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Pathogen dynamism and variability of rice yellow mottle virus in Kenya

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Abstract

Rice yellow mottle disease (RYMD) continues to constrain rice production in Africa. Rice yellow mottle virus (RYMV), which belongs to the *Sobemovirus* genus, is the causal agent of RYMD. In Kenya, previous studies on RYMV mostly focused on western Kenya, ignoring the central and coastal regions, which are also important areas for rice cultivation in the country. This has resulted in incomplete data on RYMD outbreaks. This study aimed to determine the prevalence of RYMD in the farmers' fields, and the genetic diversity of the RYMV based on the coat protein gene. Field surveys were carried out in Kenya's rice-growing regions between May and July 2023 to determine disease incidences using the IRRI Standard Evaluation Scale (SES) for rice. Symptom observations and serological tests confirmed the presence of RYMV in all surveyed rice cultivation areas. The presence of RYMV was detected in Kirinyaga, Tana River, and Taita Taveta counties of Kenya, representing the field report of its occurrence in these regions. Disease incidence and severity analysis using one-way ANOVA showed no significant difference in RYMD incidence among the rice irrigation schemes ($F(6,29) = 1.838$, $P = 0.1265$). Sequencing results of the coat protein gene (ORF4 region) revealed that the RYMV isolates in this study could be classified as S4ke, S4ug, and S4mg strains in the East Africa lineage. The discovery of the RYMV strain S4mg in Kenya runs counter to the gradual strain of breaking up and moving away that has been established in Africa over the past two centuries. Results showed that RYMV is present in all the rice-growing regions under study and exhibits intra-strain diversity in Kenya. Incorporating resistance genes into extensively grown rice cultivars is crucial for preventing the spread of the rice yellow mottle virus.

Keywords Rice yellow mottle virus, Disease incidences, CP gene, Strains, Resistance screening

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Background

Rice (*Oryza sativa* L.) is a popular cereal crop worldwide, as the grain is rich in dietary starch and protein (Darko Asante et al. 2017; Mabele et al. 2020). Over 50% of the global population relies on rice as their primary food source, with 150 million hectares dedicated to its cultivation worldwide (Atera et al. 2018). Rice is the second-most significant contributor to caloric intake in Sub-Saharan Africa (SSA) after maize. Tsujimoto et al. (2019) predicted that rapid population expansion and urbanization would further increase the demand for rice. In Kenya, this cereal crop is considered the third-most significant cereal following maize and wheat, and therefore plays a crucial role in ensuring food security

(Njuguna Ndirangu and Oyange 2019). Most rice producers are small-scale farmers in areas located in the following counties: Busia (Bunyala), Homa Bay (Kagan, Nyagweso), Migori (Lower Kuja), Kirinyaga (Mwea), Kisumu (West Kano, Ahero), Kwale (Vanga), Siaya (Dominion), Taita Taveta (Buruma), and Tana River (Bura and Hola) (GOK-NRDS 2020). Mwea, near Mt. Kenya, with over 26,000 acres under irrigation, is Kenya's largest irrigation scheme, while the rest of the schemes have an acreage below 5000 acres (Vishnu and Mukami 2020).

Both biotic and abiotic stresses hinder rice production in Africa (Odongo et al. 2021). Banwo et al. (2004) and Fargette et al. (2002) have identified the rice yellow mottle virus (RYMV) as a prominent plant pest that poses a substantial threat to the well-being of rice plants. Rice yellow mottle disease (RYMD), caused by RYMV results in significant reductions in rice crop production, ranging from 10 to 100%, and ultimately leading to the death of the infected plants (Kouassi et al. 2005). RYMV is the primary pest constraint and is endemic to rice in sub-Saharan Africa (Alkali et al. 2017; Kanyeka 2000; Kouassi et al. 2005). Closer to home in Kenya, a survey in 2015 in western Kenya found that RYMD incidences in some farmers' fields were high, resulting in 100% yield losses (Adego et al. 2017). Symptoms of RYMV include streaking, yellowing, mottling, reduced tillers, spikelet discoloration, and plant death (Sereme et al. 2016).

RYMV genome consists of a positive-sense RNA (King et al. 2018). The genome consists of approximately 4550 nucleotides, which are organized into four open reading frames (ORFs) (Rakotomalala et al. 2013). The protein encoded by ORF1 is crucial for both the process of infection and the silencing of genes. ORF2 plays a role in the synthesis of the central polyprotein and contains two overlapping ORFs. ORF2a codes for a serine protease and VPg, while ORF2b codes for RNA-dependent RNA polymerase through a ribosomal frameshift. The coat protein (CP) gene, encoded by ORF4, is expressed from the 3' end of the sub-genomic RNA (Fargette et al. 2008). In general, molecular typing of the coat protein gene of RYMV isolates in Africa is diverse with seven major RYMV strains reported (S1–S7). These are S1, S2, and S3 in West and Central Africa, and S4, S5, S6 (Pinel et al. 2000), and S7 (Ndikumana et al. 2017) in East and Southern Africa. The initial RYMV cases were documented in 1966 in Kisumu close to Lake Victoria (Bakker 1974). Nucleotide sequencing revealed that these isolates belonged to the S4lv lineage of the strain S4, as reported by Abubakar et al. (2003) and Pinel-Galzi et al. (2009). RYMV samples collected from 1997 to 2007 in other countries surrounding Lake Victoria such as Uganda and Tanzania belonged to strain S4lv (Pinel-Galzi et al. 2009). In the few years that followed this S4lv lineage was also reported in Rwanda and Burundi (Ochola et al. 2015). A new lineage named S4ke was discovered in 2012 (Adego et al. 2018) in samples collected at the initial site in Kisumu where RYMV was first reported. Mabele et al. (2020) also reported strain S4ke in Kisumu confirming that strain S4lv had been replaced by strain S4ke. In other East African countries, other lineages of strain S4 have been reported, such as S4ug in Uganda (Ochola et al. 2015), S4mg in Madagascar (Rakotomalala et al. 2013), and S4lm in Rwanda (Ndikumana et al. 2017), which are yet to be reported in Kenya.

RYMV is transmitted mechanically through agronomic practices and naturally by insect vectors, primarily chrysomelid beetles (Sokpé Longué et al. 2016). Additionally, there has been evidence of its transmission by grazing animals such as donkeys, cows, and grass rats (Sarra et al. 2007). The management of RYMV is primarily by controlling insect vector populations (Sereme et al. 2016). The predominant rice cultivars in Africa belong to the Asian species (*Oryza sativa)*, which is highly susceptible to RYMV infection (Omiat et al. 2023). According to Rakotomalala et al. (2008), RYMV resistance has been observed in the Gigante variety of *O. sativa* and a few varieties of *O.glaberrima* from the Togo series. Three resistance genes namely RYMV1, RYMV2, and RYMV3, have been identified as effective against RYMV infection (Thiémélé et al. 2010; Pidon et al. 2017).

The existing genetic diversity data for RYMV in Kenya may aid in breeding for resistance; however, it is still limited (Mabele et al. 2020). Kenya borders countries with diverse RYMV strains including S4lm, S4lv, S4ug, S5, S6w, and S6c (Uke et al. 2016; Hubert et al. 2017; Ramathani et al. 2023). With the expansion and intensification of rice cultivation in Central and Coastal parts of Kenya (GOK-NRDS 2020), we hypothesize that RYMV strains in Kenya could be diverse. This study involved the collection of samples from Kenya's primary rice growing areas, particularly in locations where RYMV has not yet been documented (Central and Coastal Kenya). The objective of the study was to assess the geographic prevalence of RYMV in Kenya and to determine the genetic variation of the CP gene through sequencing. Additionally, the study analyzed the isolates at a molecular level using phylogenetic analysis and determined their serotype by examining the coat protein's amino acid sequence. The findings from this study will improve the epidemiological monitoring of RYMV and aid in the development of sustainable control methods for RYMD in Kenya.

Results

Occurrence and RYMD field symptoms

In most rice irrigation schemes, land sizes for rice cultivation were divided into blocks containing several units of 1 acre. Most farmers were small-scale cultivators who utilized the irrigated lowland rice-producing system. We determined Kenyan farmers' preferences for several rice genotypes. The two most popular genotypes in Kenya were IR2793-80–01 and Komboka (Additional file 1: Tables S1, S2). In the western part of Kenya around Lake Victoria, farmers prefer the IR2793-80–01 genotype, while Komboka is more common along the coast (Tana River and Taita Taveta). Rice genotype Basmati 370, commonly known as Pishori, was popular in central Kenya near Mt. Kenya (Mwea). However, it faces competition from other genotypes as farmers embrace alternative genotypes such as Komboka (Additional file 1: Tables S1, S2).

A total of 280 leaf samples were collected (Additional file 2: Table S3) in all the surveyed counties, exhibiting typical symptoms such as yellow leaf streaks, yellow leaves, stunting, mottling, leaf necrosis, and reduced tillering. The symptoms closely resemble those caused by RYMV (Fig. 1a). RYMV-infected rice fields may resemble those with iron or nitrogen deficiencies due to their color alterations. However, insect vector-transmitted RYMV resulted in a patchy infection in the field (Fig. 1a), while nutrient deficiencies typically manifest over extensive areas in the field. The detection of RYMV in Kirinyaga, Migori, Taita Taveta, and Tana River in this study constitutes the first report of this virus in these counties.

All surveyed areas in this study had incidences of RYMD, with an overall mean disease prevalence of about 50%. The disease prevalence of RYMD across the surveyed regions varied from 25% in Taita Taveta in coastal Kenya to 67% in the Lower Kuja irrigation scheme in Migori in the western part of Kenya (Fig. 1b).

We conducted a one-way analysis of variance (ANOVA) on disease incidences in rice cultivation regions in Kenya. Through the ANOVA test ($F(6, 29) = 1.838$, $P = 0.126$), it was found that there were no significant differences between the means of the disease incidences in the western, coastal, and central rice cultivation regions. Disease severity was evaluated using the IRRI standard evaluation scale for rice (IRRI 2013) based on RYMV symptoms on a scale between 1 and 9, and the highest average severity score of 7 was observed in Lower Kuja followed by a score of 6 in Bunyala and West Kano (Additional file 1: Table S3). Overall Pearson's correlation coefficient of RYMD incidence and severity revealed a strong positive correlation between incidence and severity ($r = 0.849$, $P = 0.000$, CI = 95%).

Serological analysis

Among the 280 samples tested via DAS-ELISA, 223 (approximately 80%) were positive for RYMV (Additional file 1: Table S3). The DAS ELISA classified 71 positive samples as strongly positive (Table 1). The viral load was estimated by absorbance readings, and it varied from 1.267 to 0.046, with an average of 0.5226 ± 0.1573 (mean ± standard deviation, $n = 280$). The positive reaction ranged from 1.267 to 0.15 while those less than 0.1418 were attributed the negative reaction. There was a significant difference in the absorbance values among the rice-growing areas in Kenya ($P = 0.013$).

PCR detection and sequence analysis

Reverse Transcription-PCR (RT-PCR) technique was utilized to detect the presence of RYMV in leaf samples using primers that specifically target the RYMV coat protein gene (ORF4). The viral RNA amplification resulted in an amplicon size of 720 bp (Additional file 2: Figure S1). This confirmed the existence of the coat protein gene of the RYMV in the samples. The detection of RYMV using RT-PCR confirmed DAS ELISA results. A total of 55 RYMV isolates were sequenced using Sanger sequencing. Of these, 37 isolates (Additional file 1: Table S8) were selected because they were of excellent quality, long, and covered the whole CP gene. Each isolate had a uniform amplicon length of 720 bp, with none displaying a length of 723 bp, which is typically associated with the strain S5 of RYMV, and were subsequently deposited in the NCBI GenBank.

The BLAST search results indicated that the RYMV isolates obtained from this study were closely related (above 99%) to isolates reported in Madagascar, Uganda, and Tanzania (Table 2). The E-value, a statistical measure based on the expectation value, was zero, indicating a strong match between the query sequence and the sequence from the database.

Genetic distance and identity estimation

The analysis revealed a significant percentage of identity between the RYMV isolates in this study and those from Kenya, Madagascar, Uganda, and Tanzania already deposited in the NCBI reference database. For example, an isolate from Madagascar, named Mg64 (Rakotomalala et al. 2013), presented 99.4%, 99.4%, and 99.3% similarity with isolates Ke502, Ke503, and Ke508, respectively (Additional file 1: Table S4).

Isolate U14 which was collected from Mbale-5 in Eastern Uganda in 2011 (Uke et al. 2016) had a percentage identity of 99.16% with the isolate Ke506 from Busia. Similarly, isolate Tz615 of Tanzania in Kilimanjaro (Hubert et al. 2017) had a 98.60% identity with the isolate

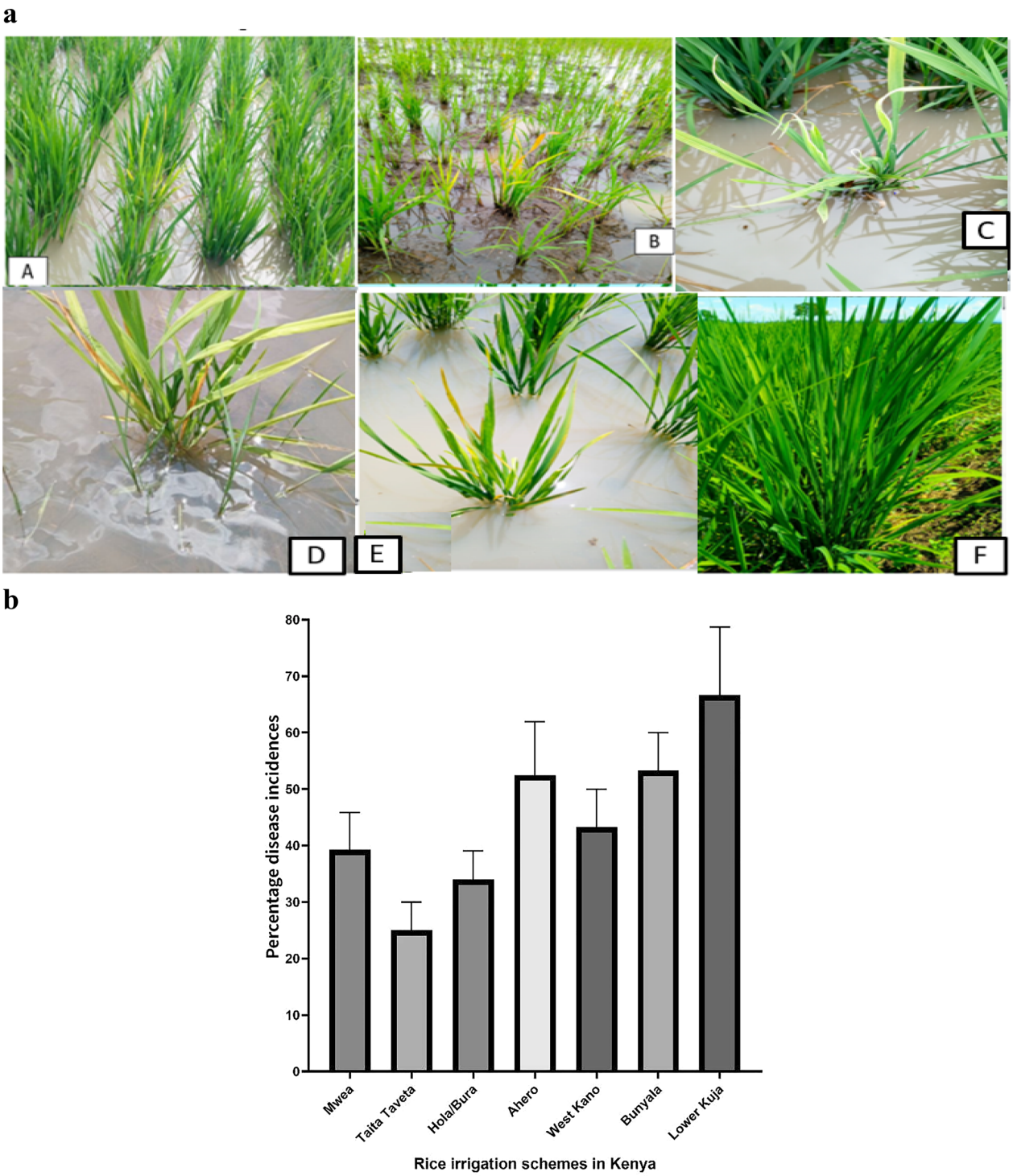

Fig. 1 **a** Symptoms of RYMV in diseased rice plants observed in the field during the 2023 survey in Kenya. **A**-leaf yellowing (Ahero), **B**-leaf-necrosis (Mwea), **C**-leaf mottling and stunting (Bunyala), **D** and **E**-yellowing, yellow-green spots and brown discoloration (Hola-Tana River), **F**-an RYMV-free rice plant (Taita Taveta). **b** Percentage of RYMD disease incidences across different rice irrigation schemes in Kenya. Error bars represent the standard deviation of the means

Table 1 Distribution of positive samples by DAS-ELISA in all sampled rice schemes

Scheme/area	No. of isolates	No. of strong positive +++	No. of positive ++	No. of weak positive +	% No. of positive	No. of negative −	% No. of negative
Mwea	103	26	62	9	94.2	6	5.8
West Kano	27	5	20	2	100	0	0
Bunyala	27	0	21	5	96.3	1	3.7
Ahero	28	16	6	5	96.4	1	3.6
Lower Kuja	28	23	5	0	100	0	0
Taita taveta	26	1	4	12	65.4	9	34.6
Bura and Hola	41	0	0	5	12.2	36	87.7
Total	280	71	118	38	80.8	54	19.2

+++ (Strong positive; ≥ 0.7 to 1.267), ++ (Positive; ≥ 0.4 to < 0.6999), + (Weak positive 0.15 to ≤ 0.3999), − (Negative ≤ 0.1418)

Table 2 Comparison of the coat protein gene percentage identity between selected RYMV isolates in Kenya under this study and those from other East African countries accessed from the NCBI database

Query isolate (this study)	NCBI ref. isolate	Country	Query cover	E-value	% identity	Acc length	Acc. No
Ke502	Mg64	Madagascar	100%	0	99.4	720	JX961575.1
Ke506	U14	Uganda	100%	0	99.2	720	AB980010.1
Ke525	TZ612	Tanzania	100%	0	99.6	720	MF447523.1
Ke525	TZ601	Tanzania	100%	0	99.4	720	MF447520.1
Ke524	TZ612	Tanzania	100%	0	99.2	720	MF447523.1
Ke591	Ke105	Kenya	100%	0	99.2	4448	MG599278.1
Ke538	KeB4	Kenya	100%	0	99.2	720	LC547479.1
Ke510	KeB4	Kenya	100%	0	99.2	720	LC547479.1
Ke504	KeB4	Kenya	100%	0	99.7	720	LC547479.1
Ke547	Ke105	Kenya	100%	0	99.7	4448	MG599278.1

[1] Kenya (Ke), [2]Madagascar (Mg), [3]Uganda (U), [4]Tanzania(TZ)

Ke524 from Taita Taveta (Additional file 1: Table S4). Finally, isolates Ke563, Ke514, Ke553, Ke563, Ke517, and Ke548 had a high percentage homology of over 98% compared with previous Kenyan isolates (Ke105, KeB1, and KeA1) reported by Mabele et al. (2020). The results of the BLAST analysis and the computation of genetic distance and percentage identity using DNASTAR's MegaAlign Pro 17 software were consistent.

Phylogenetic relationships and identification of the RYMV strains

Phylogenetic analysis showed that Kenyan isolates in this study belong to RYMV strains S4ke, S4mg and S4ug with strong bootstrap support (Fig. 2) when compared with earlier RYMV isolates (Additional file 1: Table S5). Five isolates (two isolates (Ke524, Ke535) from Taita Taveta, two isolates (Ke501, Ke506) from Busia and one isolate (Ke512) from Lower Kuja) clustered with RYMV strain S4ug isolates (TZ601, TZ612, and TZ615) of 2014 from Kilimanjaro, Tanzania (Hubert et al. 2017) and were assigned to S4ug. Surprisingly, three isolates from Busia (Ke502, Ke503, and Ke508) clustered with isolates from Madagascar Mg31, Mg 58, and Mg64 and were assigned to the S4mg strain based on previous reports (Rakotomalala et al. 2013, 2019). Other isolates in this study, notably from central (Mwea-Ke538, Ke540), coast (Hola-Ke591, Bura-Ke589), and western Kenya (Ahero-Ke518, Ke556, West Kano-Ke554, Ke548, Lower Kuja-Ke553) were assigned monophyletic lineage strain S4Ke of RYMV (Fig. 2). These group clustered together with other S4ke isolates (KeA1, KeB1, and KeB4) reported by Mabele et al. (2020).

Spatial distribution of RYMV strains in Kenya

In the present study, the occurrence of RYMV strain S4ke was recorded in the central region of Kenya, particularly in Mwea (Kirinyaga) the largest irrigation scheme in the country (Fig. 3). The strain S4ke was also detected in the Hola and Bura irrigation schemes located in the coastal region of Kenya in Tana River County. Furthermore,

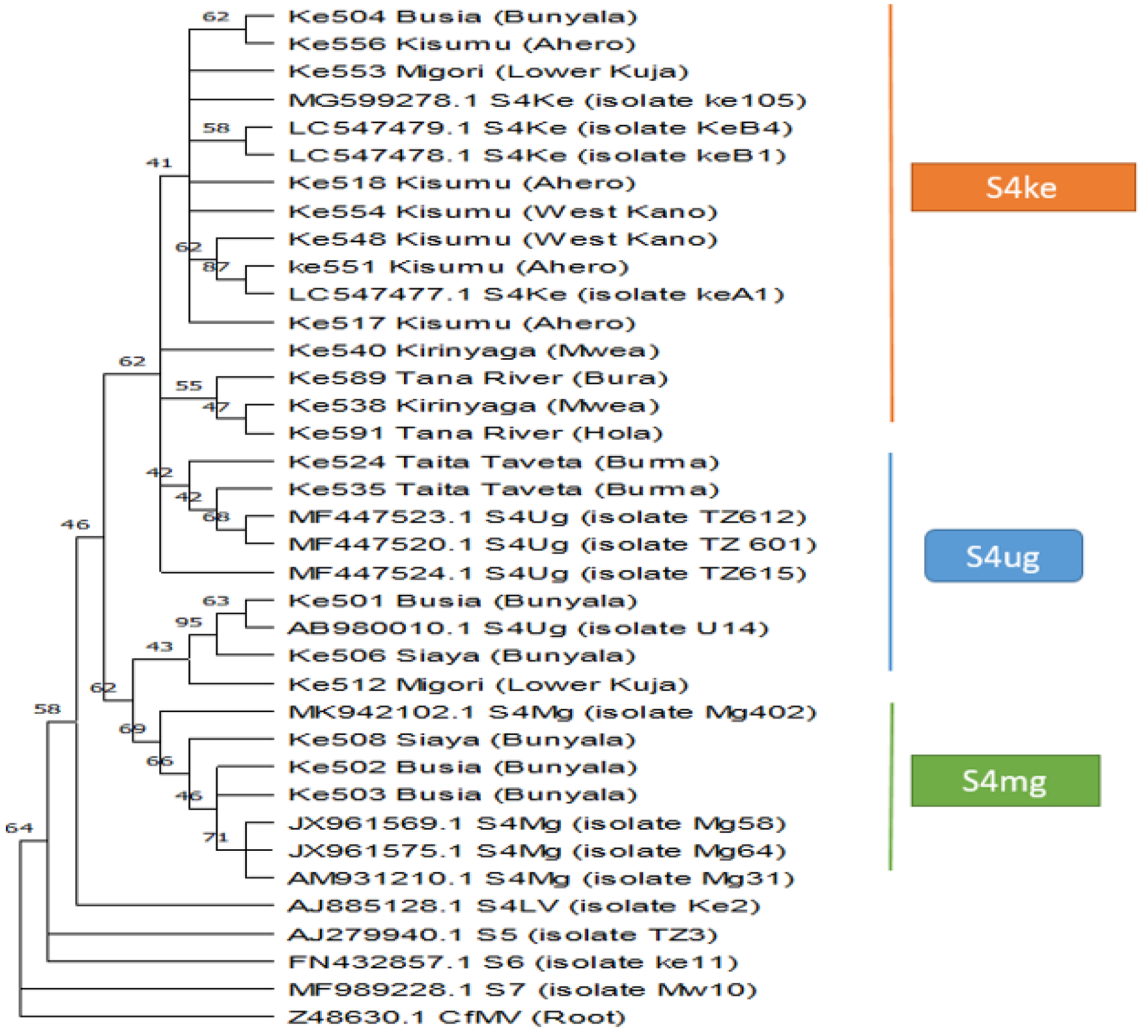

Fig. 2 Phylogenetic analysis of RYMV Kenyan isolates and selected isolates from GenBank; neighbour-joining tree showing the relationship of 20 isolates of RYMV collected in Kenya in 2023 (Ke500 series) with other reported isolates from Kenya (Ke), Madagascar (Mg), Tanzania (Tz), Malawi (Mw), and Uganda (U) constructed on basis of the coat protein gene nucleotide sequences. Cocksfoot mottle virus isolate (CfMV) was used to root the tree

the was found in schemes around Lake Victoria namely Lower Kuja (Migori), West Kano and Ahero (Kisumu), and in the Bunyala irrigation scheme in Busia (Fig. 3). The most recent report of this strain was by Mabele et al. (2020) at the Ahero irrigation scheme in western part of Kenya (Additional file 1: Table S5).

The current study reports strain S4ug of RYMV in Taita Taveta, coastal part of Kenya for the first time (Fig. 3). This strain was previously reported in Bunyala and Busia of western Kenya in 2016 by Adego et al. (2018) (Additional file 1:Table S5). This strain was also reported in Tanzania in 2013 and 2014 (Hubert et al. 2017). Strain S4mg was detected in Bunyala in the current study and is similar to that found in Madagascar (Rakotomalala et al. 2013). The first RYMV report in Kenya was in 1966 as documented by Abubakar et al. (2003) and Fargette et al. (2008), and the isolates were found to belong to strain S4lv. Contrarily, the present study did not detect strain S4lv in the samples collected from all surveyed areas. Previous studies by Pinel-Galzi et al. (2009) reported that the strain S6 was detected in coastal Kenya, however, the present study did not report any strain S6 in Kenya, similar to strains S5, S7, and S4lm of the Eastern African lineage.

Historical trends of RYMV in Kenya

We investigated and analysed the temporal signal intensity of a dataset consisting of 50 RYMV sequences. The

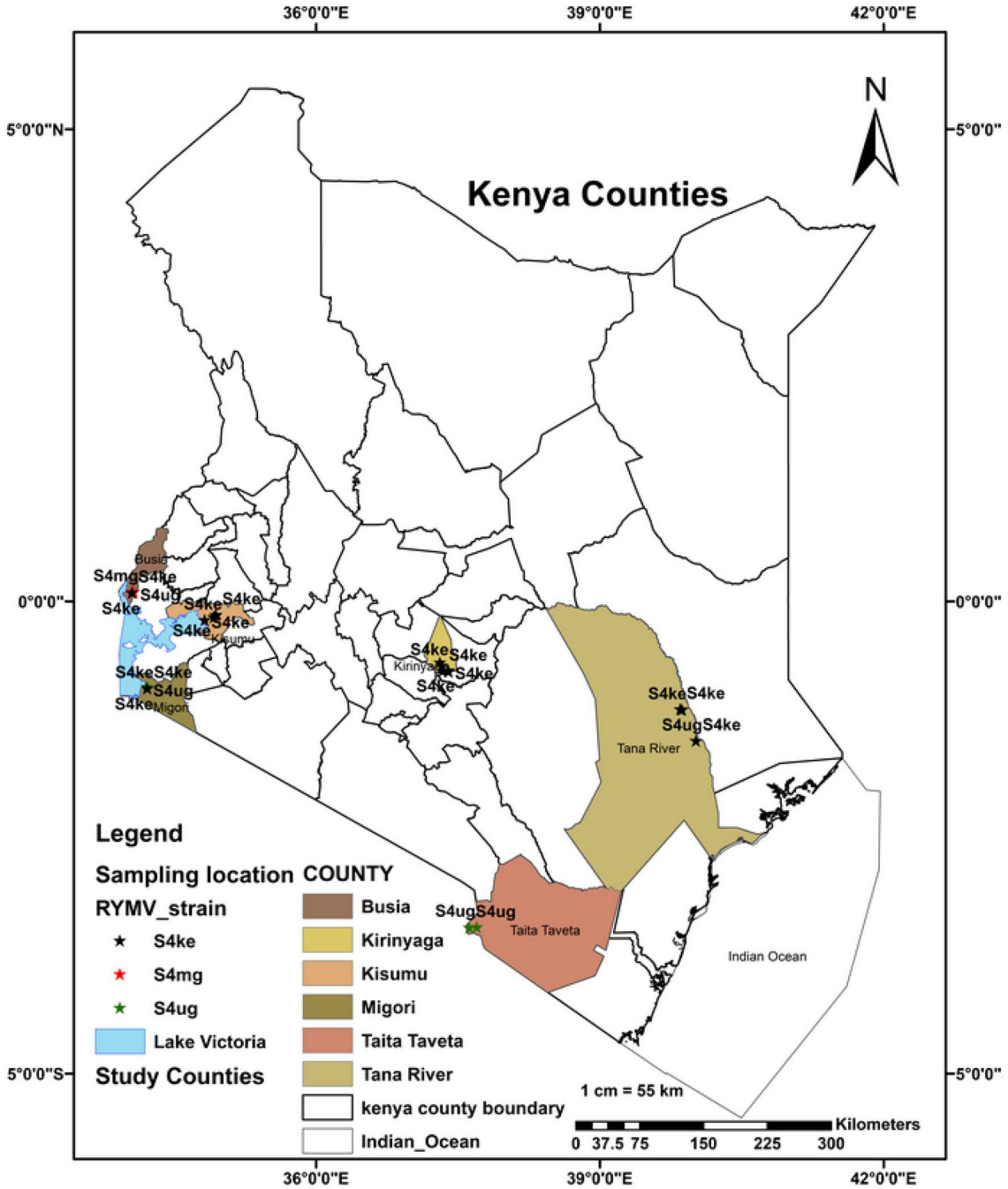

Fig. 3 A geographical map indicating major rice-growing counties (colored) in Kenya, where RYMV was reported in 2023 survey. RYMV strains identified in the current study are shown using stars

entire ORF4 dataset was analysed using linear regression and demonstrated a clear positive association between genetic divergence and sampling time spanning more than 50 years (1966–2023). These findings provide insights into the epidemic histories and potential transmission routes of RYMV strain S4lv in Kenya (lake Victoria strain S4) represented by isolates Ke1, Ke2, and Ke3 which are the oldest RYMV isolates in Kenya (Fig. 4a). Linear regression analysis was conducted to examine the relationship between the root-to-tip genetic distance and sample time in years, and the results showed a consistent temporal pattern, with a weak correlation

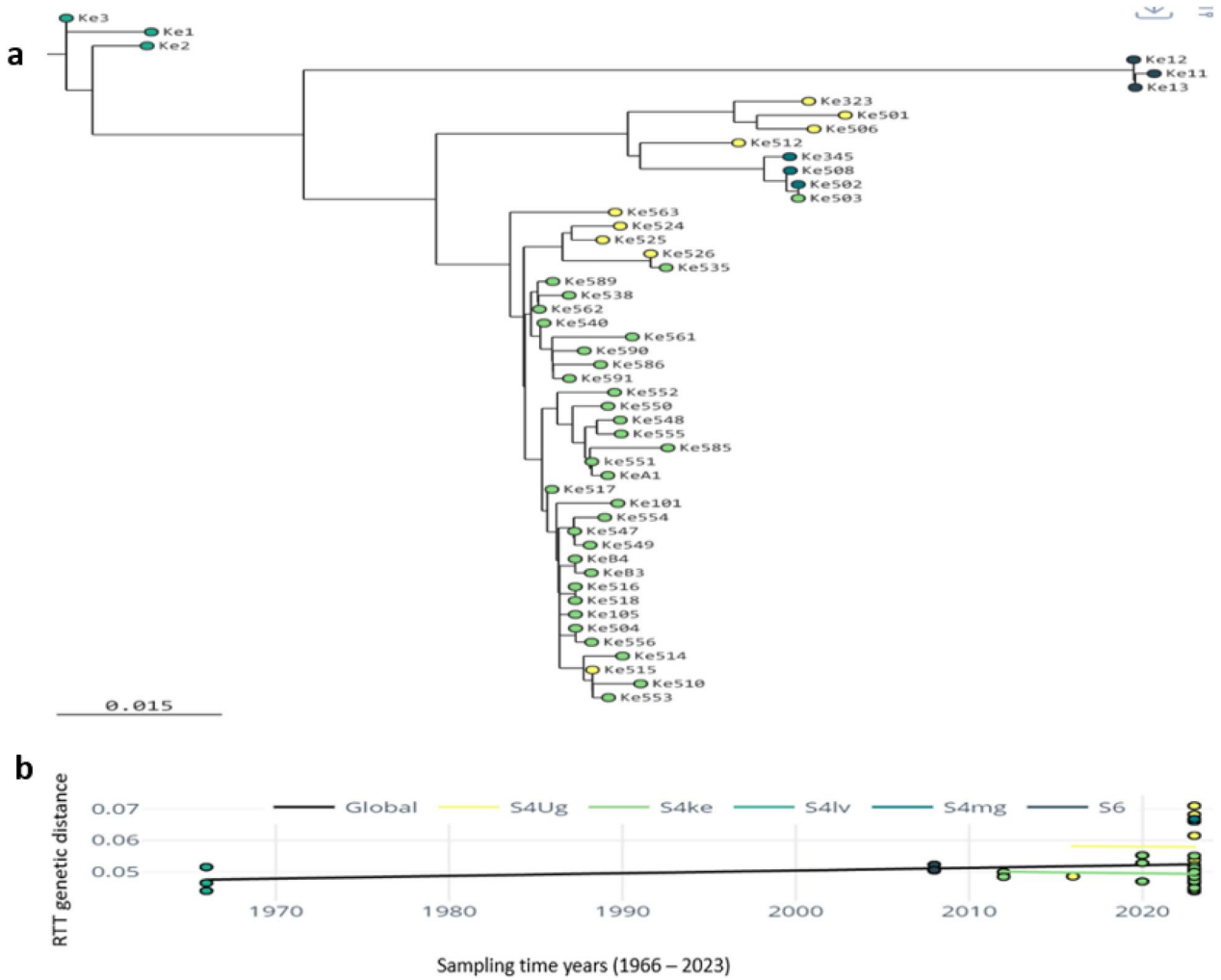

Fig. 4 Root-to-tip regression analyses. **a** A maximum clade credibility (MCC) tree constructed by continuous evolutionary inference with 50 RYMV isolates collected in Kenya from 1966 to 2023. **b** Plot of the root-to-tip (RTT) genetic distance (subs/site) against sampling time (1966–2023) estimated from the alignment of 50 RYMV isolates (1966–2023). The solid dark line in the regression panel denotes the global clock where all samples are pooled. RYMV strains are shown by different color codes in both the MCC tree and RTT graph plot as indicated by the key above with the year 2023 samples grouped as S4ke (green), S4ug (yellow), and S4mg (blue)

coefficient ($R2 < 0.027$) (Fig. 4b). The solid dark line in the regression panel denotes the Kenyan global clock where all samples are pooled. The current study detected strains S4ke, S4mg, and S4ug in 2023, with S4ke being the dominant in Kenya (Fig. 4b).

Immunological typing

With reference to other RYMV isolates that had been collected before from East and West Africa (Additional file 1: Table S6), we categorized various isolates of the RYMV according to variations in their amino acid sequences. The Kenyan RYMV isolates were identified as serotype 4 (Ser 4), as illustrated in Fig. 5. These findings align with the previously reported results of Uke et al. (2016). The Kenyan isolates were classified as part of the East African serotype group, Ser4 (Additional file 2: Figure S2), which was different from serotypes 1, 2, and 3 found in West Africa (Pinel et al.2000). The discovery of a notable serotype 4 lineage indicates a trend of specialization within serotype 4, which is the prevailing variant in East Africa (Ramathani et al. 2023).

Evaluation of rice susceptibility under greenhouse conditions

Our findings revealed that all the rice genotypes screened for their reaction to RYMV were susceptible based on symptom appearance and DAS ELISA results as they tested positive for RYMV (Table 3) fulfilling Koch's

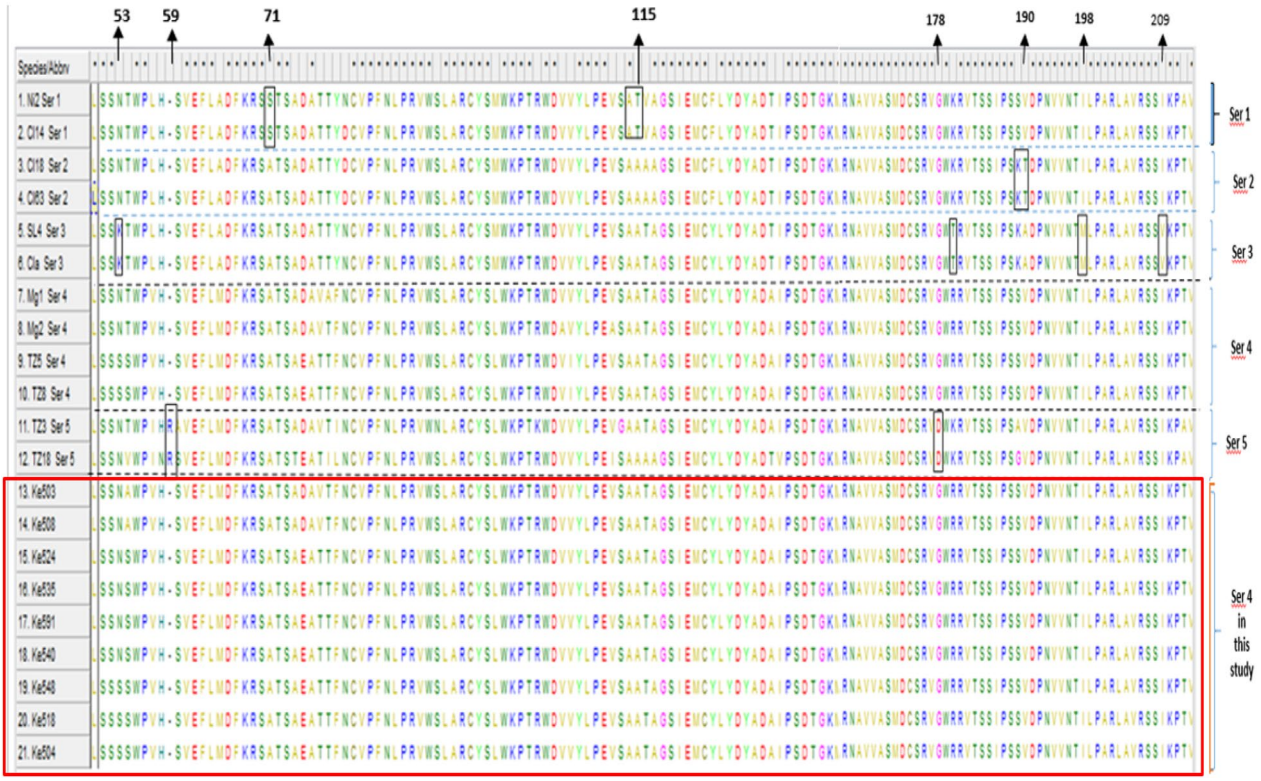

Fig. 5 Alignment of a portion of the CP gene sequence of RYMV using inferred amino acid sequences; nine (9) isolates from Kenya (marked Ser 4 in this study) belonged to serotype 4 (Ser 4). Uke et al. (20,016) used amino acid locations (framed in black) to distinguish specific serotypes (Ser 1, Ser 2, Ser 3, and Ser 5), while Ser 4 is shown in plain letters. Key: Kenya (Ke), Madagascar (Mg), Tanzania (Tz), Ivory Coast (CI), Nigeria (Ni), and Sierra Leon (SL)

postulates. The eight genotypes showed systematic symptoms of RYMV including leaf yellowing, mottling, and leaf necrosis (Additional file 1: Table S7). The genotypes showed high disease incidence and severity scores. Highly susceptible genotypes (such as IR2793-80-01) had a disease incidence of 100% and a disease severity score of 9 based on the SES scale (Additional file 1: Table S7). Basmati 370 had a disease severity score of 5 and the lowest ELISA OD value (Table 3) and was classified as moderately susceptible (Additional file 1: Table S7). The results were consistent with observations made in the field regarding RYMV symptoms. The Pinel-Galzi et al. (2018) protocol classified all the rice genotypes tested as susceptible because of their high viral loads which confirmed observations reported in the farmers' fields. The positive absorbance (optical density/OD) values in the DAS ELISA results indicated the phenotypic expression of susceptibility.

Discussion

This study examined the occurrence of RYMV in different rice cultivation regions in Kenya and assessed its diversity at both the serological and molecular levels. The detection of RYMV by RT-PCR proved to be a more sensitive and specific method for detecting RYMV than ELISA (Abrokwah et al. 2024).The present study revealed that RYMD poses a considerable risk to rice production in lowland rice ecologies. The disease appears to be more widespread in the western countries of Kisumu (Ahero, West Kano) and Busia (Bunyala), supporting earlier findings (Adego et al. 2018; Mabele et al. 2020). Our survey results further confirmed that RYMV dissemination was wider than previously thought, as it is currently found to occur in new locations such as Migori (Lower Kuja), Kirinyaga (Mwea), Tana River (Bura and Holara), and Taita Taveta. Our results strongly imply that RYMV management should be implemented in these rice-growing areas to prevent additional further spread of RYMV as stated by Uke et al. (2016) in a comparable study conducted in Uganda. Our current findings on RYMV status in Kenya confirmed the hypothesis (Pinel-Galzi et al. 2015) that RYMV is present in all African rice-growing regions and is the most significant biotic constraint (Séré et al. 2013).

RYMV was diagnosed first by visual symptom observation in the field, followed by lab-based diagnosis. Lab-based diagnosis (for instance, ELISA) had higher

Table 3 The data analysis of DAS-ELISA results conducted using two replicates per rice genotype under test, a non-inoculated genotype as control, and four replicates for the PBST control

Sample/Genotype	OD_{405nm}	OD_{cor}*	Mean OD_{cor}	Conclusion**
PBS-T control	0.039	0.00175	0	
	0.038	0.00075		
	0.037	−0.00025		
	0.035	−0.00225		
Non-inoculated control	0.346	0.30875	0.28625	Negative
	0.301	0.26375		
Komboka	1.15	1.11275	0.99625	Positive
	0.917	0.87975		
IR 64	1.163	1.12575	1.10575	Positive
	1.123	1.08575		
AT 054	1.005	0.96775	0.98625	Positive
	1.042	1.00475		
Basmati 217	0.953	0.91575	0.85625	Positive
	0.834	0.79675		
Basmati 370 (Pishori)	0.802	0.76475	0.77975	Positive
	0.832	0.79475		
BW 196	1.31	1.27275	1.20925	Positive
	1.183	1.14575		
IR2793-80–01	1.473	1.43575	1.42075	Positive
	1.443	1.40575		
Pwani Gold	1.227	1.18975	1.06475	Positive
	0.977	0.93975		
Positive control (commercial)	1.454	1.41675	1.36625	Positive
	1.353	1.31575		

* OD_{cor} corresponds to the OD_{405nm} of the sample minus the mean of OD_{405nm} of the PBST controls

** Samples are considered positive if the mean (OD_{cor}) is greater than 0.1 and twice that of the non-inoculated control (0.5725)

incidences (positivity rate) than visual symptom observation in the field for most study sites including Taita Taveta where we had 25% visual observation incidence and 65% positivity rate by ELISA. This is expected as field observation may underestimate the virus, and the virus may be present even in asymptomatic plants, as was the case for this study. Lab-based ELISA test revealed a higher positivity rate compared with field observation in all study sites except Bura and Hola. Mabele et al. (2020) obtained similar results, revealing higher incidences of lab-based diagnosis than field observation. The ELISA positivity rate was further confirmed by PCR, which is more sensitive than ELISA (Uehara-Ichiki et al. 2013). Meanwhile, for Bura and Hola, we suspect that the ELISA test was not sensitive enough for samples from this site, as most tested negative despite showing typical RYMV symptoms in the field. The viral concentration in these samples may have been low below the detectable threshold and hence failed to be detected by ELISA. Several isolates that tested negative in ELISA exhibited characteristic signs of RYMV. Issaka et al. (2012, 2021) observed a similar tendency in samples from the Niger Republic and Benin, respectively, and possibly attributed it to a low viral load in the samples.

There has been no information on RYMV in Kenya's coastal and central regions, which are known for having the largest rice production schemes and the only available data come from western Kenya (Adego et al. 2018; Mabele et al. 2020). In this study, we obtained samples from almost all rice cultivation areas in Kenya and analysed 720 nucleotides in the ORF4 of the CP gene (Pinel-galzi et al. 2009). Sequences of 37 isolates of RYMV from this study (Additional file 1: Table S6) were deposited in the NCBI GenBank. Our results indicated that RYMV strains S4ke, S4ug, and S4mg are found in Kenya, which verified prior findings indicating that RYMV strain S4 isolates are solely found in East Africa (Pinel et al. 2000). Strain S4mg previously reported in Madagascar by Rakotomalala et al. (2013) was found in Busia, Kenya. Strain S4mg is quite distant from strain S4ke and S4ug and is a collection of genetic fragments of lineages ancestral to the S4ug (Uganda) and S4Et (Ethiopia) strains created by several

recombination events (Rakotomalala et al.2013). The significant distance separating Madagascar from the African mainland suggests that the transmission mode is not the typical insect-vector-driven RYMV dispersion. The last decade has witnessed a rise in the flow of seeds between Madagascar and East African countries, including Kenya, Tanzania, and Uganda (Oyeniran 2022). Rakotomalala et al. (2019) suggested that long-distance virus transmission for instance from Madagascar to the African mainland and vice versa is aided by human activities such as trade. On the other hand, findings by Jones (2018) suggested that other plant virus transmission pathways such as contaminated seeds have been neglected yet are very important in plant virus epidemiology. With the evidence of Konate et al. (2001) and Salaudeen (2012) that RYMV is not seed-transmitted, we may link the long-distance transmission of strain S4mg to the trade and exchange of contaminated seeds (Rakotomalala et al. 2019). Abo and Sy (1997) reported that they detected RYMV in rice leaf debris and empty spikelets contaminants of rice seeds. RYMV may later be transported in rice sacks containing contaminated and partially winnowed rice seeds to distant locations, facilitating the long-distance spread of the virus (Alkali et al. 2017). This explains the possible transmission of RYMV strain S4mg from Madagascar to Kenya owing to the trade and exchange of seeds that may be contaminated as demonstrated by Rakotomalala et al. (2019).

This study identified additional strains in Bunyala Busia, namely S4ke and S4ug, which confirmed the findings of Adego et al. (2018) and made Busia the most diverse RYMV area in Kenya. The strain S4ug was first documented in Eastern Uganda by Uke et al. (2016) and Ochola et al. (2015) through extensive surveys, and its detection in Kenya verifies its spatial progression. The proximity of Eastern Uganda and Western Kenya (Busia) strongly indicates that RYMV strain S4ug crossed the border into Kenya. The East Africa lineage strain S4ke is highly prevalent in Kenya, having been detected in many regions including western, central, and coastal (Tana River) areas of the country, and has replaced strain S4lv, the very first strain of RYMV in Kenya around Lake Victoria (Abubakar et al. 2003; Pinel-galzi et al. 2009). RYMV strain S4ke is a recombination event between S4lv and S4ug (Adego et al. 2018) and appears to be aggressive as it has replaced S4lv in Kenya.

Regression of root-to-tip genetic distance versus sampling time served as a basic diagnostic instrument for molecular clock models (Rambaut et al. 2016). Clockor2 web-based tool enabled the application of Root-to-Tip (RTT) regression in temporal signal analysis. This allowed for the fitting of global and local clocks, particularly to RYMV strains, indicating clockwise evolution (Featherstone et al. 2024). The dataset exhibited a positive connection between genetic divergence and sampling time on a global scale. This dataset seemed appropriate for conducting phylogenetic molecular clock analysis using BEAST, with an R^2 value of 0.027 (Rambaut et al. 2016). Temporal signal was observed in the RYMV sub-strain S4ke dataset at a correlation coefficient of $R^2 = 0.002$, however, it was not observed in other sub-strains such as S4mg, S4ug, and S4lv. The most suitable fit was deduced using a solitary universal clock, as it provides the most economical model of the evolutionary rate of a tree (Featherstone et al.2024).

The study of differences in the amino acid sequences of the RYMV isolates created serological profiles and made it easier to find amino acid patterns specific to each serotype. There was a clear separation of isolates in terms of serotypes in the Parsimony phylogenetic analysis which allowed the identification of specific serotypes as the isolates grouped into West African isolates and East African isolates confirming earlier studies by Hebrard et al. (2005). Serotypes 1–3 are found in West Africa while serotypes 4 and 5 were found in East Africa (Pinel et al. 2000). This investigation categorized the novel 2023 RYMV isolates as serotype 4 (Ser 4) for the first time, utilizing the amino acid alignment rather than monoclonal antibodies (MAbs), which exhibited indications of conservation (Fig. 5). The serotype 1 isolates were identified by the presence of the amino acid serine at position 71 (S71) and threonine at position 115 (T115). In contrast, serotype 2 isolates had a Threonine at position 190 (T190). For isolates of serotype 3, they have peptide substitution of Arginine (R), Threonine (T), and Valine (V) at positions 53, 180, and 209 respectively, and were the most diverse. Lastly, serotype 5 had a peptide substitution Arginine (R59) and Aspartic acid (D178) which agreed with the findings of Hebrard et al. (2005). The antibodies used for ELISA analysis were raised from a Madagascan isolate, Mg1 (serotype 4) which explains why DAS ELISA worked well for our experiment.

Survey data revealed that the rice genotypes IR2798-80–01 and Komboka are the popular genotypes in Kenya. Farmers in Kenya's coastal region, specifically those in the Tana River and Taita Taveta, prefer the Komboka rice genotype. In central Kenya (Mwea), the Basmati 370 rice genotype still dominates, but farmers in these regions are gradually adopting Komboka. Basmati 370 genotype is popular in Mwea because of its good aroma and high market value (Atera et al.2018). Komboka was introduced in Kenya in 2013 by the Kenya Agricultural Research Organization (KALRO) and the International Rice Research Institute (IRRI). The fast adoption of the Komboka genotype may be attributed to its high productivity, semi-aromatic nature, strong tiller production ability, and

superior grain quality (Ng'endo et al. 2022). The genotype IR2793-80–01 was found to be popular in the western part of Kenya confirming the previous studies by Mabele et al. (2020) in the Ahero irrigation Scheme. According to our findings, all commonly farmer-preferred rice genotypes were susceptible to RYMV, as evidenced by both field surveys and greenhouse experiments conducted in this study. Eight rice genotypes were assessed for resistance to RYMV using mechanical RYMV inoculation in a greenhouse setting. Inoculated rice genotypes were evaluated based on the manifestation of RYMV symptoms and the ELISA test. All genotypes exhibited characteristic RYMV symptoms, which were validated by the ELISA test. All the rice genotypes tested were susceptible to the disease, and almost all had disease severity scores of up to 9. This shows that mechanically inoculating RYMV is very effective (Pinel-Galzi et al. 2018). It is imperative to develop rice varieties resistant to RYMD to minimize losses (Onasanya et al. 2004). The results of screening rice genotypes for RYMV resistance are consistent with earlier studies, which revealed a correlation between the presence of symptoms and an increase in ELISA absorbance values in infected leaves throughout the entire system (Albar et al. 1998; Ndjiondjop et al. 1999). The screening of rice genotypes Basmati 370 and Basmati 217 for resistance to RYMV in Uganda, conducted by Odongo et al. (2019), yielded comparable outcomes, with both genotypes exhibiting a significant susceptibility to the virus (Odongo et al. 2019). The transmission of this virus in Kenya could be attributed to farmers' agricultural practices, including the ongoing cultivation of susceptible genotypes and the practice of ratoon farming.

Conclusions

This study provided data on the prevalence of RYMD in Kenya, revealing that the disease is currently present in virtually all major rice cultivation regions of the country and we also document the first report of RYMD occurrence in Kirinyaga, Migori, Taita Taveta and Tana River, and Migori counties of Kenya. Farmers' preference for susceptible rice genotypes may have facilitated the spread of the RYMV. From this research, we confirm that the RYMV strains S4ke, S4ug, and S4mg, which belong to the East Africa lineage, are present in Kenya. The discovery of several RYMV strains in Kenya substantiates Bakker's (1974) hypothesis that Kenya is the origin of RYMV. It is critical to educate farmers about the basic RYMV transmission means in integrated efforts to curb its spread. The widespread occurrence and rapid mutation of this virus require proactive strategies to manage RYMD transmission. All popular rice genotypes cultivated in Kenya were susceptible to RYMV infection, highlighting the urgent need to develop and deploy resistant genotypes in areas with high disease incidences.

Methods

Sample collection and disease survey

A field survey was carried out between May and July 2023 to collect samples and evaluate the occurrence of RYMD in the main rice-growing counties in Kenya (Fig. 4). The target areas were in the following counties: Busia (Bunyala), Kirinyaga (Mwea), Kisumu (Ahero, West Kano), Migori (Lower Kuja), Taita Taveta (Majengo, Burma, and Kimorigo) and Tana River (Hola, Bura) (GOK-NRDS 2020). The areas were predominantly irrigated lowland ecology fields, accounting for 80% of rice cultivation in Kenya (Kega et al. 2015). We documented disease incidences and severity data, GPS coordinates at each leaf sample collection point, and details on farm sizes and the rice genotype cultivated in each survey field. Leaves exhibiting symptoms of both symptomatic and asymptomatic leaf samples were collected from each rice field visited. The samples were stored in khaki bags in a cooler box before being shipped to the laboratory for analysis.

Detection of RYMV by enzyme-linked immunosorbent assay (ELISA)

Leaf samples of approximately 0.1 g were crushed with 1 ml of phosphate-buffered saline with 0.5% Tween-20 (PBST 1X) in a 1.5-ml tube at a ratio of 1:10 (weight to volume), using a Qiagen TissueLyser II. A double antibody sandwich ELISA (DAS-ELISA) test was performed using antisera against RYMV using an ELISA kit supplied by Sediag SAS, France. The experiment followed the protocol as described by Pinel et al. (2000). Duplicate wells were utilized for each sample, and the absorbance was measured at a wavelength of 405 nm using a spectrophotometer after incubation periods of 1 h and 2 h, respectively (Uke et al. 2016). The results were validated using both positive and negative controls. The BioTek ELx808 Ultra Microplate Reader was used to measure the absorbance values and determine the viral titers, as described by Hubert et al. (2017). The intensity of the reaction was classified as strong positive (+++), mild (++), or weak (+) based on the absorbance value when absorbance value ≥ 4 times, $3 \leq 4$, $3 \leq 2.5$ or $2 \leq 2.5$, respectively of healthy control samples (Alkali et al. 2015).

RNA extraction and reverse transcription -PCR detection of RYMV pathogen

Following the manufacturer's instructions, we extracted total RNA from about 100 mg of the freeze-dried samples using a GeneJET plant RNA purification kit (Cat. No. K0801, Thermo Fisher Scientific, USA). The quality and concentration of extracted RNA were assessed using

a NanoDrop spectrophotometer. RYMV Coat Protein (CP) gene-specific primers Forward 5' CGCTCAACA TCCTTTTCAGGGTAG 3' and Reverse 5' CAAAGA TGGCCAGGAA 3' were used to amplify the RYMV CP gene and a 720 bp PCR product was obtained (Pinel et al. 2000; Longué et al. 2014). The cDNA synthesis and PCR amplification steps were combined into a single reaction using the OneTaq® One-Step RT-PCR Kit (New England Biolabs, USA). A total of 1 µg of total genomic RNA was added to 25 µL of OneTaq One-Step Reaction Mix (2X), 2 µL OneTaq One-Step Enzyme Mix (25X), 2 µL each of Gene-specific Forward and Reverse Primer (10 µM) and 17 µL nuclease-free water. The PCR mixture was incubated in a thermocycler with the following amplification conditions: reverse transcription at 48°C for 15 min, first denaturation at 94°C for 1 min, denaturation at 94°C for 15 s, annealing at 52°C for 30 s, extension at 68°C for 1 min, and final extension at 68°C for 5 min. The Biometra TRIO 48 thermal cycler (Analytik Jena, Germany) was utilized for RT-PCR. The PCR products were visualized in 1% agarose gel electrophoresis stained with SYBR Safe in a 1×Tris–acetate-Ethylenediaminetetraacetic acid (TAE) buffer for 30 min at 100 V (Mabele et al. 2020).

Sanger sequencing of the RYMV coat protein gene

We selected isolates from RT-PCR results for further sequencing analysis. A total of 55 isolates were selected based on positive PCR results and geographical distribution to ensure the representation of all rice schemes and geographical regions. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) following the manufacturer's protocol. The purified products were sequenced directly using the Sanger chain termination method outsourced to Segolip company, based at the International Livestock Research Institute (ILRI), Nairobi. Sequencing was done using the primers (forward 5' CGCTCAACATCCTTTTCAGGGTAG 3' and reverse 5' CAAAGATGGCCAGGAA 3') with an Applied Biosystems genetic analyzer. Nucleotide sequences were trimmed and cleaned using Unified Genome Analysis Environment (UGENE) version 49.1 and Bioedit Version 7.7.1. The 37 RYMV sequences (Additional file 1: Table S8) of CP gene (720 bp) obtained through the Sanger technique were deposited in the NCBI GenBank (Accession numbers PP944536 to PP944572).

Genetic diversity and phylogenetic analysis

Basic Local Alignment Search (BLAST) was used in the sequence homology search to obtain library reference sequences (Altschul et al. 1990). BLASTN analysis tool was used to find CP sequences of RYMV isolates (Additional file 1: Table S5), which were subsequently utilized in the phylogenetic analysis (Ramathani et al. 2023). The 37 CP sequences (Additional file 1: Table S8) obtained in this study were compared with 25 sequences available in the NCBI database (Additional file 1: Table S4), which included sequences from the main rice-growing countries in East Africa (Pinel et al. 2000). Multiple sequence alignment was performed using Clustal W with default parameters (Uke et al. 2016). Phylogenetic tree was inferred using the Neighbor-joining method with Molecular Evolutionary Genetic Analysis 11 (MEGA 11) software using the substitution model, Tamura 3-parameter model, and 1000 bootstrapping replications (Hubert et al. 2017). Genetic diversity of the CP sequences was assessed using MEGA 11's best-fitted nucleotide substitution model with 1000 replicate bootstraps. Isolates that were sequenced and geo-referenced (Additional file 1: Table S2). DNASTAR's MegaAlign Pro 17 was utilized to evaluate the genetic percentage identity between selected representatives of Kenyan 2023 RYMV isolates and other isolates from Eastern Africa.

Testing the temporal signal of RYMV isolates

We used an exploratory linear regression technique to evaluate the degree of temporal signal in the CP gene of the RYMV isolates in Kenya (Featherstone et al. 2024). Based on the Neighbour-joining tree reconstructed with the coat protein gene dataset, the temporal signal was visualized. We plotted the Root-To-Tip (RTT) genetic distance of Kenyan RYMV isolates against the sampling time (1966–2023) for phylogenies estimated from the alignment of 50 RYMV isolates. First, we imported a rooted tree containing RYMV isolates collected in Kenya between 1966 and 2023. Sampling dates in years were then parsed from tip labels (Rambaut et al. 2016). Clockor2 tool (https://clockor2.github.io/) was used to identify the most suitable root by evaluating the R^2 value or Residual Mean Square (RMS) of the Root-To-Tip (RTT) regression (Featherstone et al. 2024).

Serotyping isolates using amino acid sequences

Clustal W algorithm for sequence alignment was used to align serotypes for amino acids inferred from nine (9) representative RYMV isolates following the Uke et al. (2016) method. The CP gene amino acid sequences of 12 isolates (Additional file 1: Table S6) representing five serotypes were obtained from the NCBI database. These sequences were compared with nine (9) Kenyan RYMV isolates in the current study (Uke et al. 2015). The phylogenetic tree of amino acids (Additional file 2: Figure S2) was inferred using the maximum parsimony algorithm (Hebrard et al. 2005).

Validation of RYMV incidences observed in various rice genotypes in the field

To verify the RYMV occurrence on different genotypes in the field during the survey, a screening experiment was established in a greenhouse at Jomo Kenyatta University of Agriculture and Technology. Rice seeds of farmer-preferred rice genotypes (Komboka, Basmati 217, Basmati 370, IR2793-80–01, BW 196, AT 054, and Pwani Gold) were grown in planting containers replicated thrice. We included an additional set as an un-inoculated control. We used the IR64 rice genotype as an RYMV susceptible reference (Arra et al. 2024). An RYMV isolate from Kisumu was propagated on a rice susceptible genotype (IR64) and used for mechanical inoculation of the genotypes. Mechanical inoculation was performed on two-week-old seedlings following the Pinel-Galzi et al. (2018) protocol. We observed symptom development and disease progression for each rice genotype from 2 days post-inoculation (2dpi) to 4 weeks after- inoculation (4wai). A Standard Evaluation Scale for rice (IRRI 2013) was used for severity scoring based on RYMV symptoms as described by Ndikuryayo et al. (2020) protocol. We estimated the disease incidence for each genotype using the following formula:

$$\mathrm{DI}\ (\%) = \frac{Npi}{Npt} \times 100$$

where DI = disease incidence percentage, Npi = Number of infected plants, and Npt = Number of inoculated plants according to Sereme et al. (2016) method. The resistance and susceptibility of rice genotypes were assessed using symptom scoring and viral concentration through DAS ELISA (Ndjiondjop et al. 1999). Data analysis was performed using the optical density (OD) measurements at 405 nm. For the DAS ELISA test, each sample was duplicated, while the PBST control was repeated four times (Pinel-Galzi et al. 2018).

Abbreviations

ANOVA	Analysis of variance
BLAST	Basic local alignment search tool
bp	Base pair
CP	Coat protein
DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
GPS	Global positioning system
IRRI	International Rice Research Institute
KALRO	Kenya Agricultural and Livestock Research Organization
MEGA	Molecular evolutionary genetics analysis
NCBI	National Centre for Biotechnology Information
OD	Optical density
ORF	Open reading frame
PBST	Phosphate buffered saline with tween 20
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
RTT	Root-to-tip
RYM	Rice yellow mottle disease
RYMV	Rice yellow mottle virus
SES	Standard evaluation scale

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-025-00329-1.

Additional file1. Table S1. List of rice varieties preferred by farmers in Kenya. **Table S2.** RYMV representative isolates, rice scheme where they were collected, and county representative of the geographical distribution during the 2023 study of RYMV in Kenya. **Table S3.** Data on the number of samples collected, and analyzed as well as disease incidence and Severity. **Table S4.** Genetic distance and identity calculation in RYMV isolates using DNAstar MegaAlign Pro software. **Table S5.** Name, sampling year, and gene accession number of the 21 isolates obtained from NCBI belonging to Rice Yellow Mottle virus collected during a 54-year span in five African nations. **Table S6.** Isolates of Rice yellow mottle virus utilized in the amino acid sequence analysis for serotyping. **Table S7.** Disease severity and incidences for genotypes inoculated with RYMV under greenhouse conditions. **Table S8.** Accession numbers of 37 isolates of RYMV under this study that have been deposited in the NCBI GenBank.

Additional file2. Figure S1. RT-PCR detection of RYMV in diseased plants giving the expected amplicon size of 720 bp. **Figure S2.** Maximum parsimony tree showing the relationship of selected Nine (9) Kenyan isolates (Ke500 Series) in this study with other serotypes reported in Kenya (Ke), Madagascar (Mg), Tanzania (Tz), Malawi (Mw), Uganda (Ug), Rwanda (Rw), Burundi (Bu), Ivory Coast (CI), Nigeria (Ni), and Sierra Leon (SL) constructed from the coat protein gene amino acid sequences.

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Author contributions

AK, SR, RM, EMN, HRP, and EMA formulated and designed the experiments. AK and EMN conducted survey studies and lab-work analysis. AK and HRP wrote the original manuscript draft and performed the phylogenetic analysis. Each author read and gave comments and suggestions for improvement of the final manuscript.

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Availability of data and materials

A dataset of 37 isolates of RYMV geo-referenced sequences has been deposited in GenBank under the accession numbers PP944536 to PP944572.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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