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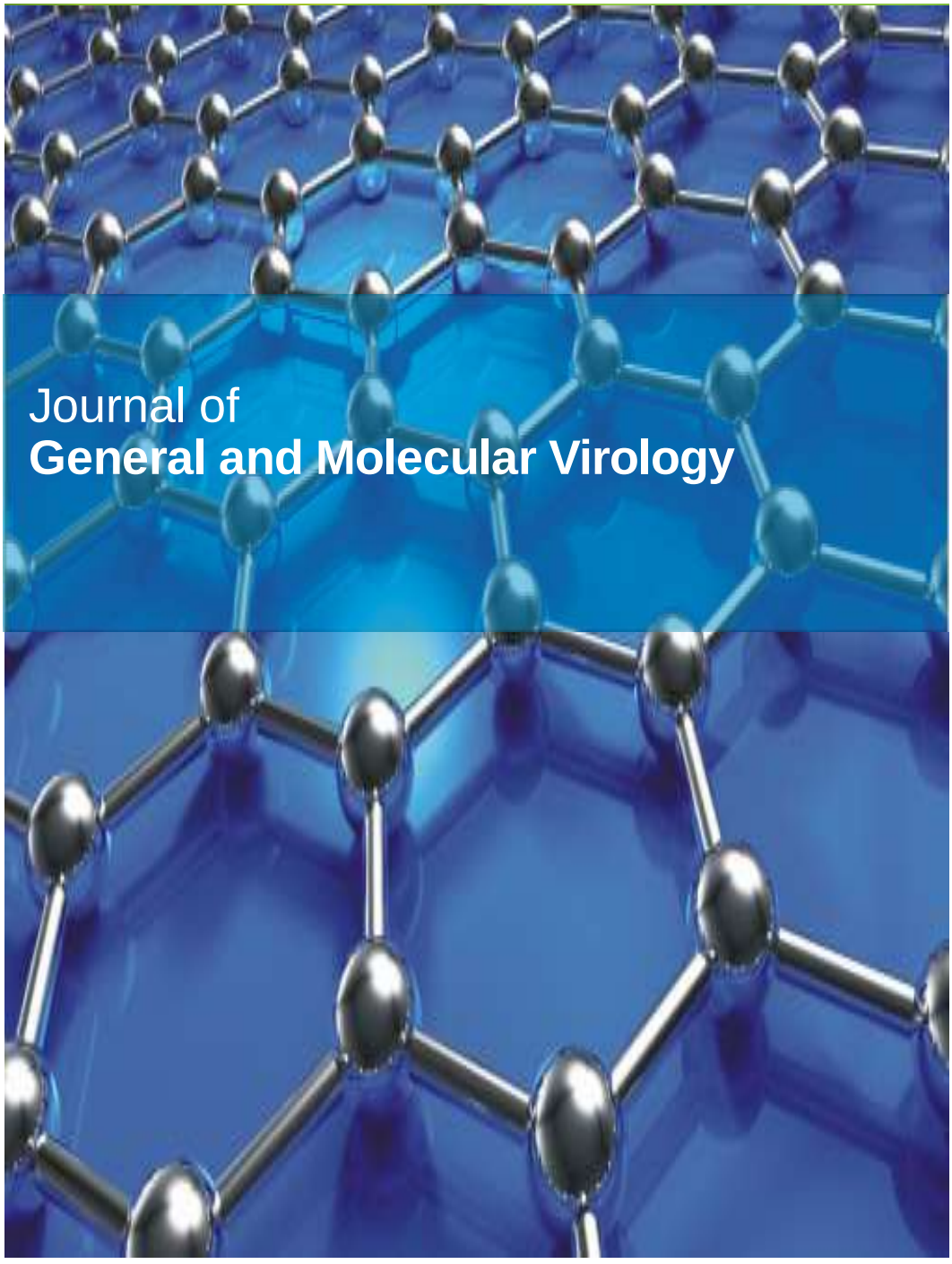

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Journal of General and Molecular Virology

Full Length Research Paper

Genetic diversity of rice yellow mottle virus from Niger Office and Selingue Development Rural Office in Mali

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Virus is the most constraint of rice production in West Africa. Genetic diversity of rice yellow mottle virus (RYMV) was assessed in two rice growing areas of Mali. Forty-three (43) infected rice leaf samples were collected from five locations within the Niger Office and Selingue Development Rural Office rice's growing areas. The infected samples were confirmed by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) using primers pair Prymv1 and Prymv2. A total of twenty-six RYMV isolates were obtained and classified into two genotypes A and B with Prymv1 and one genotype with Prymv2. Sequencing of the cDNA fragments with Prymv2 shared isolates into three distinct groups I, II and III. Most of the isolates were under Group I whereas isolates from the Selingue Development Rural Office area and Moussa-Were (Niger Office) area constituted respectively Groups II and III. This study provided baseline data for monitoring the genetic diversity of rice yellow mottle virus in the two rice-growing areas of Mali which could contribute to strategies development for the disease control.

Key words: Genetic diversity, reverse transcriptase polymerase chain reaction (RT-PCR), rice yellow mottle virus (RYMV), sequencing, Mali.

INTRODUCTION

Yellow mottle disease of rice is caused by a virus that belongs to the group sobemovirus (Hull and Fargette, 2005) and is endemic to only the African continent (IRD, 2014). This disease is more severe in irrigated rice-growing areas located in sub-Sahara Africa and the most damaging disease in rice cultivation (APCAM, 2004; IRD, 2014; Sere et al., 2013). It was first recorded on IET 2911 and BG 90-2 varieties in 1991 at Kogoni Agricultural Research Sub-Station and Niger Office rice growing area in the Niono sector in Mali (Anonymous, 1992). The incidence of the disease varied from 64 to 100% in Niger Office zone (WARDA, 2000) and 25 to 80% in Selingue Development Rural Office depending on the date of infection and the susceptibility of rice cultivars (APCAM, 2004). Most rice cultivars are susceptible to this virus (APCAM, 2004). Some varieties such as Gigante (*Oryza*

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sativa) and Tog series (*Oryza glaberrima*) showed natural resistance (Thottappilly and Rossell, 1993; Ndjiondjop et al., 1999; Pidon, 2016). In rice, the resistance against this virus is governed by three genes which are rymv1-2 (*O. sativa*), rymv1-3 and rymv1-4 (*O. glaberrima*) (Thiemele et al., 2010). Other developed resistant varieties like Moroberekan, Faro 11 and IR47686-15-1-1 (Soko et al., 2015; Amancho et al., 2009), WITA9 and WAT316 (Michel et al., 2008) showed some level of susceptibility to few virus isolates.

Emergence of the virulent pathotypes capable of overcoming one or two resistance alleles has often occurred (Hebrad et al., 2018; Pidon et al., 2017; Pinel-Galzi et al., 2016). A hypervirulent pathotype was discovered in West and Central Africa by Hebrad et al. (2018). Knowledge and mapping of viral diversity are important to reduce disease incidences through improvement of local varieties susceptible to rice yellow mottle virus (RYMV). The diversity of RYMV strains has been well documented in Africa (Pinel-Galzi et al., 2015; Trovào et al., 2015; Hubert et al., 2017; Longue et al., 2017). In Mali, there is little information on the diversity of rice yellow mottle virus. RT-PCR and sequencing are the most appropriate tools for assessing the genetic variability of rice yellow mottle virus in Mali since it is RNA virus. In this study, we assessed the genetic diversity of 26 Rice Yellow Mottle Virus (RYMV), isolated from rice leaves collected in Niger Office and Selingue Development Rural Office rice growing areas in Mali.

MATERIALS AND METHODS

Sampling

Sampling was done from rice fields (Niono 7, Koyan N'peguena and Moussa Were) at the Niger Office and Selingue Development Rural Office (Selingue). A total of forty-three (43) leaves samples, suspected to be infected by rice yellow mottle virus, were collected which was composed of nineteen from Niono 7, eighteen from Moussa-Were, one from Koyan, two from N'peguena and three from Selingue. In each study area, diseased rice samples were collected at tillering in the four corners and the middle of each infected parcel, by harvesting leaves bearing typical yellow mottle symptoms. All samples were stored in ice for analysis at the Microbiology and Biotechnology Research Laboratory at the Faculty of Sciences and Techniques at the University of Sciences, Techniques and Technologies of Bamako (USTTB).

Identification of rice yellow mottle virus by RT-PCR

Extraction of total viral RNA

RNA was extracted from leaf samples using the Qiagen RNeasy Mini Kit Promega. The concentration of different RNA was measured in ng/µl using Biophotometer Eppendorf.

Amplification by RT-PCR

Samples were amplified by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) with two specific primer pairs PRymv1 (F: 5'-TGCCAATACCTATCTCCACCA-3'; R: 5'-TCACCTCTAGCGTTTGGTACG- 3') and PRymv2 (F: 5'-CCCGCAGGACCATACTAACGA-3'; R: 5'-GGGCTTCGTCACCTCTAGC-3') in addition to the reagents of the Access Kit RT-PCR system according to manufacture instructions. The mixture was distributed in PCR Flat Cap 0.2 ml BIOLOGIX tubes with a negative control (mixed without RNA) and amplified using the Applied Biosystems thermocycler based on the following programs : reverse transcription at 48°C for 45 min, inactivation of Reverse transcription (RT) at 94°C for 2 min, denaturation at 94°C for 30 s, hybridization at 61°C for 1 min, elongation at 68°C for 2 min, final extension at 68°C for 7 min and 4°C for conservation forever. The denaturation, hybridization and elongation steps were repeated in 45 times.

Electrophoresis of RT-PCR products

RT-PCR products were run on Agarose D1 Low EEO 2% (w/v) gel which was prepared with a solution of TBE (Tris, Borate, EDTA) and mixed with 30 µl of 10% ethidium bromide (1 mg/ml). The gel products were visualized under Ultra-Violet (UV) rays and photographed with the Gel Documentation System E-BoX.

Sequencing

All positive samples identified with Prymv2 primer pair were directly sent at Inqaba biotec (South Africa) for sequencing.

Data analysis

The size of amplified cDNA fragments was determined in base pair (bp) with the E-cap software in comparison with the standard 50 bp DNA Step Ladder Marker Cat # G4521 for Prymv2 primer and with the Bench standard 100 bp DNA Step Ladder marker Cat # G8291 for Prymv1 primer. Sequence alignment was performed with the MEGA.7 software. Phylogenetic tree was constructed from the genetic distance between RYMV isolates with MEGA.7 software (Dao et al., 2018; Kumar et al., 2016).

RESULTS AND DISCUSSION

The forty-three (43) suspected infected rice leaf samples collected from the different was confirmed by RT-PCR using primer pairs Prymv1 and Prymv2. Twenty-six out of 43 samples were amplified between 250 and 1100 bp. The amplified samples from Prymv2 primer pair were purified and sequenced. Variability was observed among the forty-three (43) samples with 60% of RYMV infected rice samples identified by both primers. Additional two migration profile, A and B, were obtained with Prymv1. Profile A was formed from a band of 300 bp and profile B from three bands of 300, 500 and 1100 pb (Figure 1). These two profiles corresponded to two (2) isolates of rice yellow mottle virus which were identified at Niger Office area whilst isolate A was identified only at Selingue Development Rural Office area. Isolate A was the most predominant with 96% out of the whole isolate while genotype B consisted of only VE7 isolate.

All the twenty-six (26) isolates showed a single

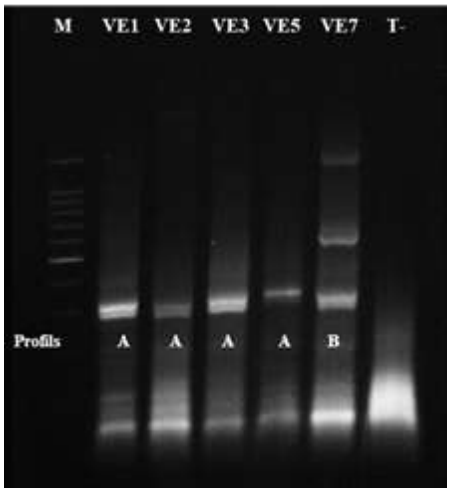

Figure 1. Profile of RT-PCR products from five rice yellow mottle virus isolates with Prymv1 primer.

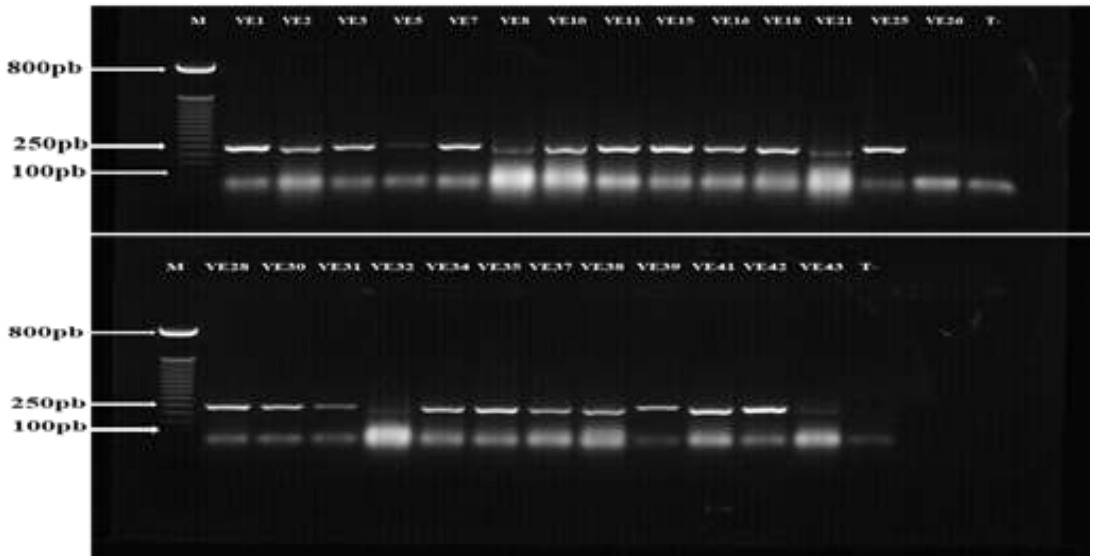

Figure 2. Profiles of RT-PCR products of rice yellow mottle virus with Prymv2 primer.

migration pattern composed of a single band of approximately 250 bp with Prymv2 (Figure 2).

Information on positive samples is recorded in Table 1. Fifty-eight percent (58%) or 23 samples from the Niger Office area as well as the 3 samples from the Selingue Development Rural Office positively confirmed the presence of the disease which was previously reported by WARDA (2000) and APCAM (2004).

This information indicates the persistence of the disease in these different rice growing areas for about thirty years. The number of groups revealed by Prymv1 is similar to that of N'Guessan et al. (2001) who worked on 9 isolates from Mali by immunological and molecular characterization. Similarly, Onasanya et al. (2006) revealed the existence of two pathogenic groups on 10 strains of RYMV in Mali by greenhouse screening. However, Sere et al. (2005) found three groups with isolates collected in Mali using serological analysis. Alkali et al. (2015) and Issaka et al. (2012) generated three groups using also serological technic with isolates collected from the northern of Nigeria and Niger. Isolates from Central Africa Republic were classified into three distinct pathogenic profiles by mechanical inoculation of six rice cultivars (Longue et al., 2018). According to

Table 1. List of positive samples identified by RT-PCR.

No.	Sample code	Rice variety	Locality	Sampling area
1	VE1	Kogoni 91-1	Niono 7	NO
2	VE2	Kogoni 91-1	Niono 7	NO
3	VE3	Kogoni 91-1	Niono 7	NO
4	VE5	Kogoni 91-1	Niono 7	NO
5	VE7	Kogoni 91-1	Niono 7	NO
6	VE8	Kogoni 91-1	Niono 7	NO
7	VE10	Kogoni 91-1	Niono 7	NO
8	VE11	Kogoni 91-1	Niono 7	NO
9	VE15	Kogoni 91-1	Niono 7	NO
10	VE16	Kogoni 91-1	Niono 7	NO
11	VE18	Kogoni 91-1	Niono 7	NO
12	VE21	Kogoni 91-1	Niono 7	NO
13	VE25	Kogoni 91-1	Niono 7	NO
14	VE26	Kogoni 91-1	Niono 7	NO
15	VE28	Kogoni 91-1	Niono 7	NO
16	VE30	Wassa	Moussa Were	NO
17	VE31	Wassa	Moussa Were	NO
18	VE32	Wassa	Moussa Were	NO
19	VE34	Wassa	Moussa Were	NO
20	VE35	Wassa	Moussa Were	NO
21	VE37	*Oryza longistminata*	Moussa Were	NO
22	VE38	Wassa	Moussa Were	NO
23	VE39	Wassa	Moussa Were	NO
24	VE41	Wassa	Selingue	SDRO
25	VE42	Adny 11	Selingue	SDRO
26	VE43	Wassa	Selingue	SDRO

NO: Niger Office; SDRO: Selingue Development Rural Office.

Sérémé (2009), these different groups are related to the proteins involved in the mechanism of "silencing" to circumvent the defense system of the plant. This information reported intra- and inter-regional variability of RYMV. The sequencing of the RT-PCR products using Prymv2, showed a high nucleotide variability of the envelope protein gene of the isolates (Figure 3). The order of nucleotide sequences allowed knowing more information on the genetic diversity of the isolates.

According to similarity level, the study samples were classified into major groups: I, II and III (Figure 4). Group I, with two clusters (I1 and I2), was composed of genotypes from Niono 7, Moussa-Were (Niger Office area) and those from Selingue. This group constituted 95% of isolates from Niger Office rice growing area and more than 85% of total study's materials. Isolate VE7, which was genotype B with Prymv1, showed high similarity with isolates VE1, VE2, VE3, VE5, VE7, VE8, VE11, VE31, VE32 and VE34 under cluster I1 in accordance with the results of Prymv2 cDNA fragment sequencing. This indicates a strong phylogenetic similarity between the isolates, although they were collected from different localities. This result was aligned with those from Adego et al. (2017) founding diversity of isolates within the same S4 group in western Kenya by serological and molecular typing. Groups II and III were respectively composed of isolates from Selingue and Moussa Were. No virus was detected on N'peguena and Koyan samples. Results from current study revealed genetical and regional diversities of RYMV isolates collected from different locations of Niger Office (Niono 7, Koyan N'peguena and Moussa Were) and Selingue Development Rural Office (Selingue) rice's growing areas. Abubakar et al. (2003) reported relationships between genetic and geographical distances of rice yellow mottle virus strains. The three isolates from Selingue located about 395 km away from the Niger Office area showed genetic dissimilarity with respect to all isolates. However, one isolate (VE43) from this group was classified in Group I and the other two (VE41 and VE42) in Group II. According to Abubakar et al. (2003), the greater the geographical distance, the higher the genetic distance. Classification of the virus based on the nature of the proteins predicts the complexity to

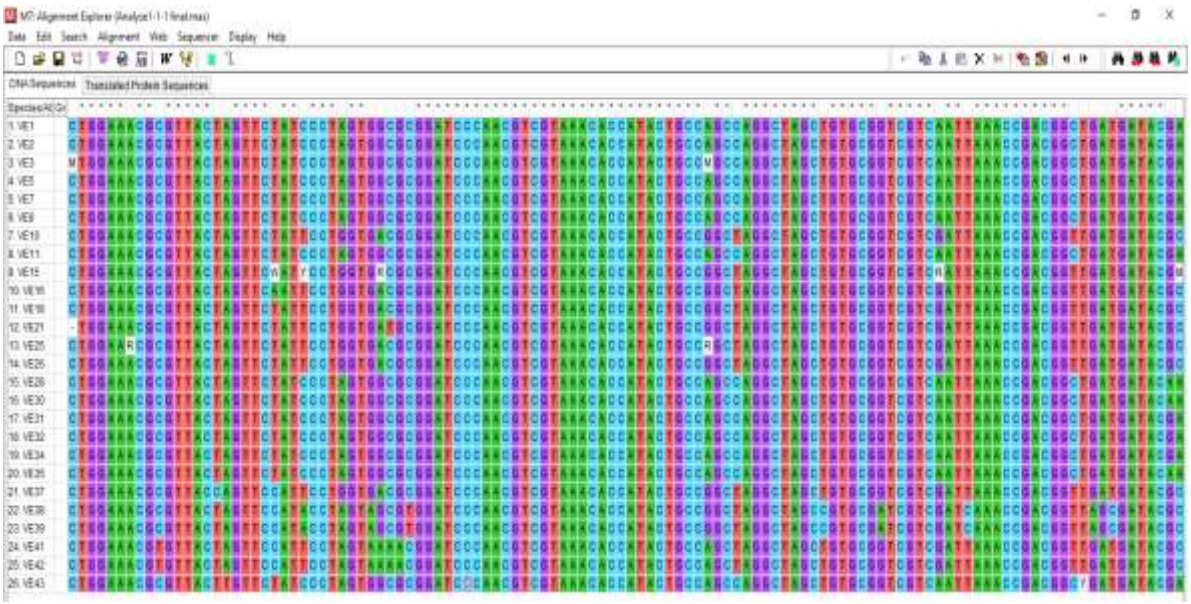

Figure 3. Partial alignment of the cDNA sequences of rice yellow mottle virus isolates from the Niger Office (VE1 to VE39) and the Selingue Development Rural Office (VE41 to VE43).

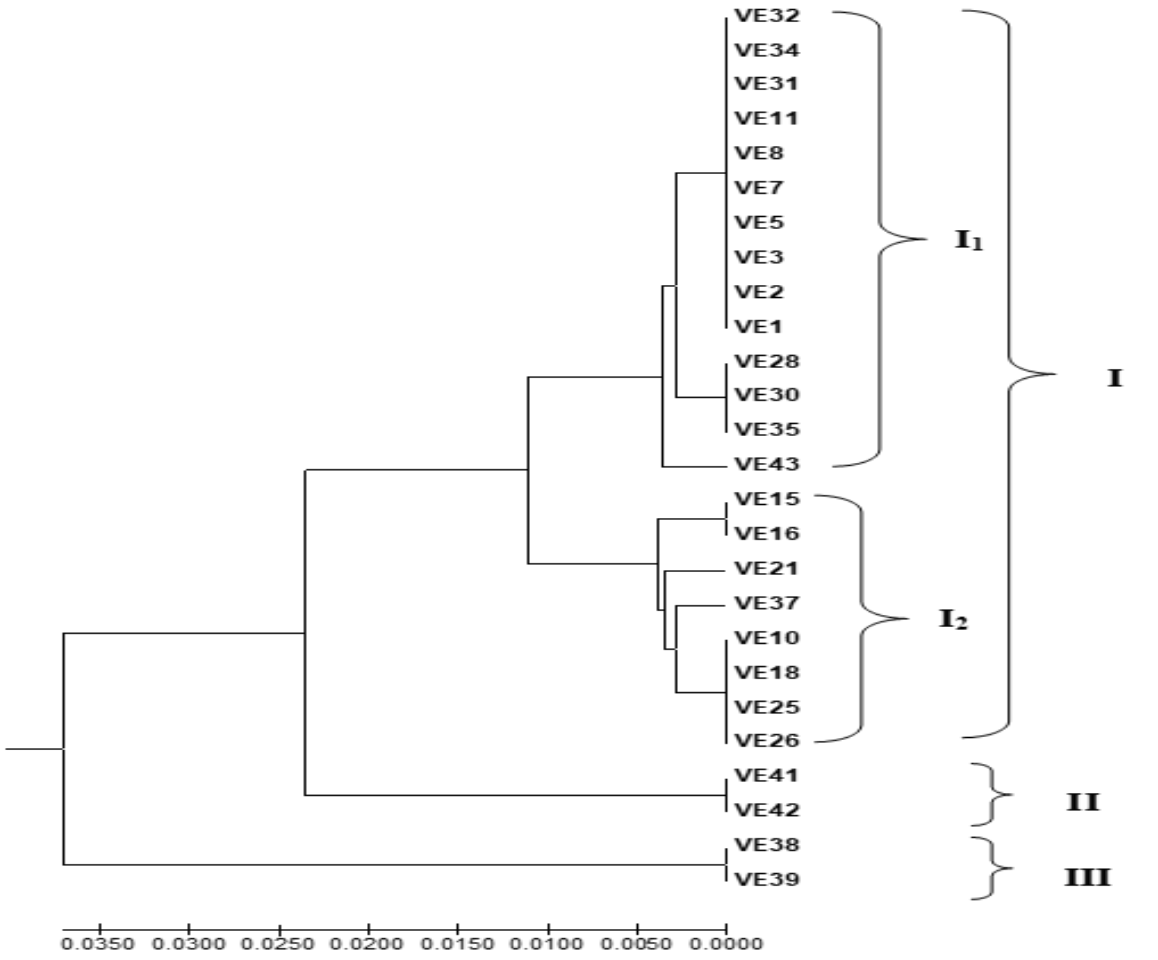

Figure 4. Similarity levels of 26 virus isolates based on Prymv2.

overcome it. Due to frequent genetic mutations, microorganisms have the ability to adapt and grow within the new environments which facilitates their emergence at any time and becoming more virulent or more contagious (Hubert et al., 2017; Herzog, 2016). Virulent pathotypes overcoming one or two resistance alleles have been reported (Longue et al., 2018; Hebrad et al., 2018; Pidon et al., 2017; Pinel-Galzi et al., 2016). N'Guessan et al. (2001) obtained two strains of RYMV at 6% divergence in nucleotide sequence of the capsid gene showing no difference in term of pathogenicity. According to Hubert et al. (2017), knowledge about the genetic diversity of RYMV is based on a limited number of protein sequences in the envelope. The results obtained in the current study provide basic information for monitoring the evolution of yellow mottle virus genetic diversity within rice-growing areas in Mali.

Conclusion

Rice yellow mottle virus is present within the most growing areas of rice in Mali (Niger Office and Selingue Development Rural Office). High genetic dissimilarity was noticed from different areas based on several genotypes showing genetic variability related to geographical distance. There is therefore a complication to control this disease. Information generated on the variability of the RYMV capsid gene sequences is a good starting point of database creation for RYMV in Mali with a goal to set up a disease control program.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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