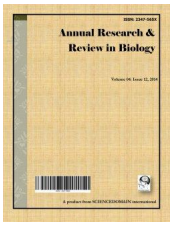

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Molecular Detection of Ugandan Passiflora Virus Infecting Passionfruit (*Passiflora edulis* sims) in Rwanda

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Authors' contributions

This work was carried out in collaboration between all authors. The whole team jointly designed the study and developed the protocols. Author WWB wrote the first draft of the manuscript, while authors CKD, KM and JMM reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The study aimed at identifying the pathogen associated with passionfruit woodiness disease 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 leaf samples exhibiting virus-like symptoms were collected from farmer's fields in Nyamagabe, Ngororero and Gicumbi district found in South, West and North provinces of Rwanda, respectively. Virus detection was done using enzyme-linked immunosorbent assay and reverse-transcription polymerase chain reaction. Virus-like symptoms observed in the field included; leaf mosaic, crinkle,

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distortion, fruit woodiness and malformations. Ugandan passiflora virus was detected in 70% of the positive samples and other unidentified potyviruses. The incidence of virus infection was highest in North at 45.8% and lowest in West province at 18.7%. Partial amino acid sequences of the coat protein of 169 residues were used to determine the identity of the associated virus. Sequences obtained were highly similar and displayed features typical of potyviruses with 93-100% identity. Comparisons of these sequences with those of other existing potyviruses indicated highest identity (94-100%) to Ugandan passiflora virus isolates from Uganda. Sequences of four Rwandan isolates are deposited in Genbank: isolate RW10 (Accession No. MK132862), RW23 (MK132863), RW104 (MK132864) and RW140 (MK132865). This study confirms presence of the Ugandan passiflora virus in the country. This necessitates the need for 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, Fischer and Rezende [3] reported a 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 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 identifying the causal pathogens of the PWD in Rwanda. The results obtained will offer a platform for breeding passionfruit resistant varieties.

2. MATERIALS AND METHODS

2.1 Collection of Passionfruit Leaf Samples

Passionfruit leaves observed with virus-like symptoms such as leaf mosaic, crinkle, distortion, fruit woodiness and malformations were collected from 66 farmers' fields at Gicumbi (North), Ngororero (West) and Nyamagabe (South province) districts of Rwanda on September 2012. These districts are among major passionfruit production areas in Rwanda [1]. In addition, samples were collected from a passionfruit field belonging to Rwanda Agriculture Board (RAB), Huye district in South province. Twenty-two (22) passionfruit fields per district, at least 2 km apart, were randomly selected and observed for the presence of plants bearing viral-like symptoms. From each field, 3 plants (2 diseased: 1 healthy) were randomly selected and 3-5 leaves collected from the growing points. A total of 201 samples were collected, bagged and preserved over silica gel. These samples were stored at room temperature in the laboratory until analysed.

2.2 Detection of Virus by Serological Assays

The samples were previously ground in liquid nitrogen to a fine powder and approximately, 200 mg of each sample was homogenised in 1 ml of extraction buffer by vortexing for 1 min. These samples were stored at -20°C until analysed.

Antigen-coated-plate enzyme-linked immunosorbent assay (ACP-ELISA) was used to detect potyviruses in the samples, double antibody sandwich (DAS-ELISA) [11] was used to detect *Cucumber mosaic virus* (CMV) and *Cowpea aphid borne mosaic virus* (CABMV) using commercial kits acquired from Deutsche Sammlung Von Mikroorrganismen und Zellkulturen (DSMZ) Germany following manufacturer's protocol. Healthy passionfruit leaves were used as negative controls while, diseased passionfruit leaves supplied with the kits were used as positive controls. In addition, a blank containing only extraction buffer was also used as a control. All samples were tested in duplicate and optical density values were read at A405nm. Samples with absorbance A405nm values greater than 2 times the average of negative control were considered positive.

2.3 RNA Extraction and cDNA Synthesis

The samples were previously ground in liquid nitrogen to a fine powder and stored at -80°C until analysed. Total RNAs were extracted from 100 mg of frozen powdered passionfruit leaves using ZR plant RNA MiniprepTm Kit, (catalogue No. R2024; Zymo Research, USA), following manufacturer's instructions. Virus complementary deoxyribonucleic acid (cDNA) was synthesised from 1 µg of total RNA using Maxima first Strand cDNA synthesis kit for RT-PCR, (catalogue No. K1642; Thermo Scientific) as per manufacturer's instructions.

2.4 Primer Used for Virus Amplification

PCR products were generated using potyvirus universal primer U335 and D335 [12] which amplify a fragment of ~335 bp from the central conserved region of coat protein (CP) gene (Table 1). In addition, primer sets UPVF2/R2 position 558-581/1307-1329, and UPVF4/R4 position 919-938/1099-1118 in CP gene were used to amplify fragments of ~772 and 200bp, respectively. These set of primers were designed using the nucleotide sequence of the UPV Ugandan isolate accession no. FJ896003.

2.5 Virus Amplification

PCR reaction were set up in 20 µl volumes and contained 1x reaction buffer, 1.5mM MgCl2, 250µM dNTP, 1 unit of Taq polymerase (Bioneer, USA), either 10 pmol of UPVF/UPVR or 5 pmol of U335/D335 (Langeveld et al. 1991) primer sets. The following cycling conditions were used: for primer pair U335/D335- 95°C for 5 min, 30 cycles of 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 set UPVF/UPVR- 95°C for 5 min, 30 cycles of 94°C for 30 s, 48°C for 1 min, 72°C for 30 s, followed by a 7 min extension at 72°C. Amplification of cDNAs in polymerase chain reaction (PCR) was done using thermocycler (GeneAmp PCR system 9700). DNA fragments were separated on a 1% agarose gel/0.5X-TBE stained with 0.25X GelRed and extracted using GeneJET Gel Extraction Kit (catalog No. K0692; Thermo Scientific) following manufacturers' instructions. Purified DNA fragments were sequenced at BecA-ILRI hub.

2.6 Sequence Data Analysis

Deduced amino acid sequences (169 aa) of partial coat protein gene obtained were compared to other sequences of potyviruses strains available from Genbank using Basic Local Alignment Search Tool at the National Centre for Biotechnolgy Information (Table 2). Multiple sequence alignments were obtained with CLUSTAL W [13]. Phylogenetic tree was obtained with MEGA version 6.0 [13] using neighbour-joining with unweighted pair group method averages (UPGMA). Tree branches were bootstrapped 1000 replications.

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

Primer	Sequence	DNA amplification size (bp)	Reference
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*

Table 2. Known potyvirus strain sequences obtained from the genbank and used for sequence comparison

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 were observed (Fig. 1). Collected passionfruit leaves samples were tested for presence of Potyviruses, *Cucumber mosaic virus* (CMV) and *Cowpea aphid borne mosaic virus* (CABMV). Out of the 198 symptomatic and asymptomatic samples collected, 44 (22.2%) tested positive for potyvirus (Table 3). All the samples tested negative for CMV and CABMV.

3.1.2 Detection of Rwandan UPV isolates

Out of the 198 samples, 45.5% tested positive for the potyvirus, while 54.5% were negative (Table 3). Further testing of the 90 positive samples with specific primers showed that 31.8% were positive for Ugandan passiflora virus (UPV). Occurrence of virus infection was highest in Gicumbi district, North province at 57.6% followed by Nyamagabe district, South province at 47% and the lowest in Ngororero district, West province at 31.8%. Result of amplification reactions with the UPVF2/R2, U335/D335 and UPVF4/R4 primer sets are shown in Figs. 2, 3 and 4, respectively.

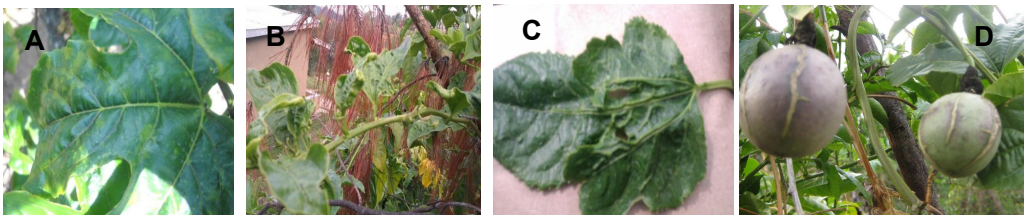

Fig. 1. Common symptoms observed in the passionfruit field in Rwanda. (A) Leaf mosaic, (B&C) Leaf rolling, crinkle and distortion, (D) Fruit woodiness and malformations

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
Northern	Gicumbi	22	66	26	0	0	38	27
Southern	Nyamagabe	22	66	8	0	0	31	22
Western	Ngororero	22	66	10	0	0	21	14
			198	**44**	**0**	**0**	**90**	**63**

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	

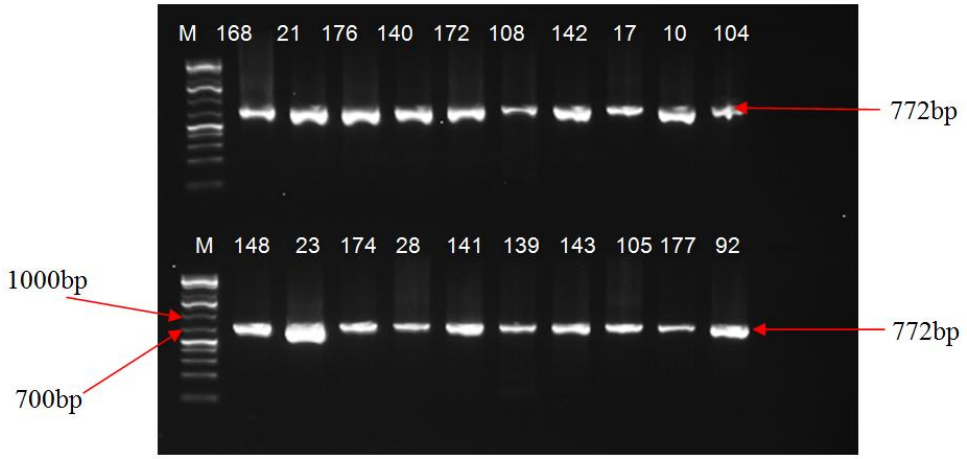

Fig. 2. Amplification of a 772bp fragment with primer set UPVF2/R2 on twenty passionfruit samples. 1 % agarose gel containing gel red, 100 volts for 35 mins, M= 1Kb^{+} DNA ladder

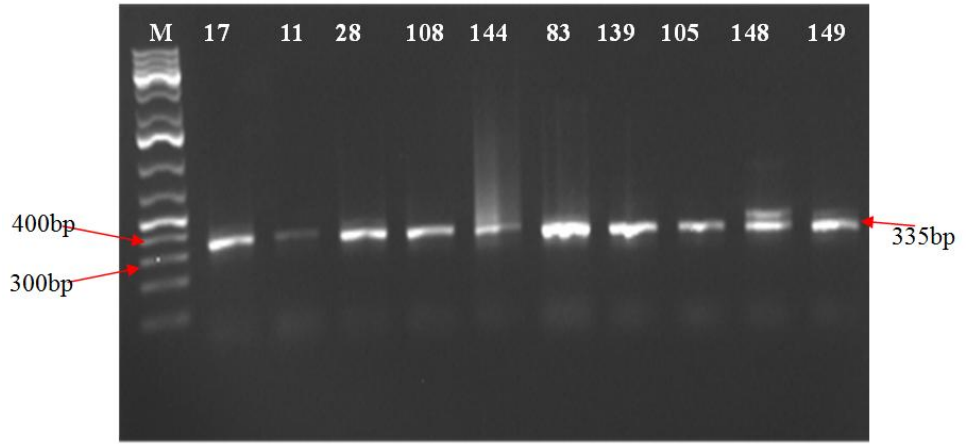

Fig. 3. Amplification of a 335 bp fragment with primer set U335/D335 on ten passionfruit samples. 1 % agarose gel containing gel red, 100 volts for 35 mins, M= 1Kb^{+} DNA ladder

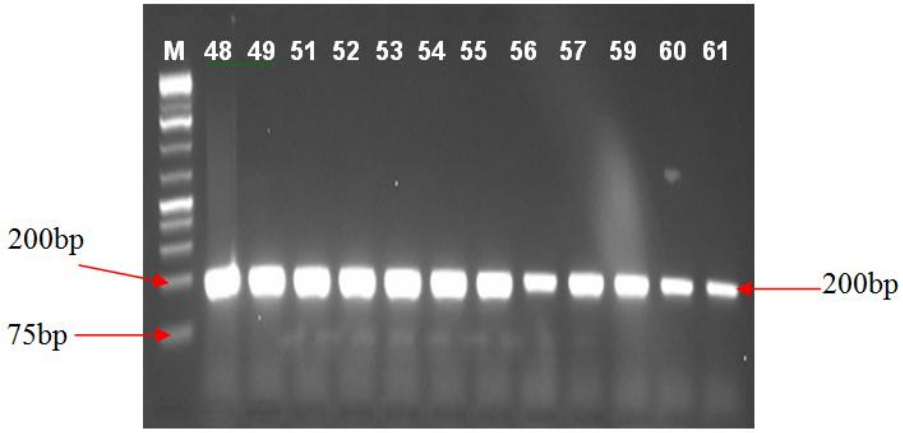

Fig. 4. Amplification of a 200 bp fragment with primer set UPVF4/R4 on twelve passionfruit samples. 1 % agarose gel containing gel red, 100 volts for 35 mins, M= 1Kb^{+} DNA ladder

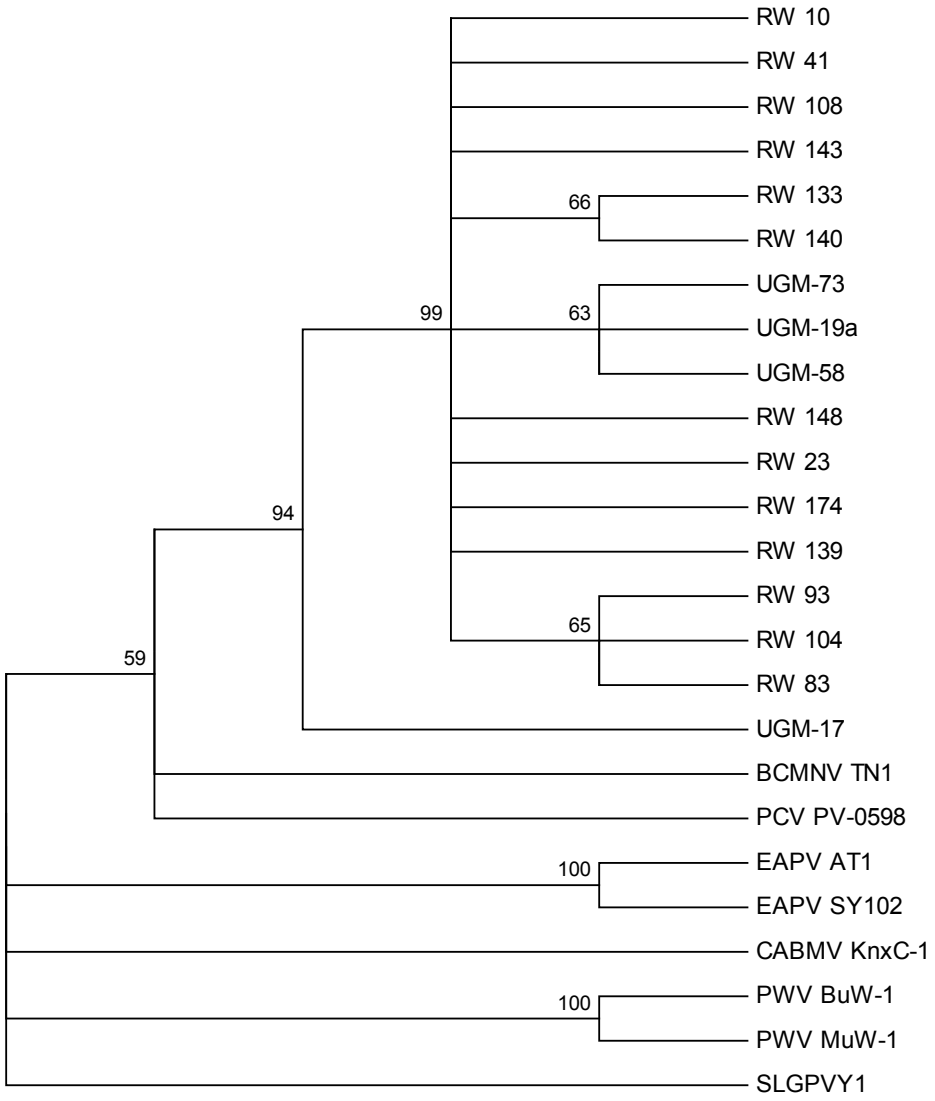

Fig. 5. Phylogenetic tree of thirteen Ugandan passiflora virus isolates from Rwanda and other representative potyvirus strains reported on passionfruit worldwide

The tree was based on alignments of the predicted amino acid of the partial coat protein gene and Potato virus Y (SLGPVY) was used as the outgroup taxa.
Key: RW- Rwanda Isolates; UGM-Uganda Isolates

3.1.3 Sequences analysis of Rwandan UPV isolates

Similarity studies were done between the deduced amino acids (169 aa) sequences of Rwandan isolates with those of other selected potyviruses in Genbank; *Passionfruit woodiness virus*, *East Asian passiflora virus*, *Cowpea aphid borne mosaic virus*, *Passiflora chlorosis virus*, *Bean common mosaic necrosis virus*, *Potato Y virus* and Ugandan passiflora virus (Table 1). The Rwandan isolates showed highest similarity of 94-100% aa with various isolates of Ugandan passiflora virus (UGM-19a, UGM-58, UGM-73) from Uganda with evolutionary divergence values between 0.00-0.06 (Table 4). These similarities are higher than the species demarcation criteria of <82% amino acid identity proposed by Adams et al. [14], confirming that the Rwandan and Ugandan isolates are same species. The similarity with other potyviruses was; 45.2-46.8% BCMN-TN1, 36.8-37.6% PCV-PV-0598, 36.2-38.7% EAPV-AT1 and EAPV-SY102, 33.0-37.6% CABMV-M3 and CABMV-Knxc-1, 36-39.2%

PWV-BuW-1 and PWV-MuW-1, and the lowest was 11.7-13.2% PVY-SLGPVY1. The sequences of four Rwandan isolates are deposited in Genbank: isolate RW10 (Accession No. MK132862), RW23 (MK132863), RW104 (MK132864) and RW140 (MK132865).

3.1.4 Phylogenetic relationships between Rwandan UPV isolates and other representative potyvirus strains

A close relationship between the Rwandan and Ugandan isolates is clearly indicated by phylogenetic tree (Fig. 5) based on the partial CP aa sequences. Rwandan and Ugandan isolates were grouped in a monophyletic cluster with 100% bootstrap value, clearly distinct from the PWV, CABMV, EAPV isolates. The most closely related Ugandan isolates are; UGM-19a (Accession no. FJ896000), UGM-58 (Accession no. FJ896001) and UGM-73 (Accession no. FJ896002). PWV, CABMV and EAPV isolates were grouped in separate clusters with a 100% bootstrap value. PWV, CABMV and EAPV isolates were grouped in separate clusters with a 100% bootstrap value.

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, [4,5,6,7,9,10,15]. 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 other 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.

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. The detection of the UPV strains in Rwanda can be attributed to introduction of infected passionfruit plant materials from one country to another perhaps also explaining why 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.

Ugandan passiflora 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 RHODA [1]. Most of the Rwandan passion fruit growers traditionally recycle planting materials (seeds) either sourced from their old orchards or neighbours' field or market which are of poor quality [16]. 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 [17] 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 emphasise on local quarantine to minimise pathogen spread and disease incidences.

Our results also demonstrated that, RT-PCR was more sensitive than ELISA method. This is because the higher percentage of positive samples was detected when using RT- PCR compared to ELISA. Although ELISA is a commonly used technique in the 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 [18,19,20,21,22]. 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 Ugandan passiflora virus which could be the primary pathogen causing PWD disease. Whether passionfruit woodiness virus 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rwanda Horticulture Development Agency (RHODA). A survey report on the status of horticulture in Rwanda. 2008;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. 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;123.
8. Wylie SJ, Jones MGK. The complete genome sequence of a passionfruit 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 passion fruit in Brazil and causes passion fruit woodiness disease. Arch Virol. 2006;151:1797-1809.
   DOI: 10.1007/s00705-006-0755-6
10. Iwai H, Yamashita Y, Nishi N, Nakamura M. 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(4):811-818.
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. 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. Adams MJ, Antoniw JF, Fauquet CM. Molecular criteria for genus and species discrimination within the family Potyviridae. Arch Virol. 2005;150:459-479.
15. Taylor RH, Greber RS. Passion fruit woodiness virus. CMI/AAB Descriptions of Plant Viruses. 1973;122.
16. 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;36. Available:http://nileis.nilebasin.org/system/files/CBT%20Final%20Report%20by%20HCA.pdf Accessed on 14/3/2018
17. Rwanda Agribusiness Development Assistance (ADAR). Diagnostic mission to determine constraints to production and exportation of high quality fruit (Passion fruit, Physalis and Apple banana) from Rwanda. 2002;33. Available:http://pdf.usaid.gov/pdf_docs/Pnadj079.pdf (Accessed on 14/3/2018)
18. Varveri C. Potato Y potyvirus detection by immunological and molecular techniques in plants and aphids. Phytoparasitica. 2000;28:2.
19. 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.
20. 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.
21. 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
22. Gawande SJ, Shukla A, Chimote VP, Kaushal N, Kaundal P, Garg ID, Chimote KP. Development of PCR-based techniques for the detection of immobilized Potato virus Y virions. Journal of Plant Pathology. 2011;93(1):127-132.

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