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Article

Genomics Unravels Passion Fruit Viral Disease Complexity in Kenya

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Abstract: Passion fruit virus diseases (PWDs) pose a significant threat to Kenya's passion fruit industry. To unravel the complexity of these diseases, comprehensive virus surveys were conducted across major passion fruit-growing counties. PWD symptoms like fruit hardening, chlorotic mottling, and leaf distortion, were prevalent. The study unveiled the first 23 complete genomes of Ugandan Passiflora virus (UPV) and two East Asian Passiflora distortion virus (EAPDV) in Kenya. UPV showed 99% nucleotide (nt) match to a UPV genome from Uganda and 66% nt identity match to EAPDV. Phylogenetic analysis revealed distinct lineages (I-III), indicating potential multiple introductions into Kenya. Recombination analysis detected no significant breakpoints. However, the study proposed the renaming of EAPDV to passiflora distortion virus (PDV) and UPV to passiflora virus (PV) for neutral nomenclature. Additionally, the study highlighted the role of coinfections in symptom expression, suggesting a synergistic relationship between UPV, EAPDV (PDV), and other viruses. The results recommend for stringent management strategies and enhanced surveillance to mitigate the economic impact of these viruses to Kenyan passion fruit industry. Overall, the study highlights, need to strengthen phytosanitary measures and border surveillance to safeguard Kenya's agriculture from the threat of emerging plant viral diseases.

Keywords: passionfruit woodiness disease; metagenomics; viruses

1. Introduction

Passion fruit (*Passiflora edulis*) is a dicotyledonous perennial plant with shallow roots, and woody vines which climbs by use of tendrils. It belongs to the family *Passifloraceae* with a wide genetic base of approximately 525 species [1]. There are about 60 species of passion fruit producing edible fruits and the most important include the yellow passion fruit (*Passiflora edulis var. flavicarpa*) and the purple passion fruit (*Passiflora edulis var. purplar*) [2]. Passion fruit is an important fruit crop due to its aromatic (unique fragrance and flavor) edible fruits, medicinal properties (as sedatives, antiplasmodic, and antibacterial) and ornamental use. It is an important source of minerals and vitamins (such as ascorbic acid), phytoconstituents and phenolic compounds [3,4]. Passion fruit is thought to have originated within the latitudes of Tropical America probably in southern Brazil, Paraguay and Northern Argentina where it grows on the edges of rain forests [5,6]. The crop was introduced into England in 1810 and later spread to Australia, Hawaii, and South Africa in 1880 and Kenya in 1920. Today, passion fruit is widely cultivated in nearly all tropical and subtropical regions in the world [7].

The production potential of passion fruit in Kenya is estimated at 24 to 30 ton ha^{-1} [8,9]. However, the Kenya average yield is low (8 ton ha^{-1}) mainly due to a significant number of pests and diseases and inadequate clean planting materials [9,10]. Important diseases of passion fruit include dieback

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(*Fusarium* spp and *Phytopthora* spp), brown spot (*Alternaria passiflorae*), collar and stem rot (*Fusarium solani*), anthracnose (*Colletotrichum passiflorae*), bacteriosis caused by *Xanthomonas campestris pv. Passiflorae* and woodiness viral disease complex [11]. Yield losses of 80-100% have been attributed mainly to multiple occurrences of those diseases [10,12,13] which contribute to lack of certified disease-free planting materials [14]. These constraints have also led to the reduction of the lifespan of the plants in the field from 7 years to an average of 1 to 2 years [9,15]. Of these constraints, viral diseases are the most devastating to the production of passion fruits.

Viruses are a major cause of diseases in passion fruit production and have posed a significant threat to fruit yields and quality [16]. Currently, more than 25 different viruses belonging to the genus *Potyvirus*, *Cucumovirus*, *Begomovirus*, *Tymovirus*, *Cilevirus*, *Carlavirus*, *Tobamovirus* and *Nepovirus* have been identified and characterized in passion fruit plants [7,17–27]. Of these, cowpea aphid borne mosaic virus (CABMV, genus *Potyvirus*), ugandan passiflora virus (UPV, genus *Potyvirus*) in East Africa and have been reported to be the causal agents of the most limiting disease (woodiness disease) in passion fruit production [20,21,28]. Other viral pathogens from other parts of the world have been designated as putative etiological agents of woodiness disease including *passion fruit woodiness virus* (PWV) in Australia and Brazil [29], East asian passiflora virus (EAPV) in Japan [29], South African Passiflora virus (SAPV) in South Africa [30], which was later found to be synonymous with CABMV [22,31]. Despite reports of passion fruit woodiness disease (PWD)-complex being the most important constraint in all passion fruit production areas in Kenya, affecting both the purple and the yellow forms [28,32,33], limited studies have been made to identify the viruses responsible for its epidemics. The most recently identified virus infecting passion fruit in Kenya is the *potyvirus* CABMV [21,28].

Given that a broad diversity of passion fruit-infecting viruses causing PWD has been found in other parts of the world [29,34,35], more etiological agents of PWD and passion fruit virome complexity remain to be undiscovered in East Africa. In addition, the lack of a holistic information on viruses infecting passion fruit in Kenya hinders the development of diagnostic tools, effective control measures, such as cross protection and creation of broad-spectrum virus-resistant passion fruit varieties through conventional breeding. High through-put sequencing (HTS) has immense potential in identifying unknown plant viruses, identify diverse strains or new variants [36–39]. This study explored: (i) the discovery and understanding of viruses causing passion fruit woodiness disease complex in major passion fruit growing regions of Kenya, (ii) provide first complete genomes of passion fruit related virome in Kenya and investigate whether any genetic relationship exist with other viruses in global database, (iii) whether any genetic recombination exist within the newly discovered virus and its role in driving virulence.

2. Materials and Methods

2.1. Collection of Passion Fruit Leaf Samples

Virus like and non-symptomatic passion fruit leaf samples were collected from small holder farms in major passion fruit growing areas in Kenya between August and November 2014 (Table 1; Figure 1). Five plants were sampled per field by collecting three leaf samples in each plant (from upper, middle, and lower leaves within a vine). The samples from each plant were bulked for virus testing. A total of 22 bulked samples were collected from Rift valley, (Nakuru (1), Trans Nzoia (3), Elgeyo Marakwet (5), Uasin Gishu (2)), Central (Kirinyaga (3), Muranga (2), Kiambu (5)) and Western (Kakamega (1) regions in Kenya placed and sealed in zip lock bags containing silica gel and preserved for at least one month prior to RNA extractions.

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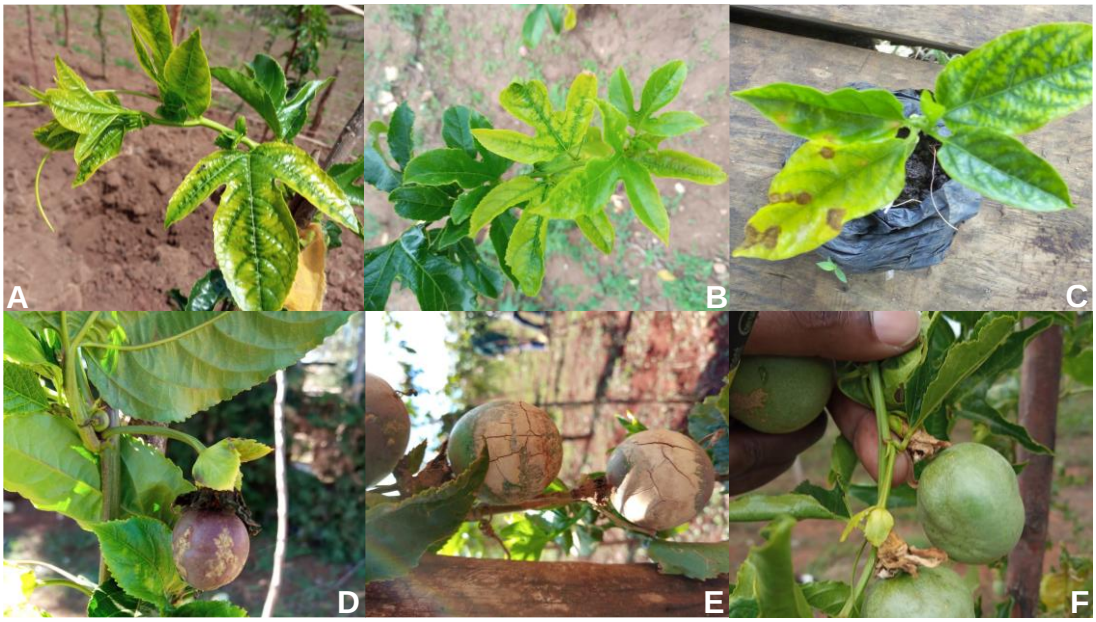

Figure 1. Diversity in passion fruit virus symptoms observed in the field. (**A**) Chlorosis, mottling, (**B**) severe mosaic on the upper leaves, rugosity and distortion, (**C**) nursery seedling with leaf necrosis, mosaic on the lower surface of the leaves, (**D**) fruit deformation and discolored rind, (**E**) thick hard and woody rind, cracked fruits (**F**) deformed fruits.

2.2. RNA Extraction

The RNA was extracted from the 22 samples preserved in silica gel using TRIzol reagent (Sigma-Aldrich, St. Louis, MO, USA), followed by RNase-free DNase (Invitrogen) treatment as described in [21]. The RNA quality control was performed using a Qubit (Invitrogen), and the integrity was further confirmed using RNA screentape (TapeStation 2200; Agilent Technologies). The extracted RNA was stored at -80 °C awaiting Library preparation.

2.3. High Throughput Sequencing

The extracted RNA with a concentration ranging from 0.1 - 1µg of total RNA was used for library preparation, using the TruSeq stranded Total RNA Sample Preparation kit with Ribozero Plant (Illumina, San Diego, CA). This involved subjecting the RNA to depleting Ribosomal RNA using rRNA binding beads. The RNA was then fragmented and primed with random hexamers. First strand cDNA synthesized using a mixture of Superscript II and Actinomycin D to allow RNA dependent synthesis and inhibit DNA dependent synthesis, respectively. The second strand cDNA synthesis was done using dUTP instead of dTTP to create stranded cDNA. The libraries were then adenylated at the 3'end using set A and B adaptors. The cDNA fragment was enriched by 15 cycles of PCR. The final size and concentration of each library was verified using Qubit and D1000 ScreenTape (TapeStation 2200, Agilent Technologies). Ten nanomolar library pools were prepared by mixing the libraries to achieve an equal molar concentration. The libraries were pooled and multiplexed in one lane, and a 1% PhiX v3 spike was included. Sequencing was conducted using MiSeq cycle 2 x 251 v3 kit (Illumina) to generate paired-end reads at Biosciences eastern and central Africa-International Livestock Research Institute (BecA-ILRI), Nairobi Kenya.

2.4. Sequence Analysis

Sequence reads were analyzed by VirusDetect v1.7 using default setting and plant virus database v232 and identified viral contigs extracted. In parallel the RNA-Seq raw reads were first trimmed using Trim Galore [40], with minimum sequence length set to 50bp and minimum required adapter overlap (stringency) set to 1bp. The de novo assembly was performed using the metaSPAdes

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version 3.13.0 genome assembler [41] with default settings. In addition, a second assembler, CLC Genomics Workbench 20 (CLCGW) (CLC bio, Qiagen, Redwood City, CA) was used with the quality scores limit set to 0.01, maximum number of ambiguities to two and removing any reads with <50 nt. De novo assembly was performed using CLCGW with settings of automatic word size, automatic bubble size, minimum contig length 800, mismatch cost two, insertion cost three, deletion cost three, length fraction 0.5 and similarity fraction 0.9 [42–44]. Contigs were sorted by length, examined individually and then subjected to a BLAST and PASC sequence comparisons [45,46]. In addition, trimmed reads from CLCGW were also imported into Geneious Prime (Biomatters Ltd, Auckland, New Zealand) [47], for reference mapping using minimum overlap 10%, minimum overlap identity 80%, allow gaps 10% and fine-tuning set to iterate up to 10 times. Virus coding regions were improved by aligning nt sequences to the aligned deduced amino acid sequences using MUSCLE available in Geneious. Open reading frames (ORFs) were predicted, and annotations made using Geneious. Consensus viral sequences assembled by both approaches were compared and all reads were re-mapped to the consensus in each sample to resolve conflicts and generate final consensus sequences by visual inspection using Geneious software. Reads were mapped back again at highest stringency to the final consensus sequences to determine read count and genome coverages.

2.5. Phylogenetic and Recombination Analysis

All available complete genome sequences of potyviruses reported to infect passionfruit were downloaded from NCBI and aligned with the potyvirus consensus sequences found in this study using the Muscle algorithm as implemented in MEGA-X [48]. Pairwise sequence distances and the optimal evolutionary model were then calculated for each alignment using MEGA-X. The optimal model for each alignment was then applied for phylogenetic analysis using the Maximum likelihood method in MEGA-X. Recombination analysis was performed using RDP version 4.97 [49] to detect putative recombination break points between 21 UPV and CABMV sequences. Any detected recombination signals flagged by RDP 4.97 as 'potentially arising through evolutionary processes other than recombination' were disregarded. Default parameters were used for the seven recombination detection methods implemented: RDP5.2 [50], GENECONV [51], Bootscan [52], MaxChi [53], Chimaera [54], 3Seq [55], and SiScan [56]. Only potential recombination events with an associated Bonferroni-corrected P value <0.05 of more than four recombination detection methods were considered credible evidence of recombination [57]. The BURT method of [49] was used to infer the locations and 95% confidence intervals of break point locations. Recombination breakpoint locations and origins of sequence regions potentially transferred during recombination were verified individually using the phylogenetic tools implemented in RDP 4.97.

3. Results

3.1. Virus Identification

A total of reads ranging from 1,465,364 and 3, 200,244 million reads were obtained from the HTS of different samples collected in Kenya passion fruit growing regions (Table 2). After *de novo* assembly, the contigs of interest had 4,424-232,782 reads mapping to the virus genome of interest, with an average depth of 61.9-3,159 and a normalized depth of 24.3-1,529. A total of 21 Uganda passiflora virus (UPV) and two east Asian Passiflora distortion virus (EAPDV) complete genomes were obtained. UPV and EAPDV coinfections were detected in sample 2 and 7. The UPV genomes collected from different Kenyan counties had 81 - 100% nucleotide identity (nt). Isolates 1KC, 3KE, 6KF, 7KH, 7KG and 8KI (Central region) and 11KJ, 12KK, 14KL, 18KN, 19KO, 12KD (Rift valley region) had 99 - 100% nt identity match between the isolates. Isolate KW from Uasin Gishu and isolate KM from Elgeyo Marakwet (Rift valley region) were identical but shared 86 % nt to any other closest isolate, and 81% nt identity match to KO and KN all from Rift valley region. In addition, isolate KW and KM shared 81% nt identity to 5 isolates from Central region. The highest UPV genome matched GenBank accession MK110656 from Uganda with 99% nucleotide identity (nt) to Rift valley and Central region isolates, but 81% nt identity to 9 isolates from Rift valley region. The two new EAPDV

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sequences (KA and KB) were both detected in Kirinyaga and Muranga county in Central region and had 65-66% nt identity match to the any of the new UPV genomes, but 77.2% nt identity to GenBank Japanese isolate PY-AK. Moreover, UPV variants and two partial passion fruit green spot virus (PFGSV) sequences and one partial (passiflora emaravirus) segment RNA1-5 (a new allexivirus and a new emaravirus, respectively (Supplementary Figure 1 and Figure 2) were detected. Passiflora virus A (PVA) was also detected co-infecting with UPV in samples 15 and 22 (Table 2). The complete genomes have been deposited in Genbank with accession MW355818 to MW355840.

3.2. Phylogenetic and Recombination Analysis

When all the new 21 UPV and two EAPDV (KA 23 and KB 22) genomes were compared with other closely related passiflora associated potyviruses from GenBank, the phylogenetic analysis several revealed distinct lineages (Figure 3). A distinct phylogroup consisted of UPV (I-III), EAPDV, passion fruit woodiness virus, passion fruit, severe mottle virus, bean common mosaic virus, soyabean mosaic virus, watermelon mosaic virus, and east Asian passiflora virus species. The UPV phylogroup I had sequences from Central, Rift valley and UPV-MK110656 from Uganda. The minor phylogroup II and III had all the UPV sequence from Rift valley. Notably, CABMV and bean common necrosis virus grouped separately forming a distinct phylogroup. The CABMV isolate 11KP from Kenya [21], grouped closely together with other eight CABMV sequences from different parts of the world (Figure 3). There was no significant putative recombination break points detected when all the new 21 UPV and the two EAPDV (KA 23 and KB 22) genomes obtained from this study.

3.3. Symptoms

The characteristic of virus-like symptoms of the passion fruit leaves samples included wrinkling, mosaic, chlorosis, vein clearing, ring spots and distorted leaves (Figure 2). However, these symptoms varied significantly within a region. For instance, the sample collected from Trans Nzoia revealed leaf yellow spots, flecks or mottling associated mosaic, rugosity and distortion which was associated with UPV-I. Kiambu samples had mosaic on the lower and upper surface of the leaves which was associated with confection of CABMV and UPV-I. In addition, passion fruit seedlings in Kiambu had mosaic on the leaves and found to be associated with UPV-I. The Muranga samples had thick hard and woody rind, cracked fruits which was observed and associated with UPV-I, KPV and CABMV. There were severe deformed fruits symptoms in the samples collected from Elgeyo Marakwet and found to be linked to UPV-I and UPV-II infections. In the neighboring Country of Uasin Gishu, severe mosaic on the upper leaves of passion fruit plant samples was observed and found to be associated with UPV-I, UPV-II and CABMV.

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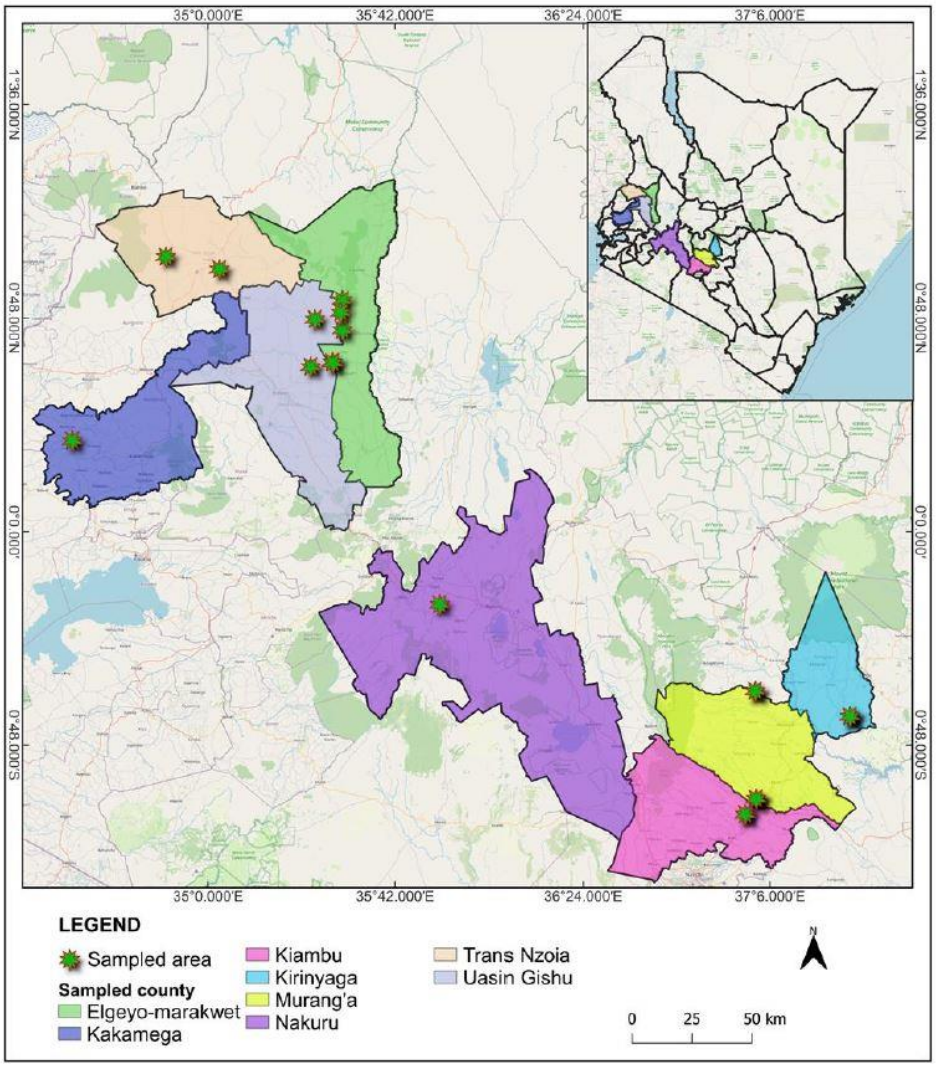

Figure 2. Map of Kenya showing the geographical field locations (counties) where all the passion fruit leaf samples with virus like symptoms were collected**.**

Table 1. Cultivar type, symptoms and geographical information of passion fruit leaf samples that were used to uncover passion fruit viromes using high throughput sequencing (HTS) in Kenya. The samples were collected between August and November 2014.

Sample No.	HTS No.	Cultivar	Fruit type	Viral like disease symptoms	County	Region
K1	1_S1_L001	KPF 11	Yellow	Symptomatic	Kirinyaga	Central
K2	2_S2_L001	KPF 11	Yellow	Symptomatic	Kirinyaga	Central
K3	3_S3_L001	KPF4	Yellow	Symptomatic	Kirinyaga	Central
K4	4_S4_L001	KPF 12	Yellow	Symptomatic	Kiambu	Central
K5	5_S5_L001	KPF 4	Yellow	Symptomatic	Kiambu	Central
K6	6_S6_L001	KPF4	Yellow	Asymptomatic	Muranga	Central
K7	7_S7_L001	Unknown	Hardy shell	Symptomatic	Muranga	Central
K8	8_S8_L001	Unknown	Purple	Symptomatic	Kiambu	Central
K9	9_S9_L001	Unknown	Sweet yellow	Symptomatic	Kiambu	Central
K10	10_S10_L001	Unknown	Sweet yellow	Symptomatic	Kiambu	Central
K11	11_S11_L001	Unknown	Purple	Symptomatic	Nakuru	Rift Valley
K12	12_S12_L001	Unknown	Purple	Symptomatic	Uasin Gishu	Rift Valley

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K13	13_S13_L001	Unknown	Purple	Symptomatic	Uasin Gishu	Rift Valley
K14	14_S14_L001	Unknown	Yellow	Symptomatic	Elgeyo Marakwet	Rift Valley
K15	15_S15_L001	Unknown	Purple	Symptomatic	Elgeyo Marakwet	Rift Valley
K16	16_S16_L001	Unknown	Purple	Symptomatic	Elgeyo Marakwet	Rift Valley
K17	17_S17_L001	Unknown	Purple	Symptomatic	Elgeyo Marakwet	Rift Valley
K18	18_S18_L001	Unknown	Purple	Symptomatic	Elgeyo Marakwet	Rift Valley
K19	19_S19_L001	Unknown	Grafted	Symptomatic	Trans Nzoia	Rift Valley
K20	20_S20_L001	Unknown	Grafted	Symptomatic	Trans Nzoia	Rift Valley
K21	21_S21_L001	Unknown	Grafted	Symptomatic	Trans Nzoia	Rift Valley
K22	22_S22_L001	Unknown	Grafted	Symptomatic	Kakamega	Western

Table 2. Viruses identified in different sample including total number of reads obtained, length of assembled genome sequences, number of sequences reads mapped, average and normalized sequencing depth.

Sample	Raw reads	Virus identified	Contig length	Sequence reads mapped	Average depth	normalized depth
1_KC	1,545,706	UPV-I	9,684	13,294	183	118.6
2_KA	1,509,132	EAPDV	9,638	17,410	232.5	154.1
2_KD		UPV-I	9,679	175,623	2,307.	1,529
3_KE	3,200,244	UPV-I	9,670	36,941	516.8	161.5
6_KF	2,547,696	UPV-I	9,650	4,424	61.9	24.3
7_KH	1,149,924	UPV-I	9,779	66,140	939.9	817.4
7_KB		EAPDV	9,638	11,276	162	141
8_KI	1,465,364	UPV-I	9,874	85,227	1,217	830.7
11_KJ	2,578,474	UPV-I	9,669	9,217	117.9	45.8
11_KP		UPV-II	9,654	14,188	181.7	70.5
		CABMV (Munguti et al. 2020)	9,846	14,888	185.9	72.1
12_KK	2,706,154	UPV-I*	9,650	75,058	1,039.6	384.2
12_KQ		UPV-II*	9,669	32,348	431.3	159.4
13_KR	1,779,280	UPV-II	9,877	81,444	1,151.8	647.4
13_KW		UPV-III	9,683	43,291	620.2	348.6
14_KL	2,974,246	UPV-I*	9,629	9,318	131.3	44.1
14_KS		UPV-II	9,771	220,180	3,062.3	1,029.6
15_KT	1,706,154	UPV-II	9,658	77,940	1,044.6	612.2
15_KM		UPV-III	9,667	127,649	1,696.5	994.3
16_KU	1,627,772	UPV-II	9,677	41,050	557	342.3
17_KG	2,400,688	UPV-I	9,651	232,782	3,159	1,315.9
18_KN	2,395,822	UPV-I	9,671	185,274	2,619	1,093.3
18_KV		UPV-II	9,680	126,554	1,792.6	748
19_KO	1,847,440	UPV-I	9,636	55,234	735.9	398.4

* Additional variant of the virus identified, but could not be fully assembled. UPV - Uganda passiflora Virus (UPV). EAPDV - East Asian passiflora distortion virus. CABMV - Cowpea aphid borne mosaic virus. 2 -(Passiflora virus A) samples 4, 5, 9, 10, 20, 21 (incomplete UPV), 22 - (incomplete UPV and passion fruit green spot virus).

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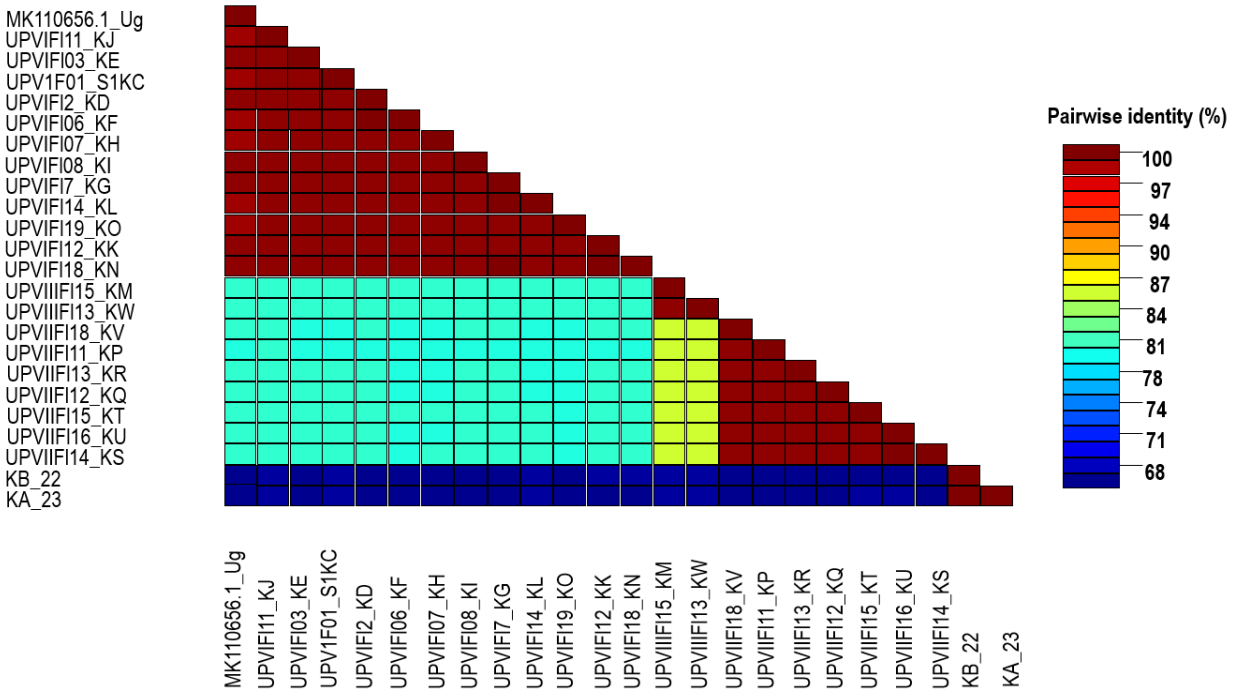

Figure 3. Species demarcation tool (SDT) interface of the new passiflora virus genomes sequences obtained in this study. The colour-coded matrix of pairwise identity scores, show species nucleotide pairwise identity scores of the new 21 genomes and the Ugandan isolate currently available in GenBank.

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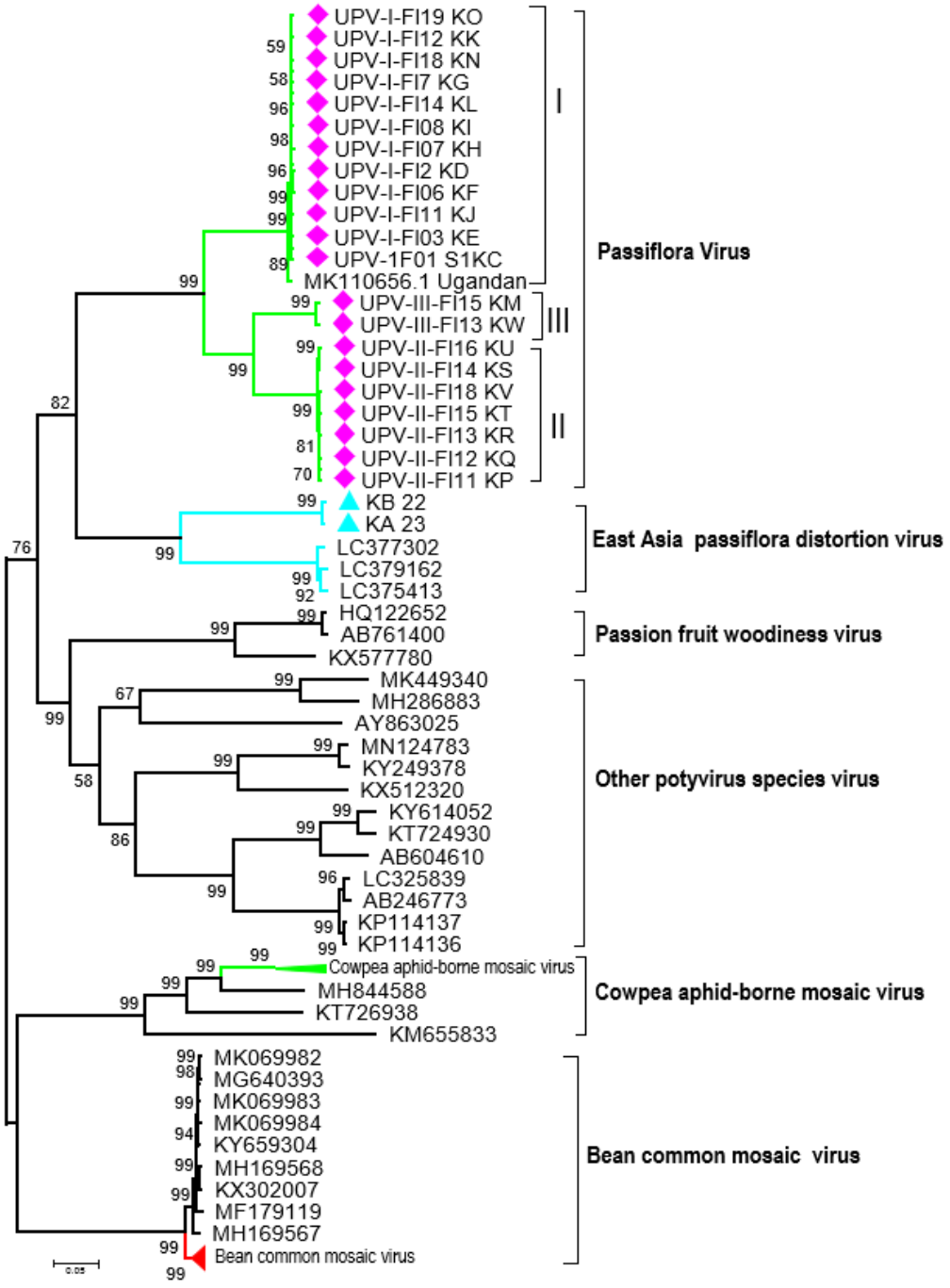

Figure 4. Phylogenetic tree analysis of 21 new UPV and two EAPDV (KA 23 and KB 22) isolates, along with other closely related potyviruses from GenBank. The tree was generated using MEGA using maximum likelihood method, and bootstrap values (≥ 50%) are shown at the nodes. Scale bar indicates evolutionary distance.

4. Discussion

To elucidate the passion fruit virus disease complexity in Kenya, virus surveys were conducted across major passion fruit growing Counties. Symptoms resembling PWD were prevalent, including conspicuous hardening and cracking of fruits, chlorotic mottle, and leaf distortion. Plant virus metagenomics has the capacity to detect viruses either as single or co-infections and can reveal the presence of novel or unsuspected plant viruses [39,58]. The study revealed the first full genomes of UPV and EAPDV in symptomatic passion fruit samples obtained from farms in Kenya. We detected mixed infections between UPV and EAPDV in two out of the 22 samples analysed by HTS. However,

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establishing associations between the specific viral infections detected and the symptoms expressed is complicated by mixed virus infections. Plant viruses co-infecting in the same host may interact either in a synergistic or antagonistic way [59] and this remains to be determined for viruses detected in passion fruit in Kenya. The discovery of UPV in major passion fruit growing regions of Kenya concurs with earlier studies reporting the presence of UPV in Uganda [20]. The detected UPV and EAPDV have close genetic link to a single UPV genome from Uganda. The UPV and EAPDV sequences grouped separately from the other passion fruit and potyviruses. A distinct lineage was formed when the 21 new UPV and EAPDV genomes were evaluated against other closely related potyvirus that are associated with the genus *Passiflora*. Recombination analysis found no significant putative recombination break points detected when all the new UPV and two EAPDV genomes were analysed. However, the CABMV had strong recombination hotspot towards the 5'UTR region of the virus.

Our results enabled the delineation of three UPV minor phylogroups named I – III. The minor phylogroup 1 had new sequences obtained from Central, Rift valley and Uganda, and the minor phylogroup II and III had all the UPV sequence from Rift valley only. The new EAPDV grouped separately to UPV but together with other four Japanese EAPDV sequences. All the other potential sequences of viruses associated passion fruit diseases retrieved from NCBI grouped separately from the Kenyan sequences. Since the UPV sequence nt identities of the samples from different passion fruit growing counties in Kenya ranged from 81 - 100%, support that the current Kenya UPV isolates belong to the same potyvirus species [20,60]. EAPDV sequences had 65 - 66% nt identity to the UPV genomes but 77.2% to the closest Japanese genome sequence isolate. Thus, neither the complete genome nor the CP comparisons found any evidence of close genomic relationship between the new UPV and EAPDV. Our analysis suggested that the observed nucleotide identities shared by the new UPV and EAPDV isolates from Kenya support the classification criteria of the new EAPDV from Kenyan as a distinct species within the genus potyvirus as previously found by [29].

The presence of UPV genomes sequences from Kenya within three minor phylogroup suggest multiple introductions might have been introduced to Kenya from the adjacent neighbouring countries, or elsewhere and the vice versa. The previous reports of UPV, genome sequences from Uganda and its high similarity to Kenyan isolate, could suggest its introduction was most likely from Uganda either directly or indirectly or vice versa. [61] reported occurrence of UPV from passionfruit virus infected samples from Rwanda with a high similarity to the UPV from Uganda based on the coat protein gene. This could be attributed to the complexity of countries in the East African Region geographic positions, such as land borders. There is therefore a need to strengthen quarantine measures due to the nature of the porous borders which could be playing a key role in the cross-border movement of the viruses with infected planting materials. For example, the Kenyan Western region borders with neighbouring countries such as Uganda with a porous land crossing border, where traders from both countries frequently interact. Unregulated trade practices, exemplified by activities such as cyclers or pedestrian trade transporters, may have inadvertently facilitated the dissemination of infected seeds or seedling planting materials along unmonitored routes, then dispersed to the rest of the country as seen in other parts of the world [61,62]. These reports of UPV infecting passion fruit in Kenya, Uganda and Rwanda suggests that UPV strains are spreading in East Africa and may be a problem more likely to emerge more widely in Africa. This indicates a threat to the passion fruit industry thus there is need to reinforce the control of PWD. Nonetheless, there is need for more sequencing to avail more complete genomes from East Africa region and other parts of the world. This will uncover UPV and EAPDV global distribution and enhance accurate determination of its origins, evolution, and molecular epidemiology towards improved passion fruit disease management. Further, availability of these genomes will facilitate development of sensitive diagnostic assays for use in virus indexing of planting materials in nurseries during certification programs as well as to support diagnostic laboratories that undertake plant health regulation mandate.

The study proposes that new currently known EAPDV "East Asian Passiflora distortion" be appropriately named as "Passiflora distortion virus (PDV)" to avoid geographical association.

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Likewise, UPV was previously detected in Uganda, and a neutral name "Passiflora virus" (PV) was proposed [20]. The new UPV sequences will equally follow the name (PV). This study has adopted latinised numerals in naming the phylogroups of these new sequences and other closely related species. Adopting neutral virus names deter misleading geographical names such as country names [37,63]. Neutral naming, hereafter such as "PV and PDV" allow a consideration of the only detected virus sequence that identifies the new virus, without a biased notion, associated with the first geographical detection locality, as the primary origin of the virus. This is because in several cases, very little surveillance and diagnostic is conducted prior to such naming being adopted. Consequently, it translates to lack of a broad spectrum understanding of the etiology of the new virus. For example, sweet potato virus East African strain was first identified in East Africa, and "East Africa (EA)" was adopted as strain name [64,65]. The "EA" is the phylogroup's distribution originally considered restricted for this virus only, leading to the speculation that the EA is the subcenter of this virus diversity and evolution [66,67]. After 13 years, this virus was identified in East Timor grouping with many other sequences from southern Africa and Southeast Asia, and neutral naming (I) was adopted [68]. As such, in the current study we propose geographically neutral naming such as "PV and PDV", since these viruses might be circulating in many other parts of the world and are yet to be detected. Notably, due to the increasing world trade in plants and plant products, it is only a matter of time before UPV and PDV are detected in additional countries. Therefore, such neutral names will minimize the unnecessary implications in virus research, i.e. evolution analysis, viral disease management, stigmatization of a particular region, international trade implications.

Thus, molecular, serological, and host range studies indicate the viral disease symptoms on passion fruit in Kenya are caused by a confection of multiple viruses with a limited molecular diversity among UPV isolates. Sequences corresponding to Normalized read depth between samples varied significantly indicating differences in virus titers in the plants. The PV occurring in Kenya was isolated from passion fruit leaves showing chlorotic mottle, distortion, thick, hard and woody cracked fruits, which are indicative of presence of PWD in Kenya. These symptoms have been previously found in passion fruit crops in Kenya [34] and neighboring countries including Uganda [7] and Rwanda [61]. Our findings suggest that these PWD distinct symptoms are contributed by synergistic coinfections of PV, CABMV and the EAPDV (PDV) within Kenya passion fruit production systems. Notably, CABMV has been associated PWD, which is linked to mosaic, blistering, and distortion on leaves, fruit woodiness, reduction in juice yield, and shorter lifespan [21,69]. Similarly, these viruses were also genetically distinct from other potyviruses infecting passion fruit as seen within the phylogenetic analysis. We therefore suggest that the new PV and PDV are distinct virus species associated with passion fruit woodiness disease, which has led to significant economic damages in Brazil, Japan, Taiwan, and East African countries [19,20,29,70]. The propagation nature of passion fruit in Kenya which mostly involves grafting also leads to accumulation of viruses [34]. As such stringent management measures including virus indexing of planting seedlings at nursery multiplication blocks is key to prevent further virus dissemination. This should be implemented to deter further impacts of these viruses and enhance passion fruit production. In addition, epidemiological studies are needed to understand the role or cowpea (*Vigna unguiculata*) crops in spreading CABMV to adjacent passion fruit growing hedges.

Recombination plays a pivotal role in viruses [71]. Most plant RNA viruses have high rates of recombination leading to an emergence of new virus species and their variants, a crucial factor in the emergence of resistance-breaking strains, virulence increase and change of host range as an adaptation strategy [37,72,73]. Despite, the critical role of recombination process in RNA viruses, the current new PV and PDV genomes isolated from Kenya passion fruit crops had no significant recombination break points. Although, recombination process varies extensively among viruses [71], genetic, biological, and epidemiological factors can affect the probability of virus recombination between different strains or species. This may include genetic homology, existing virus population size in the host, co-circulation in the same geographical area, and coinfection rate. The current sequences insufficient genetic diversity of 99-100% nt, could be attributed to the source of the nursery seedlings from a common seed stock multiplication and nursery centres then subsequently

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distributed to the rest of the country passion fruit growing regions. Furthermore, the large diversity of 34% nt genome difference between the new PDV and almost 20% nt between new PV isolates is likely to yield no viable offspring when recombining due to the large genetic distances. Importantly, viral recombination is replication-dependent, as such high viral titre increases the chances of exchanging genetic strands [74]. However, despite the remarkable symptoms observed in the field samples, it is unclear if the PWD viruses detected in this study existed with an overall high viral load. Future studies are required to decipher PWD viral load and its role in recombination.

5. Conclusions

These findings underscore the need to strengthen the current Kenya phytosanitary and border surveillance activities. This should include stringent quarantine measures over importation of plants and plant products to safeguard Kenya agriculture, and for horticultural industry to remain secure and free from new and severe viral diseases, that has potential to cripple the country's economic backbone. Moreover, monitoring activities could prevent the spread of potential viral vectors through natural arrivals due to the unpredictable effects of climate change, as has already been the case with arrival of damaging insects like locusts.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Phylogenetic analysis of the new viruses and other potyviruses, (A and B) phylogeny of the new allexiviruses and cilevirus. Figure S1: Histograms of sequence coverage of consensus viral genomes (top) and variant assemblies with variant regions showing many folds higher sequence coverage. Bars at the bottom of the figure indicate position of variant regions.

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