Original Research Article

Molecular detection of *Ugandan passiflora virus* infecting passionfruit (*Passiflora edulis* sims) in Rwanda

ABSTRACT

The study aimed at identifying the specific pathogen associated with the passionfruit woodiness in Rwanda. Field work was conducted in Rwanda while, laboratory aspects were carried out in Biosciences for eastern and central Africa-International Livestock Research Institute Hub, Nairobi, Kenya. Duration of the study was from September 2012 to May 2013. Two hundred and one samples of leaves were randomly collected from diseased and healthy passionfruit plants in Nyamagabe, Ngororero and Gicumbi district found in south, west and north province of Rwanda, respectively. Laboratory analysis using enzyme-linked immunosorbent assay and reverse- transcription polymerase chain reaction was carried out to determine the virus present. Virus-like symptoms observed in the field included; leaf mosaic, crinkle, distortion, fruit woodiness and malformations. *Ugandan passiflora virus* (UPV) was detected in 70 % of the positive samples (90) and other unidentified general potyviruses in 25.6 %. Incidence of virus infection was highest (45.8 %) in north and the least was 18.7 % in west province. Partial sequences of the coat protein gene were used determine the identity of the virus present. Sequences obtained were highly similar and displayed features typical of potyviruses 93 to 100% identity. Comparisons of these sequences with those of other existing potyviruses indicated highest identity to UPV (94-100 %) strains from Uganda. This study confirms the presence of the *Ugandan passiflora virus* in the country. This necessitates the need for the production and use of virus-free planting materials, development of virus resistant genotypes and adoption of efficient seed certification systems.

Keywords: Passionfruit, detection, Ugandan passiflora virus, Rwanda

1. INTRODUCTION

Globally, passionfruit (*Passiflora edulis* Sims) is an important economic crop as an income earner and for food and nutrition security. In Rwanda, it ranks fourth by production and acreage only after banana, avocado and pineapple [1]. About 46% of the crop is grown in the Western, 43% in the Northern, 10% in the Southern and 1% in the Eastern part of the Rwanda [1].

Viral diseases cause significant losses in production of this crop especially if the plants are infected while still young [2]. The implication is not only on yield but also on the crop lifespan, [3] reported reduction of passionfruit crop lifespan from five years to one year due to viral diseases. Over 19 viruses worldwide have been documented to infect passionfruit [3] and five of these occur in Africa [3] [4] [5] [6] [7].

One of the most destructive viral diseases infecting the crop is passionfruit woodiness disease (PWD) which is associated with four potyviruses; *Passionfruit woodiness virus* (PWV) reported in Australia [8], *Cowpea aphid-borne mosaic virus* (CABMV) in Brazil and Kenya [7] [9], *East asian passiflora virus* (EAPV) in Japan [10] and *Ugandan passiflora virus* (UPV) in Uganda [6]. However, it is not clear whether the four or more of the passionfruit viruses reported in elsewhere are present in Rwanda.

Selection and breeding of resistant varieties is the surest way to curb disease problems. Thus, identification of the specific pathogens associated with these viral diseases is of importance, as it

provides crucial information required for breeding resistance varieties. This study was aimed at identify the causal pathogens of the PWD in Rwanda. The results obtained will offer a platform for breeding passionfruit resistant varieties.

2. MATERIAL AND METHODS

2.1 Collection of samples

Samples of leaves were collected from 66 fields in three major passionfruit production areas; Northern (Gicumbi district), Western (Ngororero district) and Southern (Nyamagabe district) in Rwanda. In addition, samples were collected from a passionfruit field belonging to Rwanda Agriculture Board (RAB) in south (Huye district). Twenty-two (22) passionfruit fields (atleast 2 km apart) were randomly selected per district and observed for presence of plants with viral-like symptoms. Fresh leaf samples were collected from 3 (2 diseased: 1 healthy) randomly selected plants/farm. For each sample 3 young leaves were selected from the growing points. Two hundred and one (201) including both diseased and healthy leaf samples were collected, bagged and preserved in silica gel. These samples were stored at room temperature in the laboratory until analyzed.

2.2 Preparation of samples

Using mortar and pestle, samples were homogenized by grinding the leaves in liquid nitrogen. Approximately 200 mg of each sample was weighed and transferred into 1.5 ml eppendorf tubes in duplicates and 1 ml of coating buffer (for ACP-ELISA samples)/ extraction buffer (for DAS-ELISA samples) was added, vortexed for 1 min and stored at -20□C until analyzed. To 1.5 ml eppendorf tubes, remaining powdered samples were added and stored at -80°C for total RNA extraction.

2.3 Detection of virus by serological assays

Previously preserved samples at -20°C were removed from freezer and left to thaw. Antigen-coated-plate enzyme-linked immunosorbent assay (ACP-ELISA) was used to detect potyviruses in the samples, while *Cucumber mosaic virus* (CMV) and *Cowpea aphid borne mosaic virus* (CABMV) were detected by double antibody sandwich (DAS-ELISA) [11] using commercial kits acquired from Deutsche Sammlung Von Mikroorrganismen und Zellkulturen (DSMZ) Germany following manufacturer's protocol. The controls included negative (healthy), positive (diseased passionfruit leaves supplied with ELISA kit) and a blank (coating or extraction buffer only, ACP- and DAS-ELISA respectively). Samples with absorbance A405nm values greater than 2 times the average of negative control were considered positive. ELISA test was carried out with two repetition including positive and negative controls.

2.4 Extraction of ribonucleic acid and synthesis of complementary deoxyribonucleic acid

Approximately 100 mg of the frozen powdered leaf sample was used in extraction of ribonucleic acid (RNA) using ZR plant RNA MiniprepTm Kit, (catalog No. R2024; Zymo Research, USA), following manufacturer's instructions. One (1) µg of total RNA was used to synthesize complementary deoxyribonucleic acid (cDNA) using Maxima first Strand cDNA synthesis kit for RT-PCR, (catalog No. K1642; Thermo Scientific). To a nuclease free micro-centrifuge tube 1µg of template RNA, 4µl of reaction mix (supplied with the kit) and 2µl (10pmol/µl) of enzyme mix were added and completed to 20 µl with sterile distilled water. The tubes were vortexed gently for 1 min to mix the contents and briefly spined. The tubes were incubated at 25□C for 10 min and at 50°C for 30 min and lastly heated at 85°C for 5 min to inactivate reverse transcriptase (RT). cDNA Synthesized was stored at -20°C and used for downstream applications.

2.5 Primer used for polymerase chain reaction amplification -

Polymerase chain reaction (PCR) amplification was done using six primer pairs (Table 1).

Table 1. **Primers used in confirmation of general potyviruses and *Uganda Passiflora virus***

Primer	Sequence	Fragment	Reference

		size (bp)	
U335	5' -GAATTCATGRTNTGGTGYTHGANAAYG -3'	335	[12]
D335	5' -GAGCTCGCNGYYTTCATYTGNRHDWKNGC -3'	335	[12]
UPVF2	5'- GCACGAAATTCAAGAATACCTTAG -3'	772	*
UPVR2	5'- GACTTCATAAAATCAAATGAGTA -3'	772	*
UPVF4	5' - CAATTTGCATCGTGGTATGA – 3'	200	*
UPVR4	5' - GTTGGTTTTGCATTTTCCAC - 3'	200	*

** Primers developed during this study*

2.6 Amplification of complementary deoxyribonucleic acid

The cDNAs were further amplified in polymerase chain reaction (PCR) by using thermocycler (GeneAmp PCR system 9700). PCR reaction mixture contained 1x reaction buffer, 1.5mM MgCl2, 250µM dNTP, 1 unit of Taq polymerase (Bioneer, USA) and 10 pmol of UPVF/UPVR or 5 pmol of U335/D335 primer. Water was added to make a final volume. The following cycling conditions were used: primer pair U335/D335- 95□C for 5 min, 30 cycles at 94□C for 1 min, 56□C for 5 min, 72□C for 30 s, followed by a 10 min extension at 72□C and; primer pair UPVF/UPVR- 95□C for 5 min, 30 cycles at 94□C for 30 s, 48□C for 1 min, 72□C for 30 s, followed by a 7 min extension at 72□C.

2.7 Gel electrophoresis

Amplified products were separated in 1% agarose/0.5X-TBE stained with 0.25X GelRed. Ten (10) µl of the sample was mixed with 2 µl of loading dye (6X) and loaded into the gel. One (1) kb Plus of DNA ladder was used as a marker and water as the negative control. The samples were run in TAE buffer for 35 min at 100 V in a 150 ml BIO RAD electrophoretic apparatus. The products visualized and photographed under ultraviolet (UV) light. GeneJET Gel Extraction Kit (catalog No. K0692; Thermo Scientific) was used to purify the products according to the manufacturers' protocol. After elution, 50 µl products were saved in the tubes and DNA concentration in the purified PCR products was estimated using a spectrophotometer (Nanodrop, ThermoScientific, South Africa). Five (5) µl of each sample was used for sequencing and remaining products were preserved at -80°C.

2.8 Sequence analysis

Partial nucleotide sequences of coat protein obtained were compared with other sequences of potyvirus strains available in the Genbank using Basic Local Alignment Search Tool at the National Centre for Biotechnolgy Information. Multiple nucleotide alignments with the available sequences were carried out using CLUSTAL W in MEGA version 6.0 [13]. The alignment files created by Clustal W were bootstrapped 1000 times for generating neighbour-joining phylogenetic tree using unweighted pair group method averages (UPGMA).

Table 2. Partial coat protein nucleotide sequences of known strains obtained from the genebank

Virus	Isolate	Host	Origin	Accession no.
Passionfruit woodiness virus	PWV-BuW-1	Passionfruit	Australia	JF427623
Passionfruit woodiness virus	PWV-MuW-1	Passionfruit	Australia	JF427620
East asian passiflora virus	EAPV-AT1	Passionfruit	Japan	AB690439
East asian passiflora virus	EAPV-SY102	Passionfruit	Japan	AB690447
Cowpea aphid borne mosaic	CABMV-M3	Passionfruit	Brazil	AV434454
Cowpea aphid borne mosaic	CABMV-Knxc-1	Cowpea	Australia	JF427592
Passiflora chlorosis virus	PCV	Passionfruit	USA	DQ860147
Bean common mosaic necrosis	BCMN-TN1	Bean	USA	U37076
Potato Y virus	PVY-SLGPVY1	Potato	India	JX945850
Ugandan passiflora virus	UGM-73	Passionfruit	Uganda	FJ896002
Ugandan passiflora virus	UGM-58	Passionfruit	Uganda	FJ896001
Ugandan passiflora virus	UGM-19a	Passionfruit	Uganda	FJ896000
Ugandan passiflora virus	UGM-17	Passionfruit	Uganda	FJ896003

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Serological analysis

Symptomatic and asymptomatic passionfruit leaf samples were collected from three districts of Rwanda. In all the districts, virus-like symptoms such as leaf mosaic, crinkle, distortion, and fruit woodiness and malformations (Fig.1) were observed. Collected leaf samples were tested for three viruses using polyclonal antibodies ELISA which included Generic Potyvirus, CMV and CABMV. Out of the 198 symptomatic and asymptomatic samples collected, 44 (22.2%) tested positive for potyvirus (Table 1). No samples detected with CMV and CABMV.

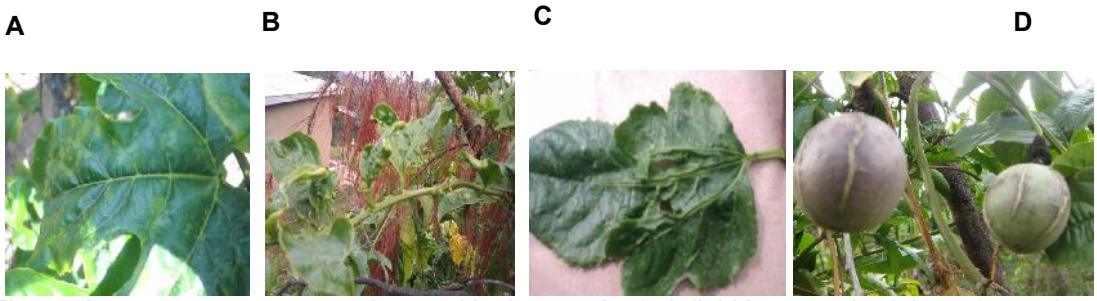


Figure 1: Common symptoms observed in the passionfruit field in Rwanda. (A) Leaf mosaic, (B&C) Leaf rolling, crinkle and distortion, (D) Fruit woodiness and malformations

3.1.2 Polymerase chain reaction analysis

Analysis using RT-PCR technique confirmed, 90 (45.5%) out of the 198 samples tested positive for the potyvirus while 54.5% were found to be negative (Table 3). Further testing of the 90 positive samples with specific primers showed that 63(31.8%) were positive for *Ugandan Passiflora Virus* (UPV). Other viruses were an unidentified potyvirus 27(13.6 %) samples. Occurrence of virus infection was high (57.6%) in Gicumbi district of the Northern Province followed by (47 %) Nyamagabe district of the Southern province and the least was (31.8% %) in Ngororero district of the Western province.

Table 3. Summary of incidence of Potyvirus and *Uganda passiflora virus* in three districts of Rwanda

LOCATION		SAMPLING		ELISA			RT-PCR		
Province	**District**	**Field**	**Samples collected**	**Potyvirus**	**CABMV**	**CMV**	**Potyvirus**	**UPV**	**Unidentified potyvirus**
Northern	Gicumbi	22	66	26	0	0	38	27	11
Southern	Nyamagabe	22	66	8	0	0	31	22	9
Western	Ngororero	22	66	10	0	0	21	14	7
			198	**44**	**0**	**0**	**90**	**63**	**27**

3.1.3 Sequences analysis

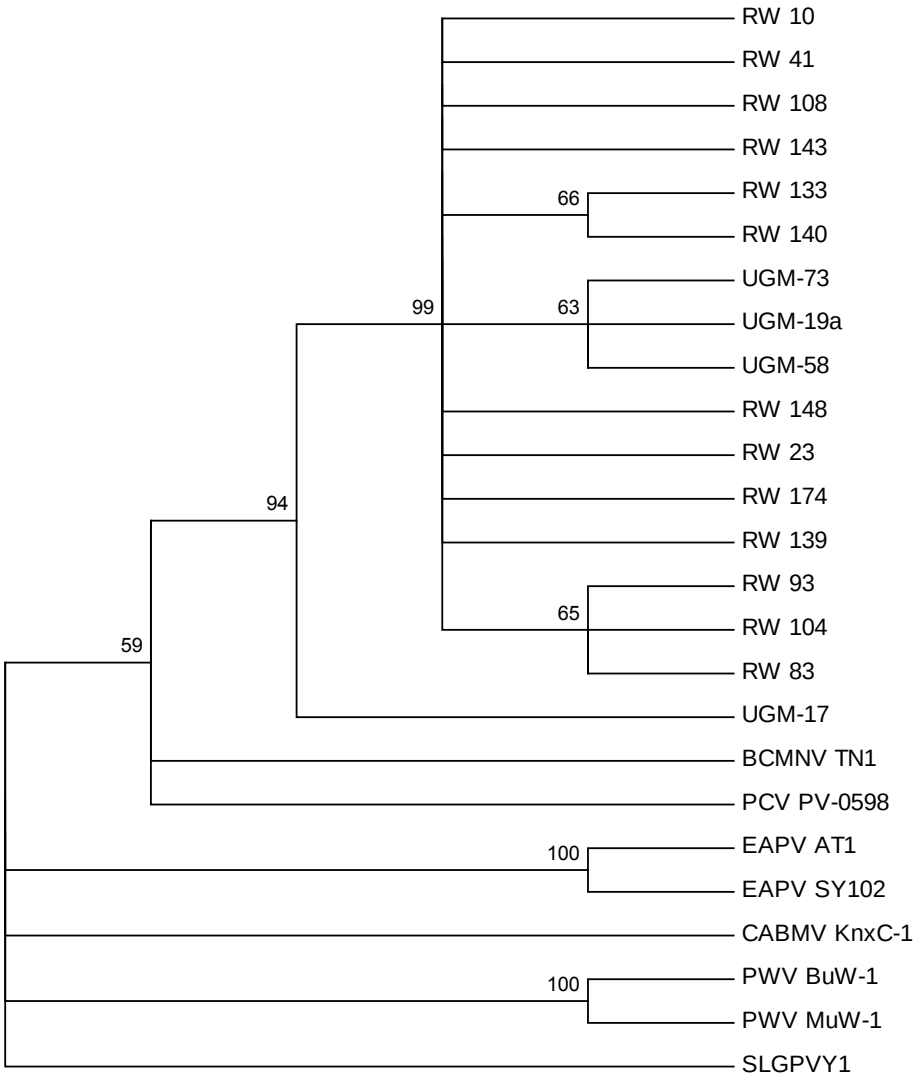
Deduced amino acids (aa) sequence analysis of partial CP gene of Rwandan isolates and other selected Potyvirus from Gene bank (Table 4) showed highest similarity (94-100% aa) with various strains (UGM-19a, UGM-58, UGM-73) of *Ugandan passiflora virus* from Uganda with evolutionary divergence values between 0.00-0.06 (Table 4). However, one isolates from Uganda (UGM-17) was exceptional and shared (56-60%) similarity, divergence values between 0.42-0.50. From USA, isolate BCMN-TN1 and PCV-PV-0598 shared (45.2-46.8% and 36.8-37.6%) similarity, respectively. From Japan, EAPV-AT1 and EAPV-SY102 shared (36.2-38.7%) similarity, divergence values between 0.96-1.05, while isolates CABMV-M3 from Brazil and CABMV-Knxc-1 from Australia shared (33.0-37.6%) and divergence values between 0.93-1.05. Australia isolates PWV-BuW-1 and PWV-MuW-1 shared (36-39.2%) divergence values between 0.90-1.01. The least was isolate PVY-SLGPVY1 from India (11.7-13.2%), divergence values between 2.03-2.21.

Table 4. Deduced amino acids percentage identities of Rwandan isolates of the *Ugandan passiflora virus* and related potyvirus species and their estimates of evolutionary divergence

No.	Virus isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	RW1		0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.04	0.45	0.98	1.01	0.73	0.98	0.96	1.01	1.01	0.98	2.11
2	RW201	100		0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.04	0.45	0.98	1.01	0.73	0.98	0.96	1.01	1.01	0.98	2.11
3	RW68	100	100		0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.04	0.45	0.98	1.01	0.73	0.98	0.96	1.01	1.01	0.98	2.11
4	RW72	100	100	100		0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.04	0.45	0.98	1.01	0.73	0.98	0.96	1.01	1.01	0.98	2.11
5	RW158	100	100	100	100		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.04	0.45	0.98	1.01	0.73	0.98	0.96	1.01	1.01	0.98	2.11
6	RW141	99	99	99	99	99		0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.06	0.43	0.98	1.01	0.70	0.98	0.93	0.98	1.01	0.96	2.11
7	RW169	99	99	99	99	99	100		0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.06	0.43	0.98	1.01	0.70	0.98	0.93	0.98	1.01	0.96	2.11
8	RW177	99	99	99	99	99	100	100		0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.06	0.43	0.98	1.01	0.70	0.98	0.93	0.98	1.01	0.96	2.11
9	RW10	98	98	98	98	98	99	99	99		0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.06	0.43	0.98	1.01	0.70	0.98	0.93	0.98	1.01	0.96	2.11
10	UGM-73	98	98	98	98	98	99	99	99	100		0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.06	0.43	0.98	1.01	0.70	0.98	0.93	0.98	1.01	0.96	2.11
11	UGM-19a	98	98	98	98	98	99	99	99	100	100		0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.06	0.43	0.98	1.01	0.70	0.98	0.93	0.98	1.01	0.96	2.11
12	UGM-58	98	98	98	98	98	99	99	99	100	100	100		0.01	0.00	0.01	0.01	0.01	0.01	0.06	0.43	0.98	1.01	0.70	0.98	0.93	0.98	1.01	0.96	2.11
13	RW41	98	98	98	98	98	98	98	98	99	99	99	99		0.01	0.02	0.02	0.02	0.02	0.07	0.45	1.01	1.05	0.68	1.01	0.96	1.01	1.05	0.98	2.21
14	RW103	98	98	98	98	98	99	99	99	100	100	100	100	99		0.01	0.01	0.01	0.01	0.06	0.43	0.98	1.01	0.70	0.98	0.93	0.98	1.01	0.96	2.11
15	RW23	98	98	98	98	98	98	98	98	99	99	99	99	98	99		0.02	0.02	0.02	0.07	0.42	0.98	1.01	0.73	0.98	0.93	0.98	1.01	0.93	2.21
16	RW133	98	98	98	98	98	99	99	99	98	98	98	98	98	98	98		0.02	0.02	0.07	0.43	0.98	1.01	0.70	0.98	0.93	0.98	1.01	0.96	2.03
17	RW93	98	98	98	98	98	98	98	98	99	99	99	99	98	99	98	98		0.00	0.07	0.42	0.96	0.98	0.68	0.96	0.90	1.01	1.05	0.98	2.11
18	RW83	98	98	98	98	98	98	98	98	99	99	99	99	98	99	98	98	100		0.07	0.42	0.96	0.98	0.68	0.96	0.90	1.01	1.05	0.98	2.11
19	RW60	97	97	97	97	97	96	96	96	95	95	95	95	94	95	94	95	94	94		0.50	0.98	1.01	0.70	0.96	0.96	0.98	1.01	0.98	2.21
20	UGM-17	59	59	59	59	59	60	60	60	60	60	60	60	59	60	60	60	60	60	56		0.90	0.93	0.77	1.01	0.82	0.98	0.93	0.93	2.72
21	EAPV_SY102	37	37	37	37	37	37	37	37	38	38	38	38	37	38	38	37	39	39	37	38		0.02	0.82	0.98	1.08	0.96	0.93	0.98	2.56
22	EAPV_AT1	36	36	36	36	36	36	36	36	37	37	37	37	36	37	37	36	38	38	36	37	97		0.87	0.98	1.11	0.93	0.96	1.01	2.56
23	BCMNV_TN1	45	45	45	45	45	46	46	46	46	46	46	46	47	46	45	46	47	47	46	41	41	39		0.80	0.87	1.01	0.93	0.96	2.31
24	PWV_BuW-1	37	37	37	37	37	37	37	37	37	37	37	37	36.0	37	37	37	38	38	38	35	36	36	44		0.17	0.98	0.96	1.08	2.56
25	PWV_MuW-1	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	39	39	38	40	32	31	42	87		0.98	0.85	0.98	2.56
26	PCV_PV-0598	37	37	37	37	37	38	38	38	38	38	38	38	37	38	38	38	37	37	38	36	38	40	35	38	39		1.11	0.85	2.56
27	CABMV_knxc-	34	34	34	34	34	34	34	34	34	34	34	34	33	34	34	34	33	33	34	38	36	35	35	34	38	32		0.45	2.43
28	CABMV-M3	34	34	34	34	34	35	35	35	35	35	35	35	34	35	36	35	34	34	34	35	32	31	33	30	34	38	60		3.41
29	PVY_SLGPVY	13	13	13	13	13	13	13	13	13	13	13	13	12	13	12	13	13	13	12	8.6	8.6	8.6	10	9.4	8.6	7.8	7.0	4.8	

3.1.4 Phylogenetic analysis

A close relationship between the Rwandan and Ugandan isolates is clearly indicated by phylogenetic tree (Fig. 2) based on the partial CP aa sequences. Rwandan and Ugandan isolates were grouped in a monophyletic cluster with 99% bootstrap value, clearly distinct from the PWV, CABMV, EAPV isolates. The most closely related Ugandan isolates are; UGM-19a, UGM-58 and UGM-73. PWV, CABMV and EAPV isolates were grouped in separate clusters with a 99% bootstrap value.



KEY: RW- Rwanda Isolates UGM-Uganda Isolates

Fig. 2. Phylogenetic tree of thirteen *Ugandan passiflora virus* isolates from Rwanda and other representative potyvirus strains reported on passionfruit worldwide. The tree was based on alignments of the predicted amino acid of the partial coat protein gene and rooted on the sequence of *Potato virus Y*.

3.2 Discussion

Passionfruit woodiness disease (PWD) is one of the most important challenge limiting passionfruit production in the world and infect various passiflora species. Symptoms and pathogenesis of PWD have been described in several countries, including Uganda, Kenya, Nigeria, South Africa, Australia, Brazil and Taiwan, [4] [5] [6] [7] [9] [10] [14]. The present study, aimed at identify cause of PWD in Rwanda, which is among the major challenging constraint in passionfruit production. Molecular analysis has demonstrated presence of the *Ugandan Passiflora Virus* (UPV) strains and unidentified Potyviruses in the main passion fruit-producing regions. UPV was first described in Uganda, where it molecular studies demonstrated that it can also cause PWD in passion fruit [6]. UPV has a wide host range and this reveals a threat to the passionfruit industry reinforcing the need to control PWD. CABMV and CMV were not present.

Present study indicate that all the Rwandan isolates have a high degree of similarity among themselves and with UPV isolates (except UGM-17) from Uganda. Thus, they are probably sharing a common evolutionary ancestor. The high significant homology with the Ugandan isolates can be attributed to introduction of infected passion fruit plant materials from one country to another. The infected plant material could be the cause of introduction of the virus in new habitat and this might be one of the reasons that the isolates from different locations clustered together. The sub grouping within the main cluster suggests some variation among the isolates, possibly strain differences. Incidentally, our analysis reinforced the idea that isolate UGM-17 designated as UPV was different from other Ugandan isolates as previously noted [6]. While the virus isolates UGM-19a, UGM-73 and UGM-58 were almost identical (94-100% aa), Isolate UGM-17 display 56-60% identity to the Rwandan isolates. This indicates some degree of genetic diversity among the UPV strains, which could complicate the process of breeding resistant varieties. Thus, further research is recommended to substantiate the diversity within the UPV strains.

The virus was detected in all the surveyed areas, signifying how widely the disease is distributed in the country. The high incidence of potyvirus and specifically UPV in Gicumbi (North) compared to Nyamagabe (South) and Ngororero (West) district suggest that the virus may be more serious in Northern Province where passionfruit is mainly produced as reported by [1]. Most of the Rwandan passion fruit growers traditionally recycle planting materials (seeds) either sourced from their old orchards or neighbors' field or market which are of poor quality [15]. In addition, lack of a certification scheme for planting materials, free movement of infected material from one area to another, lack of a method to clean up the infected material in the field and establishing orchards near the old ones as highlighted by [16] may have a role to play in disease spread and the high incidence which was observed in this study. This indicates a potentially high reduction in yield and quality of this crop and hence, there is a need to emphasize on local quarantine to minimize pathogen spread and disease incidences.

Our results also demonstrated that, RT-PCR was 2 times more sensitive in detection of potyvirus than ELISA method. Although ELISA is a commonly used technique in analysis of large volumes of samples, PCR techniques should be used for verification especially in the cases when the virus is expected to appear at a very low concentration in the host plant. These observations support the application of PCR-based techniques in detection of potyvirus species, as demonstrated by others [17] [18] [19] [20] [21]. Thus, there is a strong necessity for the use of highly sensitive methods to detect viruses and differentiate between species. This necessity has grown with the tendency for global plant material exchanges and increasingly stringent plant material certification regulations.

4. CONCLUSION

In conclusion, the evidence gathered in this study indicates that the virus isolated from passionfruit in Rwanda is a potyvirus comprising a strain of UPV which could be the primary pathogen causing PWD disease. Whether *passionfruit woodiness virus* (PWV) is also present remains to be demonstrated. Given that there were some unidentified potyvirus in present study, there is a need for further research to identify and establish their role in disease development. Passionfruit woodiness disease remains one of the major challenges affecting production of this crop in Rwanda, and thus breeding programs should aim at developing varieties that are resistance to UPV.

CONSENT (WHERE EVER APPLICABLE)

Not applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Not applicable, the study did not deal with human or animal subjects. The research involved plant issues.

REFERENCES

1. Rwanda Horticulture Development Agency (RHODA). A Survey Report on the Status of Horticulture in Rwanda. October 2008.Pp 85.

2. Booker HM, Umaharan P, McDavid CR. Effect of Cowpea severe mosaic virus on crop growth characteristics and yield of cowpea. Plant Dis.2005; 89:515-520.

3. Fischer IH, Rezende JAM. Diseases of passion flower (*Passiflora spp.*). Pest technology. 2008; 2 (1): 1-19.

4. Brand RJ, Burger JT, Rybicki EP. Cloning, sequencing, and expression in Escherichia coli of the coat protein gene of a new Potyvirus infection South African. Passiflora. Archives of Virology. 1993; 128, 29-41.

5. McKern NM, Strike PM, Barnett OW, Dijkstra J, Shukla DD, Ward CW. (1994). Cowpea aphid-borne mosaic virus - Morocco and South African Passiflora virus are strains of the same potyvirus. Archives of Virology. 1994;136: 207-217.

6. Ochwo-Ssemakula M, Sengooba T, Hakiza JJ, Adipala E, Edema R, Redinbaugh MG, et al. Characterization and distribution of a Potyvirus associated with passion fruit woodiness disease in Uganda. Plant Disease. 2012; 96: 659-665.

7. Kilalo-Ngeranwa DC. Molecular detection of viruses associated with passionfruit (Passiflora edulis sims) woodiness disease, monitoring and management of aphid vectors in Kenya. PhD thesis, University of Nairobi. 2012; Pp 123.

8. Wylie SJ, Jones MGK. The complete genome sequence of a Passion fruit woodiness virus isolate from Australia determined using deep sequencing, and its relationship to other potyviruses. Arch Virol. 2011; 156: 479. DOI:10.1007/s00705-010-0845-3

9. Nascimento AVS, Santana EN, Braz ASK, Alfenas PF, Pio-Ribeiro G, Andrade, GP, et al. *Cowpea aphid-borne mosaic* virus (CABMV) is widespread in passionfruit in Brazil and causes passionfruit woodiness disease. Arch Virol. 2006; 151:1797-1809. DOI 10.1007/s00705-006-0755-6

10. Iwai H, Yamashita Y, Nishi N, Nakamura M, et al. The potyvirus associated with the dappled fruit of *Passiflora edulis* in Kagoshima prefecture, Japan is the third strain of the proposed new species East Asian Passiflora virus (EAPV) phylogenetically distinguished from strains of Passion fruit woodiness virus. Arch Virol. 2006 151: 811.

11. Clark MF, Adams AN. Characteristics of the microplate methods of Enzyme- linked immunosorbent assay for the detection of plant viruses. Journal of general virology. 1977; 34: 475-483.

12. Langeveld SA, Dore JM, Memelink J, Derks AFLM, Vander Vlught CIM, Asjes CT et al. Identification of potyviruses using the polymerase chain reaction with degenerate primers. Journal of general Virology. 1991; 72:1531-1541.

13. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA 6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution. 2013; 30: 2725-2729.

14. Taylor RH, Greber RS. Passionfruit woodiness virus. CMI/AAB Descriptions of plant viruses 122. 1973

15. Horticultural Council for Africa (HCA). Integrating the horticultural council for Africa in the Nile basin initiative's analysis and sustainability of cross border trade in fruits and vegetables along the selected corridor. Final report November, 2012. Pp 36. http://nileis.nilebasin.org/system/files/CBT%20Final%20Report%20by%20HCA.pdf. Accessed on 14/3/2018

16. Rwanda Agribusiness Development Assistance (ADAR). Diagnostic mission to determine constraints to production and exportation of high quality fruit (Passionfruit, Physalis and Apple banana) from Rwanda. March-April 2002. Pp33. http://pdf.usaid.gov/pdf_docs/Pnadj079.pdf Accessed on14/3/2018.

17. Varveri C. Potato Y potyvirus detection by immunological and molecular techniques in plants and aphids. Phytoparasitica.2000; 28: 2.

18. Moury B, Cardin L, Onesto JP, Candresse T, Poupet A. Enzyme-linked immunosorbent assay testing of shoots grown in vitro and the use of the immunocapture-reverse transcription-polymerase chain reaction improve the detection of *Prunus necrotic ring spot virus* in rose. Phytopathology. 2000; 90: 522-528.

19. Dovas CI, Hatziloukas E, Salomon R, Barg E, Shiboleth Y, Katis NI. Comparison of methods for virus detection in *Allium spp*. Journal of Phytopathology. 2001;149: 731-737.

20. Lunello P, Ducasse D, Conci V. Improved PCR detection of potyviruses in Allium species. European Journal of Plant Pathology.2005;112:371-378. DOI 10.1007/s10658-005-6232-3.

21. Gawande SJ, Shukla A, Chimote VP, Kaushal N, Kaundal P, Garg ID, et al. Development of PCR-based techniques for the detection of immobilized *Potato virus Y* virions. Journal of Plant Pathology. 2011; 93 (1):127-132.