

Characterization of *Rice yellow mottle virus* in north-eastern Tanzania

By

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Summary : Rice (*Oryza sativa* L.) is an important cereal-grain in some African countries and known to be attacked by *Rice yellow mottle virus* (RYMV). Rice plants with virus like symptoms were collected from lower Moshi and other areas in the north-eastern region of Tanzania. RYMV was detected by DAS-ELISA, RT-PCR and/or electron microscopy from almost all collected samples. Some RYMV isolates were sequenced and identified as strain S4. Also, three of them, Tz-12-20, Tz-12-22 and Tz-10-36, were clustered as a new group named S4-mk (Mt. Kilimanjaro). Cluster of S4-mk formed a monophyletic group of isolates in strain S4-lv reported from the Tanzanian side of the Lake Victoria. However, S4-mk and S4-lv lineages are separated from each other and S4-mk has the characteristic three amino acid substitutions. The isolates of S4-mk were also closely related to that of strain S4-mg. Amino acid sequences of the coat protein of the isolates in group S4-mk showed over 96% identity with those in other strain S4 varieties, S4-lv, S4-mg and S4-lm. The phylogenetic tree also indicated the possibility of strain S4-mk to have dispersed from south-western Kenya to north eastern Tanzania. As for the serotyping, followed by FARGETTE *et al.* (2002), these three isolates in S4-mk were classified into serotype 4.

Key words : Phylogenetic analysis, Rice, *Rice yellow mottle virus* (RYMV), Serotype, Tanzania

Introduction

Rice (*Oryza sativa* L.) is an important cereal crop and staple food at least in 17 countries in the Asia-Pacific Region and 8 countries in Africa, supplying about 20% of total calorie intake in developing countries¹⁾. The crop is estimated to feed about 3.5 billion people maintaining their livelihood by rice farming and allied businesses²⁾.

Rice blast caused by *Pyricularia oryzae* and rice yellow mottle disease (RYMD) caused by *Rice yellow mottle virus* (RYMV) are the most devastating diseases in sub Saharan Africa, presently^{3, 4)}. RYMV, in particular, poses a serious threat due to its serious damage and rapid spread. RYMV is transmitted by insect vectors such as *Chaetocnema* and *Chrysispa* spp, via virus contaminated soil and water, and by contact with RYMV-infected rice plants⁵⁾. Rice plants infected with RYMV suffer mottling,

yellowing and stunted growth and possible total yield loss depending on the cultivar, age of plant at the time of infection and rice growing ecosystems : paddy or upland⁶⁾.

In Tanzania, RYMV was first reported in 1982⁷⁾. Since then, surveys have been carried out throughout the country to explore distribution and characteristics of the virus^{8, 9)}. Upon these surveys, Tanzanian RYMV-isolates were serologically characterized into two serotype (Ser)s : Ser 4 and Ser 5 by monoclonal antibodies. Later, the isolates were molecularly classified into three strains named S4, S5 and S6. The geographical distribution of Tanzanian RYMV serotypes and strains are documented by KANYEKA *et al.* (2007). Despite all the surveys already conducted, information regarding the prevalence, characteristics and epidemiology of RYMV is still needed for successful management of the virus, especially in recently infested areas such as the Lower Moshi irrigation scheme

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(LMIS), the most advanced and productive lowland rice production area in north-eastern Tanzania and neighboring area.

LMIS was established in 1987 mainly with a Japanese ODA loan. Regrettably, some of the rice production in Lower Moshi is now seriously threatened due to the emergence of a new disease that typically shows yellowing and stunting symptoms. Our preliminary study, Mgonja *et al.* (2010) identified the pathogen of this disease in this area as RYMV by RT-PCR.

In this study, further identification, serotyping and molecular characterization of RYMV in LMIS and other rice production areas of the north-east in Tanzania were conducted.

Materials and methods

Disease survey and sample collection

Three areas in LMIS (Upper Mabogini, Kikwatani and Rau Kati), four nearby areas (Mandaka, Kaloleni, Majani Mapana) and Kilimanjaro Agricultural Training Center in Lower Moshi were surveyed in 2010 and Lower Moshi and other five rice-production area (Musa Mwijanga, Ndungu, Mombo, Mhande and Leki Tatu) were surveyed in 2012. All of these areas surveyed in this study are located in north-eastern Tanzania. The areas represent irrigated-lowland ecology, which is more prone to RYMV infections compared to other ecosystems¹¹. By the surveys guided by local rice producers, farmers and/or researchers, a total of 87 samples, mostly with RYMD symptoms, were collected and saved as dried or frozen leaves. Among them, 38 samples were further tested (Table 1).

Serological detection by ELISA using polyclonal antibody

Based on field observations and the preliminary results of inoculation experiment, *Rice yellow mottle virus* (RYMV) was suspected as the causal pathogen. Accordingly, DAS-ELISA was carried out on collected samples with antisera against RYMV in ELISA kit from NEOGEN Europe Ltd., Scotland, UK, following the company's protocol. Test wells were observed for color changes at 60 and 120 minutes of incubation and absorbance at 405 nm was measured. Samples with absorbance value at least two times greater than that of corresponding negative control (healthy rice leaf) were considered positive.

Molecular detection and sequencing

RT-PCR was carried out on some selected samples for further confirmation of RYMV infection. Total RNA was extracted from fresh or frozen leaf samples using TRIzol[®] Reagent (Invitrogen, NY, USA). First-strand cDNA was

synthesized using ReverTra Ace- α [®] kit (TOYOBO, Osaka, Japan) and specific reverse primer. After the reaction, the first-strand cDNA was amplified by PCR using TaKaRa Ex[™] kit (TaKaRa, Otsu, Japan) according to the protocol. Primers: 3PRYF (A3) 5'-CAAGATGGC-CAGGAA-3' and 3PRYR (B3) 5'-GTGGGTGCCATTCCCC-ACATGG-3' that amplify the CP gene (720 bp) and 3' untranslated region (3'UTR) of RYMV¹² were used. Although the 3PRYF primer is a CP forward primer, the 3PRYF primer's sequence overlapped with RYMV coat protein (CP) gene, thus a new primer, A'3, 5'-GTTGTGG-TCAGACCTCGCG-3', was designed. Amplification was performed under initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min and then a final extension at 72°C for 10 min. Some of the PCR products were purified and cloned into the plasmid. Recombinant plasmids were transformed into competent *Escherichia coli* strain DH5 α cells (Invitrogen, NY, USA), and five clones of each of the isolates were sequenced.

Phylogenetic analysis

Nucleotides (720 bp) and their amino acid sequences of CP of 33 RYMV isolates presented in Table 2 were retrieved from the NCBI-GenBank.

Trees were constructed by Neighbor joining method. Confidence in clades from the resulting topology was tested by analyzing 1,000 bootstrap replications.

Serotype

RYMV amino acid sequences of 14 isolates representative of the five serotypes were retrieved from GenBank provided by the NCBI. These sequences are the ones described by FARGETTE *et al.* (2002). Then, sequences were aligned together with three Tanzanian isolates in this study using Clustal W. Lastly, alignments were double checked by BioEdit software.

Electron microscopy

Transmission electron microscope (Hitachi E-102 TEM, Japan) was used to examine a leaf sample (Tz-10-36) for morphology of the pathogen by phosphate-tungstic acid (PTA) staining.

Results

Field observations

RYMD symptoms were observed in almost all surveyed areas in this study (Table 1), although the severity varied considerably among the schemes/fields in surveys in 2010 and 2012. In the second survey in 2012, the incidences of RYMD appeared to have intensified in Upper Mobogini and Duma areas of Lower Moshi and Mahande

Table 1 Detection of RYMV from selected samples by ELISA and RT-PCR.

Sample code ^a	Site of collection	Cultivar	Symptom (s) ^b	ELISA ^c	RT-PCR ^d
Tz-10-11	Lower Moshi (Upper Mabogini)	Unknown	Y, M, St.	+	+
Tz-10-15	Lower Moshi (Upper Mabogini)	IR64	Y, M	+	+
Tz-10-28	Lower Moshi (Upper Mabogini)	IR64	Y, M	+	nt.
Tz-10-31	Lower Moshi (Upper Mabogini)	IR64	Y, M	+	nt.
Tz-10-4	Lower Moshi (Kikwateni)	TXD306	Y, M	+	+
Tz-10-34	Lower Moshi (Kikwateni)	TXD306	Y, M, St.	+	nt.
Tz-10-36	Lower Moshi (Rau Kati)	TXD306	Y, M, St.	+	+
Tz-10-56	Lower Moshi (Rau Kati)	TXD306	Y, M, St.	+	nt.
T-10-24	Lower Moshi (Rau Kati)	Unknown	Y, M	+	nt.
Tz-10-37	Lower Moshi (Majani Mapana)	TXD306	Y, M	+	nt.
Tz-10-38	Lower Moshi (Kaloleni)	TXD306	Y, M, St.	+	+
Tz-10-3	Lower Moshi (Mandaka)	Unknown	Y	+	nt.
Tz-10-57	Lower Moshi (Mandaka)	IR64	Y, M	+	nt.
Tz-10-58	Lower Moshi (KATC)	IR64	Y, M, St.	+	nt.
Tz-10-54	Mombo	Local	Y, M	+	nt.
Tz-10-55	Mombo	Unknown	Y, M	+	nt.
Tz-10-52	Ndungu	Unknown	Y, M	+	nt.
Tz-10-53	Ndungu	Unknown	Y, M, St.	+	nt.
Tz-10-43	Leki Tatu	Unknown	Y, St.	-	nt.
Tz-10-15	Leki Tatu	Unknown	Y, St.	-	nt.
Tz-10-46	Leki Tatu	Unknown	Y	-	nt.
Tz-10-49	Mahande	Kihogo	Y,M	+	nt.
Tz-10-50	Mahande	Unknown	Y, M, St.	+	nt.
Tz-12-7	Lower Moshi	IR64	Symptomless	-	nt.
Tz-12-8	Lower Moshi	IR64	Symptomless	-	nt.
Tz-12-9	Lower Moshi	IR64	Y, M, St.	+	nt.
Tz-12-10	Mombo	Unknown	Y	-	nt.
Tz-12-11	Ndungu	Unknown	Y	-	nt.
Tz-12-12	Ndungu	IR54	Y, M, St.	+	nt.
Tz-12-13	Ndungu	IR54	Y, M	+	nt.
Tz-12-15	Ndungu	Unknown	Y, M	+	nt.
Tz-12-16	Leki Tatu	Unknown	Y	-	nt.
Tz-12-17	Leki Tatu	Unknown	Y	-	nt.
Tz-12-18	Leki Tatu	Unknown	Y	-	nt.
Tz-12-19	Mahande	TXD306	M	+	nt.
Tz-12-20	Mahande	Kihogo	Y, M, St.	+	+
Tz-12-21	Mahande	Unknown	Y, M	+	nt.
Tz-12-22	Musa Mwijanga	IR64	Y, M, St.	+	+

^aTz-10 and Tz-12 represent samples collected in 2010 and 2012, respectively. ^bY: yellowing, M: mottling, St.: stunting, ^c+: positive, -: negative, ^dnt.: not tested.

irrigation schemes, compared to the situation in the first survey in 2010. On the other hand, Mombo appeared to have recovered from serious RYMD because no RYMD symptoms were observed during the second survey, although this area was found to be infested in the first survey (Table 1). Moreover the popular varieties in Lower Moshi, IR64 and TXD306, showed severe RYMD symptoms (data not shown).

Detection

By ELISA, RYMV was detected in 28 out of 38 tested

samples, confirming the virus incidence in five surveyed area excluding Leki Tatu. In addition, DNA of selected samples from Lower Moshi, including Tz-10-15 and Tz-10-36 from Upper Mabogini and Rau Kati respectively, was amplified with the expected size of 1,400 bp by RT-PCR confirming the presence of CP gene and 3'UTR of RYMV genome in the samples. Electron microscopic observation of infected leaf-sap of a sample (Tz-10-36) revealed abundant spherical particles with a diameter of about 28 nm (data not shown).

Molecular analysis and topology

The PCR products of three isolates, Tz-12-20 from Mahande, Tz-12-22 from Musa Mwijanga and Tz-10-36 from Lower Moshi, which was collected 2 years earlier than above mentioned 2 isolates, were cloned and sequenced. Based on the molecular characterization of RYMV following PINEL *et al.* (2009), these three isolates were identified as strain S4 (Fig. 1). They appeared to belong to a monophyletic group of isolates different from isolates in S4-lv¹⁴ collected in the Lake Victoria area of the Tanzanian side and named as S4-mk because they are from areas at the foot of Mt. Kilimanjaro. The isolates of S4-mk were also closely related to those of S4-mg (Fig. 1). More precisely, three isolates representing

S4-mk, Tz-12-20, Tz-12-22 and Tz-10-36, have characteristic difference from S4-lv and another variant of the strain S4 : S4-mk with substitutions of I₁₀₇→V, S₁₆₁→N, and T₁₆₄→L (Fig. 2).

Molecular variability was assessed with amino acid sequences since they are more conserved and hence highly informative about gene evolution than nucleotides¹⁵. Accordingly, divergence between the isolates of S4-mk and other strain S4 groups : S4-lv, S4-mg (Madagascar) and S4-lm (Lake Malawi) were computed. The nucleotide sequences of the three isolates in S4-mk from Tanzania in this study showed high homology (99.1%~99.5%) (data not shown). Also, homology between the three isolates from Tanzania in this study and 37 isolates in strain S4

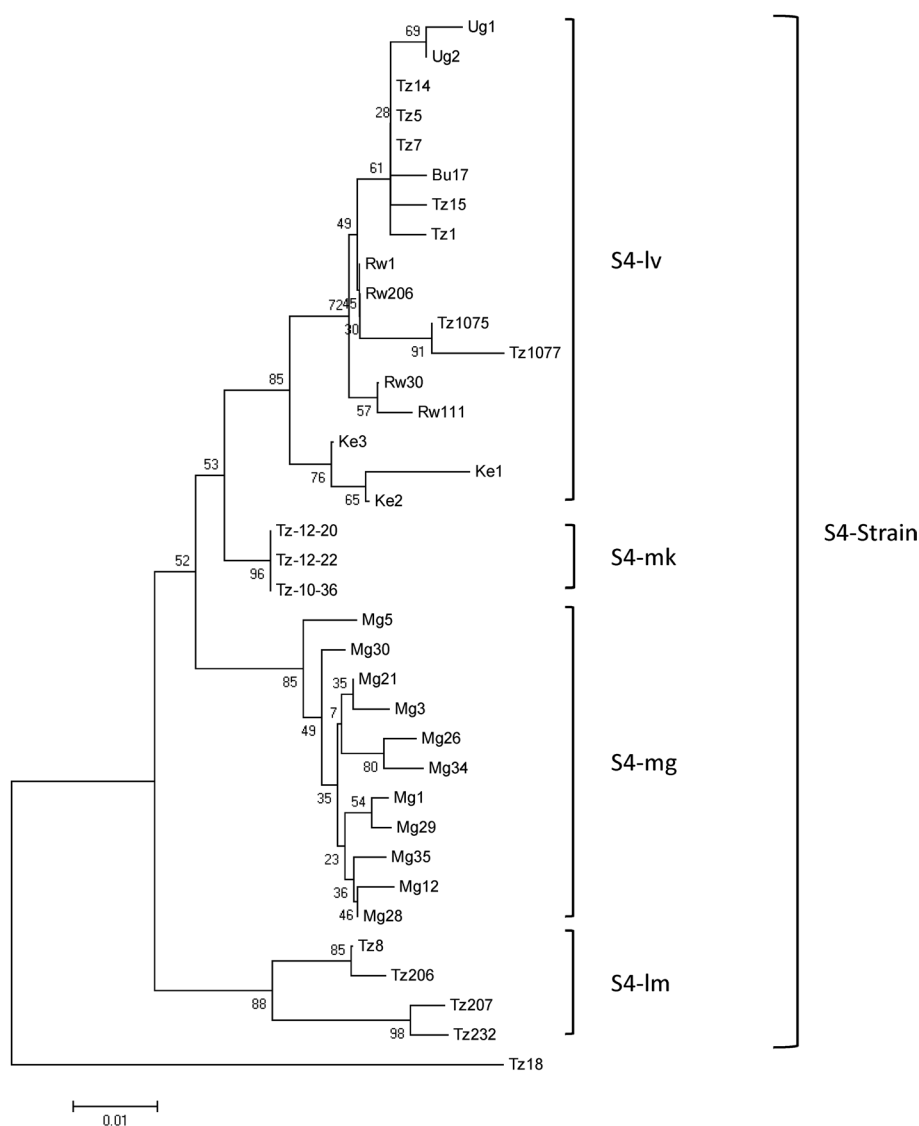


Fig. 1 Neighbor-joining (NJ) tree depicting the relationships of RYMV isolates collected from Tanzania with 33 isolates reported in Africa constructed from the amino acid sequences of coat protein gene. S4-lv : S4 Lake Victoria, S4-mk : S4 Mount Kilimanjaro, S4-mg : S4 Madagascar, and S4-lm : S4 Lake Malawi. An isolate Tz18 of strain S5 was used as an out group. The tree shows S4-mk (in this study) the fourth sub-clade of S4 strain.

	100	110	120	130	140	150	160	170	
Tz-12-20	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	INGGTLARNAV	
Tz-12-22	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	INGGTLARNAV	S4-mk
Tz-10-36	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	INGGTLARNAV	
Bu17	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Tz1	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Tz15	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Tz1077	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Ke1	WSFGXCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	S4-lv
Ke2	WSFARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Rw1	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Rw111	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Ug1	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVSSS	VWYGAEGCHL	ISGGTARNAV	
Ug2	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Tz8	WSLARCYSMW	KPTQWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Tz206	WSLARCYSMW	KPTQWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	S4-lm
Tz207	WNLARCYSMW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Tz232	WNLARCYSMW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	ISGGTARNAV	
Mg1	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	INGGTSARNAV	S4-mg
Mg3	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	INGGTSARNAV	
Mg12	WSLARCYSLW	KPTRWDVYVL	PEVSAATAGS	IEMCYLYDYA	DAIPSDTGKM	SRTAGFVTSS	VWYGAEGCHL	INGGTSARNAV	

Fig. 2 Comparison of the amino acid sequences of S4 groups between S4-mk, plane box, and S4-lv, S4-lm, and S4 mg, dotted box. Numbers reflect the amino acid position in the coat protein.

groups (Table 2) from GenBank was analyzed and it was found that Tz-10-36, Tz-12-20 and Tz-12-22 showed over 96% identity to the isolates of RYMV from other strain S4 groups at the amino acid (aa) level (Table 3). Furthermore, the divergences among all S4-lv isolates were small (1.7% to 3.8%).

Serotype

The comparison of amino acid sequences indicated that isolates Tz-10-36, Tz-12-20 and Tz-12-22 from Lower Moshi, Mhande and Musa Mwijanga respectively, belonged to serotype 4 (Ser 4) isolates described by FARGETTE *et al.* (2002) (Fig. 3). However, this is the first serotyping of isolates from the north-eastern region of Tanzania not by monoclonal antibodies but by amino acid sequences.

Discussion

The present study has revealed the disease by RYMV as a serious threat to rice production in Lower Moshi, the most advanced and productive lowland in Tanzania. This rice-disease, which has emerged as recently as 2008 by observation of farmers (OIZUMI, unpublished data), is now seriously devastating in this area. Preliminary results by one of the co-authors indicated that the disease is mechanically and contact transmissible to rice varieties of IR64 and TXD306, the widely cultivated varieties in Lower Moshi (OIZUMI, unpublished data). In this study, the pathogen was confirmed as RYMV by a few different and reliable measures such as ELISA, RT-PCR and electron microscopy firstly in this area.

So far, ELISA is the most reliable serological method for identification of plant viruses, and viral protein can be credibly detected through the assay using specific antibodies. With RYMV-antisera from NEOGEN, this test detected RYMV in almost all the Lower Moshi and north-eastern area samples tested. Likewise, PCR is now the most powerful molecular diagnostic method and RT-PCR tests amplified the DNA products of some RYMV isolates from Lower Moshi and north-eastern area to the expected 1,400 bp indicating that RYMV-genome was present in rice plants. As the conclusion, RYMV was present in Lower Moshi, Ndungu, Mombo, Mahande, and Musa Mwijanga, except Leki Tatu. This strongly suggests that RYMV control should be conducted in these promising rice production areas to combat further infection. The abundance of rice-ratoons in Upper Mabogini, Mandaka and Kaloleni, especially during off seasons, may play a significant role in RYMV-epidemics in Lower Moshi. In these areas, paddy fields remain extremely wet during off seasons, mainly because of poor management of irrigation water. Such watery soil condition supports the growth of ratoons and ratoons may serve as a potential reservoir for RYMV and primary source of infections for the next crop.

SARRA (2005) and TRAORÉ *et al.* (2006) demonstrated that transplanting RYMV-infected seedlings can considerably amplify the virus infections in the paddy fields. However, it has been a common practice in some parts of Lower Moshi to set up rice seedbed beside the paddy fields. This practice greatly exposes the seedlings to RYMV infections. This may be one of the reasons for serious

Table 2 List of RYMV sequences used in phylogenetic tree, Fig. 1 and Fig. 2.

Isolate	Strain	Country of origin	Reference	Accession no.
Bu 17	S4-lv	Burundi	Ndikumana <i>et al.</i> , 2012	HE654717
Ke 1	S4-lv	Kenya	Abubakar <i>et al.</i> , 2003	AJ511805
Ke 2	S4-lv	Kenya	Traoré <i>et al.</i> , 2005	AJ885128
Ke 3	S4-lv	Kenya	Fargette <i>et al.</i> , 2008	AM931178
Mg 1	S4-mg	Madagascar	Fargette <i>et al.</i> , 2004	AJ608210
Mg 3	S4-mg	Madagascar	Pinel <i>et al.</i> , 2000	AJ279923
Mg 5	S4-mg	Madagascar	Pinel <i>et al.</i> , 2000	AJ279925
Mg 12	S4-mg	Madagascar	Fargette <i>et al.</i> , 2008	AM931192
Mg 21	S4-mg	Madagascar	Fargette <i>et al.</i> , 2008	AM931200
Mg 26	S4-mg	Madagascar	Fargette <i>et al.</i> , 2008	AM931205
Mg 28	S4-mg	Madagascar	Fargette <i>et al.</i> , 2008	AM931207
Mg 29	S4-mg	Madagascar	Fargette <i>et al.</i> , 2008	AM931208
Mg 30	S4-mg	Madagascar	Fargette <i>et al.</i> , 2008	AM931209
Mg 34	S4-mg	Madagascar	Fargette <i>et al.</i> , 2008	AM931213
Mg 35	S4-mg	Madagascar	Fargette <i>et al.</i> , 2008	AM931214
Rw 1	S4-lv	Rwanda	Unpublished	GQ470541
Rw 30	S4-lv	Rwanda	Ndikumana <i>et al.</i> , 2011	HE654709
Rw 110	S4-lv	Rwanda	Ndikumana <i>et al.</i> , 2011	HE654711
Rw 111	S4-lv	Rwanda	Ndikumana <i>et al.</i> , 2011	HE654710
Rw 205	S4-lv	Rwanda	Ndikumana <i>et al.</i> , 2012	HE654722
Rw 206	S4-lv	Rwanda	Ndikumana <i>et al.</i> , 2012	HE654723
Tz 1	S4-lv	Tanzania	Pinel <i>et al.</i> , 2000	AJ279938
Tz 4	S4-lv	Tanzania	Abubakar <i>et al.</i> , 2003	AJ511793
Tz 5	S4-lv	Tanzania	Fargette <i>et al.</i> , 2004	AJ608217
Tz 6	S4-lv	Tanzania	Abubakar <i>et al.</i> , 2003	AJ511795
Tz 7	S4-lv	Tanzania	Abubakar <i>et al.</i> , 2003	AJ511796
Tz 8	S4-lm	Tanzania	Fargette <i>et al.</i> , 2004	AJ608218
Tz 13	S4-lv	Tanzania	Abubakar <i>et al.</i> , 2003	AJ511802
Tz 14	S4-lv	Tanzania	Abubakar <i>et al.</i> , 2003	AJ511803
Tz 15	S4-lv	Tanzania	Traoré <i>et al.</i> , 2005	AJ885156
Tz 18	S5	Tanzania	Hébrard <i>et al.</i> , 2005	AJ877020
Tz 206	S4-lm	Tanzania	Fargette <i>et al.</i> , 2008	AM931225
Tz 207	S4-lm	Tanzania	Fargette <i>et al.</i> , 2008	AM931226
Tz 232	S4-lm	Tanzania	Fargette <i>et al.</i> , 2008	FNF432879
Tz 1075	S4-lv	Tanzania	Pinel <i>et al.</i> , 2009	FN432882
Tz 1077	S4-lv	Tanzania	Pinel <i>et al.</i> , 2009	FN432892
Ug 1	S4-lv	Uganda	Pinel and Fargette, 2006	AM114523
Ug 2	S4-lv	Uganda	Pinel and Fargette, 2006	AM114524

and sudden outbreaks of RYMV in Upper Mabogini, Mandaka, and Kaloleni because farmers have individual nurseries in their plots despite the high incidence of RYMV.

Phylogenetic analysis clarified the identity and probable source of RYMV that has emerged in north-eastern Tanzania. Trees constructed from 33 isolates of RYMV reported in Africa grouped Tz-10-36 from Lower Moshi,

Tz-12-20 from Mahande and Tz-12-22 from Musa Mwijanga as S4 with the rest of isolates in strain S4.

More importantly, the isolate of Lower Moshi, Mahande and Musa Mwijanga formed a distinct clade that was clearly separated from a monophyletic group of strain S4-lv. This suggests that the clade may be representing a new distinguishable group of strain S4, which we named S4-mk (S4-Mt. Kilimanjaro). By phylogenetic

Table 3 Coat protein sequence identities (in percent) between RYMV isolates collected from Tanzania (this study) and reported from African regions (Tanzania, Burundi, Rwanda, Uganda, Kenya, and Madagascar)

Country	nt/aa	Sample code		
		Tz-12-20	Tz-12-22	Tz10-36
Tanzania	nt	95.8-96.2	95.6-96.1	95.5-95.9
	aa	96.2-97.9	96.2-97.9	96.2-97.9
Burundi	nt	96.2	96.1	95.9
	aa	97.0	97.0	97.0
Rwanda	nt	95.6-95.9	95.5-95.8	95.6-95.9
	aa	97.4-97.9	97.4-97.9	97.4-97.9
Uganda	nt	95.2-95.5	95.1-95.4	95.0-95.2
	aa	96.6-97.0	96.6-97.0	96.6-97.0
Kenya	nt	95.2-96.1	95.1-95.9	95.0-95.8
	aa	96.2-98.3	96.2-98.3	96.2-98.3
Madagascar	nt	94.8-95.6	94.3-95.1	94.8-95.6
	aa	96.6-97.9	96.6-97.9	96.6-97.9

Accession numbers of references isolates are S4 strains in Table 2. Samples are from Tanzania, Burundi, Rwanda, Uganda, Kenya, and Madagascar. Kenya isolates are S4-lv and S4-lm, Madagascar isolate is S4-mg.

tree (Fig. 1) and the year of first RYMV reports, Kenya in 1966²⁴⁾ and Tanzania in 1982⁷⁾, suggest that strain S4-lv has dispersed from south-eastern Kenya to the Lake Victoria region in north-eastern Tanzania, leading to the current epidemics in some irrigated lowlands, including Lower Moshi. At the same time, the strain might have mutated to S4-mk because of changing ecology and this could have happened very recently as RYMV evolves rapidly according to FARGETTE *et al.* (2008).

Kenya is the probable source of S4-mk since Kenyan isolates (Ke1, Ke2, and Ke3) were genetically closest to that of S4-mk, among all 33 isolates analyzed, including many from Tanzania (Table 3). In fact, Ke1, Ke2, and Ke3 originate from the southern part of the country^{8, 18, 19)}. The epidemics of RYMD are long known in this area^{24, 25)} and the area is very close to north-eastern Tanzania where we observed the outbreak of RYMV recently. Apparently, there is no large physical barrier in between, except the rift valley which is about 50-100 Km wide. Strain S4-lv, therefore, could easily move from one side to another by vectors or other ways. Contrary to the above hypothesis, strain S4 might have existed in north-eastern Tanzania for quite a long time. This is because all the natural means of RYMV transmission, especially

insect vectors, cannot move over a long distance²⁶⁾. Moreover, the virus is not seed borne in either cultivated²⁷⁾ or wild rice species²⁸⁾. RYMV, therefore, is likely to have been existing even in Lower Moshi for many years, before the recent epidemics, but with extremely low incidence. The epidemics have been possibly triggered by the continuous cultivation of susceptible varieties (cvs. IR64 and TXD306) without dry spell gaps or rotation with non RYMV-host. This has now become the common practice in some parts of Lower Moshi. Many experts, including Ochola and Tusiime^{29, 30)}, warn that typical rice cultivation practices can activate devastating epidemics of RYMD. At the same time, field-studies need to be conducted to explore how the virus disperses over a long distance in Tanzania. Molecular epidemiological data, including that presented in this study, can be useful in such researches, leading to the successful management of RYMV, include the making of a resistant variety in the country.

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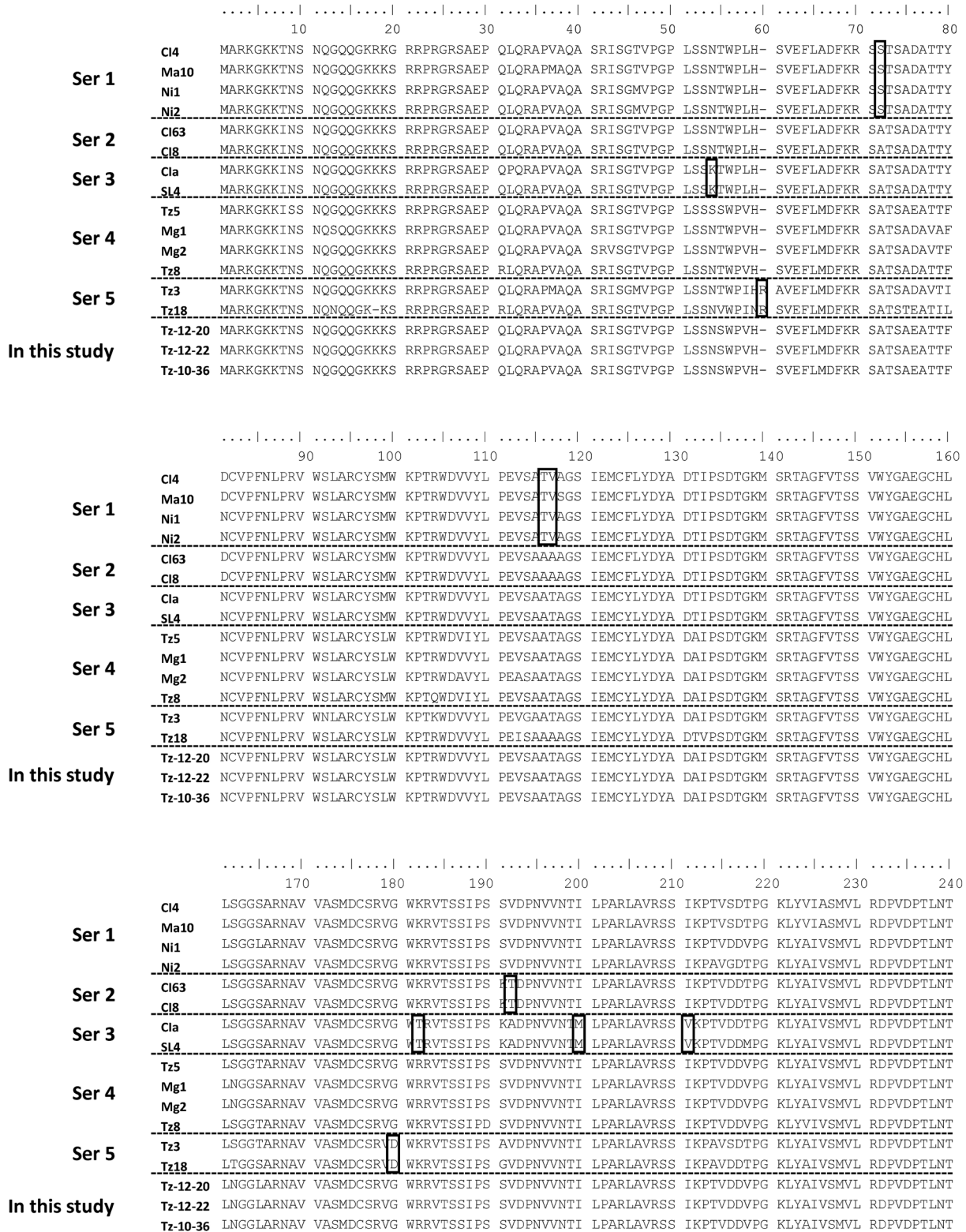


Fig. 3 Alignment of the coat protein gene constructed from the deduced amino acid sequences of 14 RYMV isolates. Isolates collected from Tanzania are Tz-10-36, Tz-12-20, and Tz-12-22. Specific serotypes (Ser 1, Ser 2, Ser3, and Ser 5) are distinguished from the amino acid position (framed), whereas Ser 4 is indicated in plain letters. Numbers reflect the amino acid position in the coat protein.

Table 4 RYMV sequences used in serotype analysis for Fig. 3.

Isolate	Country of origin	Serotype	Sequence accession no.
CI4	Cote d'Ivoire	Ser1	AJ608206
Ma10	Mali	Ser1	AJ608208
Ni2	Nigeria	Ser1	AJ608213
Ni1	Nigeria	Ser1	AJ608212
CI63	Cote d'Ivoire	Ser2	AJ608207
CI8	Cote d'Ivoire	Ser2	AJ279909
CI a	Cote d'Ivoire	Ser3	AJ608219
SL4	Sierra Leone	Ser3	AJ608214
Tz5	Tanzania	Ser4	AJ608217
Mg1	Madagascar	Ser4	AJ608210
Mg2	Madagascar	Ser4	AJ608211
Tz8	Tanzania	Ser4	AJ608218
Tz3	Tanzania	Ser5	AJ608216
Tz18	Tanzania	Ser5	CAI59706

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タンザニア北東部産 *Rice yellow mottle virus* の解析

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要約：アフリカにおいて重要な穀物のひとつであるイネ (*Oryza sativa* L.) は、*Rice yellow mottle virus* による重大な被害を受けており、タンザニアにも広がっている。水稻栽培の最も進んだ地域の一つであるローワーモシ灌漑地区を含む北東部で、ウイルス病様病徴の認められる灌漑水田において検体の採集を行った。採集した試料は、電子顕微鏡を用いたウイルス粒子の観察、DAS-ELISA 法、PCR 法による検出を行ったところ、RYMV の感染が確認された。3 つの分離株 Tz-12-20, Tz12-22, Tz10-36 の PCR 産物については、遺伝子解析を行い系統と血清型の判別を行った。その結果、S4 型 (PINEL *et al.*, 2009) 分離株であることが示され、また、系統解析から、ビクトリア湖周辺分離株である S4-lv や S4-lm, S4-mg とは異なる単一のクラスターを形成し、新しいグループ (S4-mk) であると考えられた。さらに系統樹から、S4-mk 分離株はケニアの西南地域からタンザニアの北東部において広がっている分離株であることを示した。系統樹において単一のクラスターを形成した一方、S4-mk 分離株と他の S4 型分離株 (S4-lv, S4-lm, S4-mg) 間のアミノ酸配列で、96% 以上の高い相同性を示した。また、アミノ酸配列から血清型の判別が可能 (FARGETTE *et al.*, 2002) であることから、タンザニア産 RYMV でこの手法を用いて血清型を検討したところ、類別可能な 5 種類の血清型のうち、Ser4 に分類された。

キーワード：イネ、系統解析、血清型、タンザニア、*Rice yellow mottle virus* (RYMV)

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