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Occurrence and distribution of viruses associated with papaya ringspot disease in Kenya

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Papaya ringspot disease is a serious threat to papaya production in Kenya. For effective management, it is important to determine the occurrence and distribution of the viruses associated with the disease. A survey was conducted in 2017, covering a total of 103 papaya fields in major papaya production areas in the country. To determine the disease incidence, 20 plants per field were visually inspected for symptoms associated with the disease. Disease severity was evaluated on a scale of 1 to 5, while disease prevalence was determined as the proportion of fields showing disease symptoms per county expressed as a percentage. A total of 287 leaf samples were collected from surveyed fields and tested for Moroccan watermelon mosaic virus (MWMV), cowpea mild mottle virus (CpMMV), and papaya mottle-associated virus (PaMV) using polymerase chain reaction (PCR)-based techniques. The highest (71.4%) disease incidence was recorded in Kiambu County, while the lowest was recorded in Busia County (2.8%). No symptomatic plants were observed in Siaya and Bungoma (0%) counties. Disease prevalence ranged from 0 to 100%. The highest disease severity, 4.0, was reported in Baringo County; while the lowest, 2.0, was reported in Kwale, Kilifi, and Taita Taveta counties. MWMV was the most prevalent, with 140 out of 287 samples testing positive and also widespread, having been detected in 11 out of the 22 counties surveyed. PaMV was the second most prevalent, detected in 39 out of 287 samples collected and in 9 out of 22 counties. CpMMV was the least prevalent, detected in 7 out of 287 samples and in three counties. The occurrence of both MWMV and PaMV was detected in five counties, while the occurrence of PaMV and CpMMV was detected in three counties. The presence of MWMV, PaMV and CpMMV was detected in one county. Viruses associated with papaya ringspot disease in Kenya are widespread in papaya-growing regions, with some counties reporting 100% disease prevalence. The development and implementation of control strategies for the disease in the country are of paramount importance. In the future, it is important to identify factors influencing disease spread in the country for effective management.

Key words: Incidence, viral diseases, control strategies, farmers, interventions.

INTRODUCTION

Carica papaya L. is an important fruit crop in Kenya, grown by small and large-scale farmers for subsistence, local, and export markets. However, statistics regarding its production in the country are unsatisfactory. For instance, there has been a steady increase in the area of papaya production over recent years with no substantial increase in yields (HCDA, 2021). The low papaya yields are mostly attributed to poor agronomic practices, including the lack of improved crop varieties and crop damage by pests and diseases (Rimberia and Wamocho, 2014; Kansiime et al., 2020; HCDA, 2021).

Viral diseases threaten plant crops by impairing their growth and vigor, leading to a decrease in gross yields. These diseases also reduce the quality of produce, thereby decreasing marketable yield (Woolhouse et al., 2005). The viruses most often reported to cause diseases in papaya include papaya apical necrosis virus (PANV), papaya ringspot virus (PRSV), papaya mosaic virus (PapMV), papaya leaf curl virus (PaLCV), tobacco ringspot virus (TRSV), papaya leaf distortion mosaic virus (PLDMV), *Papaya meleira* virus (PMeV), papaya lethal yellowing virus (PLYV), and several other viruses that may not be of economic significance (Mishra et al., 2016).

Among the diseases infecting papaya, papaya ringspot disease is the most important biotic constraint worldwide. The disease is highly destructive, threatening both small-and large-scale papaya growers in various parts of Kenya (Ombwara et al., 2014; Rimberia and Wamocho, 2014; Mumo et al., 2020). The impact of the disease is being felt in the country, with farmers in different regions of Kenya abandoning papaya cultivation in favor of other crops (Mumo et al., 2021). This calls for an urgent need to develop disease management measures.

Papaya ringspot disease in Kenya has been reportedly associated with a potyvirus MWMV (Mumo et al., 2020), as well as other viruses such as cowpea mild mottle virus (CpMMV) and papaya mottle-associated viruses (PaMV and PaMMV). The occurrences and distribution of these viruses in the country are scarcely known, although this information is crucial for disease management and prevention (Gashaw et al., 2014). The objective of this study, therefore, was to establish the incidence, prevalence, severity, and distribution of the viruses associated with papaya ringspot disease in the country.

MATERIALS AND METHODS

Sampling sites and sampling procedure

Surveys of papaya fields and the sampling of papaya plants were conducted between January and April 2017 in 22 counties. These counties include Taita Taveta, Kwale, Kilifi, Kisumu, Homabay, Migori, Siaya, Bungoma, Busia, Vihiga, Nakuru, Baringo, Elgeyo Marakwet, Kiambu, Murang'a, Kirinyaga, Embu, Tharaka Nithi, Meru, Makueni, Machakos and Kitui. Fields with papaya crops, whether established as a pure stand or intercropped, were purposefully surveyed along selected routes. In each county, a specific representative route that captured the area of interest was discussed and agreed upon by the survey team and adopted. Factors considered for the selection of routes included the sample area and the availability of suitable papaya fields. When farmers resided within the same county and papaya fields were close to each other,

sampling was done at a minimum distance of 5 km between fields; otherwise, a distance interval of 10 km between fields was adopted. A transect was drawn diagonally in the field from both directions, resulting in two transects (Sseruwagi et al., 2004). During sampling, representative plants were randomly selected along X-shaped transects in each field to reduce biases. In total, 103 papaya fields were surveyed.

Incidence, severity and prevalence of papaya ringspot in selected counties in Kenya

Twenty plants per field were visually inspected for papaya ringspot symptoms on leaves, stems, petioles, and fruits. The general vigor of the inspected plants was also recorded. The disease severity scale was based on the area or proportion of symptomatic plant tissue. The scale from 1 to 5 (Ombwara et al., 2014) was adopted (Table 1). Scores of '1' (no visible symptoms) were excluded when calculating the mean severity per field to allow for a true evaluation of the degree of damage caused to the diseased plants. Disease incidence was determined as the proportion of the plants showing symptoms out of 20 examined, expressed as a percentage. The prevalence of papaya ringspot was determined in every study county as the proportion of fields with at least one diseased plant out of the total number of fields observed in that county, expressed as a percentage.

Sample collection and virus detection

Two hundred (200) symptomatic and 87 asymptomatic leaf samples were randomly collected from 2 to 5 plants per field. This involved harvesting the second youngest fully developed leaf from the shoot apex of symptomatic and asymptomatic plants using sterile forceps. The number of papaya leaf samples collected per field depended on the disease severity across the field and the plant population. The collected leaf samples were preserved in RNAlater™ (Invitrogen™) stabilization solution to prevent RNA degradation and were transported to the Biosciences Eastern and Central Africa—International Livestock Research Institute (BecA-ILRI) Hub, Nairobi laboratory, and stored at 4°C before RNA extraction.

RNA extraction and PCR process

Leaf samples were removed from the RNAlater™ solution using sterile forceps, and any remaining solution was blotted away using a sterile absorbent paper towel. Total RNA was extracted from the samples using the RNeasy® Plant Mini Kit (Qiagen, Inc.) following the manufacturer's instructions. The integrity of the extracted RNA was assessed through agarose gel electrophoresis, where 0.8% agarose was dissolved in 100 ml of 0.5X TAE (Tris-acetate-EDTA) buffer, stained with 3 µl of GelRed® Nucleic Acid Gel Stain (Biotium), and run at 100 V for 30 min in a gel tank. The gel was visualized using a gel imaging system with a UV transilluminator. The quantity of RNA was measured using the Qubit™ 2.0 Fluorometer system (Invitrogen™) following the manufacturer's instructions and normalized to 5 µg before cDNA synthesis. The cDNA was synthesized using the SuperScript™ III First-Strand Synthesis System (Invitrogen™) and stored at -20°C for use as a template in the PCR process.

Samples were screened for viruses in PCR using a set of primers specific to the respective viruses: 5' TCTCAGCTAGCACGCAACAA

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Table 1. Scale used in rating disease severity in papaya plants during field survey.

Scale	Symptoms expressed
1	No visible symptoms
2	1-25% of plant tissues portraying symptoms such as mild mottling and mild mosaic patterns on the leaves, little distortion of leaves, mild oily streaked petioles/stems, apparent but negligible stunting
3	26-50% of plant tissues portraying symptoms: moderate yellow and mosaic patterns on the leaves, moderate distortion of leaf shape, moderate oily streaked petioles/stems, moderate stunting, moderate ringspots symptoms on fruits
4	51-75% of plant tissues portraying symptoms: severe yellow and mosaic patterns on leaf, severe leaf distortion with reduced size, severe oily streaked petioles/stems severe ringspots on fruits, plant partially stunted
5	more than 75% of plant tissues portraying symptoms: very severe yellow and mosaic patterns symptoms on leaf, very severe leaf distortion and reduced size, very severe oily streaked petioles/stems, plant severely stunted and very severe ringspots symptoms on fruits.

3' and 5' CGGTGTTGAGCCAAACGAAG 3' for MWMV, 5' AGACCAAAGAGTGCTTCGGG 3' and 5 TAGGAACTCCCAGTCCCTCG for PaMV, 5' AACATGGCGACAGCTGAAGA 5 3 and GAAGAGCGACCAGTTCCCAA 3' for CpMMV (Mumo et al., 2020). These primers were designed to amplify a 315 bp fragment for MWMV, 304 bp for PaMV, and 694 bp for CpMMV.

In brief, a 10 µl PCR reaction mixture was prepared, consisting of 5 µl of AccuPower® Taq PCR 2X Master Mix (Bioneer, Korea), 3.6 µl of nuclease-free water, 0.2 µl of 10 µM each of forward and reverse primers (Macrogen), and 1 µl of cDNA (50 ng/µl). A positive control contained a sample infected with the virus, while a negative control contained nuclease-free water, used in place of nucleic acid. The PCR reactions were run on a thermal cycler (Eppendorf Mastercycler Nexus Gradient) under the following cycling conditions: 3 min at 95°C; 30 cycles of 30 s at 95°C, 30 s at 58°C, and 1 min at 72°C; and a final extension at 72°C for 5 min for all sets of primers. The amplified PCR products, alongside O'GeneRuler ™ 1-Kb plus DNA ladder (Invitrogen™, USA), were separated on 2% (wt/vol) agarose gels, and the bands were visualized under a UV transilluminator before documentation through digital photography.

Data analysis

Data on disease incidence, prevalence and severity were analyzed by computing means among the counties surveyed. The presence of viruses was scored based on the presence or absence of the right size of the amplified fragment in the gel electrophoresis.

RESULTS

Incidence, prevalence and severity of papaya ringspot in Kenya

Papaya ringspot disease was previously reported in Kenya and in this study, the disease was observed in the majority of the counties surveyed (Table 2). It was evident that the disease has a wide occurrence with an average incidence of 21.1% reported in the counties surveyed. The highest (71.4%) disease incidence was reported in Kiambu County, followed by Murang'a and Nakuru counties with means of 51.4 and 52.8%, respectively. The least incidence was recorded in Busia County with a mean of 2.8%. However, Bungoma and Siaya counties had zero incidences (Table 2). The PRSD prevalence differed within

the counties' surveyed regions with an average of 65.5%. Elgeyo Marakwet, Embu, Homabay, Kiambu, Nakuru, Kitui and Vihiga, counties recorded the highest (100%) disease prevalence, while No disease prevalence was observed in Bungoma and Siaya counties. Generally, mild disease severity (2.9) across the counties was recorded, with the highest severity of 4.0 recorded in Baringo County followed by Kirinyaga and Murang'a counties with a mean of 3.8. The least disease severities were recorded in Kwale, Kilifi and Taita Taveta counties with a mean of 2.0, while no disease severity was recorded in Bungoma and Siaya counties (Table 2).

Viruses associated with PRSD in Kenya

The viruses detected in the collected samples are shown in Table 3, and based on the detection of the respective viruses in the samples by PCR (Figure 1). A sharp band of 315, 304 and 694 bp in gel electrophoresis indicated the presence of the MWMV, PaMV and CpMMV respectively. When only one virus was detected in a sample, it is reported as a single viral detection, while in cases where more than one virus was detected in the same field surveyed and sampled, mixed infections are reported (Figure 1 and Table 3). Dual infections occurred when more than one virus was amplified in a sample.

From the PCR-based detection, 180 of 287 samples collected tested positive for at least one virus infection. MWMV was the most widespread virus detected alone, in mixed infections and dual infections. The virus was reported in 11 of 22 counties surveyed; namely, Nakuru, Busia, Homabay, Kisumu, Migori, Embu, Kiambu, Kirinyaga, Meru, Makueni, Murang'a, and Machakos, and in 140 of 287 samples collected (Table 3). PaMV was the second most widespread virus and was detected in 9 of 22 counties surveyed; namely, Baringo, Embu, Kirinyaga, Meru, Tharaka Nithi, Kitui Machakos and Taita Taveta and in 39 of 287 samples collected (Table 2). CpMMV was the least prevalent virus and was detected in only three counties including Baringo, Meru and Kitui (Table 3).

Mixed infections of MWMV and PaMV were detected in

Table 2. Incidence, prevalence and severity of papaya ringspot in major papaya-producing counties of Kenya.

County	Disease incidence (%)	Disease prevalence (%)	Disease severity
Baringo	7.7	75	4.0
Bungoma	0.0	0	1.0
Busia	2.8	50	3.0
Elgeyo Marakwet	7.2	100	2.7
Embu	35.4	100	3.7
Homabay	20.3	100	3.2
Kiambu	71.4	100	2.7
Kilifi	6.7	33.3	2.0
Kirinyaga	36.0	77.7	3.8
Kisumu	13.3	50.0	3.1
Kitui	19.4	100.0	2.9
Kwale	3.8	25.0	2.0
Machakos	33.8	90.9	3.2
Makueni	12.9	57.1	2.5
Meru	36.9	75.0	3.1
Migori	38.1	50.0	2.8
Murang'a	51.4	100	3.8
Nakuru	52.8	100	3.1
Siaya	0.0	0.00	1.0
Taita Taveta	12.4	33.3	2.0
Tharaka Nithi	11.9	45.5	2.4
Vihiga	14.3	100.0	3.3
Mean	21.1	65.5	2.9
LSD (P=0.05)	2.6		3.0

Severity was visually assessed using a scale of 1-5.

Source: Ombwara et al. (2014).

samples collected from 5 of 22 counties; namely, Embu, Kirinyaga, Meru, Machakos and Makueni while that of PaMV and CpMMV were detected in Baringo, Meru and Kitui counties. The presence of all three viruses (MWMV, CpMMV and PaMV) was obtained in Meru County (Table 3 and Figure 1D). Detections of more than one virus in fields sampled were encountered in some counties. Fifteen samples from 4 of 22 counties; namely, Embu, Kirinyaga, Machakos and Makueni had dual infections of MWMV and PaMV, while 2 of 8 samples from Kitui County were co-infected with CpMMV and PaMV (Table 3).

Detection of PRSD symptoms signified the presence of viral infection in some counties. In other instances, the presence of PRS-like symptoms was not an indicator of viral presence or absence. In Vihiga County, for instance, plants displayed symptoms and none of the three viruses was detected in them. In Baringo, Migori, Embu, Kiambu, Tharaka Nithi and Taita Taveta counties, 8/13, 10/10, 28/28, 10/10, 3/12 and 4/12 samples, respectively, displayed symptoms; however, the viruses were detected in 3/13 (1 PaMV and 2 CpMMV), 4/10 (MWMV), 17/28 (15 MWMV and 2 PaMV), 9/10 (MWMV), 2/12 (PaMV) and 3/12 (PaMV) in the respective order in each county. In some instances, the number of plants infected with viruses

was higher compared to the number of symptomatic plants. For instance, in Kirinyaga, Makueni and Kisumu counties, 28/42, 13/25 and 6/12 plants respectively displayed symptoms whereas viruses were detected in 40/42 (40 MWMV and 4 MWMV+PaMV), 19/25 (8 MWMV and 11 PaMV) and 10/12 (MWMV) plants, respectively (Table 3). The most prevalent symptoms in plants included vein clearing, mosaic patterns, mottling, leaf distortion, puckering, shoe-stringing on leaves, water-soaked marks on the petioles and stems, ringspots on fruits, and stunted growth (Figure 2).

Papaya plants singly infected with MWMV displayed puckering, vein clearing, leaf distortion, shoe stringing, mottling water-soaked marks on stems and petioles, ringspots on fruits and stunted growth. On the other hand, papaya plants infected with PaMV displayed mottling, puckering and leaf distortion symptoms (Figure 2 and Table 3). The symptoms of plants in dually infected fields with MWMV and PaMV were severer, including leaf distortion, mosaic, mottling, vein clearing, ringspots, water-soaked marks, shoe stringing, puckering and stunted growth (Figure 2 and Table 3). Papaya plants infected with PaMV and CpMMV showed mild symptoms such as mottling and stunted growth.

Table 3. Incidence (%) of viruses associated with papaya ringspot in 22 counties of Kenya as determined through PCR approach.

County	No. of samples collected ^a	No. of symptomatic samples ^b	Symptoms ^c	MWMV	PaMV	СрММV	MWMV+PaMV	CpMMV +PaMV
Baringo	13	8	Мо	-	1	2	-	-
Elgeyo marakwet	8	2	SG, M	-	-	-	-	-
Nakuru	7	7	Mo, LD, RS, SG	7	-	-	-	-
Bungoma	3	0	None	-	-	-	-	-
Busia	4	2	WS	2	-	-	-	-
Homabay	14	14	PU, VC, WS, LD, SG	14	-	-	-	-
Kisumu	12	6	Mo, PU, WS, LD	10	-	-	-	-
Migori	10	10	Mo, M, VC	4	-	-	-	-
Siaya	2	0	None	-	-	-	-	-
Vihiga	2	2	Mo, VC	-	-	-	-	-
Kiambu	10	10	Mo, RS, WS, VC, LD, SG	9	-	-	-	-
Kirinyaga	42	28	LD, Mo, VC, RS, WS, SS, PU, SG	40	4	-	4	-
Meru	13	10	VC, M, Mo, PU, LD, SS, SG, WS	4	4	2	2	-
Murang'a	16	12	LD, M, RS, Mo, VC, SS, WS, PU	12	-	-	-	-
Tharaka Nithi	12	3	Мо	-	2	-	-	-
Embu	28	28	LD, VC, PU, M, Mo, WS, LC	15	2	-	2	-
Kitui	8	8	Mo, SG	-	5	3	-	2
Machakos	26	22	Mo, PU, RS, SS, WS, SG	15	7	-	5	-
Makueni	25	13	Mo, LD, WS, M, PU, RS, SG	8	11	-	2	-
Kwale	12	6	Mo, M	-	-	-	-	-
Kilifi	8	2	Mo, LD	-	-	-	-	-
Taita taveta	12	4	Mo, PU, LD	1	2	-	-	-
Total	287	200		140	39	7	15	2

^aNumber of samples collected per county for virus detection using PCR approach. ^bNumber of samples collected from plants exhibiting papaya ringspot symptoms. ^csymptoms exhibited by plants M: Mosaic patterns on the leaves; Mo: Mottling symptoms on the leaves; VC: vein clearing; PU: Puckering; SS: shoe stringing; LD: Leaf distortion: WS: Water-soaked marks on stems and petioles; RS: Ringspots on fruits and; SG: Stunted growth of the plant. (-), not detected; MWMV, Moroccan watermelon mosaic virus; PaMV, Papaya mottle virus; CpMMV, Cowpea mild mottle virus.

DISCUSSION

Papaya ringspot disease is a major threat to papaya production in Kenya. The impact of the disease in the country is becoming serious that many growers have abandoned the fruit crop in favour of other crops (Mumo et al., 2021). This study provides information on the incidence, severity and prevalence of PRSD and maps out its distribution which is important aspects for the development of an effective management approach.

Papaya plants showing symptoms associated with the disease were observed in 20 out of 22

counties surveyed, causing minimal to severe levels of damage. Prevalence levels of up to 100% were also reported in some counties signifying the widespread and threat of the disease to papaya production in the country. The highest disease severities were reported in Kirinyaga, Murang'a Makueni, Machakos and Kiambu counties.

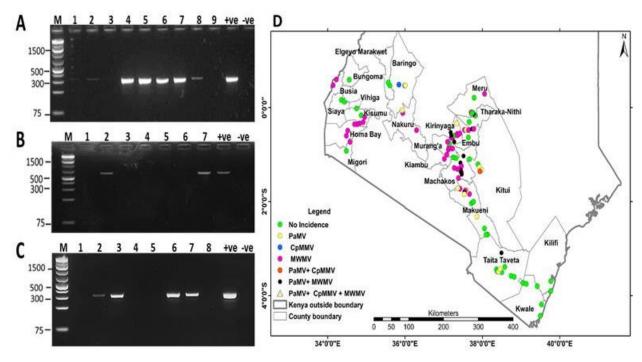


Figure 1. Gel electrophoresis for diagnostic studies of MWMV PaMV and CpMMV in Kenyan papaya. A band at 315 bp in (A), 694 bp in (B) and 304 bp in (C) show the presence of MWMV, CpMMV and PaMV, respectively. M indicates the O'GeneRuler™ 1 kb plus DNA ladder. +ve is a positive control, -ve is negative control. Numbers 1-9 = papaya samples. (D) A section of the map of Kenya showing combinations of viruses associated with the disease as determined through RT-PCR approach in selected counties in Kenya. The map was developed using QGIS software.

Source: QGIS Development Team (2019).

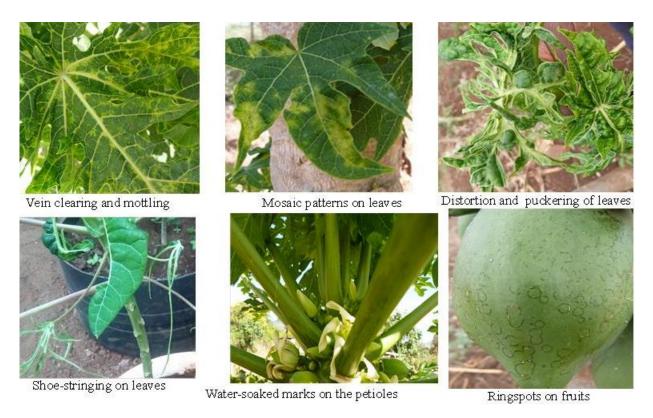


Figure 2. Symptoms displayed by papaya plants infected by viruses associated with the disease.

The situation could partly be attributed to a lack of management measures as observed during the survey due to minimal knowledge of the disease and its causal agents (Mumo et al., 2021). In these counties, some farmers also cultivated papaya as a mono-crop on large fields for commercial purposes, which could have encouraged fast disease spread because of the high host density and large size of cropped area (Piper et al., 1996; Kumar et al., 2010). Furthermore, monoculture facilitates easy movement of vectors from plant to plant during their transitional flights as they probe for a suitable host (Kumar et al., 2010), a situation that could contribute to the high disease incidences in these counties.

Three viruses, MWMV, PaMV and CpMMV were detected in both symptomatic and asymptomatic papaya samples collected during the survey in farmers' fields in the major growing counties in Kenya. MWMV was the commonest and was widely distributed. The virus is one of the most common cucurbit viruses in Africa (Lecog et al., 2001; Yakoubi et al., 2008; Ibaba et al, 2016; Kidanemariam et al., 2019). Although the virus was reported for the first time in papaya more than a decade ago in Congo (Arocha et al., 2008), its wide distribution in the country indicates that the virus is well established in papaya and there is an urgent need to develop management strategies is of paramount importance. The PaMV was recently discovered and described as a 'new' virus infecting papaya in Kenya (Mumo et al., 2020). However, little is known about its impact on papaya crops, its vectors and mode of transmission as well as alternate hosts. Nevertheless, the virus poses a serious production challenge to papaya because of its wide distribution and occurrences of dual infections with other viruses. The CpMMV infecting papaya is recombinant (Mumo et al., 2020) and its incidences in papaya production counties are very low. The detection and low incidences of CpMMV in papaya could be attributed to the recent host jump from cowpea to papaya after recombination and mutation leading to an increase in the host range (Legg and Thresh, 2000; Monci et al., 2002; Woolhouse et al., 2005). The CpMMV has been reported in leguminous and solanaceous crops in Africa (Jeyanandarajah and Brunt, 1993). During the survey, it was observed that cowpea plants were intercropped with papaya. Therefore, there is a chance that the whitefly transmitted the virus from cowpea to papaya, but this needs to be confirmed empirically.

Some plants displayed papaya ringspot symptoms (Table 3), although no viruses were detected. For example, in Baringo, Migori, Embu, Kiambu, Tharaka Nithi and Taita Taveta counties, the number of plants infected was lower compared to the number of symptomatic plants. The absence of viruses in symptomatic plants could be attributed to other viral or non-viral diseases, nutrient disorders, insect damage (Schreinemachers et al., 2015) and viral load/titer and or existence of variants which may not be detected by the primers used (Ghoshal and

Sanfacon, 2015). In other instances, the number of plants infected with the viruses was higher compared to the number of symptomatic plants (for example in Kirinyaga, Makueni and Kisumu counties). The absence of symptoms on virus-infected plants could probably be because the plants had just been infected and had not developed symptoms at the time the survey was carried out, the time of the year/season when the plant was infected, antagonisms due to co-infection with another virus or tolerance of the plant to the viruses (Mowlick et al., 2008; Kumar et al., 2010; Singh and Shukla, 2011).

The distribution of individual virus infections in Kenya is not region-specific. For instance, single PaMV infections occurred in Tharaka Nithi (Eastern) and Taita Taveta (Coast); while MWMV single infections were recorded in Kiambu, Murang'a, Nakuru, Kisumu, Homabay, Migori and Busia counties, which are either located in Central, Rift Valley or Western. The difference may be a result of the different frequencies of distribution of individual viruses. The two viruses, PaMV and MWMV were found in Kirinyaga, Embu, Makueni and Machakos Counties, which are located in the central and eastern regions. The PaMV and CpMMV were found in Baringo (Rift valley), Meru (Central) and Kitui (Eastern) Counties. No dual infection with MWMV and CpMMV was detected.

CONCLUSION AND RECOMMENDATIONS

This study has successfully determined the incidence, severity, prevalence, and distribution of papaya ringspot-associated viruses in Kenya. These viruses are prevalent across various counties and may potentially be spreading to unreported areas. Co-infections of these viruses have also been observed. Papaya ringspot exhibits peculiar patterns of prevalence and symptom development, influenced by varying weather conditions. In some instances, symptoms may be masked in infected plants, depending on the seasons (Stevens, 1983; Mowlick et al., 2008).

As a result, it is crucial to implement continuous monitoring and surveillance of these viruses to assess potential variations in symptoms and prevalence throughout the year. Simultaneously, management measures should be enforced to curb the spread of the disease. These measures include the use of virus-free planting materials, roguing of infected plants, restrictions on the movement of seedlings from one region to another, and certification for the production of clean seedlings in regions not yet infested.

Furthermore, it is essential to investigate the effects of co-infections of these viruses on papaya plants. Additionally, we should explore the possibility of other viruses causing symptoms in samples where no viruses were initially detected. While this study, conducted in 2017, has provided a valuable baseline for understanding the incidence of viral diseases, further studies conducted

over time will be necessary to establish the long-term patterns of distribution and severity of these diseases in papaya cultivation in Kenya.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Arocha Y, Vigheri N, Nkoy-Florent B, Bakwanamaha K, Bolomphety B, Kasongo M, Betts P, Monger WA, Harju V, Mumford RA, Jones P (2008). First report of the identification of Moroccan watermelon mosaic virus in papaya in Democratic Republic of Congo. Plant Pathology 57(2):387.
- Gashaw G, Alemu T, Tesfaye K (2014). Evaluation of disease incidence and severity and yield loss of finger millet varieties and mycelial growth inhibition of Pyricularia grisea isolates using biological antagonists and fungicides in vitro condition. Journal of Applied Biosciences 73:5883-5901.
- Ghoshal B, Sanfaçon H (2015). Symptom recovery in virus-infected plants: Revisiting the role of RNA silencing mechanisms. Virology 479:167-179.
- HCDA (2021). Horticulture Validated report. Nairobi, Kenya. Nairobi, Kenya. Retrieved from www.agricultureauthority.go.ke/.../Horticulture-Validated-Report-2014-Final-copy.pd
- Ibaba JD, Laing MD, Gubba A (2016). Genome sequence analysis of two South African isolates of Moroccan watermelon mosaic virus infecting cucurbits. Virus Genes 52(6):896-899.
- Jeyanandarajah P, Brunt AA (1993). The Natural Occurrence, Transmission, Properties and Possible Affinities of Cowpea Mild Mottle Virus. Journal of Phytopathology 137(2):148-156.
- Kansiime MK, Rwomushana I, Mugambi I, Makale F, Lamontagne-Godwin J, Chacha D, Kibwage P, Oluyali J, Day R (2020). Crop losses and economic impact associated with papaya mealybug (Paracoccus marginatus) infestation in Kenya. International Journal of Pest Management 0(0):1-14.
- Kidanemariam DB, Sukal AC, Abraham AD, Njuguna JN, Stomeo F, Dale JL, James, Harding MR, James AP (2019). Molecular characterisation of a putative new polerovirus infecting pumpkin (Cucurbita pepo) in Kenya. Archives of Virology 1(0123456789):0-4.
- Kumar NKK, Singh HS, Kalleshwaraswamy CM (2010). Aphid (aphididae: Homoptera) vectors of papaya ringspot virus (PRSV), bionomics, transmission efficiency and factors contributing to epidemiology. Acta Horticulturae 851:431-442.
- Lecoq H, Végétale SDP, Cedex M, Dafalla G, Medani W, Desbiez C, Wipf-scheibel C (2001). Biological and Molecular Characterization of Moroccan watermelon mosaic virus and a Potyvirus Isolate from Eastern Sudan. Plant Disease 85(5):547-552.
- Legg JP, Thresh JM (2000). Cassava mosaic virus disease in East Africa: A dynamic disease in a changing environment. Virus Research 71(1-2):135-149.
- Mishra R, Kumar RG, Patil BL (2016). Current Knowledge of Viruses Infecting Papaya and Their Transgenic Management. In R. K. G. et Al. (Ed.), Plant Viruses: Evolution and Management. New Delhi 110012, India: © Springer Science+Business Media Singapore pp. 189-204.
- Monci F, Sánchez-Campos S, Navas-Castillo J, Moriones E (2002). A natural recombinant between the geminiviruses Tomato yellow leaf curl Sardinia virus and Tomato yellow leaf curl virus exhibits a novel pathogenic phenotype and is becoming prevalent in Spanish populations. Virology 303(2):317-326.

- Mowlick S, Akther MS, Kundu BC, Akanda AM (2008). Masking behaviour and quantitative assessment of growth and and yield reduction of papaya due to papaya ringspot virus. Bangladesh Research Publications Journal 1(3):206-214.
- Mumo NN, Mamati GE, Ateka EM, Rimberia FK, Asudi GO, Boykin LM, Machuka EM, Njuguna JN, Pelle R, Francesca S (2020). Metagenomic Analysis of Plant Viruses Associated With Papaya Ringspot Disease in *Carica papaya* L. in Kenya. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.00205
- Mumo NN, Mamati GE, Ateka EM, Rimberia FK, Asudi GO (2021). Farmers' Knowledge, Perception and Management Practices af Papaya Ringspot Disease in Kenya. African Journal of Horticultural Science 18(2):31-42.
- Ombwara FK, Asudi GO, Rimberia FK, Ateka EM, Wamocho LS (2014). The Distribution and Prevalence of Papaya Ring Spot Virus (PRSV) in Kenyan Papaya. Acta Horticulturae (1022):119-124.
- Piper JK, Handley MK, Kulakow PA (1996). Incidence and severity of viral disease symptoms on eastern gamagrass within monoculture and polycultures. Agriculture Ecosystems and Environment 59:139-147.
- QGIS Development Team (2019). QGIS Geographic Information System; Open Source Geospatial Foundation Project, 2019. https://qgis.org/
- Rimberia FK, Wamocho LS (2014). Papaya industry in Kenya: Production, consumption and outlook. Acta Horticulturae (1022):181-188.
- Schreinemachers P, Balasubramaniam S, Boopathi NM, Ha CV, Kenyon L, Praneetvatakul S, Sirijinda A, Le NT, Srinivasan R, Wu MH (2015). Farmers' perceptions and management of plant viruses in vegetables and legumes in tropical and subtropical Asia. Crop Protection 75:115-123.
- Singh V, Shukla K (2011). Influence of inoculation time on severity of virus disease caused by Papaya ringspot virus. Annals of Plant Protection Sciences 19(1):142-146.
- Sseruwagi P, Sserubombwe WS, Legg JP, Ndunguru J, Thresh JM (2004). Methods of surveying the incidence and severity of cassava mosaic disease and whitefly vector populations on cassava in Africa: A review. Virus Research 100(1):129-142.
- Stevens WA (1983). Virology of flowering plants (1st ed.). Springer, Boston, MA. https://doi.org/110.1007/978-1-4757-1251-3
- Woolhouse MEJ, Haydon DT, Antia R (2005). Emerging pathogens: The epidemiology and evolution of species jumps. Trends in Ecology and Evolution 20(5):238-244.
- Yakoubi S, Desbiez C, Fakhfakh H, Wipf-Scheibel C, Marrakchi M, Lecoq H (2008). Biological characterization and complete nucleotide sequence of a Tunisian isolate of Moroccan watermelon mosaic virus. Archives of Virology 153(1):117-125.