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Diversity of Viruses and Viroids Infecting Taro in Kenya Based on Small RNA Sequencing and PCR Detection

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Abstract

Purpose: Viral diseases cause severe yield losses and quality decline in crops worldwide. Despite their economic significance, the occurrence and distribution of the major viruses and viroids infecting Taro in Kenya remain poor, limiting the development of robust disease management strategies to mitigate their spread. This study thus aimed to identify the viruses and viroids infecting Taro in Kenya as a basis for developing effective management strategies to support the prevention and control of Taro viruses.

Methodology: Viral surveys and sampling were conducted across nine Taro-growing counties with diverse agroecological conditions in Kenya to determine the incidence and distribution of viruses affecting Taro. Leaf and whole plant samples of symptomatic edible and wild Taro were collected for PCR, RT-PCR, and small RNA sequencing assays to determine the diversity of viruses and viroids infecting Taro.

Results: Disease-like symptoms, including stunting, leaf rolling, shrinkage, deformed leaves with mosaic and yellow veins, and dwarfism, were observed. An overall mean disease incidence of 32-60% was recorded in all sites surveyed. Small RNA sequencing revealed the presence of both DNA and RNA viruses. Detected DNA viruses included the *Taro Bacilliform Virus* (TaBV) and *Taro Bacilliform CH Virus* (TaBCHV), badnaviruses specific to Taro, the *sweet potato Badnavirus B, sugarcane bacilliform virus*, and *sweet potato leaf curl virus*. The RNA viruses included the sweet potato feathery mottle and *Phaseolus vulgaris alphaendornavirus*. A Citrus exocortis viroid was also detected. Interestingly, the wild relatives of Taro displayed very few viral sequence hits. This study reports the Taro viruses and viroids circulating in Kenya and is the first to describe the incidence, distribution, and sequence variability of TaBV in Kenya.

Recommendations: Future studies should focus on developing effective management strategies to support the prevention and control of Taro viruses, including genetic resources for virus-Taro interactions, removing infected crops, controlling insect vectors, and developing virus-free planting materials.

Keywords: *Diversity, Colocasia esculenta, Small RNA sequencing, Taro Bacilliform Virus (TaBV), sequence variability, viroids*

INTRODUCTION

Taro (*Colocasia esculenta*) is an ancient crop and among the most important root crops in many Sub-Saharan African countries (Akwee et al., 2015; Ndabikunze et al., 2011). It is an Araceae and is cultivated for its edible corms and leaves. Taro corms and leaves are a rich source of starch and dietary fiber and contain substantial amounts of protein, vitamins, and minerals. Taro also has some socio-economic importance and is a good export earner worldwide (Akwee et al., 2015; Ndabikunze et al., 2011). It is mainly cultivated by smallholder farmers in Kenya and plays important socio-economic, nutritional, and cultural roles (Talwana et al., 2009). Its production in Kenya is extremely low compared to neighboring countries like Rwanda and Burundi, which are Taro exporters. The production in Kenya is even extremely low compared to other root and tuber crops like cassava, sweet potato, and yams. Taro production systems in Kenya are regarded as an informal production activity managed outside the conventional market and economic channels by researchers and policymakers (Onyeka et al., 2014). It has not been given much attention despite being a food security crop because of its good adaptation to different agro-ecological conditions (Akwee et al., 2015).

It is only grown by small-scale farmers near the streams and riverbanks in the Mount Kenya region, parts of the Rift valley, western, and Nyanza because of the lack of modern irrigation facilities for upland taro cultivation. Its production is further constrained by a myriad of challenges such as pests and diseases (Ivancic, 1992), lack of proper seed systems, poor value addition, and access to organized market systems (Wanyama & Mardell, 2006). Taro is affected by viral, bacterial, and fungal diseases, including the oomycetes. Notably, viral diseases are the most predominant because of sharing and use of young suckers and sets from the previous crop as planting material because of a lack of proper seed systems (Yusop et al., 2019). Most of these materials are already infected by viruses, whereas others get infected through transmission by insect vectors such as aphids and leafhoppers, thus causing a massive disease build-up and spread (Gollifer et al., 1977; Macanawai et al., 2005; Yusop et al., 2019).

In addition, Taro is also a potential host to viroids because they are predominant in tuber crops, such as potatoes, causing stunting of the plants and elongated tubers (Hadidi et al. 2022). Viroids are single-stranded and highly structured noncoding RNAs that replicate autonomously and move systemically in host plants with the aid of the host machinery (Hadidi et al., 2022). Notably, they spread mechanically in the field by leaf damage, tuber, and stem grafts, with some evidence of insect transmission by aphids (Kovalskaya & Hammond, 2014). As such, they are important disease agents that require attention. Taro viral infections lead to severe yield reduction (Babu et al., 2011). However, taro improvement and production intensification require a robust clean seed delivery system which depends on the availability of an effective rapid virus screening protocol.

Studies in other countries and regions report the *Dasheen Mosaic Virus* (DsMV), *Taro Bacilliform Virus* (TaBV), *Colocasia bobone disease virus* (CBDV), *Taro vein chlorosis virus* (TaVCV), and *Taro reovirus* (TaRV) as the main Taro infecting viruses (Revill et al., 2005; Yusop et al., 2019). Dasheen Mosaic Virus (DsMV) and Taro Bacilliform Virus (TaBV) are the most predominant viruses (Revill et al., 2005). These Taro viruses have

varying adverse effects on taro production individually or when in synergy with some infections causing up to a hundred per cent loss (Revill et al., 2005). Although Kidanemariam et al. (2018) reported different taro viruses in East Africa, the occurrence and distribution of the major Taro infecting viruses in Kenya remain poorly characterized. Moreover, there is no report of viroids infecting Taro in Kenya and East Africa.

Traditional detection methods are challenged when plants are infected with multiple viruses belonging to several distinct families, especially when they have not been previously described or are remarkably divergent from those previously characterized. Improved virus detection strategies include molecular methods that can detect diverse members of large viral groups without requiring prior information about the viruses present. Next-generation sequencing (NGS) techniques with small RNA, total RNA or DNA, dsRNA, or mRNA have significantly broadened the possibilities of virus diagnostics by enabling the rapid discovery of new viruses and virus strains (Adams et al., 2009; Wu et al. 2015). Small RNA deep sequencing was first used in 2009 to detect five different viruses in a single plant of sweet potato 'Huachano' inoculated with *sweet potato chlorotic stunt virus* (SPCSV) and *sweet potato feathery mottle virus* (SPFMV) for complete plant viral sequence assembly and the discovery of novel plant viruses (Kreuze et al., 2009).

Cognizant of this, employing this technique in Taro viral diagnostics can provide detailed information on the occurrence and distribution of Taro viruses and viroids as a basis for developing robust management strategies to mitigate their spread. This study used PCR, RT-PCR, and small RNA sequencing techniques to identify the viruses and viroid infecting Taro in Kenya and determine their incidence and distribution.

MATERIALS AND METHODS

Viral Survey and Sample Collection

Viral surveys and sampling were conducted between August and October 2017 in Kiambu (1.0314 °S, 36.8681 °E), Murang'a (0.7839 °S, 37.0400 °E), Meru (0.3557 °N, 37.8088 °E), Nyeri (0.4197 °S, 37.0400 °E), Siaya (0.0617 °S, 34.2422 °E), Busia (0.4347 °N, 34.2422 °E), Kakamega (0.2827 °N, 34.7519 °E), Kisii (0.8067 °S, 34.7741 °E), and Machakos (1.5177 °S, 37.2634 °E) counties of Kenya (Fig. 1) to determine the incidence and distribution of viruses affecting Taro. In each county, ten fields were surveyed in which disease incidence was recorded, and leaf/whole plant samples of symptomatic edible and wild Taro were collected. The incidence of Taro viruses-like diseases was determined through visual aid by counting plants showing viral symptoms, including stunting, leaf rolling, mosaic, and down curling of the leaf blades in every ten plants picked randomly across each field. The leaf samples were preserved in tubes containing silica gel upon collection, and whole plants were placed in plastic bags to prevent dying off. The samples were then transported to the BecA-ILRI hub laboratory in Nairobi, Kenya, for planting in the screen house and *in vitro* laboratory analysis.

Viral incidence in each farm was determined using the formulae:

IC= (n/N) * 100% where; IC = incidence; n = number of plants with viruses-like symptoms; N (10) = total number of plants assessed.

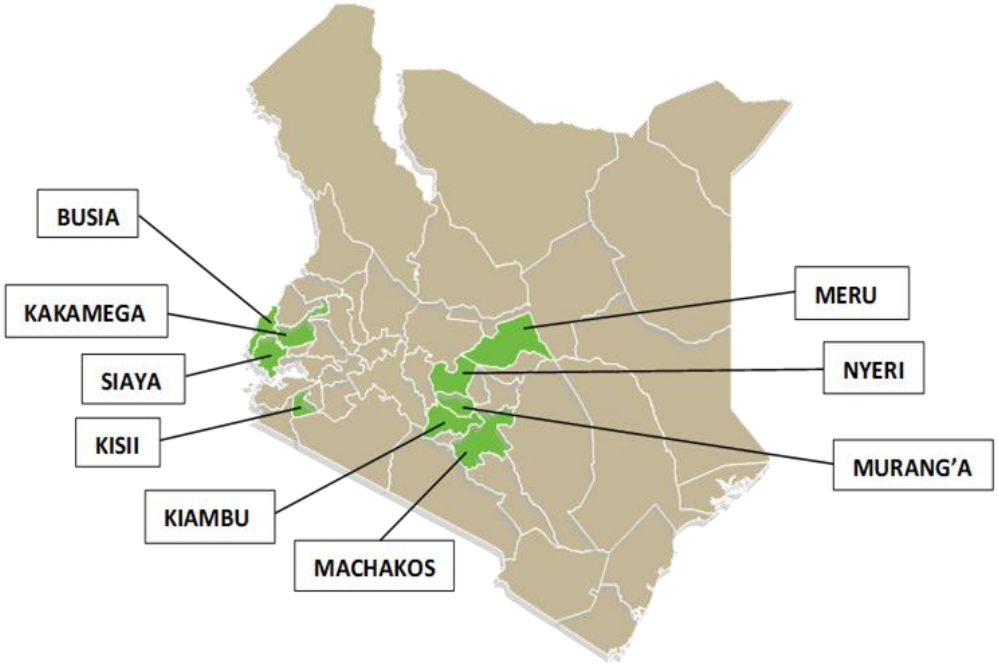

Fig. 1: Kenyan map showing the nine counties (highlighted in green and labeled) where viral survey and sampling were done

Nucleic Acids Extraction, PCR, and RT-PCR

Total plant DNA and RNA were extracted from 290 leaf samples using the Qiagen DNeasy Plant Mini Kit and the Qiagen RNeasy Plant Mini Kit, respectively. The quality and quantity of the DNA and RNA were checked using 1.0% Agarose gel electrophoresis and the NanoDrop® ND-1000 Spectrophotometer (Thermo Scientific, USA). RT-PCR and PCR were used to identify and detect the RNA and DNA viruses reported in other countries using degenerate primers (Supplementary Tables S1-S5). The extracted DNA and RNA were standardized to 50ng/µl and then subjected to PCR and RT-PCR. Amplified products were stained with 2.5µl of gel red per 100ml of Tris-borate-EDTA buffer, electrophoresed through a 1.5% agarose gel, and visualized using a UV trans-illuminator (Syngene™ Ingenius 3 Manual Gel Documentation System, Thermo Scientific, USA) to detect the expected band. PCR amplicons were submitted for sanger sequencing, and the resultant sequences were analyzed/cleaned and assembled using QIAGEN CLC Main Workbench.

Library Preparation And Small RNA Sequencing

Plantlets collected from the various farms were planted in potted autoclaved soil containing compost and were subsequently monitored for viral symptoms for two months in the screen house. A representative sample of 48 leaf samples comprising 27 edible symptomatic Taro, 9 edible asymptomatic Taro, and 12 wild Taro relatives was picked for virus detection and identification. Small RNA extraction was performed using the Qiagen MiRNeasy mini-Kit following the manufacturer's instructions. The Small RNA quality was checked using the Bioanalyzer nanochip (Agilent, Santa Clara, CA, USA), followed by small RNA library preparation using the NebNext Small RNA Library Prep Set for Illumina (New England

Biolabs, Ipswich, MA, USA). Small RNA fragments were ligated with single-stranded adapters, first at the 3′-end, then at the 5′ end, followed by reverse transcription and PCR amplification to generate the DNA template colonies. The library was then sequenced using the Illumina Miseq sequencing platform.

Small RNA Data Analysis And Virus Identification

The raw reads were pre-processed for adapter removal and quality filtering using Trimmomatic (version 0.39) (Bolger et al., 2014). The clean fastq sequences were then converted to fasta format using the seqkit toolkit (Shen et al., 2016). The reads were collapsed into single unique sequences to remove duplicates using the FASTX short reads processing toolkit (FASTX-Toolkit: FASTQ/a short-reads pre-processing tools; http://hannonlab.cshl.edu/fastx_toolkit). The sequences were then searched against the NCBI non-redundant nucleotides (nt) database to identify possible viruses in the samples.

RESULTS

Symptoms and Field Incidence Data of Taro Viral Diseases

Data collection in the field was mainly through visual observation. Plants showing viral symptoms, including stunting, leaf rolling, shrinkage, deformed leaves with mosaic and yellow veins, down curling of the leaf blades, and dwarfism (Fig. 3) in every ten plants picked randomly across each field, were counted and recorded. Viral symptoms were common in all fields surveyed, affecting 32-60% of the surveyed plants. Nyeri county had the highest average incidence at 60%, with an incidence range of 40% to 90% from farm to farm. Murang'a and Meru counties had the least average incidence at 32%, with an incidence range of 20% to 60% (Fig. 2). The other seven counties had an average incidence of between 38% and 57%, with farm-to-farm incidences ranging between 30% and 70%.

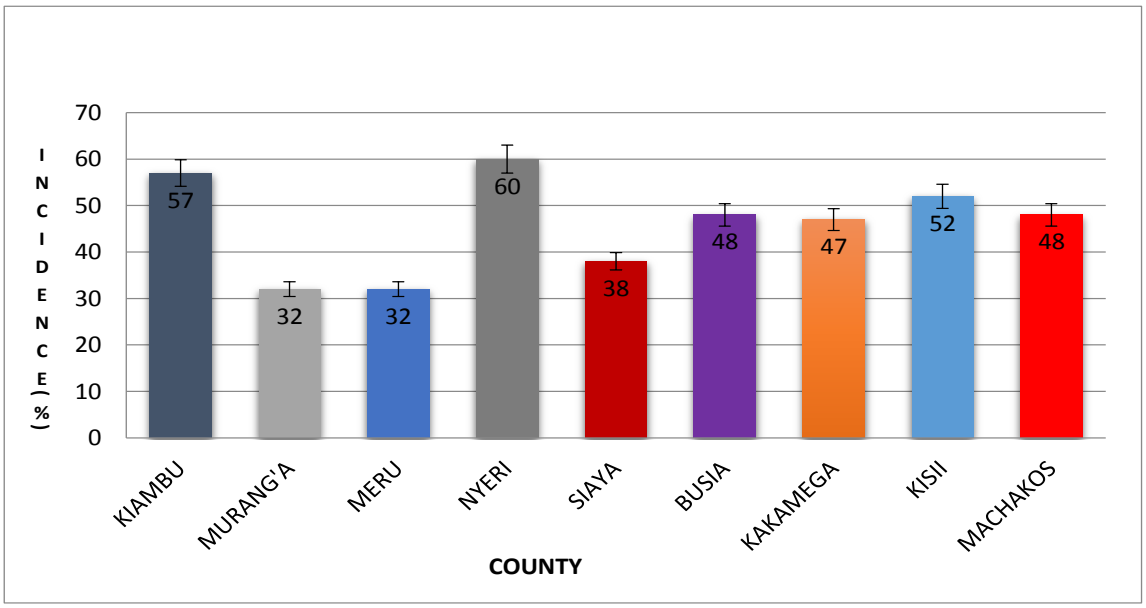

Fig. 2: Graph showing the disease incidence among the nine counties surveyed. The error bars were calculated based on the average incidence per county.

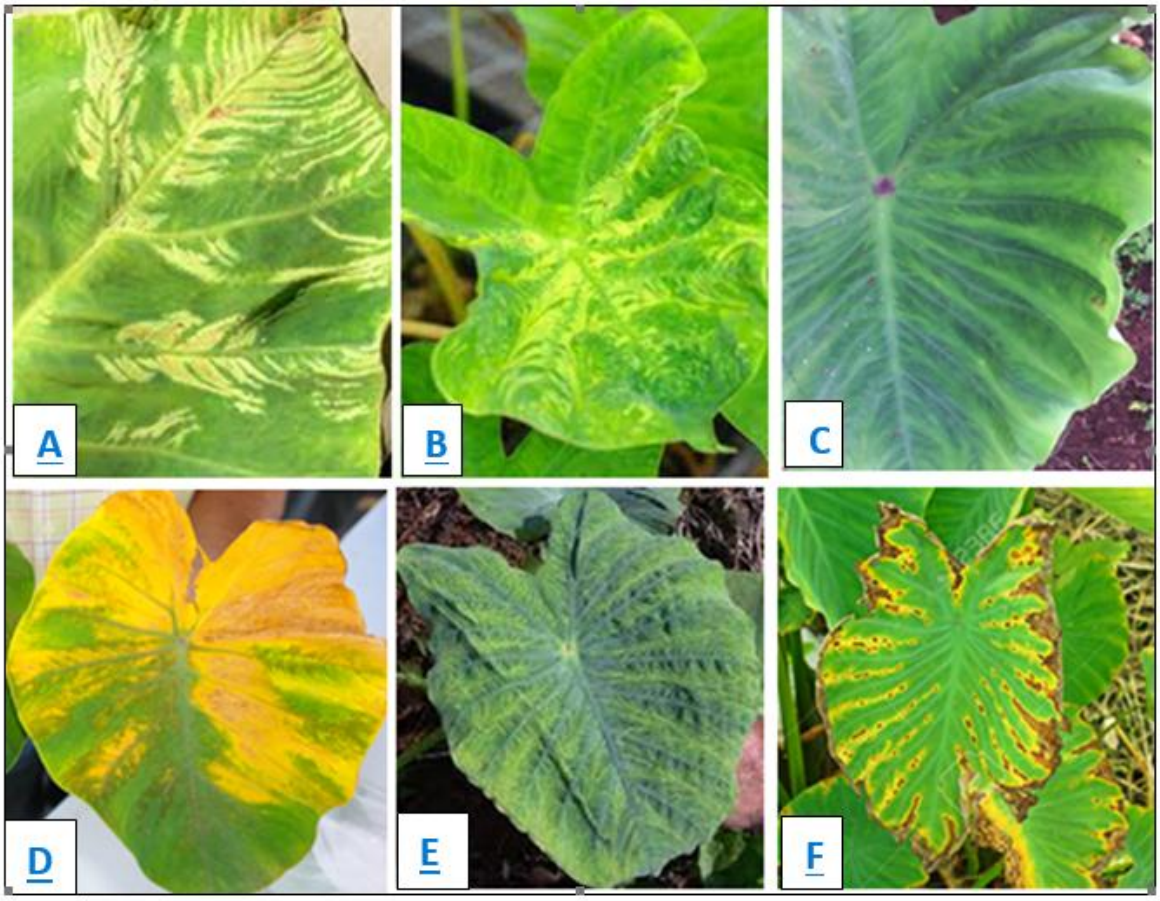

Fig. 3: Symptoms of Taro viral diseases in the field. (A & B) Deformed leaves with mosaic formations; (C) Yellowing of veins; (D) Conspicuous yellowing of the leaf with brown spots; (E) Leathery leaf; (F) Browning and drying of the leaf margin and blades.

Detection of Viruses by PCR and RT-PCR

DNA and RNA samples were subjected to PCR and RT-PCR to screen for five viruses, including Dasheen Mosaic Virus (DsMV), Taro Bacilliform Virus (TaBV), *Colocasia* bobone disease virus (CBDV), Taro vein chlorosis virus (TaVCV), and Taro reovirus (TaRV), reported to infect Taro in other countries and regions. Among the five viruses, only *Taro Bacilliform Virus* (TaBV) was detected through PCR. An amplicon of 320bps, which was the expected band size, was obtained for the positive samples (Supplementary Fig. 1). Detections were made in samples collected across the nine counties at varying incidences. Nyeri County had the highest incidence at 63%, while Machakos County had the least at 23% (Table 1). Notably, all the 20 samples collected from wild relatives of Taro tested negative for TaBV.

Table 1: Sampling locations and summary of TaBV-positive samples based on the PCR assay.

County	Ward	Number of Samples collected	The number that tested Positive	% Positive
Kiambu	Ngewa, Kikuyu, Karai, Gitaru, Nyathuna	30	14	47
Murang'a	Ngenda, Kigumo	30	15	50
Meru	Mikinduri, Nyaki, Kiguchwa, Kiamurio, Nkomo, Mitunguu, Nyagene, Maraa	30	14	47
Nyeri	Ruguru, Iriaini, Karatina, Kirimukuyu	30	19	63
Siaya	Central Alego, Yala township	30	14	47
Busia	Bunyala Central, Hajula	30	8	27
Kakamega	Mahiakalo, Butsotso East, Ingotse Matia	30	16	53
Kisii	Obaracho, Nyakoe	30	8	27
Machakos	Kathiani	30	7	23
Total	**30 wards**	**270**	**115**	**43**

Sanger sequencing of 15 amplicons drawn from samples from nine counties and subsequent bioinformatics analysis using CLC Genomics Workbench v 8.0.3 revealed a 33-94% sequence variability in the putative reverse transcriptase (RT)/ribonuclease H (RNaseH) coding region of the TaBV isolates. Sequences of the 15 isolates were deposited in the

National Center for Biotechnology Information (NCBI) database accession numbers ON853594 – ON853608; Kiambu 1 (ON853594), Siaya 10 (ON853595), Busia 12 (ON853596), Kakamega 17 (ON853597), Murang'a 2 (ON853598), Kisii 22 (ON853599), Machakos 24 (ON853600), Murang'a 3 (ON853601), Machakos 32 (ON853602), Meru 4 (ON853603), Meru 5 (ON853604), Nyeri 6 (ON853605), Nyeri 7 (ON853606), Nyeri 8 (ON853607), and Siaya 9 (ON853608). All the 15 isolates showed 94-99% similarity to four TaBV isolates isolated from East Africa; Tz24 (MG833013), Ug75 (MG017323), Tz17 (MG017322), and Ke52 (MG017321). Notably, the Tz24 isolate had been isolated from Tannia, a wild taro relative in Tanzania. Pairwise sequence comparison revealed that isolates from Nyeri county had the highest nucleotide sequence variability of up to 58%, while those from Murang'a had the least variability of up to 8% (Fig. 4). Isolates from Siaya, Kakamega, Kiambu, and one from Nyeri had the greatest nucleotide sequence identity ranging between 84 and 92%, while the remaining counties had 71-94% nucleotide sequence identity.

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Phylogenetic analysis revealed that the 15 isolates formed two clades, with those from Murang'a forming a distinct group (Fig. 5).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Busia 12	1		94.37	92.80	91.13	90.64	80.96	92.72	77.88	78.44	82.93	42.28	43.22	38.76	40.26	38.58
Nyeri 6	2	94.37		93.33	91.69	91.71	81.20	90.08	78.11	79.38	82.45	40.40	41.71	37.37	39.28	36.96
Meru 5	3	92.80	93.33		91.71	91.47	80.00	90.13	78.57	80.57	81.96	42.17	43.47	38.92	39.90	38.48
Murang'a 2	4	91.13	91.69	91.71		92.25	80.00	87.06	76.21	78.44	85.01	39.14	40.45	36.08	39.05	35.70
Murang'a 3	5	90.64	91.71	91.47	92.25		81.45	87.97	75.81	78.20	82.76	39.65	40.95	36.60	38.76	36.20
Kisii 22	6	80.96	81.20	80.00	80.00	81.45		79.28	81.06	80.52	75.18	35.55	36.76	33.02	34.51	32.49
Meru 4	7	92.72	90.08	90.13	87.06	87.97	79.28		75.58	76.07	81.02	39.75	41.21	39.15	41.44	38.70
Machakos 24	8	77.88	78.11	78.57	76.21	75.81	81.06	75.58		79.54	70.51	35.03	36.64	32.81	33.78	32.66
Machakos 32	9	78.44	79.38	80.57	78.44	78.20	80.52	76.07	79.54		73.46	37.73	38.46	34.34	34.64	34.63
Nyeri 7	10	82.93	82.45	81.96	85.01	82.76	75.18	81.02	70.51	73.46		38.78	40.10	35.77	38.40	35.29
Kiambu 1	11	42.28	40.40	42.17	39.14	39.65	35.55	39.75	35.03	37.73	38.78		92.35	87.77	85.22	83.99
Nyeri 8	12	43.22	41.71	43.47	40.45	40.95	36.76	41.21	36.64	38.46	40.10	92.35		85.26	85.26	84.03
Siaya 10	13	38.76	37.37	38.92	36.08	36.60	33.02	39.15	32.81	34.34	35.77	87.77	85.26		86.52	86.98
Siaya 9	14	40.26	39.28	39.90	39.05	38.76	34.51	41.44	33.78	34.64	38.40	85.22	85.26	86.52		86.67
Kakamega 17	15	38.58	36.96	38.48	35.70	36.20	32.49	38.70	32.66	34.63	35.29	83.99	84.03	86.98	86.67	

Fig. 4: Pairwise sequence comparison of the 15 TaBV isolates.

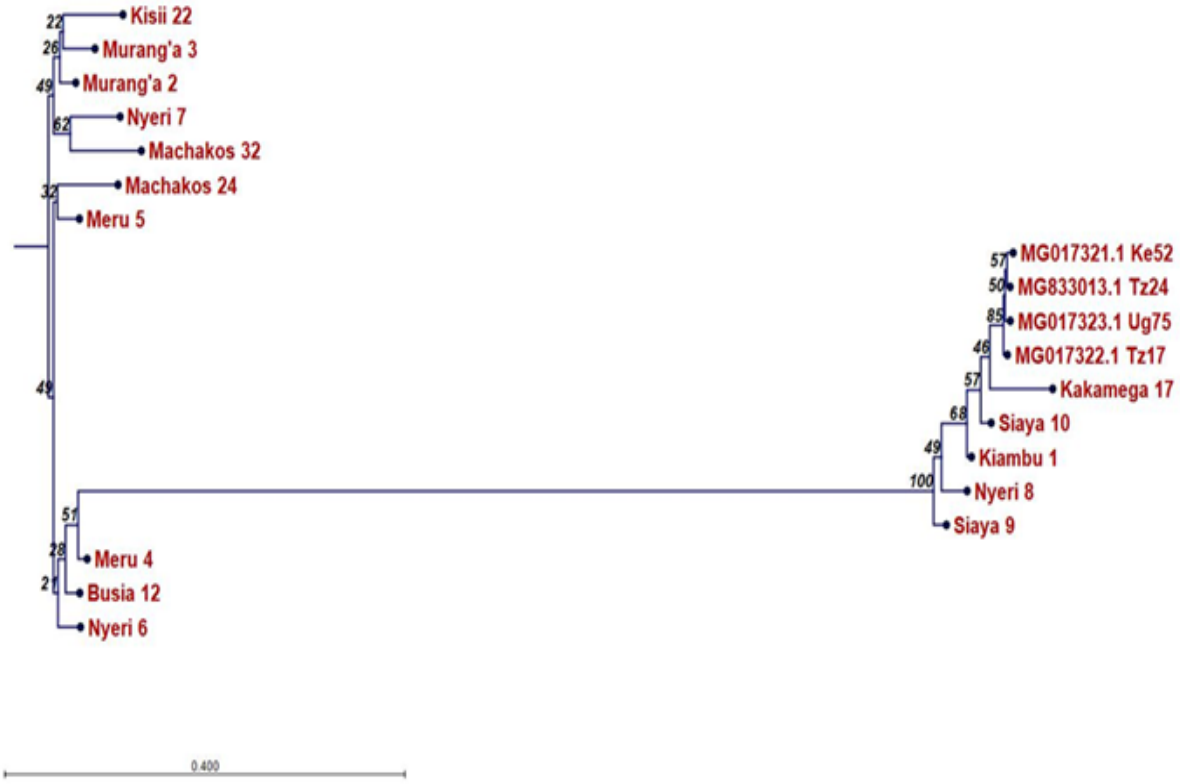

Fig. 5: Phylogenetic analysis of the 15 isolates and 4 TaBV isolates isolated from East Africa (all with bootstrap numbers) based on the 320bp RT/RNase H-coding sequence ORF 3. The maximum-likelihood method and a Jukes-Cantor parameter model with 1000 bootstrap replications were employed.

Small RNA Sequencing of Symptomatic and Asymptomatic Taro Plants

Sequencing of the edible and wild Taro libraries produced a total of 25,707,591 SmallRNA reads. After trimming adapters and low-quality reads, 24224023 SmallRNA reads comprising 17642010, 6552208, and 29805 reads from symptomatic, asymptomatic, and wildtype samples were retrieved, respectively. The sequences were then analyzed independently, and the results were reported per category. Blasting the clean reads against the NCBI non-redundant nucleotides (nt) database to identify possible viruses in the samples revealed badnaviruses, a begomovirus, potyvirus, and a rhabdovirus. Notably, there were detections of the *Taro Bacilliform Virus* and *Taro Bacilliform CH Virus*, which are badnaviruses specific to Taro. These two viruses were detected in both the symptomatic and asymptomatic samples. Other badnaviruses identified were: *Sweet potato Badnavirus B* and *Sugarcane bacilliform virus*. The S*weet potato leaf curl virus* and the *Sweet potato feathery mottle virus* were the only begomovirus and potyvirus detected, respectively. Of note, *Phaseolus vulgaris alphaendornavirus*, a rhabdovirus, was predominant in all samples except in the wild Taro relatives. The only viroid detected was the Citrus exocortis viroid. These hits were predominantly of the coding regions of the viruses and the viroid, highlighting the actual occurrence of the viruses and viroid in Taro. Notably, the wild taro relatives had no viral nor viroid hits. These results further confirmed the virus families infecting Taro in Kenya and demonstrated the reliability of the sRNA deep sequencing data in determining virus and viroid diversity. This discovery is the first report of a viroid infecting Taro.

Co-infection of Taro in Kenya

Both DNA and RNA viruses were detected in the same samples. In most samples, there were detections of *Taro Bacilliform Virus*, *Taro Bacilliform CH Virus*, and a potyvirus, a rhabdovirus, a begomovirus, or all in both symptomatic and asymptomatic plants.

DISCUSSION

Viral diseases cause severe yield losses and quality decline in Taro worldwide, thus having a huge economic impact on farmers (Babu et al., 2011). In Kenya, the occurrence and distribution of the major viruses and viroids infecting Taro remain poor, limiting the development of robust disease management strategies to mitigate their spread. Herein, viral symptoms, including stunting, leaf rolling, shrinkage, deformed leaves with mosaic and yellow veins, leaf rolling, and dwarfism, were common in all taro fields surveyed, affecting 32-60% of the surveyed plants. The incidence and distribution data obtained in this study demonstrated that taro viral infections are both highly diverse and prevalent in Kenya, necessitating a need for in-depth studies on the virus diversity in Kenyan taro fields. This study applied small RNA sequencing and PCR techniques to identify viruses infecting Taro in Kenya and is the first to report a comprehensive occurrence and distribution of the major viruses infecting Taro in Kenya. The degenerate primers of already reported taro viruses (Supplementary Tables S1-S5) only detected TaBV, and thus were not adequate to make a conclusion on viral diversity in the tested samples. Of note, TaBV was distributed in all the 30 wards across the nine counties surveyed.

The sequence variability in the putative reverse transcriptase (RT)/ribonuclease H (RNaseH) coding region from Taro bacilliform virus (TaBV) isolates from different counties ranged

between 33% and 94%. The high variability was attributed to a second Badnavirus, Taro Bacilliform CH Virus, that was recently identified to infect Taro (Kazmi et al., 2015). Degenerate primers are limited in providing a true reflection of viral diversity in the field, especially when viruses have not been previously described or are remarkably divergent from those previously characterized (Wu et al., 2015). This study thus further employed Next-generation sequencing (NGS) techniques with small RNA to broaden the possibility of taro virus diagnostics because of its superiority in detecting multiple viruses present even in the absence of disease symptoms (Adams et al., 2009; Wu et al., 2015).

NGS technologies have significantly advanced our ability to comprehensively study plant viruses, especially in vegetatively propagated crops such as Taro that are prone to virus accumulation and co-infections. NGS has been applied for viral diversity studies, detection, and virus genome assembly and reconstruction. NGS has been successfully used to detect viruses in different crop plants, such as grapevines (Coetzee et al., 2010; Jo et al., 2015), sweet potato (Kashif et al., 2012), tomato (Li et al., 2012), garlic (Wylie et al., 2014), pear (Jo et al., 2016), pepper (Jo et al., 2017), and orange (Matsumura et al., 2017). NGS has also identified new viruses infecting sweet potatoes (Gu et al., 2014). In this study, small RNA sequencing revealed badnaviruses, begomoviruses, potyviruses, and rhabdoviruses, including the Taro Bacilliform CH Virus, a badnavirus, and Colocasia Bobone Disease Virus, a rhabdovirus, to be the main viruses infecting Taro in Kenya. Moreover, the Taro Bacilliform virus (TaBV) was detected using TaBV degenerate primers, with detections spread across all the nine counties surveyed.

Dasheen Mosaic Virus (DsMV), *Taro Bacilliform Virus* (TaBV), *Colocasia* bobone disease virus (CBDV), Taro vein chlorosis virus (TaVCV), and Taro reovirus (TaRV), are the main viruses reported to infect Taro (Revill et al., 2005). Most of these viruses have been detected in Pacific Islands. Studies in Asian countries report that the Dasheen Mosaic Virus (DsMV), a potyvirus, and Taro Bacilliform Virus (TaBV), a badnavirus, are the most predominant Taro viruses (Revill et al., 2005). DsMV is found wherever Taro is grown, infecting both the edible and ornamental aroids (Zettler & Hartman, 1986, 1987; Jackson, 1980; Shaw et al. 1979). It is characterized by chlorotic and feathery mosaic patterns on the leaf, distortion of leaves, and stunted plant growth, although cultivars vary considerably in symptom expression (Zettler & Hartman, 1987).

In this study, DsMV was not detected using degenerate primers and small RNA sequencing. However, the Sweet potato feathery mottle virus, a potyvirus, was predominantly detected in both symptomatic and asymptomatic plants, suggesting a potyvirus(es) circulating in Taro growing areas in Kenya. This finding supported the speculations regarding the extent of potyviruses present in Taro due to cross-species transmission via mechanical transmission or aphid vectors that feed on different host plants (Yusop et al., 2019). TaBV is thought to occur in combination with CBDV to cause “alomae” disease (James et al. 1973). Alomae disease is considered Taro's most destructive virus disease (Jackson and Gollifer, 1975; Rodoni et al., 1994). Its symptoms include crinkling of young leaves that fail to develop normally, thickening of veins and lamina, shortening of the petioles, and irregularly shaped outgrowths on the petioles. Infected plants ultimately die because of the development of systemic

necrosis (Rodoni et al., 1994). Infection with TaBV alone results in a range of mild symptoms, including stunting, mosaic, and down-curling of the leaf blades.

In contrast, infection of Taro with CBDV alone results in bobone disease, characterized by stunting, leaf distortion, and the presence of galls on the petioles (Jackson, 1978). Herein, both the Taro Bacilliform CH Virus, a badnavirus, and Colocasia Bobone Disease Virus, a rhabdovirus, were detected through small RNA sequencing. They mostly occurred as co-infections in the analyzed samples, plus two to three other RNA and DNA viruses. Moreover, the Taro Bacilliform virus (TaBV) was detected using TaBV degenerate primers, with detections spread across all the nine counties surveyed. In the same line, other badnaviruses, including the sweet potato Badnavirus B and the Sugarcane bacilliform virus, confirmed the wide host range of badnaviruses, especially in perennial hosts that are propagated vegetatively (Bhat et al., 2016). These findings were consistent with other studies which postulate that TaBV is widespread in almost all Taro growing regions, including Kenya (Kidanemariam et al., 2018), and its occurrence alongside a putative rhabdovirus, CBDV, which leads to the lethal Alomae disease (Yang et al., 2003; Higgins et al., 2016).

To date, there is no report of begomoviruses infecting Taro worldwide. However, our small RNA sequencing strongly suggested that begomoviruses(es) could be circulating in taro fields in Kenya after several begomoviruses, including the *East African Cassava mosaic virus* and *sweet potato leaf curl virus*, were predominantly detected in both symptomatic and asymptomatic plants. Begomoviruses are single-stranded DNA plant viruses transmitted by whiteflies of the *Bemisia tabaci* complex. They are important groups of emerging plant viruses infecting numerous vegetables, root, and fiber crops in subtropical and tropical regions (Navas-Castillo et al., 2011). Their symptoms include leaf curling, mosaic, vein yellowing, and generalized leaf yellowing, often accompanied by stunting (Leke et al., 2015). These symptoms were common amongst the taro fields surveyed, further suggesting the possibility of having a begomovirus(es) circulating in Taro. Begomoviruses, such as the *East African cassava mosaic Kenya virus* (EACMKV), have already been reported in Kenya (Bull et al., 2006). Notably, they have been reported to cause significant yield losses in other root crops. For example, cassava mosaic diseases in Sub-Saharan Africa cause yield losses exceeding $2 billion annually (Thresh et al., 1997). This discovery is the first report of begomoviruses infecting Taro. Nonetheless, further studies should be done to identify whether specific begomoviruses are infecting Taro and their influence on yield. Viral detections in the asymptomatic plants suggested latency in some of these viruses or the lack of manifestation of the symptoms, possibly because of low viral titer.

Viroids are small, single-stranded, circular RNAs that induce specific diseases in higher plants despite lacking protein-coding capacity (Kovalskaya & Hammond, 2014). Their small size and distinct molecular structure make them potent molecular features for inducing resistance to viral pathogens through RNA silencing (Sano et al., 2010). The presence of viroids affects disease severity and symptom manifestation in plants and are molecular vehicles for the introduction of diseases (Natalia et al., 2014). Detection of a viroid in a host is thus useful because of its considerable economic importance. To date, there are no reports of viroids infecting Taro. However, this study detected the Citrus exocortis viroid to infect Taro. Viroids have been reported to cause diseases in many species, including *Solanum*,

causing the tubers to be small, elongated, distorted, and cracked. They are mainly transmitted through vegetative propagation and aphids (Owens et al., 2009). Their symptoms are like those of many plant virus infections, including stunting, vein discoloration and clearing, chlorotic or necrotic spots, leaf distortion and mottling cankers, and tuber malformations (Natalia et al., 2014). Of note, tuber malformation was a common symptom during our sample collection in the farmers' fields across the nine counties that were sampled. These findings allude that Taro is also affected by viroids. However, whether this was a case of cross-species transmission without any significant impact or points to the existence of taro viroids should be investigated using homology-independent approaches that combine deep sequencing of small RNAs with a computational algorithm.

Notably, the wild taro relatives had no viral nor viroid hits. These findings affirmed that wild species possess some level of tolerance to viral infections, possibly because of having a rich reservoir of resistance genes useful in breeding cultivars with a genetically controlled resistance against numerous diseases (Okoń et al., 2021). The viral symptoms observed in plants during the survey were consistent with the viruses detected. Moreover, the viral severity in the fields was also positively correlated with the number of viruses identified, as evidenced by the positive correlation between the viral incidences based on the visual identification of symptoms and the PCR results. These results demonstrate the reliability of the sRNA deep sequencing data in determining virus and viroid diversity.

CONCLUSION AND RECOMMENDATIONS

Identifying viruses and their incidence significantly aid in the development of management strategies to control their spread. Our small RNA sequencing and subsequent analyses constitute the first comprehensive report of Taro viruses and viroids in Kenya. The high incidence and co-existence of viruses previously not known to infect Taro require further exploration to determine their association, which potentially end up with supper strains capable of limiting Taro productivity. The findings of this study collectively form a basis for further studies, including genetic resources for virus-Taro interactions, and insight for developing robust management strategies to mitigate their spread. Future studies should focus on developing effective management strategies to support the prevention and control of Taro viruses, including removing infected crops, controlling insect vectors, and developing virus-free planting materials. Further studies on Taro viroids, especially in viroid-induced RNA silencing as transcriptional machinery for inducing resistance to viral pathogens, should also be conducted to decipher their role in plant-virus interactions and their potential use as effective modulators of Taro defense mechanism.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest.

The authors declare that they have no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX

Table S1: Primers and PCR conditions for *Taro Bacilliform Virus* (TaBV) (Yang et al. 2003*b*)

5 µl of DNA sample in a total 25 µl reaction	
TaBV1	5'CKSTGYAARSAACATGGTCTTG 3'
TaBV4	5' TAATCAAGYGGWGGGAGYTTCTC 3'
Product size	320 bp
PCR program (35 Cycles)	
Initial denaturation	94^0C, 2 min
Denaturation	94^0C, 30 sec,
Annealing	57^0C, 30 sec
Extension	68^0C, 1 min
Final Extension	68^0C, 10 min

Table S2: Primers and PCR conditions for *Dasheen Mosaic Virus* (DsMV) (Maino, 2003)

5 µl of RNA sample in a total 25 µl reaction	
DsMV 3F	5'AGTACAAACCTGARCAGCGTGAYA 3'
DsMV 3R	5'TTYGCAGTGTGCCTYTCAGGT 3'
Product size	540 bp
PCR program (35 Cycles)	
Reverse transcriptase step	1 cycle of 42^0C, 35 min
Initial denaturation	94^0C, 2 min
Denaturation	94^0◦C, 30 sec,
Annealing	55^0C, 30 sec
Extension	68^0C, 1 min
Final Extension	68^0C, 10 min

Table S3: Primers and PCR conditions for *Colocasia Bobone Disease Virus* (CBDV) (Revill et al. 2005)

5 µl of RNA sample in a total 25 µl reaction	
CBDVF1	5'GAGCCAAACTGTCAAAAGC 3'
CBDVR2	5' CGATGCACTGGTCTTGATC 3'
Product size	150 bp
PCR program (35 Cycles)	
Reverse transcriptase step	1 cycle of 42^0C, 35 min
Initial denaturation	94^0C, 2 min
Denaturation	94^0C, 30 sec
Annealing	58^0C, 30 sec
Extension	68^0C, 1 min
Final Extension	68^0C, 10 min

Table S4: Primers and PCR conditions for *Taro Vein Chlorosis Virus* (TaVCV) (Revill et al. 2005)

5 µl of RNA sample in a total 25 µl reaction	
TaVCV1	5' ATAATCCAGCTTTACATTCACTGAC 3'
TaVCV2	5' TGCC TGGGCTTCCTGAGATGATCTG 3'
Product size	220 bp
PCR program (35 Cycles)	
Reverse transcriptase step	1 cycle of 42^0C, 35 min
Initial denaturation	94^0C, 2 min
Denaturation	94^0C, 30 sec
Annealing	55^0C, 30 sec
Extension	68^0C, 1 min
Final Extension	68^0C, 10 min

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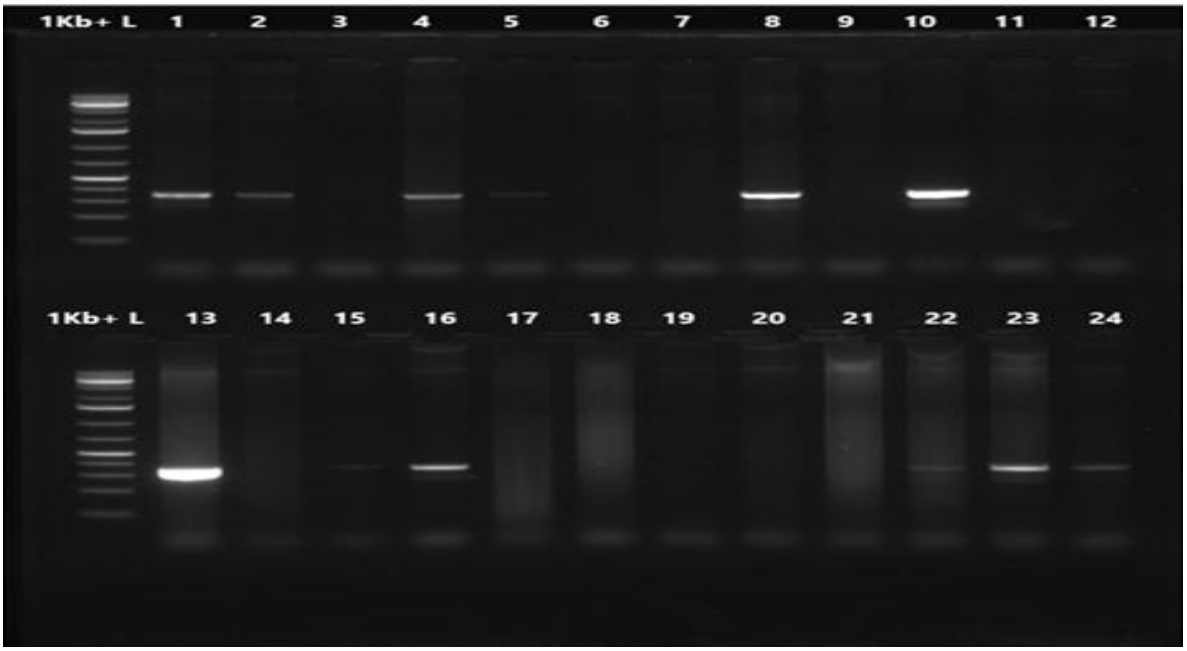
Table S5: Primers and PCR conditions for *Taro Reovirus* (TaRV) (Revill et al. 2005)

5 µl of RNA sample in a total 25 µl reaction	
TaVCV1	5’ ATAATCCAGCTTTACATTCACTGAC 3’
TaVCV2	5’ TGCC TGGGCTTCCTGAGATGATCTG 3’
Product size	750 bp
PCR program (35 Cycles)	
Reverse transcriptase step	1 cycle of 50^0C, 35 min
Initial denaturation	94^0C, 2 min
Denaturation	94^0C, 30 sec
Annealing	57^0C, 30 sec
Extension	68^0C, 1 min
Final Extension	68^0C, 10 min



Supplementary Fig. 1 Gel image showing amplicons of the expected size, 320bps of TaBV positive samples. The ladder is the Thermo Scientific GeneRuler 1 kb Plus DNA Ladder.