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Phylogenic Analysis and Serotyping of *Rice yellow mottle virus* Strains in Uganda

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Abstract *Rice yellow mottle virus* (RYMV) is a major rice (*Oryza* spp.) virus found only in Africa. In the present study, we collected samples of rice mainly from Eastern Uganda showing RYMV symptoms. The presence of RYMV was confirmed using ELISA and PCR. Some RYMV isolates in Uganda were sequenced and showed high homology levels with the Madagascar RYMV strain. They were grouped into S4ug strain in the East African lineage, showing high homology levels with isolates from neighboring countries. Serotyping based on the amino acid sequences of a RYMV coat protein gene fragment enabled to classify the Uganda isolates into Ser 4, in accordance with a previous serotyping report of other Ugandan isolates, using monoclonal antibodies. The present study revealed the widespread occurrence of RYMV in Uganda with a low diversity, irrespective of the origin of location and the rice varieties, as well as the first serotyping of Ugandan RYMV based on the amino acid sequences but not on monoclonal antibodies.

Key words: Detection, Sequencing, *Sobemovirus*, Transmission

Introduction

Rice (*Oryza* spp.) is an important staple food as well as a cash crop in sub-Saharan Africa. Rice imports account for nearly 40% of the total consumption (Seck *et al*., 2012). Recently, many African countries have decided to increase rice production to meet the high and increasing demand through the Coalition for African Rice Development (CARD) and the development of national rice strategies. Although rice had been grown in Uganda since the early 1940s, production was mainly limited to lowland agro-ecological zones using a few irrigation schemes. With the introduction of New Rice for Africa (NERICA) upland rice varieties, however, rice farming has been widely adopted in the country. The Regional Rice Research and Training Center based at the National Crops Resources Research Institute (NaCRRI) has been at the forefront of the development and expansion of rice varieties and crop management technologies. Collaboration with the Promotion of Rice Development (PRiDe) Project supported by the Japan International Cooperation Agency (JICA) enable to promote rice production and train farmers to increase productivity. However, the occurrence of *Rice yellow mottle virus* (RYMV) is becoming a serious problem in Uganda (Ochola *et al*., 2015).

RYMV, a member of the family *Sobemoviridae*, is the main disease constraint on rice production in Africa, and after first having been reported in Kenya in 1966 by Bakker (1970), it has been detected in almost all the rice-planting countries in Africa (Banwo *et al*., 2004), including Uganda. Following the first report of RYMV in Uganda (Pinel and Fargette, 2006), sequences of the coat protein (CP) genes of two isolates collected near the Lake Victoria Crescent in 2000 became available in the database, NCBI. Ochola and Tusiime (2011a, b) reported the occurrence of some isolates with different symptoms of the disease in Butaleja, Mbale, Pallisa, Namutumba, Iganga, Mayuge, Bugiri, Bukedea, Soroti, and Lira in Uganda based on phenotypic scoring and ELISA. Although the area affected by RYMV in Uganda is expanding, the characteristics of the RYMV isolates, including serotypes, are not well documented.

RYMV isolates can be separated into six serotypes (Ser) using monoclonal antibodies (MAbs) (Konaté *et al*., 1997; Mansour and Baillis, 1994; N'Guessan *et al*., 2000). Ser 1, Ser 2, and Ser 3 are found in West Africa while Ser 4, Ser 5, and Ser 6 are found in East Africa (Pinel *et al*., 2000; Fargette *et al*., 2002). Two Ugandan isolates reported in 2006 (Accessions: AM114523 and AM114524) were classified into Ser 4 using a combination of polyclonal antibodies (PAbs) and MAbs (Pinel and Fargette, 2006). Recently, Fargette *et al.* (2002) and Hébrard *et al.* (2005) have reporeted that the amino acid (aa) sequence of the CP gene of RYMV can be used to distinguish Ser 1 through Ser 5.

In the present study, we collected rice samples in Eastern Uganda where the rice production is higher

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than in other regions of the country and carried out the phylogenic analysis and serotype identification of RYMV. Some RYMV isolates were characterized on a molecular basis for phylogenic information and serotyped using the CP aa sequence.

Materials and Methods

Virus isolates

The leaf samples of rice plants with symptoms by RYMV were collected from 9 fields (collection sites) in Wakiso, Iganga, Mbale, and Lira in Eastern Uganda from 2009 to 2012 (Table 1). A total of about 60 samples were tested using double-antibody sandwich ELISA (DAS-ELISA) with an antibody kit (NEOGEN Europe Ltd., UK).

Detection, cloning, DNA sequencing and serotyping

The genomes of 10 RYMV-infected samples were further analyzed. Genome fragments containing the CP gene were transcribed and amplified by RT-PCR after the extraction of total RNA from leaves using TRIzol® (Invitrogen, NY, USA). The first strand of the cDNA primed with a specific reverse primer was synthesized using the ReverTra Ace-α-® kit (TOYOBO, Osaka, Japan). The first-strand cDNA was amplified by PCR using a TaKaRa Ex™ kit (TaKaRa, Otsu, Japan). The primers A3 and B3, designed by Pinel *et al.* (2000), were used to amplify regions, including 720 bp of the CP gene. Although the A3 primer is a CP forward primer, the A3 primer's sequence overlapped with the CP gene, and a new primer, A'3, was additionally designed in the present study (Table 2). Amplification conditions followed those of Uke *et al.* (2014). After purification by the Wizard® SV Gel and PCR Clean-Up system (Promega, WI, USA), the DNAs were cloned into the plasmid. Recombinant plasmids were transformed into competent *Escherichia coli* strain DH5α cells (Invitrogen, NY, USA), and five clones of each isolate were sequenced with an Applied Biosystems 3130/3130xl genetic analyzer (Applied Biosystems, CA, USA). Nucleotide sequence data were analyzed with Sequence scanner v2.0 and CLC Sequence Viewer 6, and a consensus sequence was constructed using the Basic Local Alignment Search Tool in NCBI. To analyze the relationships with other isolates, a multiple sequence alignment was performed and a phylogenetic tree was constructed using the maximum-likelihood method by CLUSTAL W and MEGA6.

Following the method of Hébrard *et al.* (2005), an alignment for serotypes was performed for 50 isolates, including multiple RYMV serotypes, using CLUSTAL W.

Results

Occurrence of RYMV in Eastern Uganda

We observed the rice plants with symptoms by RYMV in the rice paddy fields of Wakiso, Iganga, Mbale, and Lira. Most of the rice samples with RYMV symptoms collected in the 9 fields of these areas were found to be RYMV-positive based on ELISA. Among them, Wakiso was the area without record of RYMV based on genetic information. Since the survey by Ochola *et al.* (2015) was conducted in 2009 and 2010, we added samples in 2011 and 2012, including a new rice planting area. Most of the samples with RYMV symptoms collected in the four fields were RYMV-positive based on ELISA.

Table 1. Origin of *Rice yellow mottle virus* sequenced samples between 2009 and 2012.

Sample code	Year	Collection site	Rice variety	Accession No.
U101	2009	Wakiso-1	IR64	AB981479
Tilda D	2010	Mbale-1	Unknown	AB985686
U8	2011	Mbale-2	K85	AB980006
U10	2011	Mbale-3	WITA 9	AB980004
U12	2011	Wakiso-1	IR64	AB980003
U13	2011	Mbale-4	K85	AB980005
U14	2011	Mbale-5	K85	AB980010
U21	2011	Lira-1	Supa	AB980007
U25	2011	Lira-2	Supa	AB980008
U39	2012	Iganga	Supa	AB980009

Table 2. Primers used in RT-PCR amplification of the coat protein of the *Rice yellow mottle virus* isolates.

Primers	Position	Sequence	Reference
A3*	3442-3457	5'-CAAAGATGGCCAGGAA-3'	Pinel *et al.*, 2000
A'3	2901-2919	5'-GTTGTGGTCAGACCTCGCG-3'	Present study
B3*	4298-4320	5'-CCATGTGGGGAATGGCACCCAC-3'	Pinel *et al.*, 2000

*for detection.

Phylogenetic analysis

CP genes from all the isolates tested showed the highest homology with a Madagascar isolate (AJ279924). Although Madagascar isolates and Uganda isolates in the present study (hereafter referred to as Ugandan new isolates) showed high homology levels and were classified into members of the East African lineage, all the Ugandan new isolates formed a distinct cluster from the Madagascar isolates as well as the Ugandan old isolates (Ug1 and Ug2) collected in 2000 (Pinel and Fargette, 2006) (Fig. 1). In the present study, our Ugandan isolates were also classified into S4ug group excluding TildaD by phylogenetic analysis (Fig. 2).

The Ugandan new isolates shared a high homology regardless of their local origins and varieties. More interestingly, the Ugandan old isolates showed a > 95% homology with the Ugandan new isolates at the aa sequence level. When compared to isolates from three neighboring countries (Kenya, Burundi, and Rwanda), the Ugandan isolates also shared a high homology level with them (Table 3).

Serotype identification

Based on the aa sequence (Table 4.), the Ugandan new isolates were classified into Ser 4 (Fig. 3), in accordance with a previous study (Pinel and Fargette, 2006) in which the Ugandan old isolates were serotyped by MAbs.

Discussion

As the rice planting area expands, the detection of RYMV in each locality by symptom observation, as well as ELISA confirmation, is becoming more important. Farmers need practical information about the control and management strategies. Breeders for resistant rice varieties also request information about virus diversity. In the present study, we confirmed that the spread of RYMV was wider spread than indicated in previous studies (Pinel and Fargette, 2006; Ochola *et al*., 2015), using molecular and serotype information. RYMV was detected in various rice varieties such as IR64, Supa, K85, WITA9, and other unidentified local varieties, as well as in the experimentally grown Koshihikari (*O. sativa japonica*) and some NERICA varieties such as NERICA1, NERICA4, and NERICA10 (data not shown). The occurrence of RYMV in Iganga, Mbale and Lira in Uganda had been reported by Ochola and Tusiime (2011b), and the present survey confirmed the widespread occurence of RYMV in the rice paddy fields of Eastern Uganda.

Homology studies showed that the new Ugandan RYMV isolates were very closely related to those of the East African lineage, as reported for the Ugandan RYMV isolates collected in 2000 (Pinel and Fargette, 2006). These new Ugandan RYMV isolates were, however, placed in a distinct cluster from that of Ug1 and Ug2, but in the same cluster as that with other reported Ugandan and Madagascar isolates. Although Abo *et al.* (2000) reported that beetle species in the genus *Chrysomelidae* are responsible for the long-distance transmission of RYMV, *Chrysomelidae* beetles are generally known to be short-distance flyers (Thresh, 1985). Consequently, the successful long-distance transmission of RYMV by these Coleopterous insects is likely to be rare (Traoré *et al*., 2005), and may account for the independence of East African isolates from West African ones. Moreover, based on our survey in Ugandan rice fields (Fujiie *et al*., 2014), common leaf beetles belonged to *Chaetocnema* spp., *Chrysispa viridicyanea* and *Altica* spp. and their status as RYMV vector remains unknown. Thus, the vectors which are responsible for long-distance transmission of RYMV in Uganda have not been well documented. Though the role of wild hosts of RYMV such as wild rice species or other *Poaceae* species was suggested (Ochola *et al*., 2015), wild RYMV hosts may also be important in harboring RYMV vectors.

All the Ugandan new isolates display a very high homology level irrespective of their origins, which are more than 200 km apart. This suggests that the invasion and spreading of RYMV in those areas are relatively new events related to the recent expansion of rice planting. Although Uganda old isolates from the Lake Victoria area were S4lv, both Ugandan new isolates, in the present study and the study by Ochola *et al.* (2015), were S4ug or S4mg, and these S4lv isolates formed a distinct cluster with the Ugandan new isolates. This may suggest that they have a different origin or had accumulated mutations during the last 14 years. Thus, additional surveys of RYMV in the Lake Victoria area may give us information about the existence, expansion and possible mutation rate of RYMVs in the Ugandan old isolates.

The Ugandan new isolates were classified into Ser 4 for the first time not based on MAbs. As the production of MAbs is not easy and MAbs for serotyping are not available commercially at present, serotyping by aa sequences was useful and accurate.

The sequence data of Ugandan RYMVs had not been updated until recently (Ochola *et al*., 2015). In the present study, we used newly collected samples from Uganda and sequenced 720 bp of their nucleic acids in the ORF 4 (Ngon *et al*., 1994) of the CP. As a results, the

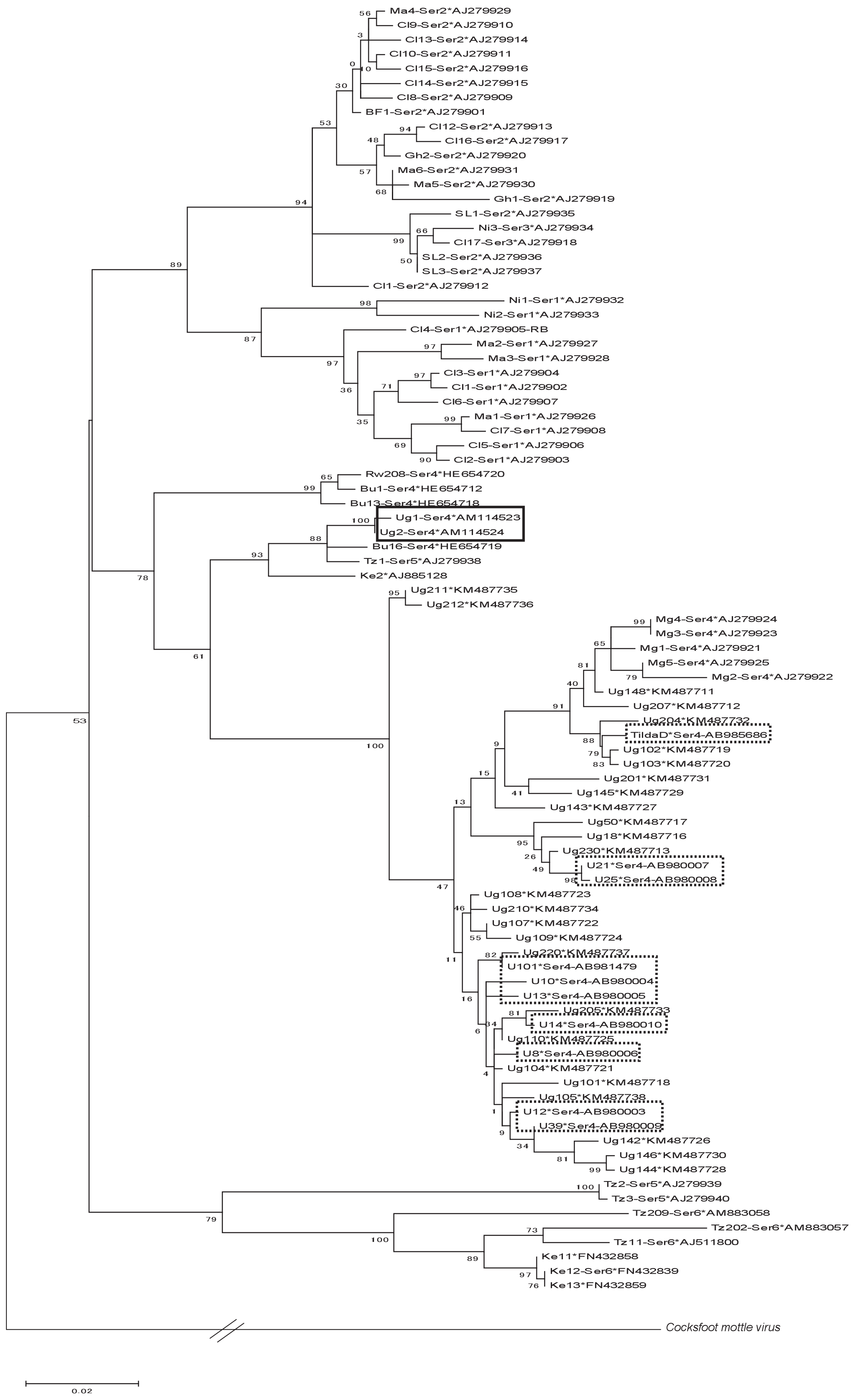

Fig. 1. Maximum-likelihood tree depicting the relationship of *Rice yellow mottle virus* isolates collected in Uganda (in the dotted box) with 90 reported isolates, including Ugandan old isolates (in the plane box), constructed from the nucleotide sequences of the coat protein gene. BF: Burkina-Faso, CI: Côte d'Ivoire, Gh: Ghana, Gu: Guinea, Mg: Madagascar, Ma: Mali, Ni: Nigeria, SL: Sierra-Leone, Tz: Tanzania, Ug and U: Uganda, Ke: Kenya, Bu: Burundi, and Rw: Rwanda.

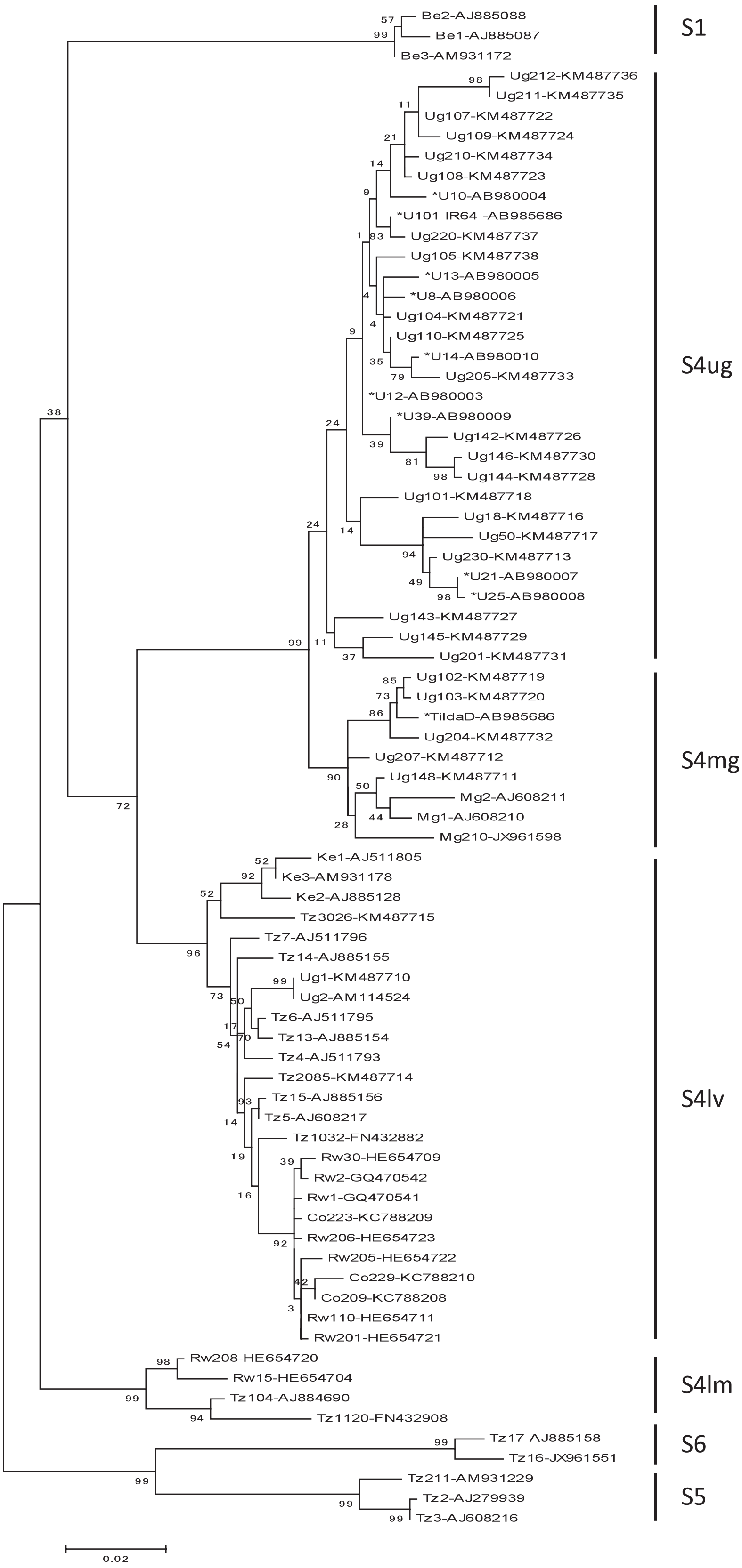

Fig. 2. Phylogeny of the S4ug and S4lv strains, and relationships with other strains. The maximum-likelihood phylogenetic tree was reconstructed from the coat protein sequences of 76 isolates. S1 strains originate from Benin (Be), West Africa and other strains, Tanzania (Tz), Uganda (Ug and U), Madagascar (Mg), Co (Congo), Rwanda (Rw), and Kenya (Ke) are from East Africa. * indicates Ugandan isolates in the present study.

Table 3. Coat protein sequence identities (in percent) between *Rice yellow mottle virus* isolates in Uganda (present study) and in other East African countries.

Isolate	Uganda		Madagascar		Kenya		Burundi		Rwanda	
	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
U8	93	96	96-97	98-99	89-93	93-96	92-96	95-97	92	95
U10	94	96	96	98-100	89-93	93-96	92-94	95-97	92	96
U12	93-94	96	96-97	98-99	89-93	93-96	92-94	95-97	92	95
U13	94	96	96-97	100	89-93	93-96	93-94	95-97	93	96
U14	93	96	96-97	98-99	90-93	93-96	92-94	95-97	92	95
U21	93	95-96	96-97	97-99	89-93	93-96	92-93	95-97	92	95
U25	92-93	95-96	96-97	97-99	89-93	93-96	92-93	95-97	92	95
U39	94	96	96-97	98-99	89-93	93-96	93-95	95-97	93	95
U101	94	96-97	96-97	98-99	89-93	92-97	93-95	96-97	93	96
TildaD	93	95-96	97-98	97-99	90-92	92-96	92-94	95-97	93	95

Accession numbers of reference isolates are; Madagascar (AJ279921, AJ279922, AJ279923, AJ279924 and AJ279925), Uganda (AM114523 and AM114524), Kenya (AJ885128, FN432858 and FN432859), Burundi (HE654712, HE654718 and HE654719) and Rwanda (HE654720).

phylogeny of RYMVs in Uganda appears to be generally stable in phylogeny, although they form two clusters belonging to the East African lineage.

Although long-distance transmission by vector insects is rare (Traoré *et al*., 2005), once RYMV reaches one area, there are various modes of transmission in and out of nearby paddy fields (Traoré *et al*., 2008; Uke *et al*., 2014). The avoidance of invasion and spreading by the removal of infected ratoons and seedlings, as well as the use of resistant varieties, is recommended. The emergence of new isolates with different genetic backgrounds, however, may break the resistant varieties or result in the development of different symptoms of the disease. With this in mind, and also to understand the evolution of RYMV, the monitoring and characterization of RYMV should be continued in all the rice planting countries in Africa.

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Table 4. *Rice yellow mottle virus* isolates used in the amino acid sequence analysis for serotyping shown in Fig. 3.

Isolate	Serotype*	Region	Country of origin	Accession no.
CI4	Ser 1	West Africa	Côte d'Ivoire	AJ608206
Ma10	Ser 1	West Africa	Mali	AJ608208
Ni1	Ser 1	Central Africa	Nigeria	AJ608212
Ni2	Ser 1	Central Africa	Nigeria	AJ608213
CI63	Ser 2	West Africa	Côte d'Ivoire	AJ608207
CI8	Ser 2	West Africa	Côte d'Ivoire	AJ279909
CIa	Ser 3	West Africa	Côte d'Ivoire	AJ608219
SL4	Ser 3	West Africa	Sierra Leone	AJ608214
Mg1	Ser 4	East Africa	Madagascar	AJ608210
Mg2	Ser 4	East Africa	Madagascar	AJ608211
Tz5	Ser 4	West Africa	Tanzania	AJ608217
Tz8	Ser 4	West Africa	Tanzania	AJ608218
Tz3	Ser 5	West Africa	Tanzania	AJ608216
Tz18	Ser 5	West Africa	Tanzania	AJ877020
Ug18	-	West Africa	Uganda	KM487716
Ug50	-	West Africa	Uganda	KM487717
Ug101	-	West Africa	Uganda	KM487718
Ug102	-	West Africa	Uganda	KM487719
Ug103	-	West Africa	Uganda	KM487720
Ug104	-	West Africa	Uganda	KM487721
Ug105	-	West Africa	Uganda	KM487738
Ug107	-	West Africa	Uganda	KM487722
Ug108	-	West Africa	Uganda	KM487723
Ug109	-	West Africa	Uganda	KM487724
Ug110	-	West Africa	Uganda	KM487725
Ug142	-	West Africa	Uganda	KM487726
Ug143	-	West Africa	Uganda	KM487727
Ug144	-	West Africa	Uganda	KM487728
Ug145	-	West Africa	Uganda	KM487729
Ug146	-	West Africa	Uganda	KM487730
Ug148	-	West Africa	Uganda	KM487711
Ug201	-	West Africa	Uganda	KM487731
Ug204	-	West Africa	Uganda	KM487732
Ug205	-	West Africa	Uganda	KM487733
Ug207	-	West Africa	Uganda	KM487712
Ug210	-	West Africa	Uganda	KM487734
Ug211	-	West Africa	Uganda	KM487735
Ug212	-	West Africa	Uganda	KM487736
Ug220	-	West Africa	Uganda	KM487737
Ug230	-	West Africa	Uganda	KM487713

*-: not described

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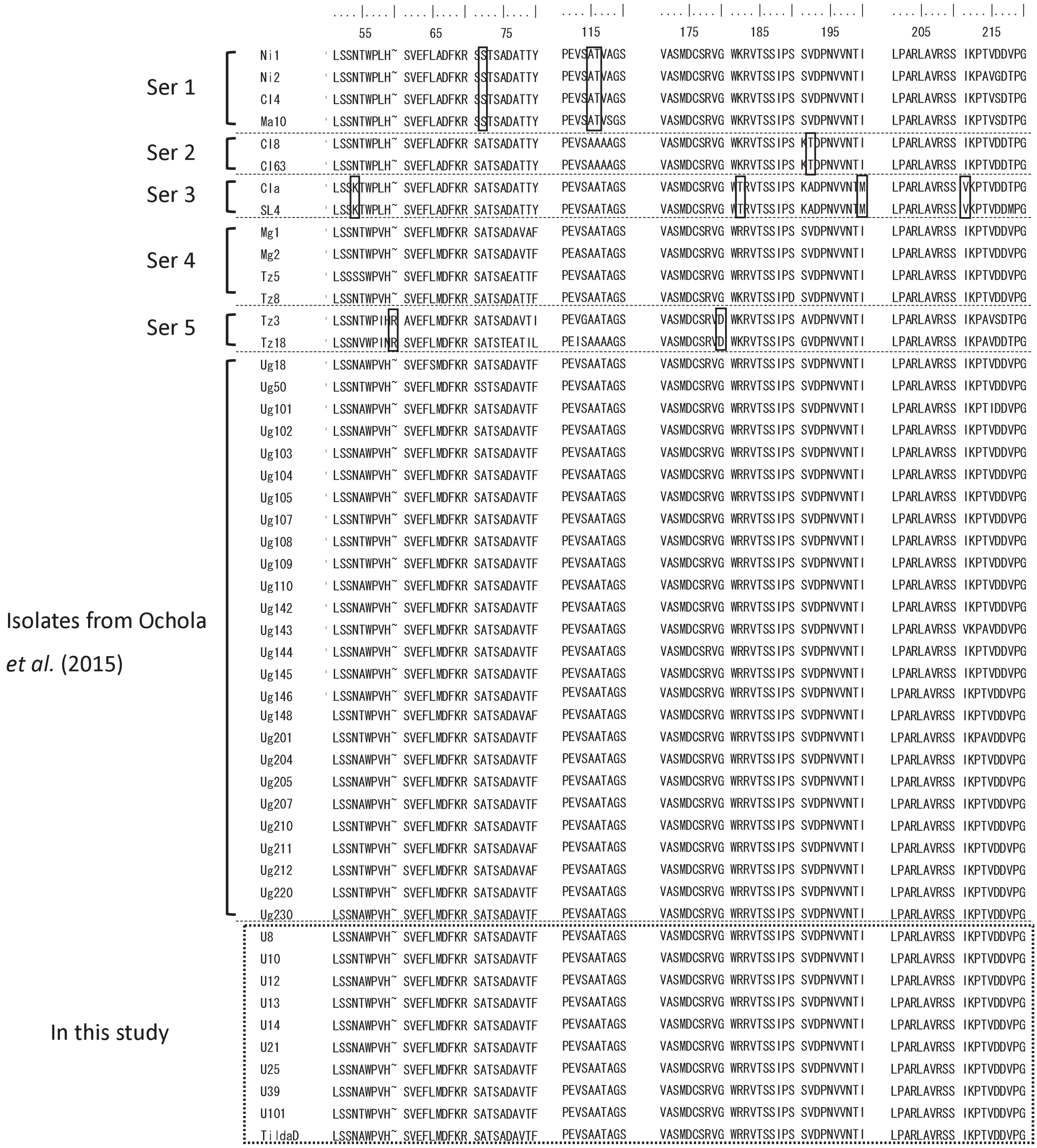

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          ....|....| ....|....| ....|....|  ....|....|  ....|....| ....|....| ....|....|  ....|....| ....|....|
              55         65         75          115         175        185        195          205        215
Ni1       LSSNTWPLH~ SVEFLADFKR SSTSADATTY  PEVSATVAGS  VASMDCSRVG WKRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ni2       LSSNTWPLH~ SVEFLADFKR SSTSADATTY  PEVSATVAGS  VASMDCSRVG WKRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPAVGDTPG
Cl4       LSSNTWPLH~ SVEFLADFKR SSTSADATTY  PEVSATVAGS  VASMDCSRVG WKRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVSDTPG
Ma10      LSSNTWPLH~ SVEFLADFKR SSTSADATTY  PEVSATVSGS  VASMDCSRVG WKRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVSDTPG
Cl8       LSSNTWPLH~ SVEFLADFKR SATSADATTY  PEVSAAAAGS  VASMDCSRVG WKRVTSSIPS KTDPNVVNTI  LPARLAVRSS IKPTVDDTPG
Cl63      LSSNTWPLH~ SVEFLADFKR SATSADATTY  PEVSAAAAGS  VASMDCSRVG WKRVTSSIPS KTDPNVVNTI  LPARLAVRSS IKPTVDDTPG
Cla       LSSKTWPLH~ SVEFLADFKR SATSADATTY  PEVSAATAGS  VASMDCSRVG WTRVTSSIPS KADPNVVNTM  LPARLAVRSS VKPTVDDTPG
SL4       LSSKTWPLH~ SVEFLADFKR SATSADATTY  PEVSAATAGS  VASMDCSRVG WTRVTSSIPS KADPNVVNTM  LPARLAVRSS VKPTVDDMPG
Mg1       LSSNTWPVH~ SVEFLMDFKR SATSADAVAF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Mg2       LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEASAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Tz5       LSSSSWPVH~ SVEFLMDFKR SATSAEATTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Tz8       LSSNTWPVH~ SVEFLMDFKR SATSADATTF  PEVSAATAGS  VASMDCSRVG WKRVTSSIPD SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Tz3       LSSNTWPIHR AVEFLMDFKR SATSADAVTI  PEVGAATAGS  VASMDCSRVD WKRVTSSIPS AVDPNVVNTI  LPARLAVRSS IKPAVSDTPG
Tz18      LSSNVWPINR SVEFLMDFKR SATSTEATIL  PEISAAAAGS  VASMDCSRVD WKRVTSSIPS GVDPNVVNTI  LPARLAVRSS IKPAVDDTPG
Ug18      LSSNAWPVH~ SVEFSMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug50      LSSNTWPVH~ SVEFLMDFKR SSTSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug101     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTIDDVPG
Ug102     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug103     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug104     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug105     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug107     LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug108     LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug109     LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug110     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug142     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug143     LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS VKPAVDDVPG
Ug144     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug145     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug146     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug148     LSSNTWPVH~ SVEFLMDFKR SATSADAVAF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug201     LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPAVDDVPG
Ug204     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug205     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug207     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug210     LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug211     LSSNTWPVH~ SVEFLMDFKR SATSADAVAF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug212     LSSNTWPVH~ SVEFLMDFKR SATSADAVAF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug220     LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
Ug230     LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
U8        LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
U10       LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
U12       LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
U13       LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
U14       LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
U21       LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
U25       LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
U39       LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
U101      LSSNTWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
TildaD    LSSNAWPVH~ SVEFLMDFKR SATSADAVTF  PEVSAATAGS  VASMDCSRVG WRRVTSSIPS SVDPNVVNTI  LPARLAVRSS IKPTVDDVPG
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Fig. 3. Alignment of the coat protein genes constructed from the deduced amino acid sequences of 10 *Rice yellow mottle virus* isolates. Isolates collected from Uganda are enclosed in the dotted box. Specific serotypes (Ser 1, Ser 2, Ser 3, and Ser 5) were distinguished following the procedure of Hébrard *et al.* (2005) using amino acid positions (framed), whereas Ser 4 is indicated in plain letters.