) and 2019B (March-July, 2019). Details of the site description refer to section 5.2.1 in chapter five.

Plant materials: Hot pepper variety Scotch bonnet belonging to *Capsicum chinense* was used. For the companion crops, maize (Var. ZM607), sorghum (Var. IS21219), sunflower (Var. Fedha) varieties were used. The seeds were obtained from maize, sorghum and horticulture programs in RAB. Hot pepper seeds were raised in trays containing sterilized sandy: loam soil (1:2) under screenhouse conditions. At 2-3 leaf stage, the seedlings were transplanted into plastic potting bags containing steam-sterilized sandy-loam-soil and maintained in the screenhouse for one and half months before transplanting to the field.

Experimental design: Two field experiments were carried out in parallel that is border crop and intercrop. Each experiment was laid out in a Randomized Complete Block Design (RCBD) with four treatments replicated thrice. The replicates were separated by a gap of 2 m while plots within a replicate were separated by 1 m. Treatments used in border crop experiment comprised of pepper/maize border, pepper/sorghum border, pepper/sunflower border and sole pepper (control), in which a row of maize, sorghum or sunflower plants were grown as a strip, surrounding the pepper plants (Fig. 6.1A). Each experimental plot measured 2.7×3.6 m with four rows of pepper spaced 60 cm between rows and 45 cm within row. Sixteen (16) hot pepper plants per plot. Sorghum, maize and sunflower were spaced 20, 30 and 50 cm apart within a row, respectively. Spacing between the border row and pepper was 60 cm.

Treatments used in the intercropped experiment were pepper/maize intercrop, pepper/sorghum intercrop, pepper/sunflower intercrop and sole pepper crop as control. Arrangement pattern included a row of companion crop (maize, sorghum or sunflower) alternating with two rows of pepper (Fig. 6.1B). Each plot measured 2.7×4.2 m with 3 rows of companion crop and 4 rows of pepper, spaced at 60 cm. Twenty-four (24) hot pepper plants per plot. The spacing used within a row for each crop is as mentioned in the border crop experiment.

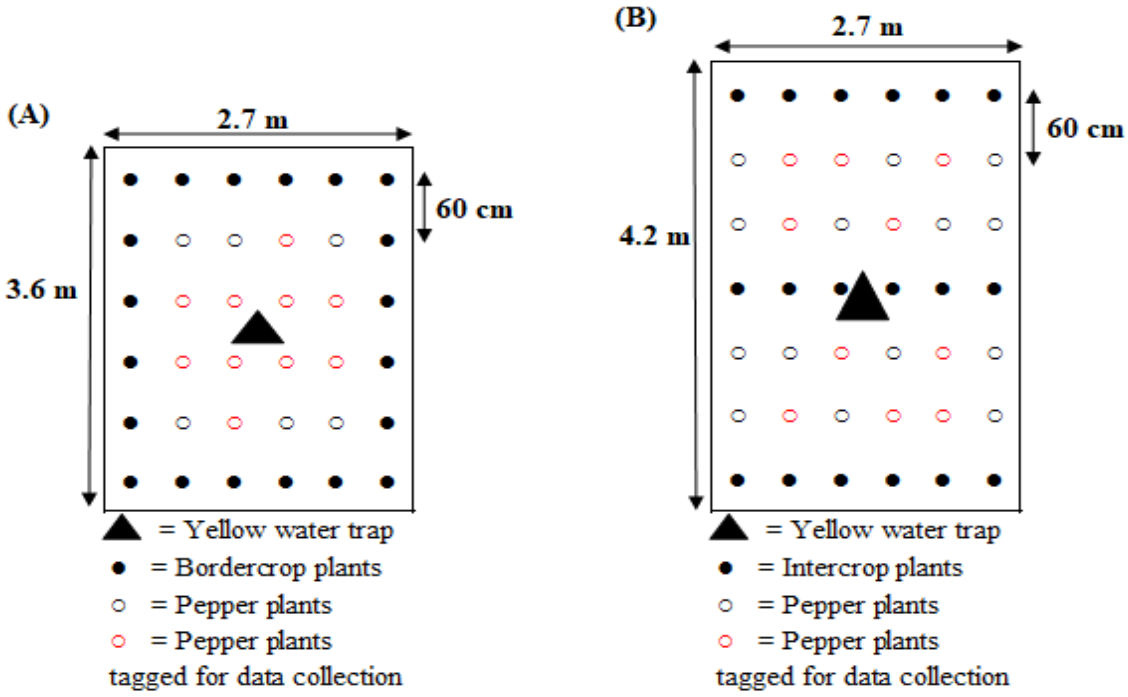

Figure 6.1. Illustration of the plot layout for (A) Border crop experiment and (B) Intercrop experiment

Crop establishment and maintenance of the experimental fields: The land was manually prepared to a medium tilth using hoes and garden rakes. For both experiments, companion crops were planted two weeks before in their respective plots to allow them to establish before transplanting hot pepper. Planting holes were manually dug using hoes, and organic

manure at a rate of 500g/plant was incorporated into the soil at the time of planting in both pepper and companion crops. Sorghum was drilled and later thinned, when the seedlings had attained a height of about 10 cm maintaining a spacing of 20 cm between plants. For maize and sunflower four seeds/hole were planted and 2-weeks later they were thinned leaving two seedlings/hole. The plants were top-dressed with granular fertilizer at 15 g of NPK 17-17-17/plant one week after transplanting and 5 g of urea one month after transplanting. When necessary, copper oxychloride, and metalaxyl-M 4% w/w and mancozeb 64% w/w were sprayed at a rate of 2.5 g/l of water for control of fungal diseases. Application of insecticide was not done throughout the study so that the effects of the companion crops on aphid populations could be assessed. Weeding was done two times in a month. The plots were rain-fed and the experiments were conducted in two seasons in separate fields adjacent to each other.

Assessment of the incidence and severity of viral diseases in hot pepper: Ten plants of pepper were randomly selected per plot. The number of infected plants was recorded fortnightly for 12 weeks starting from 4 weeks after planting (WAP). The incidence and severity of viral diseases were assessed following procedures described in section 4.2 of chapter four. The AUDPC values were estimated following formula elaborated in section 5.2.1 of chapter five.

Assessment of aphid population on hot pepper: Monitoring of aphids was done on a fortnight basis starting from 4 to 12 WAP. Both un-winged and winged aphids were monitored following procedures described in section 5.2.1 of chapter five. Collected aphids were counted and identified under a stereo dissecting microscope in Phytopathology Laboratory at RAB, Rubona. Existing entomological keys were used to aid in identifying

different species of aphids based on their morphological features as described by Martin (1983), and Blackman and Eastop (2000).

Assessment of growth and yield components of hot pepper: Plant height, stem girth and number of branches were measured on ten hot pepper plants randomly selected from the interior rows per plot. Height of plant was measured from base to the tip of the longest branch using a meter rule. The girth was measured at the base of the stem at soil level. The average number of marketable fruits per plant was counted and fruit weight per plant measured. The yield per hectare was obtained by adding up marketable fruit yields from different plots of the same treatment and converting it to hectares.

Detection of viruses in the experimental plots: The detection of specific viruses of interest in the experimental plots was carried out using DAS-ELISA and RT-PCR. At 12 WAP, young leaves from diseased pepper plants were collected per plot, placed in a cool box containing ice and transported to Phytopathology Laboratory at RAB Rubona, to identify the viruses present. Based on the symptoms appearance, a total of 24 leaf samples were collected from the entire experiment for both border and intercrop for two seasons (12 samples per practice) and tested for the presence of PVY, CMV and PVMV. Specific polyclonal antiserums were used from Deutsche Sammlung Von Mikroorrganismen und Zellkulturen (DSMZ, Germany) following manufacturer's instructions. Extraction buffers were used as negative controls. The absorbance values were read at 405 nm using a microplate reader (BioTek ELX800, USA). A sample was regarded as positive when the absorbance value exceeded the average of negative controls by a factor of two.

The RT-PCR was used to confirm the results from DAS-ELISA. Total ribonucleic acid (RNA) was extracted from each leaf sample using CTAB method described by Allen *et al.* (2006) with modifications as explained in section 4.2 of chapter four. The resultant RNA was

stored at -80°C until analysis. The CMV and PVMV primers were used for the amplification. Details of the primers are provided in Table 4.2 of chapter four.

The RT-PCR procedures were performed using One Taq One-step RT-PCR Kit (Catalogue E531S5, New England Biolabs Inc.), following manufacturer instructions. Thermal cycling conditions were: 48°C at 15 mins for RT; followed by 1 min at 94°C for initial denaturation; 40 cycles of 94°C at 15 secs for denaturation, 54°C at 30 secs for annealing and 68°C at 45 secs for the extension, respectively. The final extension was at 68°C for 5 mins. These conditions were similar for the two viruses. The amplified products were separated by electrophoresis in 1.2% agarose gel stained with ethidium bromide at 100 V for 40 mins in 1 × Tris-Acetate-EDTA (TAE) buffer. Gels were visualized under UV light.

Data analysis: Data from border and inter-crop experiments, sites and seasons were analyzed separately. The disease incidence and severity scores data were square-root transformed [sqrt (X+1)] and, aphid counts were log-transformed [log (2X+1)] to ensure normal distribution of the data before subjecting to the analysis of variance (ANOVA). Growth and yield data were subjected to ANOVA. Comparison of means was done by the Fisher's protected least significant difference (LSD) test at 5% level of probability, using Statistical Analyzing System (SAS Institute, Cary, NC) program.

6.3. Results

Effect of border and inter-crops on virus disease incidence and AUDPC: The incidence of symptomatic plants increased with time across all treatments for both border and inter-crops experiments (Table 6.1 & 6.2). In season one, the effect of the border and inter-crop treatments on virus disease incidence in pepper plants had a significant ($p<0.05$) effect from 8 to 12 weeks after planting (WAP) in Table 6.1. Pepper plants from the control plots recorded

the highest disease incidence through the growing period. In season two, significant ($p<0.05$) effects of the border treatments on virus disease incidence were observed at a later stages of crop growth i.e. 10 to 12 WAP (Table 6.2). At 12 WAP, use of maize, sunflower or sorghum as border crops significantly reduced ($p<0.05$) the incidence of viral diseases by 24, 31 and 38% compared with the control in season one and by 32, 54 and 46% in season two.

Unlike the border experiment, effects of intercrop treatments on disease incidence was observed at an early stage 8 WAP. Similar to season one, the highest disease incidence was recorded in control plots. Percentage reduction on incidence of viral diseases in pepper plants intercropped with sorghum, maize or sunflower was 24, 35 and 27% in season one, and 39, 39 and 32% in season two, respectively at 12 WAP.

Table 6.1. Percentage virus disease incidence in hot pepper as influenced by border and inter-crops overtime during first season from December 2018 to April 2019, Rwanda

	Weeks after transplanting				
Treatment	4	6	8	10	12
Maize border	0.0±0.0^{b}	16.7±3.3	20.0±5.8^{b}	23.3±3.3^{b}	73.3±6.7^{b}
Sunflower border	0.0±0.0^{b}	10.0±5.8	13.3±6.7^{b}	26.7±3.3^{b}	66.7±3.3^{b}
Sorghum border	0.0±0.0^{b}	10.0±5.8	13.3±8.8^{b}	20.0±11.5^{b}	60.0±20.0^{b}
Sole pepper	6.7±3.3^{a}	26.7±3.3	50.0±5.8^{a}	73.3±8.8^{a}	96.7±3.3^{a}
LSD	5.4	15.4	22.4	24.9	22.2
P value	0.0519	0.1092	0.0155	0.0034	0.0479
Maize intercrop	0.0±0.0	16.7±3.3	23.3 ±8.8^{b}	26.7±12.0^{b}	73.3±6.7^{b}
Sunflower intercrop	3.3±3.3	16.7±8.8	16.7±8.8^{b}	20.0±5.8^{b}	63.3±12.0^{b}
Sorghum intercrop	0.0±0.0	13.3±6.7	23.3±6.7^{b}	43.3±12.0^{ba}	70.7±12.0^{b}
Sole pepper	6.7±3.3	26.7±3.3	50.0 ±5.8^{a}	73.3±8.8^{a}	96.7±3.3^{a}
LSD	7.7	19.6	24.9	32.6	20.3
P value	0.2192	0.4725	0.050	0.0223	0.0376

Values represent the means and standard error of un-transformed data. For each column, means with the same letter are not significantly different ($p\leq0.05$, LSD test), n=30.

Table 6.2. Percentage virus disease incidence as influenced by border and inter-crops overtime during second season from March to July 2019, Rwanda

	Weeks after transplanting				
Treatment	4	6	8	10	12
Maize border	6.7±3.3	33.3±8.8	36.7±6.7	53.3±8.8^{b}	63.3±6.7^{b}
Sunflower border	0.0±0.0	10.0±0.0	16.7±3.3	30.0±0.0^{b}	43.3±6.7^{b}
Sorghum border	3.3±3.3	23.3±12.0	33.3±17.6	43.3±20.2^{b}	50.0±20.8^{b}
Sole pepper	6.7±3.3	20.0±5.8	26.7±8.8	80.0±5.8^{a}	93.3 ±3.3^{a}
LSD	9.4	26.1	34.4	25.7	27.7
P value	0.363	0.2993	0.578	0.0424	0.0421
Maize intercrop	10.0±10.0	23.3±14.5	23.3±12.0^{b}	46.7±16.7^{b}	56.7±12^{b}
Sunflower intercrop	3.3±3.3	10.0±0.0	23.3 ±3.3b	33.3 ±3.3^{b}	56.7±6.7^{b}
Sorghum intercrop	0.0±0.0	26.7±8.8	30.3±6.7^{b}	50.0±5.8^{b}	63.3±3.3^{b}
Sole pepper	6.7±3.3	33.3±6.7	55.0±5.8^{a}	76.7±12^{a}	90.0±5.8^{a}
LSD	18	29.8	21.9	25.2	24.9
P value	0.6294	0.3846	0.0420	0.0368	0.0435

Values represent the means and standard error of un-transformed data. For each column, means with the same letter are not significantly different ($p \leq 0.05$, LSD test), n=30.

For border crop experiment, the intensity of the diseases differed significantly ($p<0.05$) among the treatments in season one (Table 6.3). Pepper plots without a border crop had the highest AUDPC value (290.7) while plots bordered by sorghum had the least value of 94. Though no significant differences observed in season two, the intensity of the diseases was high in sole pepper plots. A significant ($p < 0.05$) reduction in the amount of diseases was observed in pepper plots with intercrops compared with plots without intercrops in both seasons (Table 6.3). In both seasons, pepper plants intercropped with sunflower had the least AUDPC value followed by maize and sorghum intercrops. The intensity of the disease was highest in control plots planted with sole pepper.

Table 6.3. Area under disease progress curve of hot pepper as influenced by border and inter-crops during two cropping seasons

Treatment	AUDPC*	
	December 18-April 2019	March - July 2019
Maize border	131.3±18.7[b]	255.3±48.2
Sunflower border	99.3±20.3[b]	112.7±4.4
Sorghum border	94.0±45.7[b]	201.3±39.7
Sole pepper	290.7±21.1[a]	275.3±29.9
$LSD_{(0.05)}$	93.6	173.4
P Value	0.0039	0.2130
Maize intercrop	154.7±20.1[b]	151.3±35.9[b]
Sunflower intercrop	132.0±41.3[b]	138.0±6.1[b]
Sorghum intercrop	157.3±44.7[b]	223.3±48.7[ba]
Sole pepper	280.7±12.2[a]	336.0±40.5[a]
$LSD_{(0.05)}$	106.4	174.5
P Value	0.0450	0.0498

Values represent the means and standard errors. For each column, means with the same letter are not significantly different ($p \leq 0.05$, LSD test), n=30. *Area under disease progress curve.

Effect of border and inter-crops on aphid population: Aphid species identified were *M. euphorbiae* and *A. gossypii*, and the latter was the most abundant throughout the experimental period. The number of alate aphids captured on YWT differed significantly ($p < 0.05$) between the two seasons and thus, data were analysed separately for both border and inter-crops experiments (Table 6.4). However, the treatments did not have any significant ($p > 0.05$) effect on the number of alate aphids landing on protected pepper compared with unprotected plots for both experiments. Similarly, the number of apterous aphids on pepper leaves did not differ significantly among the treatments (Table 6.5).

Table 6.4. Number of aphids captured in yellow water traps in hot pepper plots as influenced by border and inter-crops during two cropping seasons in Rubona Research Station, Rwanda

Treatment	December 18-April 2019		March - July 2019	
	A. gossypii	*M. euphorbiae*	*A. gossypii*	*M. euphorbiae*
Maize border	67.8±10.0	41.7±2.7	11.4±2.8	8.3±2.2
Sunflower border	37.0±3.8	43.2±6.8	14.0±4.5	7.8±1.8
Sorghum border	36.3±3.9	52.2±6.3	17.4±6.7	10.2±3.5
Sole pepper	52.3±11.0	48.5±10.5	27.5±11.8	14.7±4.9
P Value	0.0668	0.7113	0.1650	0.1641
Maize intercrop	74.8±8.0	44.8±7.9	15.9±3.4	9.7±3.6
Sunflower intercrop	70.8±4.4	58.7±11.7	10.8±2.4	13.3±3.8
Sorghum intercrop	88.7±7.4	49.2±8.8	26.2±9.7	14.4±4.3
Sole pepper	67.3±7.8	41.8±5.9	23±7.6	16.2±6.2
P Value	0.1871	0.5692	0.3498	0.3846

Values represent the means and standard errors of untransformed data, n=12.

Table 6.5. Number of aphids per plant in hot pepper plots as influenced by border and inter-crops during two cropping seasons in Rubona Research Station, Rwanda

Treatment	December 18-April 2019		March - July 2019		Mean of total aphids
	A. gossypii	*M. euphorbiae*	*A. gossypii*	*M. euphorbiae*	
Maize border	9.8 ± 2.6	2.7 ± 1.3	1.2 ± 0.8	2.3 ± 1.2	8 ± 1.8
Sunflower border	4.4 ± 3.6	3.1 ± 1.4	1.3 ± 0.5	1.8 ± 1.8	5.3 ± 1.9
Sorghum border	24.8 ± 14.4	1.6 ± 0.9	1.4 ± 0.7	2.2 ± 1.5	15 ± 7.7
Sole pepper	7.7 ± 4.2	2.4 ± 1.6	3.9 ± 1.8	5.7 ± 2.9	9.8 ± 2.6
P Value	0.2852	0.8664	0.2475	0.4958	0.4410
Maize intercrop	9.3 ± 4.4	0.8 ± 0.6	3.4 ± 3.4	5 ± 3.6	9.3 ± 3.1
Sunflower intercrop	20.4 ± 11.9	3.7 ± 2.8	0.8 ± 0.4	3.3 ± 1.8	14.1 ± 6.2
Sorghum intercrop	30.4 ± 14.9	7.1 ± 6	6.2 ± 2.7	4.4 ± 4.3	24.1 ± 9.1
Sole pepper	7.4 ± 4.3	2.4 ± 1.6	13 ± 7.6	6.2 ± 4.2	14.6 ± 4.4
P Value	0.3498	0.6133	0.2509	0.9533	0.3846

Values represent the means and standard errors of untransformed data, n=4 replicated thrice.

Effect of border and inter-crops on growth and yield of hot pepper: Utilization of maize, sorghum or sunflower as border crops did not have significant effects on growth and the yield of hot pepper during the two cropping seasons (Table 6.6 & 6.7). Similarly, the growth of hot pepper plants was not significantly affected by the intercrops in season one (Table 6.6). However, in the second season, hot pepper plants intercropped with sunflower were significantly shorter and thinner compared with those of the control. The number of fruits/plant, yield/plant and yield/hectare were not significantly different, except for hot pepper plants intercropped with maize in the first season (Table 6.7). Hot pepper plants intercropped with maize yielded more and heavier fruits than those of the rest of the treatments.

Table 6.6. Plant height, stem girth and number of main branches of hot pepper as influenced by border and inter-crops during two cropping seasons

Treatment	December 18-April 2019			March - July 2019		
	Height (cm)	Girth (cm)	No. of Branches	Height (cm)	Girth (cm)	No. of Branches
Maize border	73.9±7.8	4.4±0.4	5.9±0.3	72.3±2.4	4.1±0.4	5.7±0.3
[1]Sun border	68.4±0.1	4.1±0.0	6.9±0.3	50.5±5.8	3.6±0.3	4.7±0.3
Sorghum border	79.8±10.9	4.3±0.3	7.4±1.4	65.5±9.3	4.2±0.8	6.3±0.7
Sole pepper	75.3±10.9	5.1±0.9	8.1±1.6	56.7±5.6	3.9±0.3	5.7±0.3
$LSD_{(0.05)}$	28.3	1.7	3.6	20.5	1.6	1.4
P Value	0.8293	0.5493	0.6001	0.1493	0.8375	0.1404
Maize intercrop	67.5±10	4.0 ±0.3	6.7±1.0	53.1±3.0[a]	3.8±0.1[a]	5.3±0.3
Sun intercrop	83.7±11	4.2 ±0.2	8.3±1.0	40.6±5.0[b]	2.9±0.4[b]	4.3±0.3
[2]Sorg intercrop	97.1±18	4.2±0.8	9.2±3.0	62.1±2.0[a]	4.0±0.1[a]	5.7±0.3
Sole pepper	75.6±11	4.4 ±0.3	8.4 ±2.0	55.3±2.0[a]	3.9±0.2[a]	5.0±0.6
$LSD_{(0.05)}$	42.4	1.5	5.6	9.9	0.7	1.3
P Value	0.4641	0.9605	0.7407	0.0068	0.0252	0.2011

Values represent the means and standard errors. For each column, means with the same letter are not significantly different ($p \leq 0.05$, LSD test), n=30.[1]sunflower and [2]sorghum.

Table 6.7. Yield of hot pepper as influenced by border and inter-crops during two cropping seasons

Treatment	December 18-April 2019			March - July 2019		
	Fruits/ plant	Yield/ plot (kg)[1]	Yield/ ha (tons)*	Fruits/ plant	Yield/ plot (kg)	Yield/ ha (tons)
Maize border	19.7±4.3	3.1±0.5	3.2±0.5	32.8±6.1	4.4±0.7	4.5±0.7
[1]Sun border	14.1±1.3	1.9±0.2	2.0±0.2	25.6±6.7	2.9±0.7	3.0±0.7
[2]Sorg border	15.6±6.5	2.1±0.8	2.2±0.9	25.6±7.4	3.6±1.5	3.7±1.6
Sole pepper	23.7±1.1	2.3±0.2	2.4±0.2	33.5±1.2	3.5±0.2	3.6±0.2
$LSD_{(0.05)}$	12.9	1.6	1.7	19.2	2.9	3.1
P Value	0.3743	0.4172	0.4185	0.6613	0.7231	0.7237
Maize intercrop	28.7±1.0	5.0±0.2[a]	4.4±0.1[a]	23.2±2.5	3.2±0.2	3.9±0.2
Sun intercrop	18.1±5.1	3.2±0.4[b]	2.9±0.4[b]	18.8±4.9	2.5±0.4	2.2±0.4
Sorg intercrop	17.3±2.0	4.0±0.6[ba]	3.6±0.6[ba]	26.1±2.6	3.7±0.7	3.3±0.6
Sole pepper	23.7±1.2	2.9±0.2[b]	2.6±0.2[b]	27.7±5.1	4.4±0.9	3.9±0.9
$LSD_{(0.05)}$	9.2	1.3	1.2	13.1	2.1	1.9
P Value	0.0683	0.0265	0.0261	0.4636	0.2925	0.2915

Values represent the means and standard errors. For each column, means with the same letter are not significantly different ($p \leq 0.05$, LSD test), n=30. *Estimation of hot pepper yield per ha was done excluding the area dedicated to companion plants,[1]Sunflower and [2]Sorghum.

Detection of viruses: *Cucumber mosaic virus*, PVY and PVMV were detected by DAS-ELISA in the samples. Of the 24 samples analysed, the most prevalent virus was CMV detected in 91.7% of the samples followed by PVY in 70.8% and the least was PVMV detected in 20.8%. Mixed viral infections of CMV + PVY was a common occurrence in 70.8% of the samples while CMV+PVMV occurred only in two samples (8.3%). Triple infections were also rare, CMV+PVY+PVMV occurring only in three samples (12.5%). The CMV and PVMV fragments size of each 502 bp was successfully amplified from the virus-infected hot pepper leaf samples using RT-PCR technique (Fig.6.2). Amplification of PVY by RT-PCR technique was not done.

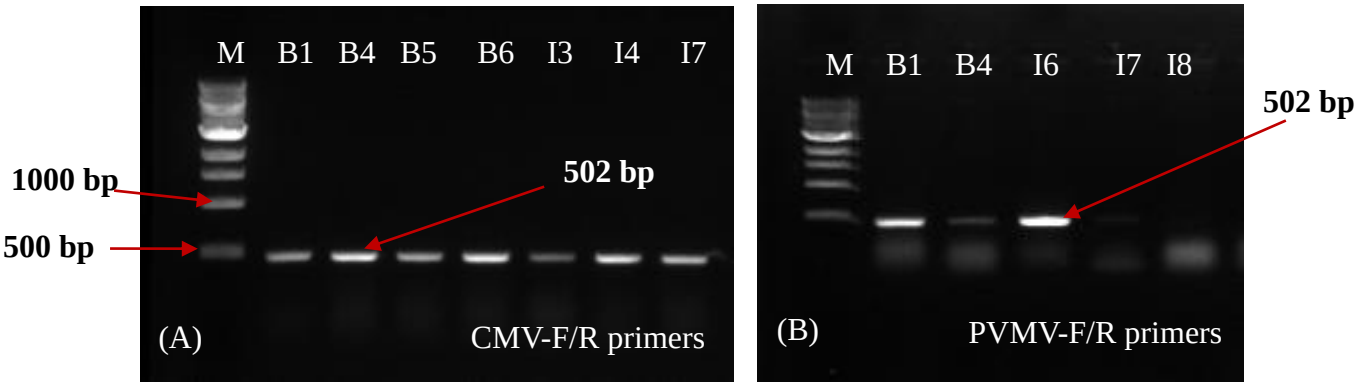

Figure 6.2. Detection of *Cucumber mosaic virus* and *Pepper veinal mottle virus* from diseased leaf samples by RT-PCR using (A) CMV-F/R and (B) PVMV-F/R primers respectively. Amplified RT-PCR products analysed by electrophoresis on 1.2% agarose gel at 100 volts for 40 minutes. Lane M - 1 Kb DNA ladder; lanes B1, B4, B5, B6 samples collected from the border crop experiment and lanes I3, I4, I6, I7, I8 samples from the intercrop experiment. Each primer set amplified a ~502bp product from infected leaf samples.

6.4. Discussion

The use of companion crops (maize, sorghum and sunflower) as crop borders and as intercrops resulted in reduced incidence of aphid-transmitted viruses of hot pepper with significant reductions in the AUDPC. Border crop and intercrop treatments have had positive effects on virus disease control in previous studies (Olubayo *et al*., 2009; Fajinmi and Fajinmi, 2010; Ashenafi *et al*., 2014). The reduction in the incidence of viral diseases observed with companion cropping treatments in the present study might be attributed to reduced possibility of infective aphids landing and probing on the hot pepper through their interception on the border crops and the intercrops. The non-host companion crops intercept the infective aphids and while probing they lose their virus content possibly due to cleansing their mouthparts (Hooks and Fereres, 2006). As a result, their ability to transmit the viruses to the primary crop at the centre of the plots is reduced. Previous studies by Fereres, (2000) reported more than 50% reduction in transmission of *Potato virus Y* and *Cucumber mosaic*

virus to pepper when aphids had access periods to either corn or sorghum, before moving to pepper.

Results from the experiments also indicate that maize, sorghum or sunflower did not reduce the number of aphids that landed on protected pepper in relation to the control plots. This agrees with the finding of Fereres, (2000) who reported no difference between the number of aphids landing in pepper plots unprotected and plots surrounded by sorghum, vetch and maize crops. Thus, concluded that the border crops acted as natural sinks for viruses of pepper. Several other previous studies have also suggested that intercrops or border crops may act as a 'sink' for the NPVs rather than as a mechanical or physical barrier (Dhanju *et al*., 1995; Difonzo *et al*., 1996). Our data disagree with the hypothesis that use of tall border crops or intercrops may act as mechanical barriers that interfere or impede insect pests on flight from reaching the host plants (Mannan, 2003; Muindi *et al*., 2009). The present findings support the virus-sink hypothesis, whereby, maize, sorghum and sunflower crops may have acted as sinks for the NPVs leading to reductions in virus disease incidence of hot pepper.

Aphids are prevalent in small scale farmers' fields. Two species of aphids were identified from the experimental plots i.e. *M. euphorbiae* and *A. gossypii* and, the latter was the most abundant during the two seasons. Both species are polyphagous and their composition reflected the cropping system in the adjacent surroundings. The main crops surrounding the experimental fields consisted of hot pepper, potatoes, amaranth, kales, stevia and other common weeds such as blackjack, which are all preferred hosts of these species. The composition is similar to that which was observed by Djieto-Lordon *et al*., (2014) and Rajput *et al*., (2017) in pepper fields. Both species and in particular, *A. gossypii* are efficient vectors of CMV, PVY and PVMV viruses identified in this study (Pernezny *et al*., 2003; Fajinmi *et*

al., 2011). These aphid species probably contributed to the spread of the virus infections observed in the hot pepper experimental plots. A limitation of our study was that aphid landing in the companion crops was not monitored in our study and obvious edge effects were not observed within plots.

Despite the reduced incidence of viral diseases observed with border crop and intercrop treatments in our experiments, impact on growth and yield of hot pepper were not generally observed. The yield of hot pepper plants intercropped with maize was significantly greater than the control only for the first season. Reduced growth of hot pepper plants was observed for intercrops of sunflower, only in the second season. Previous results indicate that different companion crops differ in their effects on growth and yield of the primary crop. Fereres, (2000) and Muindi *et al*., (2009) demonstrated that companion crops may modify the microclimate conditions within the plots, thus affecting the protected crop through competition for light, moisture and nutrients. Such factors may increase or decrease the final yield of the protected crops despite the potential advantage of the reduced incidence of viral diseases. In this study, the variety of sunflower used was tall, giant, with wider leaves and with a heavy vegetative growth. This may have probably affected the growth of hot pepper crop because of shading effects leading to undesirable competition for light, nutrients and water. The increase in yield when pepper is intercropped with maize is also reported elsewhere (Fajinmi and Odebode, 2010; Ashenafi *et al*., 2014; Degri and Ayuba, 2016). However, the mechanism leading to increased yield in plots intercropped with maize in the present study is not well understood.

The present results have shown the effectiveness of sorghum, maize and sunflower in managing non-persistent virus diseases when used as crop borders or as intercrops. The strategy is appropriate for the farmers, environmentally friendly and easily adaptable.

Therefore, it can be used together or complementing other control strategies to help maintain the non-persistent viruses transmitted by aphids at levels below the economic threshold. Since no one method of control is likely to completely eliminate the vectors and viruses, an integrated approach to the management of these pests is the most sustainable solution.

CHAPTER SEVEN:

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1. General discussion

Hot pepper is an important economic crop for poverty alleviation and any efforts towards improving its production will have a direct effect on livelihoods of the resource-poor farmers. The current study aimed to establish the farmers' perception and knowledge of viral diseases; determine the presence and distribution of six selected viruses across the major hot pepper production areas; to determine the prevalence of six selected viruses associated with hot pepper; to screen hot pepper genotypes for reaction to viruses and aphids; and to evaluate the effect of companion crops on aphids and associated-viral diseases. This information is crucial for the designing of sustainable management strategy for the viral diseases.

The results showed that majority (two-thirds) of the farmers lacked knowledge and understanding of diseases and insect-pests especially the viral diseases (chapter 3). Only few (one-fifths) of the farmers had access to extension services or training. This observation is consistent with previous reports (Nagaraju *et al*., 2002; Schreinemachers *et al*., 2015), suggesting that there is a need to strengthen seed systems and extension services to educate farmers.

From the survey in chapter three, farmers indicated that viral diseases are serious hindrances to hot pepper farming. This was confirmed by the results from chapter four which revealed that all tested viruses were present and prevalent in the major areas where hot pepper is cultivated in the country. The high prevalence recorded could be attributed to many factors such as climatic conditions, flora diversity and insect-vectors (Thresh *et al*., 2003b; Kenyon *et al*., 2014). Farmers' lack of knowledge on viral diseases and their management coupled

with poor agronomic practices as revealed during the survey (chapter 3) might have greatly contributed to the spread of the viruses in all AEZs (Waweru *et al.*, 2020a). The Rwandan isolates of CMV, PVMV, PeVYV displayed low diversity as confirmed by phylogenetic analysis. The primers designed in this study successfully detected the target viruses and therefore they can be useful in indexing of the virus-free hot pepper plants.

The CMV, PVMV and PVY which are mainly transmitted by aphids, were the most predominant viruses in decreasing order of importance. During survey (chapter 3) and field experiments (chapter 5&6) carried out in Rwanda, it was evident that among the insect-vectors, aphids (*A. gossypii* and *M. euphorbiae*) were the most predominant in pepper fields (Waweru *et al.*, 2020a, b; 2021). The three viruses (CMV, PVMV, PVY) are efficiently transmitted by *A. gossypii* and *M. euphorbiae* in a non-persistent manner (Palukaitis *et al.*, 1992; Mello *et al.*, 2011), and therefore might have contributed to the spread of viruses.

Though the incidence of PMMoV and TMV (seed-borne transmitted viruses) were low compared to aphid-transmitted viruses, they were also present in all AEZs (chapter 4). In chapter three, two-fifths of the farmers indicated that they normally recycle or used uncertified planting materials from neighbours and local markets. These practices might have provided a means to propagate the diseases. In addition, both viruses could have been spread unknowingly by farmers as they work in the fields or through infected seeds (Genda *et al.*, 2005).

Although there are known resistant/tolerant hot pepper varieties, considerable variation among them in their susceptibility to viruses have been reported (Li *et al.*, 2020). Hence in chapter 5 of this study 18 hot pepper genotypes were assessed for their resistance to viral diseases in the field and screenhouse. Two local genotypes (00802PPR; *C. baccatum*

00767PPR) and three introduced genotypes (*C. annuum* PBC 462, PP9950-5197 and ICPN 18-7) were found resistant to viral diseases. The CMV was the most prevalent viruses infecting hot pepper in chapter 4. Out of the 14 hot pepper genotypes evaluated against artificially inoculated CMV in the screenhouse, four genotypes (0802PPR, *C. baccatum* 00767PPR, *C. annuum* PBC 462 and PP9852-170) were found resistant to CMV. Several previous studies have investigated for sources to resistance against PVMV, TMV, CMV, PMMoV and *Chili veinal mottle virus* (Suzuki *et al*., 2003; Fajinmi *et al*., 2013; Appiah *et al*., 2014; Rahman *et al*., 2016). The findings from this study revealed some additional sources for resistance against viral diseases that may be utilized in the breeding programs to improve the existing germplasm and also develop new varieties that are resistant.

Aphids and associated viral diseases are serious problems that hinder progress in pepper farming as revealed in chapter 3 and 4. Application of live barriers in the form of border and intercrops was effective in reducing the incidence of non-persistent-aphids-transmitted viruses in hot pepper. The study revealed that sole pepper plots had a higher incidence of viral diseases compared to pepper plots that were intercropped and had crop borders. The CMV, PVMV and PVY were the viruses detected in the field while for aphid species, it was *M. euphorbiae* and *A. gossypii*. Previous reports have provided evidence that utilization of border and inter-crops has a role to play in reducing virus disease incidence in various crops (Hooks and Fereres, 2006; Fajinmi and Fajinmi, 2010; Olawale *et al*., 2015). The efficacies of maize, sunflower and sorghum whether utilized as border or inter-crops were similar and thus, any of the three crops can be selected by farmers and use in combination with other options for better protection of pepper fields against insects that are known to transmit viruses.

7.2. Conclusions

This study has revealed that virus-induced diseases are widespread and an important constraint to hot pepper production in Rwanda. The farmers are not well equipped with knowledge of the cause, spread and management of the viral diseases. A majority of the farmers rely mainly on the farmer-to-farmer interactions for information. Awareness creation on viral diseases and integrated disease management through farm-level training is needed. Our findings provide fundamental information for designing long-term management options for virus-induced diseases in hot pepper production being promoted for export diversification. Six plant viruses that are prevalent in hot pepper production in Rwanda were detected and identified including CMV, PVMV, PVY and PeVYV transmitted by aphids, and PMMoV and TMV mainly transmitted via seeds. The most prevalent were CMV followed by PVMV and PVY.

Three hot pepper genotypes 00767PPR, 00802PPR and PBC 462 were 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.

The study also showed that farmers may opt for use of companion cropping as another option to the management of viral diseases. The sorghum, maize and sunflower have the potential to control non-persistently transmitted viral diseases whether utilized as crop border or inter-crops. The aphid species identified in the experimental fields were *Macrosiphum euphorbiae* and *Aphis gossypii* which are potential vectors of most of the hot pepper viruses. However, use of sorghum, sunflower and maize as border or inter-crops did not control the population of aphids landing on pepper crop. For sustainable management of viral diseases, there is need

for strategies that are compatible with companion cropping for integrated disease management.

Extension materials focusing on information generated from this study will be developed and disseminated to farmers and extension agents through existing extension approaches such as Farmer field schools (FFS), farmer promoters and 'TWIGIRE Umuhinzi' model. In order to reach wider audience seminars targeting policy makers, agricultural scientists, university researchers and extension agents will be organized. In addition, reports, copy of the thesis and scientific publications will be shared through different platforms.

7.3. Recommendations

From the results obtained in this study, it is recommended that:

1. The government should strengthen extension services such as the farmer field schools, farmer promoters and plant health clinics which are already in place to help in improving farmers' knowledge on diseases and pests' management.
2. The use of sunflower, maize and sorghum as live barriers in the form of border and intercrops for reducing the incidence of non-persistent viruses in hot pepper fields is recommended to be used with other management strategies to reduce diseases entry and spread.
3. The new hot pepper genotypes namely PBC 462, HP 0117, PP9852-170, PP9950-5197 and ICPN 18-7 from the WVC, that showed resistance to viral diseases are recommended for adoption by growers while, the two local genotypes 00767PPR and 00802PPR are recommended for breeding programs.
4. Further studies should be carried out to determine the economic significance of viruses identified as well as to identify other viruses not targeted in this study.

5. Further research is necessary to establish the optimal distance between pepper and the companion crops that is effective in reducing the virus infection without adversely affecting the yields of the primary crop.
6. Detailed experiments are needed to identify and validate resistance of the tested genotypes to aphids under broader environmental conditions.

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APPENDICES

Appendix 1. Questionnaire on hot pepper viral diseases survey

Section A: Identification

Province……District……. Sector……
Cell……...Village…….……. Date……
GPS Coordinates: Longitude…… Latitude…… Altitude…… m.a.s.l
Name of enumerator…….
Name of respondent…… Age (yrs)……Sex: a. Male b. Female

Section B: Farm characteristics

1. Size of farm……Area under hot pepper……Age of hot pepper field……
2. Land ownership: a. Solely Owned, b. Family, c. Rented, d. Cooperative, 4. Others….
3. List 5 major vegetable crop enterprises including pepper (rank them in order of importance 1st is most important, 5th is least important)

Crop	Rank

Section C: Hot pepper production systems and input usage

1. Variety of hot pepper planted: a. Scotch bonnet (yellow/red/beige), b. Habanero, c. Cayenne, d. Bird-eye, e. Others (specify)……
2. Source of seeds: a. Own, b. Neighbour, c. Cooperative/Company, d. Agro dealers, e. NGOs, f. Government institution, g. General market, 9. Others (specify)……
3. Plant spacing used…….
4. Farming system practiced: a. Mono-cropping, b. Mixed cropping, c. Intercropping
5. If mixed or intercropped, crop(s) used……..……..…….....
6. Do you weed: a. Yes, b. No
7. If yes, how often per season: Weekly/ Fortnightly/Once a month/Others……
8. Do you mulch: a. Yes, b. No
9. If yes, type of mulch used: a. Crop residues, b. Grasses, c. Others (specify)…….
10. Do you use agricultural inputs: a. Yes, b. No
11. If yes, please list them: a. Organic fertilizer, b. Inorganic fertilizer, c. Pesticide, d. Both (a & b), e. Both (a & c), f. Both (b & c), g. All (a, b, c)
12. Type of in-organic fertilizer used: a. NPK, b. DAP, c. urea, d. Others……
13. Source of in-organic fertilizer: a. Neighbor, b. Market, c. Agro-dealer, d. Government institution, e. Other(specify)……
14. Type of pesticide used: a. Insecticide, b. Fungicide, c. herbicide, d. Others (Specify)……
15. Source of pesticide: a. Neighbour, b. Market, c. Agro-dealer, d. Government institution, e. other(specify)……
16. If you use traditional products, Which ones…….…….……
17. Main reason for growing hot pepper: a. Consumption, b. sale, c. both (a &b)

d. processing

18. How do you sell your hot pepper: b. Green, 2. Dried

Section D: Hot pepper production constraints

1. List the current major problems (from the most to the least important contributing to the low hot pepper yields:

No.	Constraint	Coping strategies
1		
2		
3		
4		

Section E: Farmer perceptions of viral diseases and control

1. If diseases are cited (section D), please list major diseases from the most to the least devastating:

No.	Diseases	Coping strategies
1		
2		
3		
4		
5		

2. Are you aware of viral disease in hot pepper: a. Yes, b. No

   Note:(If no show pictures for familiarity and re-ask the question)

3. When first notice viral diseases in your farm on pepper (month/year) ……
4. Which season do these viral diseases occur in the field: a. Rainy, b. Dry, c. Year-round
5. How has the incidence of viral diseases evolved since you first noticed it on your farm: a. Increased, b. Stayed same, c. Decreased
6. Which varieties in your opinion performed well under viral infection…….
7. What do you think could be the cause of viral diseases?

Causes	Rank them in order of importance (1^{st} most important, 5^{th} is least important)
Seed	
Insect vectors	
Bad weather	
Poor soil	
Others (specify)…….	

8 How have you been coping with these viral diseases:

a. crop rotation, b. Used a different variety, c. Sprayed the fields with insecticide,

d. Maintained a clean hot pepper field through regular and timely weeding,

e. Maintained a clean hot pepper field through the removal of crop residues and burning or burying them, f. Use of clean quality seeds, g. Did nothing

9 Insects that are commonly seen on hot pepper: a. Aphids, b. Whiteflies, c. Others…….
10 Do you control insects: a. Yes, b. No
11 If yes, the method used: a. Pesticide, b. Cultural practices, c. Traditional products
12 If you use pesticides, which ones: a. Cypermethrin, b. Rocket, c. Dudu,
d. Others (Specify)……
13 If you use cultural practices, Which ones…….
14 Did you receive any information on viral diseases of hot pepper: a. Yes, b. No
15 If yes, from which sources: a. Agric. Extension (government), b. Seed companies,
c. Neighbours, d. Farmer organizations, e. Agro dealer, f. Plant clinic forum,
g. Others (specify)…….
16 Do you expect to lose yields due to viral disease this season: a. Yes, b. No
17 If yes, proportion expect to be lost: a. < 25%, b. 25-50%, c. 50-75%, d. >75%

Section F: Assessment of the field

1. Crop season as the time of visit……
2. Crop growth stage: a. Before flowering, b. At flowering, c. Early fruiting
d. At the maturity stage
3. General on-farm sanitation: a. Very Good, b. Good, c. Bad, d. Very Bad
4. Is the surrounding environment bushy/weedy: a. Yes, b. No
5. Is the farm weedy at the time of the visit: a. Yes, b. No
Common weeds observed in the field……

Appendix 2a. List of *Cucumber mosaic virus* isolates sequenced, out of a collection from hot pepper production sites in Rwanda and known isolates retrieved from the Genbank and used for phylogenetic analysis and sequence comparison

Virus Isolate	Origin	Host	Accession No.
Cucumber mosaic virus 1	Rwanda	Pepper	[1]MW080679
Cucumber mosaic virus R10	Rwanda	Pepper	[1]MW080681
Cucumber mosaic virus G11	Rwanda	Pepper	[1]MW080680
Cucumber mosaic virus	Rwanda	Pepper	MG470800.1
Cucumber mosaic virus	Japan	Pepper	D12499.1
Cucumber mosaic virus	South Korea	Pepper	KP033526.1
Cucumber mosaic virus	South Korea	Pepper	MN422338.1
Cucumber mosaic virus	South Korea	Pepper	KC527774.1
Cucumber mosaic virus	South Korea	Pepper	MN422335.1
Cucumber mosaic virus	China	Pepper	KT004544.1
Cucumber mosaic virus	India	Pepper	KU947031.1
Cucumber mosaic virus	India	Pepper	KM272275.1
Cucumber mosaic virus	India	Pepper	MN495621.1
Cucumber mosaic virus	Italy	Pepper	HE962480
Cucumber mosaic virus	Australia	Pepper	AJ585522.1
Cucumber mosaic virus	Australia	Pepper	KX525738.1
Cucumber mosaic virus	USA	Pepper	MK440591.1
Alfalfa mosaic virus *	Canada	[2]*S. tuberosum*	MF990286.1

[1]The sequences were generated for this study; [2]*Solanum tuberosum;* *Isolates used as an outgroup sequences.

Appendix 2b. List of *Pepper vein yellows virus* isolates sequenced, out of a collection from hot pepper production sites in Rwanda and known isolates retrieved from the Genbank and used for phylogenetic analysis and sequence comparison

Virus Isolate	Origin	Host	Accession No.
Pepper vein yellows virus I4	Rwanda	Pepper	[1]MT445648
Pepper vein yellows virus R13	Rwanda	Pepper	[1]MT445647
Pepper vein yellows virus G12	Rwanda	Pepper	[1]MT445649
Pepper vein yellows virus	Rwanda	Pepper	MG470802.1
Pepper vein yellows virus	China	Pepper	KP326573.1
Pepper vein yellows virus	Japan	Pepper	LC126031.1
Pepper vein yellows virus	Japan	Pepper	LC126045.1
Pepper vein yellows virus	Japan	Pepper	AB594828.1
Pepper vein yellows virus	Israel	Pepper	HM439608.2
Pepper vein yellows virus	Spain	Pepper	KY523072.1
Pepper vein yellows virus	Australia	Pepper	KU999109.1
Pepper vein yellows virus	Malaysia	Pepper	MN337276.1
Pepper vein yellows virus	Indonesia	Pepper	LC528383.1
Pepper vein yellows virus	Brazil	Pepper	MK184554.1
*Barley yellow dwarf virus**	China	Wheat	EU332330

[1]The sequences were generated for this study*;* *Isolates used as an outgroup sequences.

Appendix 2c. List of *Pepper veinal mottle virus* and *Tobacco mosaic virus* isolates sequenced, out of a collection from hot pepper production sites in Rwanda and known isolates retrieved from the Genbank and used for phylogenetic analysis and sequence comparison

Virus isolate	Origin	Host	Accession No.
Pepper veinal mottle virus R12	Rwanda	Pepper	[1]MT445645
Pepper veinal mottle virus 28	Rwanda	Pepper	[1]MT445646
Pepper veinal mottle virus	Rwanda	Pepper	MG470801.1
Pepper veinal mottle virus	Japan	Pepper	LC438542.1
Pepper veinal mottle virus	Japan	Pepper	LC438544.1
Pepper veinal mottle virus	Japan	Pepper	LC438545.1
Pepper veinal mottle virus	Yemen	Pepper	AJ780969.1
Pepper veinal mottle virus	Ethiopia	Pepper	AJ780970.1
Pepper veinal mottle virus	Senegal	Pepper	AJ780966.1
Pepper veinal mottle virus	Cameroon	Pepper	AJ780967.1
Pepper veinal mottle virus	Ghana	Pepper	AJ780968.1
Pepper veinal mottle virus	Ghana	Pepper	NC_011918.1
Pepper veinal mottle virus	Ghana	Pepper	FM202327.1
Pepper veinal mottle virus	Mali	Pepper	GQ918275.1
Pepper veinal mottle virus	Mali	Pepper	GQ918274.1
Pepper veinal mottle virus	Taiwan	Pepper	EU719646.1
Pepper veinal mottle virus	China	Pepper	MN082715.1
Pepper veinal mottle virus	China	Pepper	KR002568.1
*Squash vein yellowing virus**	USA	Squash	DQ812125.1
Tobacco mosaic virus 198	Rwanda	Pepper	[1]MT445644
Tobacco mosaic virus	China	Pepper	KJ769107.1
Tobacco mosaic virus	China	Tomato	JX993906.1
Tobacco mosaic virus	UK	Tobacco	KY810785.1
Tobacco mosaic virus	India	Soya bean	JQ895560.1
Tobacco mosaic virus	Serbia	Tobacco	GQ340671.1
Tobacco mosaic virus	Thailand	Pepper	AY633749.1
Tobacco mosaic virus	Africa	Eggplant	AY360447.1
Tobacco mosaic virus	Germany	Tobacco	AJ429081.1
Tobacco mosaic virus	China	Tobacco	AJ239099.1
Tobacco mosaic virus	South Korea	Pepper	AF012917.1
Tobacco mosaic virus	South Korea	Pepper	L35073.1
Tobacco mosaic virus	South Korea	Petunia	AB369275.1
Tobacco mosaic virus	South Korea	Impatiens	AB354955.1
Tobacco mosaic virus	China	Tobacco	GU324660.1
*Tobacco rattle virus**			J04347.1

[1]The sequences were generated for this study; *Isolates used as outgroup sequences

Appendix 3. Percentage deduced amino acids (bottom) and nucleotide (top) identities between Rwandan isolates and related *Cucumber mosaic virus* strains retrieved from Genbank

No.	Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	MW080680_Rwanda	ID	98.6	99.3	99.3	97.3	96.7	96.7	97.3	78.9	79	77.6	78.2	86.1	86.9	80.3	16.8
2	MW080679_Rwanda	99.3	ID	99.3	99.3	97.3	96	96.7	97.3	80.1	80.2	78.8	79.4	86.1	86.9	80.3	16.8
3	MW080681_Rwanda	99.5	99.7	ID	100	98	96.7	97.3	98	79.6	79.7	78.2	78.9	86.8	87.5	81	16.8
4	MG470800.1_Rwanda	99.3	99.7	99.7	ID	98	96.7	97.3	98	79.6	79.7	78.2	78.9	86.8	87.5	81	16.8
5	MN422338.1_South Korea	98.7	99.1	99.1	99.3	ID	98.6	99.3	100	80.3	80.5	79	79.7	86.2	87	81.6	18.1
6	MN422335.1_South Korea	98.5	98.7	98.7	98.9	99.5	ID	99.3	98.6	80.3	80.5	79	79.7	84.9	85.7	80.3	18.1
7	KC527774.1_South Korea	98.5	98.9	98.9	99.1	99.7	99.7	ID	99.3	81	81.1	79.7	80.3	85.6	86.3	81	18.1
8	KP033526.1_South Korea	98.7	99.1	99.1	99.3	100	99.5	99.7	ID	80.3	80.5	79	79.7	86.2	87	81.6	18.1
9	KM272275.1_India	89.8	90.2	90.2	90.2	90.4	90.4	90.6	90.4	ID	94	97.3	89.4	76.4	77.2	75.8	17.5
10	KU947031.1_India	88.9	89.3	89.3	89.3	89.6	89.6	89.8	89.6	96	ID	92	88.1	77.1	77.9	76.4	18.1
11	MN495621.1_India	89.1	89.6	89.6	89.6	89.8	89.8	90	89.8	98.7	95.4	ID	88	75.8	75.9	74.6	17.5
12	HE962480.1_Italy	89.1	89.6	89.6	89.6	89.8	89.8	90	89.8	96	94.5	95.6	ID	77.7	75.3	75.8	16.3
13	D12499.1_Japan	92.5	92.7	92.9	92.7	92.5	92	92.3	92.5	88.3	88.1	87.7	88.7	ID	78.4	92.7	16.2
14	KT004544.1_China	93.9	94.3	94.3	94.5	94.3	93.9	94.1	94.3	88.1	88.3	87.5	87.1	89.3	ID	74	15.6
15	AJ585522.1_Australia	90.2	90.4	90.6	90.4	90.6	90.2	90.4	90.6	87.9	87.7	87.1	87.5	97.5	87.7	ID	14.4
16	MF990286.1_Outgroup	44	44	44.2	44	44.2	44	44.2	44.2	42.8	43.6	42.8	43.3	43.2	43	42.2	ID

Appendix 4. Percentage deduced amino acids (bottom) and nucleotide (top) identities between Rwandan isolates and related *Pepper vein yellows virus* strains retrieved from Genbank

No.	Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	MT445649_Rwanda	ID	94.9	94.9	94.9	91.7	94.3	93.6	94.3	96.8	89.8	87.9	94.3	92.4	76.5	20.2
2	MT445647_Rwanda	97	ID	100	98.7	89.8	92.4	91.7	94.3	92.4	87.9	87.3	93	90.5	75.3	20.2
3	MT445648_Rwanda	97	100	ID	98.7	89.8	92.4	91.7	94.3	92.4	87.9	87.3	93	90.5	75.3	20.2
4	MG470802.1_Rwanda	97.2	98.5	98.5	ID	90.5	93	91.7	94.3	92.4	87.9	87.3	93	90.5	75.3	19.6
5	LC126031.1_Japan	91.1	91.1	91.1	90.9	ID	95.5	93.6	91.1	93	88.6	85.4	89.2	91.1	76.5	19.6
6	AB594828.1_Japan	93.4	92.6	92.6	92.8	94.5	ID	95.5	93.6	94.9	90.5	87.3	91.7	93	76.5	19.6
7	LC126045.1_Japan	93.2	92.4	92.4	92.6	94.5	94.9	ID	93.6	95.5	91.7	87.3	91.1	93	78.4	20.8
8	HM439608.2_Israel	94.1	94.1	94.1	94.3	91.3	93.4	92	ID	94.3	89.8	87.3	96.2	91.7	77.2	20.8
9	KP326573.1_China	94.9	93.2	93.2	93.4	94.5	95.3	94.5	93.8	ID	91.7	89.2	93.6	93.6	79.1	22.1
10	MN337276.1_Malaysia	90.3	88.6	88.6	89.2	88.4	89.8	90.5	89.6	92	ID	83.5	87.3	89.2	80.1	20.5
11	LC528383.1_Indonesia	89.8	89.4	89.4	89.2	87.5	88.4	88.4	89	89.8	85.2	ID	87.9	87.3	80.3	22.7
12	KY523072.1_Spain	94.9	94.5	94.5	94.7	90.9	92.6	92	96.6	93.8	89.2	90.5	ID	89.8	77.2	20.2
13	KU999109.1_Australia	91.7	90.9	90.9	91.1	91.3	93.2	92.4	92	93	88.6	88.6	90.9	ID	75.9	20.8
14	MK184554.1_Brazil	76.6	76	76	76.2	76.8	76.2	78.1	77.2	77.8	80.2	79.3	77	76.4	ID	21
15	EU332330.1_Outgroup	46.7	46.9	46.9	46.9	47.1	46.1	47.5	47.1	48.4	45.6	47.5	47.1	45.6	41.3	ID

Appendix 5. Percentage deduced amino acids (bottom) and nucleotide (top) identities between Rwandan isolates and related *Pepper veinal mottle virus* strains retrieved from Genbank

No.	Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	MT445646_Rwanda	ID	98.3	98.8	76	77.7	76.3	74.6	73.9	76	76	74.1	73.6	76.3	75.5	75.8	75.8	76.5	75.5	44.4
2	MT445645_Rwanda	98.5	ID	98.5	76.5	79.1	77	75.8	75.1	76.3	76.3	74.8	74.4	76.5	75.8	76	76	76.7	75.8	44.4
3	MG470801.1_Rwanda	98.5	98.5	ID	76.3	78.4	76.3	75.1	74.4	75.5	75.5	74.1	73.6	75.8	75.1	75.3	75.3	76	75.1	44.4
4	AJ780969.1_Yemen	83.4	84.8	83.4	ID	81.6	74.2	74.9	74.4	72.7	72.7	72.9	72.4	72.7	72.7	73.4	73.4	73.2	73.6	44.2
5	AJ780970.1_Ethiopia	86.3	87	85.6	91.3	ID	77.5	78	77.5	74.8	74.8	75.1	74.8	75.3	74.6	74.8	74.8	74.8	75.5	45.6
6	AJ780966.1_Senegal	82.7	83.4	82	81.8	85.5	ID	86	85.3	86.8	86.8	85.6	85.8	86.6	86.3	86.1	86.6	87.3	87	45.2
7	AJ780967.1_Cameroon	82	82.7	81.2	83.3	84	94.9	ID	98	87.3	87.3	86.1	85.8	86.8	86.8	86.8	87.3	87.3	86.8	45.2
8	AJ780968.1_Ghana	81.2	82	80.5	82.6	83.3	94.2	97.8	ID	87.7	87.7	86.6	86.3	87.7	87.7	87.3	87.7	87.7	87.3	45.4
9	FM202327.1_Ghana	82	82.7	81.2	81.2	84.1	94.9	94.2	94.9	ID	100	94	94	98	98.8	98	99	99.5	98	44.9
10	NC_011918.1_Ghana	82	82.7	81.2	81.2	84.1	94.9	94.2	94.9	100	ID	94	94	98	98.8	98	99	99.5	98	44.7
11	GQ918274.1_Mali	82	82.7	81.2	82	84.1	94.9	94.9	95.6	98.5	98.5	ID	99	93.7	94	93.7	94.2	94	95.2	44.7
12	GQ918275.1_Mali	82	82.7	81.2	82	84.1	94.9	94.9	95.6	98.5	98.5	100	ID	93.7	94	93.7	94.2	94	95.2	44
13	LC438544.1_Japan	83.4	84.1	82.7	81.2	84.1	94.9	94.2	94.9	98.5	98.5	98.5	98.5	ID	98.8	97.6	98.5	98	98	43.7
14	LC438542.1_Japan	82.7	83.4	82	82	84.1	94.9	94.9	95.6	98.5	98.5	98.5	98.5	98.5	ID	98.3	99.2	98.8	98.3	44.2
15	LC438545.1_Japan	82.7	83.4	82	82	84.8	95.6	94.9	95.6	99.2	99.2	99.2	99.2	99.2	99.2	ID	98.5	98	98.5	45.2
16	KR002568.1_China	82	82.7	81.2	82.7	84.1	94.9	95.6	96.4	98.5	98.5	99.2	99.2	98.5	98.5	99.2	ID	99	98.5	44.7
17	EU719646.1_Taiwan	82.7	83.4	82	82	84.8	95.6	94.9	95.6	99.2	99.2	99.2	99.2	99.2	99.2	100	99.2	ID	98	44.7
18	MN082715.1_China	82.7	83.4	82	82	84.8	95.6	94.9	95.6	99.2	99.2	99.2	99.2	99.2	99.2	100	99.2	100	ID	44.7
19	DQ812125.1_Outgroup	18.7	19.4	19.4	19.4	20.8	21.5	20.8	20.1	20.1	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.1	20.1	ID

Appendix 6. Percentage deduced amino acids (bottom) and nucleotide (top) identities between Rwandan isolate and related *Tobacco mosaic virus* strains retrieved from Genbank

No.	Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	MT445644_Rwanda	ID	91.1	94.3	93.7	96.3	73.1	91.6	99.4	92.7	89.6	97.3	98.9	94.7	13.7
2	GU324660.1_China	96.2	ID	94.7	91.1	93.7	70.8	84.8	91.1	92.6	85.4	92.1	91.6	89.5	14.7
3	AB369275.1_South Korea	97.2	98	ID	94.3	96.8	72.8	88	94.3	93.7	87.6	95.3	94.7	91.7	14.7
4	AB354955.1_South Korea	97.2	96.7	97.7	ID	97.3	71.2	88	93.7	91.1	86.5	94.7	94.2	91.1	14.7
5	JX993906.1_China	98.2	97.7	98.7	99	ID	72.6	90	96.3	93.7	88.6	97.3	96.8	93.7	13.7
6	KJ769107.1_China	88.7	87.2	88	87.1	88	ID	67.3	73.1	71.5	72.9	72.6	73.5	72.3	15.8
7	AJ239099.1_China	92.7	90.3	91.3	91.3	92.1	82.6	ID	92.1	85.9	82.8	91.5	92.5	88.4	12.8
8	JQ895560.1_India	99.8	96.2	97.2	97.2	98.2	88.7	92.9	ID	92.7	89.6	97.9	99.4	95.2	13.7
9	AY633749.1_Thailand	96.9	97.1	97.4	96.4	97.4	87.7	90.9	96.9	ID	88	92.1	93.2	92.1	15.2
10	GQ340671.1_Serbia	94	92.4	93	92.4	93.3	86.6	87.7	94.1	93.3	ID	88.6	90.1	88.9	13.7
11	KY810785.1_United Kingdom	98.8	96.9	97.9	97.9	98.8	88.2	92.9	99	96.6	93.5	ID	98.4	94.2	13.7
12	AY360447.1_Africa	99.6	96.4	97.4	97.4	98.3	88.8	93	99.8	97.1	94.3	99.1	ID	95.7	13.7
13	AJ429081.1_Germany	97.4	95.3	95.9	95.9	96.9	87.4	90.6	97.4	96.2	94.4	96.7	97.5	ID	13.7
14	J04347.1_Outgroup_	41.7	42.1	41.3	42	41.7	41.2	39.4	41.7	41.2	39.7	42	41.5	40.5	ID