



Survey of Potato Virus Y infection in Tree Tomato Across Kenyan Counties

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Abstract

Tree tomato or *tamarillo* (*Solanum betaceum* Cav.) is an important subtropical tree that is cultivated for its edible fruits. The main challenges to tree tomato production are diseases, pests, and drought, with viruses causing the most significant economic losses. The plant is reported to be infected by up to 20 viruses in different parts of the world. The aim of this survey was to establish the distribution of PVY in tree tomato, and assess the prevalence. Two surveys were conducted between 2018 and 2019 across three agro-ecological zones—upper highland, lower highland and midland—within nine major tree tomato-producing counties in Kenya. Leaf samples with virus symptoms were collected from 26 farms. Farms in the midland and lower highland zones exhibited more severe symptoms. Three other viruses TMMoV, Ethiopian tobacco bushy top virus and its associated RNA satellite, and potato spindle tuber viroid were discovered using next generation sequencing. Reverse transcription PCR revealed PVY to be present in 46% of farms visited. The rest of the viruses were all found to occur in mixed infection with PVY. Partial coat protein sequences from Sanger revealed a high percentage similarity, 97% to 98%, with PVY recombinant strain N:O. There were no recombinant junctions identified on any of the sequences. These findings highlighted the widespread occurrence of PVY in Kenyan tree tomato farms. The detection of mixed infections involving PVY underscores the potential for increased disease severity and economic impact. This study provided important baseline data for the development of effective virus management strategies in tree tomato production.



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Introduction

Tree tomato (*Solanum betaceum* Cav.) belongs to the *Solanaceae* family.¹ It is considered a subtropical tree and is generally grown in well drained soils between 1000 m to 3,000 m altitude.² It is native to the Andes of Ecuador, Chile, Peru and Bolivia. In Kenya, it is mainly cultivated in Makueni, Nandi, Baringo, Laikipia, Nyeri, Tharaka Nithi, Murang'a, Nakuru, Narok, Kiambu, Kirinyaga, Meru, Elgeyo Marakwet, Uasin Gishu, and Nyandarua counties.³ It is highly sought by dieticians because of the high amounts of vitamins A and C, and minerals it contains, and due to it being low in calories.⁴ Tree tomato fruit extracts have also been observed to confer therapeutic health benefits such as helping manage cholesterol levels in obese individuals, and exhibiting cancer prevention and treatment in liver and breast cancer cells lines.^{5,6}

The main constraints facing tree tomato cultivation in Kenya are diseases.⁷ Studies have observed viruses to be the most significant phytosanitary constraint to tree tomato production.^{8,9} Prior to this study, only potato virus Y (PVY) and tomato mild mottle virus (TMMoV) have been found to infect tree tomato in Kenya.¹⁰⁻¹²

Potato virus Y is a member of the *Potyvirus* genus, from the *Potyviridae* family. It is spread by aphids in a non-persistent fashion, and has a large host range, about 405 species from 31 families, with most of these, 287 species, coming from the *Solanaceae* family.^{13,14} Because of its worldwide distribution and wide host range, it is considered to be among the top ten most important plant viruses affecting crops, mainly potato, tomato, tobacco, pepper, and eggplant.^{13,14} Potato virus Y has previously been found to cause disease in tree tomato in other countries like Colombia and New Zealand.^{9,15} Solanaceous perennials like tree tomato are considered to be natural PVY reservoirs and ideal models that foster virus evolutions.¹⁶ Recently, three novel PVY recombinant strains reported in Ecuador are thought have co-evolved in tree tomato. In Kenya, PVY was first reported to infect tree tomato using traditional diagnostic techniques—dilution end point, longevity *in vitro*, electron microscopy, thermal inactivation point and symptomatology.¹⁰ To the best of our knowledge, there is no record of any study in Kenya that has established the presence of PVY in tree tomato using modern molecular techniques.

This survey was conceptualised to determine the presence, and distribution of PVY in tree tomato growing counties in Kenya, across different agro-ecological zones (AEZ), using modern molecular techniques, including RT-PCR, and Sanger sequencing. This study intended to provide important baseline data for the development of effective virus management strategies in tree tomato production.

Materials and Methods

Survey and Sample Collection

Between December 2018 and August 2019, two surveys were conducted in nine tree tomato growing counties within the three AEZ, i.e., upper highland (UH) – 2450m – 3050m, lower highland (LH) – 1850m – 2450m and midland (ML) – 1500m to 1850m.¹⁷ The counties included Baringo, Nakuru, Nandi, Elgeyo Marakwet, Meru, Tharaka Nithi, Embu, Machakos, and Nairobi. Disease severity was determined visually and using a 0 to 5 ordinal rating scale adapted from Murphy¹⁸ as follows: 0=Symptomless, 1=mottling, yellowing on young leaves, mild mosaic, 2=obvious mottling, mosaic, yellowing on leaves from at least one of the main stems, vein clearing and banding, 3=obvious vein banding and clearing on leaves over of the entire plant, mottling, mosaic, 4=obvious vein banding and clearing, mosaic, mottling, severe stunting, and leaf malformation; and 5=obvious vein banding and clearing, mosaic, mottling, severe stunting, leaf malformation and necrosis. The mean severity per county AEZ were used for comparison. Disease incidence was expressed as a percentage of all plants examined per AEZ in a county. Disease prevalence was determined by the percentage number of fields with virus associated symptoms per county AEZ. Leaf samples from each farm were collected and preserved by pressing in paper towels followed by desiccation in zip-lock bags using silica gel. The samples were taken to the molecular laboratory at KALRO Biotechnology Centre for virus detection.

Sample Preparation and RT-PCR Analysis

Total RNA was extracted from a 1 cm² portion of each leaf sample using the CTAB protocol detailed by Gambino¹⁹ with slight modifications. A Nanodrop spectrophotometer was used to determine the concentration of RNA, followed by dilution to 0.5 µg/µl. A 10 µl RT-PCR reaction mix was prepared following the manufacturer's protocol: 5 µl of the reaction mix, 2.2 µl of sterile water, 0.4 µl of the

SuperScript™ III RT and Platinum™ Taq mix, 0.2 µl of each forward and reverse primer at 10 µM concentration, and lastly 2 µl of the RNA template. A pair of universal primers for PVY, F3 – CGTTGAAA CCAATCGTTGAGAA and B3 – GACATCCTC GGTGGTGTG were used.²⁰ The reaction was done as follows: reverse transcription was done for 30 min at 50°C; initial denaturation for 2 min at 94°C; cyclic steps included denaturation for 20 s at 94°C, annealing for 20 s at 58°C, and extension for 30 s at 68°C. A total of 35 cycles were conducted followed by a final extension for 5 min at 68°C. A 1% agarose gel was prepared to view the results. One confirmed positive sample per farm was subjected to Sanger sequencing.

Data Analysis

One-way analysis of variance was used to assess disease severity and incidence across counties in the three different AEZ with significance determined at $P < 0.01$. Where significant differences were detected, the HSD (Honestly Significant Difference) Tukey-Kramer post hoc test was applied for comparing significant differences in the means of prevalence, severity, and incidence scores.

Virus incidence was calculated using percentage number of samples in a farm which tested positive for PVY. Due to the absence of PVY in one of the AEZ, UH, a student t-test at 95% confidence level was used to compare the mean virus incidence. The distribution of PVY was mapped based on the counties from which samples were collected. Multiple sequence alignment was done using Clustal Omega, and a maximum likelihood tree was inferred using MEGA 11. Recombination on the fourth recombination junction, on which the target region lies, was tested using RDP4.

Results

Survey Data

A total of 358 leaf samples were obtained from farms located across the three AEZ: ML, LH and UP. The samples were collected from 26 farms in different counties as follows: two from Meru, three from Machakos, four from Tharaka Nithi, five from Embu, three from Elgeyo Marakwet, one from Nairobi, one from Naivasha and four from Nandi. Of the 26 farms, 10 were from ML, 11 from LH and five from UH. All orchards intercropped tree tomatoes

with either tomatoes, tobacco, potatoes, peppers, capsicum, Tea, oranges, beans, banana, sweet potato, macadamia, maize, coffee, apples, cassava, arrow roots, avocado, or kale. The farmers identified three constraints to tree tomato production, pests and diseases bad weather and weeds. At the time of the study the farmers claimed to have no access to certified virus-free planting material. All the surveyed crops were in the fruiting stage and nearing harvest. Few farmers practiced pest or disease control, and even among them, disease symptoms persisted.

A range of virus-associated symptoms were observed across the surveyed farms, including vein clearing, crinkling, leaf malformation, necrotic spots, and vein banding. Disease prevalence was 100% across all regions. Disease incidence per county AEZ ranged between 60 to 80%, whereas severity ranged between 2.1 and 3.5. The highest disease severity was recorded in Meru County, whereas the highest disease incidence was recorded in Baringo County (Table 1). A one-way ANOVA revealed a statistically significant difference in the disease severity ($p < 0.01$) among UH, ML and LH. However, the incidence was not statistically significant (Table 1). Subsequent analysis using the Tukey-Kramer test for post hoc analysis at $\alpha < 0.01$ identified a significant difference in severity scores between UH, and LH and ML. No statistically significant difference was detected in severity scores between the LH and the ML.

RT-PCR Results

The RT-PCR reactions revealed that PVY was prevalent in all the counties surveyed except Elgeyo Marakwet. Virus incidence per county ranged between 25% and 100%. A total of 12 farms out of 26 farms had samples which tested positive, representing 46% of all farms surveyed (Figure 1).

RT-PCR detected PVY in 72 individual samples (Figure 2). Samples from these farms showed various symptoms including leaf mottling, chlorosis, mosaic patterns and leaf malformation. Of these, 36 samples from seven farms were infected by PVY only (gels A-E). The other 36 samples positive for PVY shown in gels F – J were also positive for a second virus, including: TMMoV, PSTVd, and ETBTv and satRNA-E.²¹⁻²³

Table 1: Incidence and Severity scores of virus-associated symptoms in the surveyed counties across different AEZ during 2018-2019 growing season, with ANOVA results, including post hoc comparisons using the HSD Tukey-Kramer test.

County (AEZ)	Severity	Incidence
Embu (ML)	2.6	70%
Elgeyo Marakwet (UH)	2.2	70%
Tharaka-Nithi (ML)	3	65%
Machakos (LH)	2.3	70%
Nandi (LH)	2.4	75%
Nairobi (ML)	3.2	60%
Nakuru (LH)	2.1	80%
Meru (LH)	3.5	70%
Baringo Farm 23 (LH)	3.15	80%
Baringo Farms 24 and 25 (UH)	2.4	80%
Significance at P<0.01	1.406e-32	0.35
Significance	sd	nsd

Severity post-hoc analysis	ML/LH	LH/UH	UP/ML
Studentized Range (Q)	3.46	14.61	16.43
HSD ($\alpha=0.01$)		4.13	

sd - significant difference at $p < 0.01$; nsd – no significant difference; ML – midlands; LH – lower highlands; UH – upper highlands.

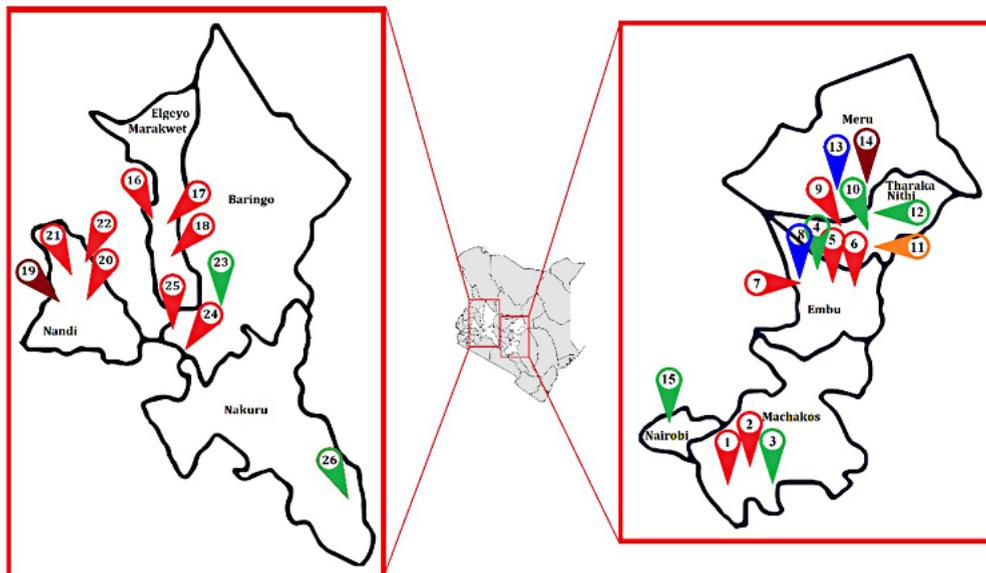


Fig.1: Map showing the location of 26 orchards that were surveyed. The Farms in red had samples which tested negative for PVY; those in green had samples which were positive for PVY only; those in blue were positive for PVY and PSTVd; the farm in orange had samples positive for PVY and ETBTv and satRNA-E; and the farms in brown had samples positive for PVY and TMMoV.

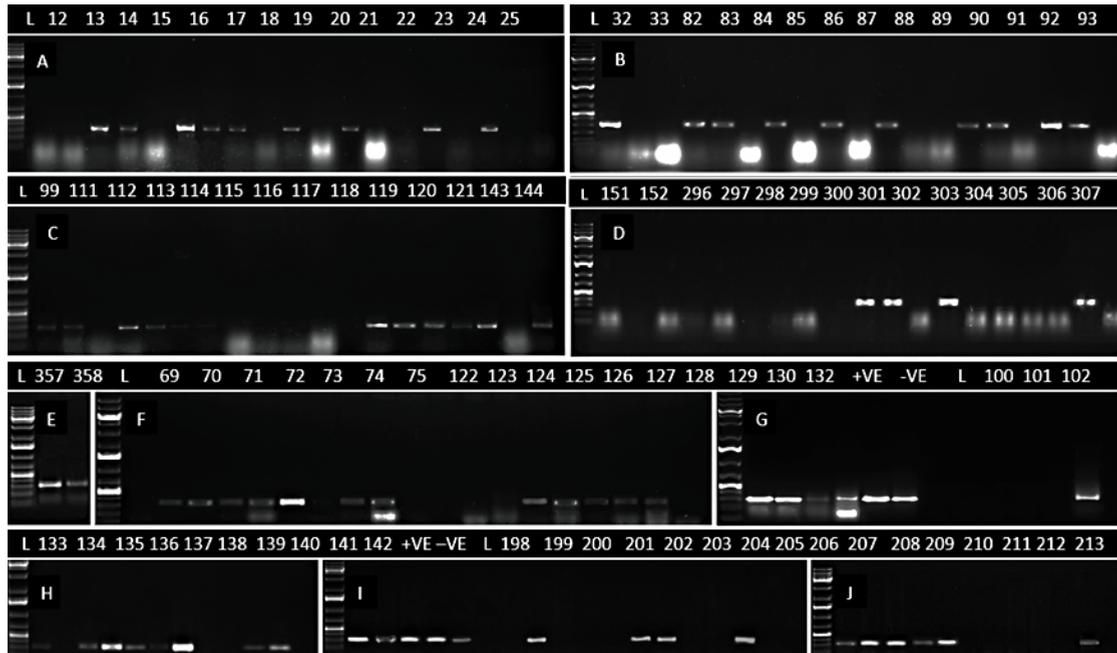


Fig. 2: Gel images showing PCR amplification of individual samples from farms that tested positive for PVY. The target region was 331 base pairs. Gels A–E represent samples from farms positive for PVY only, while gels F–J represent samples from farms positive for PVY and a second virus.

Plants in the farms which had a co-infection with PSTVd exhibited similar symptoms to those which were positive for PVY only, but with increased severity.²¹ Mottling, mild necrosis on the veins, and leaf curling were observed in farms 14 and 19 where all samples had a mixed infection of PVY and TMMoV.²³ Farm 11 which had plants with a mixed infection of PVY, and ETBTV and satRNA-E exhibited a more severe combination of symptoms, including leaf curling, malformation and vein clearing.²² There was no farm from the UH which was positive for PVY. An independent two-sample t-test showed no statistically significant difference in mean virus incidence between the ML (mean = 54.22) and LH (mean = 29.05) zones ($t = 1.26$, $p = 0.27$, two-tailed).

Nucleotide sequences from representative PVY-positive samples from the 12 farms, ON159312 - ON159323, showed a high level of nucleotide sequence similarity to each other, ranging between 98% to 100, and had a negative Tajima D value of -0.1283 . Recombination Detection Program 4 (RDP4) showed no evidence of recom-

bination on the fourth recombinant junction normally present in the CP region. The sequences showed a high percentage identity, 97 to 98% to strain N:O. Likewise, a maximum likelihood phylogenetic tree comparing Kenyan PVY isolates from tree tomato with 24 known PVY strains revealed a close relationship with the recombinant strain N:O (Figure 3).²⁴

Discussion

Detection of PVY in tree tomato using RT-PCR revealed that the virus is prevalent in all counties surveyed except Elgeyo Marakwet, and in all AEZ except the UH zones. The absence of PVY in the UH zones could be as a result of low temperatures experienced in these zones, 16°C to 20°C , which may restrict aphid population which in turn limit the spread of PVY.²⁵

The cases of mixed infection are thought to occur as PVY facilitates the aphid transmission of other viruses as follows: TMMoV by acting as a helper virus, while with PSTVd, ETBTV and satRNA-E, by encapsidation of their virion and genetic material respectively.^{26–28}

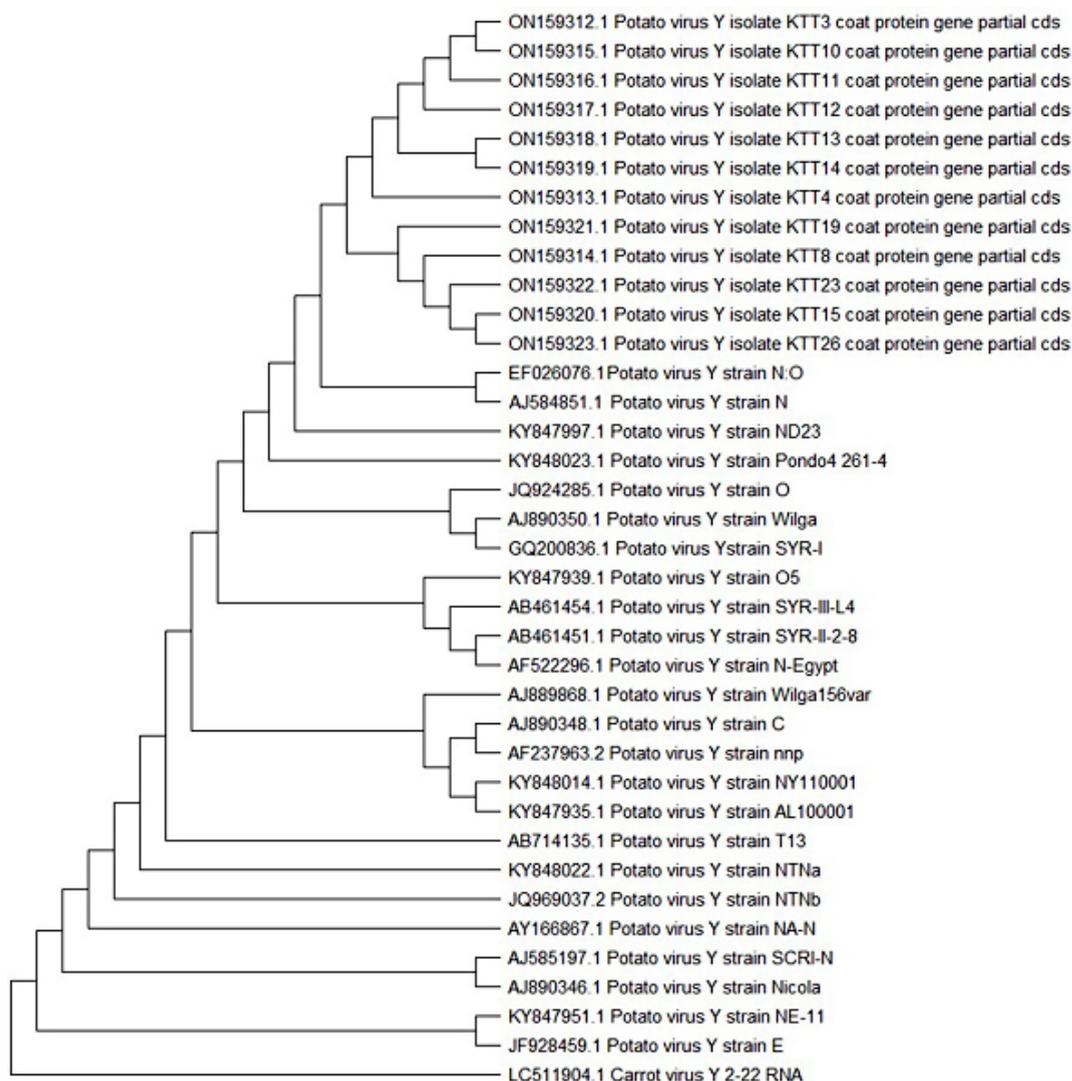


Fig. 3: A maximum likelihood phylogenetic tree was constructed using MEGA 11. The analysis included 12 nucleotide sequences of PVY isolates from this study (accession numbers ON159312 to ON159323) alongside 24 reference sequences representing known parent and recombinant PVY strains. Carrot virus Y (NC004013) was used as the outgroup.

The primers used in this study were designed to target a highly conserved region of the CP which enable the detection of both known and emerging PVY isolates. This is reflected by the negative Tajima's D value and high percentage identity observed. Consequently, the close phylogenetic relationship observed between the Kenyan isolates and strain N:O may be incidental, reflecting sequence similarity in a conserved region rather than overall relatedness. The recombinant strain N:O derives its

genome from two parental strains: Eu-N (from the 5' end to recombinant junction RJ2 at position 2390 bp) and O (from RJ2 to the 3' end).²⁴ Since the primers target a region near RJ4—where no recombination was observed in this study's sequences and which is absent in N:O—the resulting amplicons likely represent the O-strain portion of the genome.²⁴ While the primers can reliably detect PVY infections, they are not ideal for differentiating among its various strains. For accurate definition of PVY strains whole

genome sequencing and phylogenetic studies are recommended, alongside biological characterisation and symptom expression on its host.²⁹

Understanding the distribution of PVY in tree tomato, a perennial crop, provides starting point for other studies. Future research should investigate the evolution of PVY in tree tomato and other susceptible perennials across different agro-ecological zones (AEZs) in greater depth. This will help to develop effective mitigation strategies to prevent its spread to other solanaceous hosts, particularly tomato and potato, which are economically more important and widely cultivated.

Conclusion

In conclusion, this study has established the presence of PVY using RT-PCR and Sanger sequencing in eight out of nine counties. In total, 12 out of 26 farms had samples which tested positive for PVY, five of which occurred in mixed infection with other viruses, namely ETBTV and satETBTV-E, PSTVd and TMMoV. PVY was prevalent in all AEZ with the exception of UH. These findings establish a critical baseline for virus monitoring and call for integrated management strategies to mitigate the impact of viral diseases on tree tomato production in Kenya.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Permission to Reproduce Material from other Sources

Not Applicable

Author Contributions

- **Michael Njoroge Kinoga:** Conceptualization, Data Collection, Analysis, Methodology, Writing, Project Administration – Original Draft.
- **Paul Kimemia Kuria:** Conceptualization, Data Collection, Analysis, Writing, Project Administration – Review & Editing, Funding Acquisition.
- **Douglas Watuku Miano:** Visualization, Supervision, Project Administration.
- **Lusike A. Wasilwa:** Funding Acquisition, Resources, Supervision.

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