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Genetic diversity and epidemic histories of rice yellow mottle virus in Ghana

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ABSTRACT

Rice yellow mottle virus (RYMV) has persisted as a major biotic constraint to rice production in Africa. However, no data on RYMV epidemics were available in Ghana, although it is an intensive rice-producing country. Surveys were performed from 2010 to 2020 in eleven rice-growing regions of Ghana. Symptom observations and serological detections confirmed that RYMV is circulating in most of these regions. Coat protein gene and complete genome sequencings revealed that RYMV in Ghana almost exclusively belongs to the strain S2, one of the strains covering the largest area in West Africa. We also detected the presence of the S1ca strain which is being reported for the first time outside its area of origin. These results suggested a complex epidemiological history of RYMV in Ghana and a recent expansion of S1ca to West Africa. Phylogeographic analyses reconstructed at least five independent RYMV introductions in Ghana for the last 40 years, probably due to rice cultivation intensification in West Africa leading to a better circulation of RYMV. In addition to identifying some routes of RYMV dispersion in Ghana, this study contributes to the epidemiological surveillance of RYMV and helps to design disease management strategies, especially through breeding for rice disease resistance.

1. Introduction

Rice yellow mottle virus (RYMV) was first identified in East Africa in 1966 (Bakker, 1974) and subsequently in 1975 in West Africa (Fauquet and Thouvenel, 1977). Since then, this virus has progressively been reported in almost all rice-producing countries of sub-Saharan Africa and Madagascar (Abo et al., 1997; Pinel-Galzi et al., 2015). Endemic to Africa, RYMV has become one of the most important biotic constraints to rice production in Africa (Kouassi et al., 2005; Savary et al., 2019; Séré et al., 2013), where it causes large yield losses in most rice-growing agroecosystems (Agnoun et al., 2019; Suvi et al., 2018).

RYMV belongs to the *Sobemovirus* genus of the *Solemoviridae* family. It has icosahedral particles and a single-stranded positive-sense RNA genome (Hébrard et al., 2020). The RYMV has a narrow host range limited predominantly to rice species and a few wild *Poacea* (Allarangaye et al., 2007; Bakker, 1974; Traoré et al., 2009). In infected rice plants, RYMV infection shows symptoms that include mottling and yellowing of the leaves, stunting, reduced tillering, partial panicle exertion, sterility and, in severe cases, death of the plant (Attere, 1983; Bakker, 1974). RYMV is mainly transmitted at short-distance by chrysomelid beetles and by contact between plants during cultural practices (Traoré et al., 2009). No evidence of seed transmission has been found so far in rice species (Allarangaye et al., 2006; Bakker, 1974; Fauquet and Thouvenel, 1977; Konate et al., 2001)

Based on RYMV genetic data collected from 24 African countries since the 1970′s, the genetic diversity of this virus has been well described (Pinel-Galzi et al., 2015), and its evolutionary history and its global dispersion across Africa and Madagascar has started to be

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deciphered (Issaka et al., 2021; Rakotomalala et al., 2019; Trovão et al., 2015). All these genetic data revealed a spatially structured genetic diversity, with strains specifically identified in West and East Africa (Fargette et al., 2004; Pinel-Galzi et al., 2015). This structure of the genetic diversity results from an independent dynamic of dispersion of RYMV between these two regions (Trovão et al., 2015). Six strains have been specifically identified in West Africa: S1ca, S1wa, S2, S3, Sa and Sg (Hébrard et al., 2020).

Three major resistance genes (named *rymv*1, *rymv*2 and *RYMV*3) were identified in rice, mainly in African rice species, *Oryza glaberrima* (Orjuela et al., 2013; Pidon et al., 2020, 2017; Thiémélé et al., 2010). Note that these resistant genes have not been used widely for the development of commercial rice varieties, *i.e.* the deployment of resistant varieties in fields has not yet widely been performed. Almost all rice varieties cultivated in Africa currently is of the Asian species, *O. sativa,* which is highly susceptible to RYMV infection.

It has been shown in controlled conditions that RYMV is able to adapt and to overcome the resistance. The molecular mechanism underlying the resistance-breaking (RB) process has been characterized. It involved the emergence of specific point mutations on different regions of the RYMV genome (Bonnamy et al., 2022; Pinel-Galzi et al., 2016, 2007; Poulicard et al., 2014; Traore et al., 2010). Interestingly, the RYMV strains present contrasted RB abilities depending on the resistance gene. Based on these experimental results and considering the spatial distribution of RYMV strains in Africa, resistance-breaking risk maps were established (Hébrard et al., 2018). However, the validity and the sustainability of these risk maps are strictly dependent on the dispersion of the strains in field conditions and on the evolution of the local RYMV

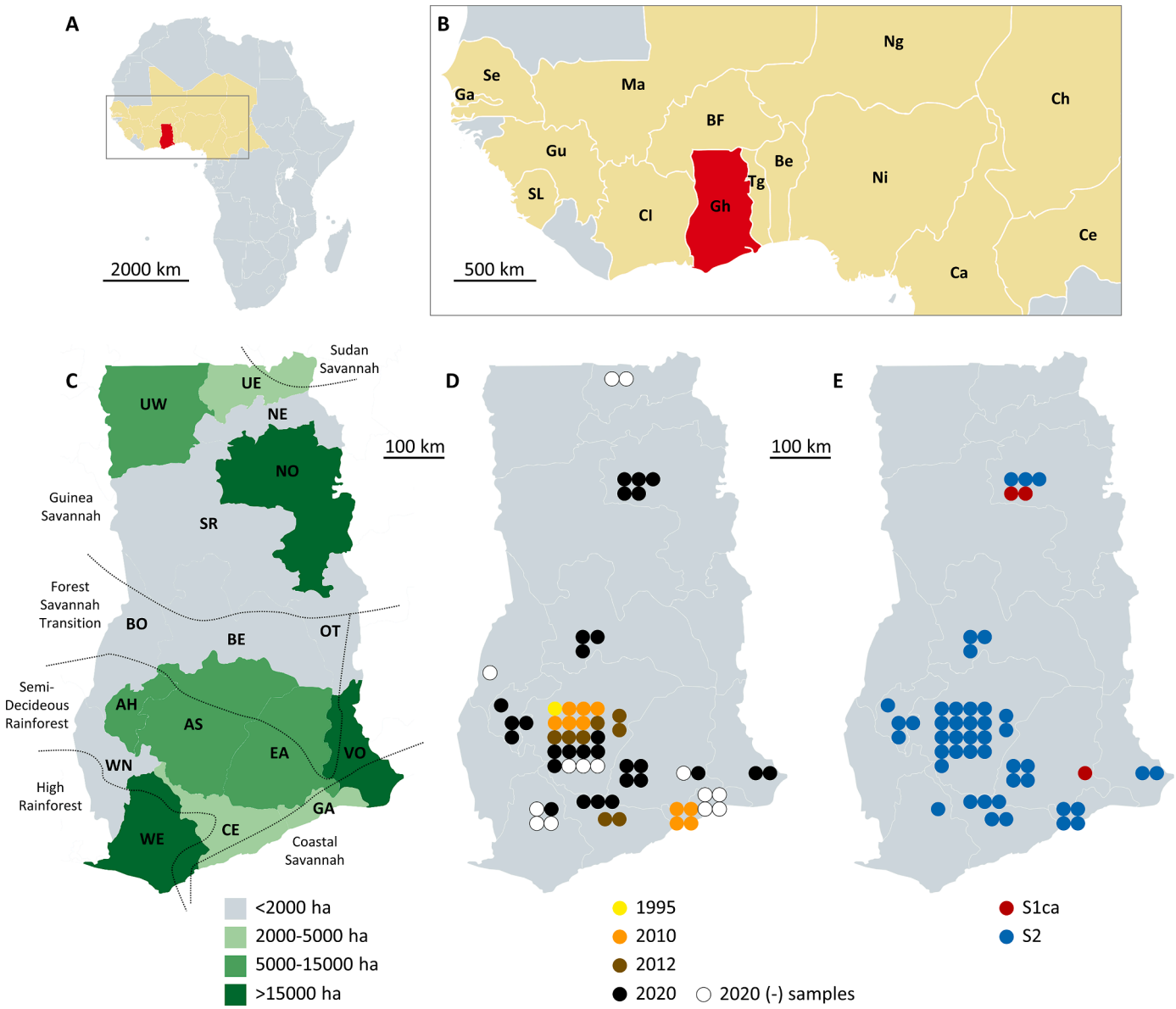

Fig. 1. Geographical localization of Ghana, distribution of rice cultivation and location of the rice samples/RYMV isolates. (A) Map of Africa and (B) focus on Ghana (in red) and the countries with RYMV genetic information used in this study (light brown): Benin (Be), Burkina Faso (BF), Cameroon (Ca), Central African Republic (Ce), Chad (Ch), Ivory Coast (CI), Gambia (Ga), Ghana (Gh), Guinea (Gu), Mali (Ma), Nigeria (Ni), Niger (Ng), Senegal (Se), Sierra Leone (SL) and Togo (Tg). (C) Rice production in Ghana. Regions are colored according to the surface (in hectares, ha) dedicated to rice cultivation. The Upper West (UW), Upper East (UE), North East (NE), Savannah Region (SR), Northern (NO), Bono (BO), Bono East (BE), Oti (OT), Ahafo (AH), Ashanti (AS), Eastern (EA), Volta (VO), Western North (WN), Western (WE), Central (CE) and Greater Accra (GA) regions and the borders of the ecological zones (dotted lines) are indicated. (D) Schematic locations of the RYMV isolates sampled in Ghana in 1995 (yellow), 2010 (orange), 2012 (brown) and 2020 (black). Samples collected in 2020 and negative to RYMV detection by DAS-ELISA are indicated by white spots. (E) Schematic location of the RYMV isolates belonging to S1ca (red) and S2 (blue) strains.

genetic diversity.

With more than 323,900 hectares dedicated to rice cultivation, Ghana (Fig. 1A-B) is an important rice producer country in Africa (FAOSTAT 2021; https://data.un.org/Data.aspx?*d*=FAO&*f*=itemCode%3A27). Distributed across 6 ecological zones and 16 regions (Fig. 1C), there are mainly three agroecosystems for rice production in Ghana, namely rainfed lowland, irrigated and upland rice ecologies (78%, 16% and 6%, respectively, of total harvested area in 2009; https://www.jica.go.jp/english/our_work/thematic_issues/agricultural/pdf/ghana_en.pdf). During the last decades, the acreage of cultivated rice is rapidly increasing and rice cultivation intensified to cope with the rising demand due to demographic and societal changes, in Africa in general and in Ghana in particular (Fig. S1). Such agricultural changes surely make rice cultivation more exposed and vulnerable to pathogen epidemics. However, very little is known about the distribution and the genetic diversity of rice pathogens in Ghana, especially RYMV. Actually, only one coat protein (CP) sequence has been reported so far from a single isolate collected in Ghana in 1995 (Pinel et al., 2000). This CP sequence indicates that the S2 strain is present in Ghana but, as Ghana is surrounded by countries where high RYMV genetic diversity have been reported (with the presence of S1wa, S1ca, and S2 strains in Ivory Coast, Burkina Faso and Togo; Pinel-Galzi et al., 2015), the genetic diversity of RYMV in Ghana could be much higher.

Thus, the aim of this study was to assess the geographical distribution of RYMV in the most important rice cultivation regions of Ghana and analyze the genetic diversity using the CP gene and complete genome sequencing. In addition, this genetic information will allow us to reconstruct the spatio-temporal dynamics of the RYMV in Ghana and to identify the ways of introduction and dispersion of the virus within Ghana and between neighboring countries. Our results will greatly contribute to the epidemiological surveillance of rice in Ghana and will facilitate the development of efficient and sustainable control strategies based on the deployment in fields of resistant varieties against RYMV to minimize the risk of emergence of RB variants.

2. Methods

2.1. Field survey and sampling

Surveys to collect symptomatic RYMV leaf samples were conducted in 2010, 2012 and 2020 during the major rice cropping season (July-September) to cover a total of 11 rice growing regions spread across different agroecological zones of Ghana, notably Ashanti, Ahafo, Bono, Bono East, Central, Eastern, Greater Accra, Northern, Upper East, Western and Volta regions (Fig. 1C). All the samples were collected from rainfed lowland fields as it corresponds to the most representative rice cultivation mode in Ghana (78% of total harvested area in 2009; https://www.jica.go.jp/english/our_work/thematic_issues/agricultural/pdf/ghana_en.pdf). The GPS coordinates were noted for leaf samples collected in each location and the rice variety plus the age of the plants were recorded during the 2020 survey (Table S1). The symptomatic leaves sampled from each plant were cut using a pair of scissors and stored in a notebook used as herbarium. The scissors were disinfected by dipping in 70% ethanol before sampling from the subsequent plants. The sample locations were reported on a map based on MapChart website (https://mapchart.net/).

2.2. Serological detection and genome sequencing

The DAS-ELISA tests were performed with polyclonal antibodies targeting the RYMV coat protein (CP) on the leaf samples as described previously (N'Guessan et al., 2000) to confirm the infection status of symptomatic leaves. To obtain the CP gene (ORF4; 720 nt) or the complete genome (*ca.* 4,45 kb) sequences, total RNA from infected leaves were first extracted using GeneJET Plant RNA Purification Kit (ThermoFisher SCIENTIFIC, MA, USA). Then, the CP genes and/or the complete genomes were specifically amplified by RT-PCR as previously described (Pinel et al., 2000). The RT-PCR products were sequenced using the Sanger technique (Genewiz, NJ, USA) and the sequences were deposited in GenBank (Accession Nos. from OQ200610 to OQ200655 for CP, from OQ225940 to OQ225946 for complete genomes; Table S2).

2.3. Genetic diversity and phylogenetic analyses

The partial and complete genome sequences obtained from Ghana were compared to those already deposited in NCBI (July 2021; *cf.* Issaka et al., 2021), *i.e.* 261 CP gene sequences and 37 complete genome sequences from isolates collected in nearly all-rice growing countries from West Africa (Fig. 1A-B). Multiple sequence alignments were performed using MUSCLE (Edgar, 2004) implemented in SEAVIEW v4.7 (Gouy et al., 2010). A search for potential recombination signals in the RYMV sequences was performed with the pairwise homoplasy test (PHI test) using SplitsTree v4.14.6 (Huson and Bryant, 2006) and the seven algorithms implemented in RDP v4.97 (Martin et al., 2015). Recombination events detected by at least 4 methods and with *P*-values below 10^{-5} were considered.

We reconstructed maximum-likelihood (ML) phylogenetic trees with SEAVIEW using the best-fitted nucleotide substitution models (Tamura 3-parameter+*G*+*I* and GTR+*G*+*I* for the CP and the complete genome datasets, respectively) determined with MEGA6 (Tamura et al., 2013) and 100 bootstrap replications. Phylogenetic trees were drawn using FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). The genetic diversity of the ORF4 dataset was estimated using the best-fitted nucleotide substitution model, with standard errors of each measure based on 1000 replicate bootstraps, as implemented in MEGA6.

2.4. Tests of temporal signal

In order to examine the degree of temporal signal in the CP dataset, we first used an exploratory linear regression approach (Duchêne et al., 2015; Murray et al., 2016). Based on ML phylogenetic tree reconstructed with the CP dataset, the temporal signal of this sequence dataset was visualized and tested in PhyloStemS (Doizy et al., 2020) to regress phylogenetic root-to-tip distances against sampling date using the root that minimized the residual mean squares.

Then, the significance of the temporal signal was evaluated by a date-randomization test. Thus, the mean rate and its 95% highest probability density (HPD) estimated with the observed sampled dates using the Bayesian Evolutionary Analysis Sampling Trees BEAST v1.10.4 package (Suchard et al., 2018, see below) were compared with a null distribution obtained by randomly permutating the tip dates 10 times (Firth et al., 2010). As previously described (Duchêne et al., 2015; Murray et al., 2016), the criterion for a significant temporal signal was that the 95% HPD for the rate estimate obtained with the observed sampled dates should not overlap with the 95% HPD for the estimate obtained with randomized sampling times.

2.5. Bayesian evolutionary inferences

We reconstructed time-calibrated epidemic histories of the RYMV in West Africa based on the CP dataset using a Bayesian statistical framework implemented in BEAST version 1.10.4 software (Suchard et al., 2018) and the BEAGLE library (Ayres et al., 2012) to improve computational performance. BEAST program uses Markov chain Monte Carlo (MCMC) integration to average over all plausible evolutionary histories for the data, as reflected by the posterior probability. The nucleotide substitution process was reconstructed using HKY85+*G* substitution model, the lognormal relaxed clock model and an initial substitution rate of 10^{-3} substitutions/site/year as previously determined for RYMV (Dellicour et al., 2018; Issaka et al., 2021; Trovão et al., 2015).

To study the geographic dispersion of RYMV in continuous time and space, we used a Bayesian SkyGrid coalescence model (Gill et al., 2013)

and a relaxed random walk model for the continuous location traits (latitude and longitude of each isolate) with a Cauchy probability distribution (according to the best model identified in Trovão et al. (2015). Bayesian inference using continuous diffusion models yield a posterior distribution of phylogenetic trees, each having ancestral nodes annotated with location estimates.

The MCMC analyses were run for 5 billion generations, sampled every 100,000th and discarding 10% as the chain burn-in until adequate effective sample sizes (ESS) were obtained (ESS>200 for all statistics). The parameter estimates were inspected using Tracer 1.7.2 (http://tree.bio.ed.ac.uk/software/tracer). A maximum clade credibility (MCC) tree was summarized from the posterior tree distribution using TreeAnnotator v1.10.4 (BEAST v1.10.4 package) and visualized with FigTree v1.3.1. The dates and the 95% HPD intervals of RYMV movements (introduction or release of RYMV to/from Ghana) were estimated as the interval between the nodes from the same clade with different geographical locations as identified in the MCC tree. The visualization of the continuous phylogeographic reconstruction of the dispersal history of RYMV into West Africa and Ghana were performed with R functions available in the package "SERAPHIM" (Dellicour et al., 2016).

3. Results

3.1. Field samplings and geographic distribution of RYMV in Ghana

A total of 63 samples showing symptoms that could be attributed to a viral infection were collected in eleven regions between 2010 and 2020 (2010: N=10; 2012: N=8; 2020: N=44; Table S1). Serological detection by DAS-ELISA confirmed that 49 of these samples were positive to RYMV. These positive samples were collected in nine regions (Ahafo, Ashanti, Bono East, Central, Eastern, Greater Accra, Northern, Volta, Western, Fig. 1D, Table S1). In addition, RYMV infection was reported in 9 rice varieties, both improved (AGRA, Bouake189, CRI-AGRA, Jasmine85, Legon1, Togo Marshall) and local varieties (Aifaifa, Anyifila and Samasa) (Table S1). During that survey, RYMV was not reported in only two regions (Bono and Upper East), although these regions are characterized by rice varieties and ecological zones similar to those where RYMV has been observed (Fig. 1C and D; Table S1).

3.2. Genetic diversity of RYMV in Ghana

All positive samples collected in 2010, 2012 and 2020 were used to analyze the genetic diversity of RYMV in Ghana. The CP gene was successfully amplified by RT-PCR and sequenced for 46 RYMV isolates (Table S2). These sequences were compared to the 261 CP gene sequences previously published (Issaka et al., 2021) coming from isolates collected in 15 West-African countries between 1975 and 2018, including one CP sequence from an isolate collected in 1995 in Ghana. This dataset is thereafter referred to as the WA307 dataset. Based on this CP dataset, phylogenetic analysis showed that almost all RYMV isolates sampled in Ghana clustered within the S2 strain (Fig. S2, Fig. 1E), a strain which was previously reported in Ghana in 1995 (Gh1 isolate) and detected in several countries surrounding Ghana (Ivory Coast, Guinea, Mali, Burkina Faso and Benin). Although S1ca and S2 strains circulate within the same regions (Fig. 1E, Table S1), no mixed infection was detected during this study. The genetic diversity of S2 RYMV isolates from Ghana is similar to that observed for the S2 isolates from Ivory Coast and Guinea, and significantly higher than those from Mali or Benin (Table 1). In addition to the S2 strain, we identified that two isolates (out of 5) from the Northern region and one isolate (out of 5) from the Eastern region belong to the S1ca strain (Fig. 1E, isolates 2020Gh113, 2020Gh139 and 2020Gh140 in Fig. S2 and Table S1). The genetic diversity of S1ca RYMV isolates from Ghana is similar to that observed in most of West-African countries (Table 1). The PHI test did not find statistically significant evidence for recombination within the WA307 dataset ($p = 0.93$).

Table 1
Genetic diversity of the S2 and S1ca strains in West- and West-Central Africa.

Strain	Group	N Seq.	d	S.E.
S2	All S2	113	0.016	0.002
	Ivory Coast (CI)	38	0.015	0.003
	Ghana (Gh)	44	0.014	0.002
	Guinea (Gu)	12	0.014	0.003
	Mali (Ma)	15	0.009	0.002
	Benin (Be)	3	0.001	0.001
	Burkina Faso (BF)	1	nd	nd
S1ca	All S1ca	126	0.042	0.006
	Nigeria (Ni)	12	0.039	0.006
	Cameroon (Ca)	10	0.039	0.008
	Ghana (Gh)	3	0.031	0.003
	Burkina Faso (BF)	5	0.029	0.009
	Central African Republic (Ce)	30	0.024	0.006
	Niger (Ng)	41	0.018	0.003
	Chad (Ch)	8	0.017	0.003
	Togo (Tg)	8	0.007	0.003
	Benin (Be)	9	0.007	0.002

d: genetic diversity estimated in substitution per site; S.E.: standard error.

Based on this CP phylogenetic tree, seven isolates representing the genetic diversity of RYMV in Ghana were selected to be fully-sequenced (S2 strain: 2010Gh47, 2010Gh55, 2020Gh106, 2020Gh109 and 2020Gh112; S1ca strain: 2020Gh113 and 2020Gh140) and then compared to the 37 complete genome sequences from West Africa. This dataset is thereafter referred to as the WA44 dataset. The reconstruction of the ML phylogenetic tree confirmed that these isolates from Ghana clustered within the S2 and S1ca strains and demonstrated that these isolates are not directly genetically related between each other (Fig. 2). No statistically significant evidence for recombination was detected in the RYMV genomes from Ghana (data not shown).

Altogether, these results showed that the genetic diversity of the S2 and S1ca isolates from Ghana is representative of those circulating in West Africa, which suggests that several introductions of RYMV to Ghana occurred and account for the genetic diversity in this country.

3.3. Epidemic histories of RYMV in Ghana

To reconstruct the evolutionary history of RYMV in West Africa and to gain information on the epidemic histories and trajectories of this virus in Ghana, we first examined the strength of the temporal signal of the WA307 sequence dataset. The linear regression exploration of root-to-tip distances as a function of sampling time suggested a weak but consistent temporal signal (Fig. S3), with a low correlation coefficient (adjusted $R^2<0.1$; $p<10^{-6}$). The presence of temporal signal was then confirmed consistently using BEAST and date-permutation tests (tip-date and clustered tip randomizations). Actually, the estimates of the permutations did not overlap those of the real (non-permutated) WA307 dataset (Fig. S4). As no signal for recombination event was detected on the WA307 dataset, we reconstructed the evolutionary and the dispersal histories of the RYMV in West Africa by Bayesian inferences. The generated maximum clade credibility (MCC) tree strengthened the topology of the ML phylogenetic tree previously obtained (Fig. 3A). In addition, this MCC tree and the projection of this MCC tree on sequential-dated maps (Fig. 3B-E; Fig. S5) highlighted that several independent introductions occurred in Ghana.

By extracting information from the MCC tree and by mapping the tip locations of the MCC tree in geographic space, we identified at least three independent introductions of isolates belonging to S2 strain (branches labelled 1 to 3 in Fig. 3A) to southern part of Ghana. Interestingly, all the S2 clusters identified in Ghana are directly genetically related to isolates from Ivory Coast (Fig. 3A), suggesting that S2 strain introductions to Ghana resulted from RYMV movements from the Ivory Coast. The date intervals between the nodes of branches 1 to 3 provide estimates of the timing of these introductions. Thus, a first S2

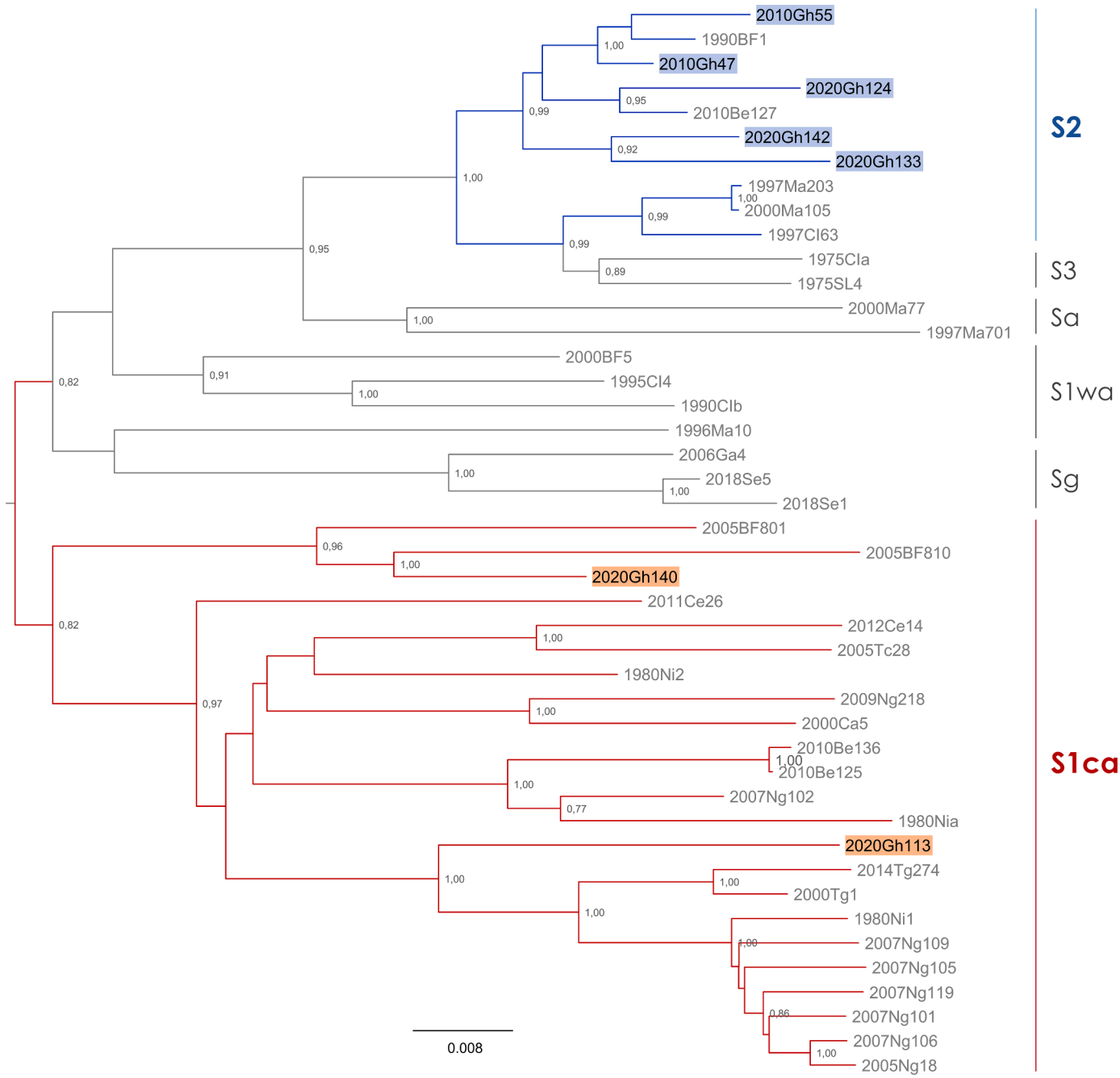

Fig. 2. Maximum-likelihood tree inferred from the WA44 complete genome sequence dataset. The names of the RYMV isolates from Ghana (Gh) are indicated in black, the fully-sequenced isolates from Ghana are highlighted, and the branches are colored according to the six RYMV strains of West and Central Africa (grey: S1wa, S3, Sa, Sg; red: S1ca; blue: S2). Number at each node indicates bootstrap values based on 100 replicates (only values above 0.60 are indicated). The country of origin of the isolates is mentioned by their names: Benin (Be), Burkina Faso (BF), Cameroon (Ca), Central African Republic (Ce), Ivory Coast (CI), Gambia (Ga), Mali (Ma), Niger (Ng), Nigeria (Ni), Senegal (Se), Sierra Leone (SL), Chad (Tc) and Togo (Tg).

introduction occurred during the early 1990′s (interval between the node 1 and the date of collection of the 1995Gh1 isolate, *i.e.* 1987.8–1995.6 with 95%HPD [1983.3–1995.6]; Fig. 3A–C; Table 2). Then, a second S2 introduction occurred during the early 2000′s (branch 2, interval between the nodes 2a and 2b, *i.e.* 1999.2–2003.5 [1993.1–2007.3]; Fig. 3A–C; Table 2). Finally, a more recent S2 introduction occurred after the 2000′s (branch 3, interval between node 3 and the date of collection of the 2020Gh133 isolate, *i.e.* 1999.8–2020.6 [1995.0–2020.6]; Fig. 3A–E; Table 2). Although S2 isolates circulated in the South since the early 1990′s, it is interesting to note that these isolates dispersed to the Northern Ghana only from the 2010′s (branch 6, interval between nodes 6a and 6b, *i.e.* 2009.9–2013.8 [2007.3–2016.8]; Fig. 3A–E; Table 2). We also identified one S2 dispersion from Ghana to Benin that occurred between 2008 and 2010 (branch 7, interval between nodes 7a and 7b, *i.e.* 2008.8–2009.6 [2007.1–2010.5]; Fig. 3A–D; Table 2).

In parallel with the dispersion of the S2 strain, the phylogeographic reconstruction showed two independent introductions of RYMV isolates belonging to the S1ca strain in Ghana (branches labelled 4 and 5 in Fig. 3A). Because there are few isolates of S1ca strain in Ghana, the range of dating estimates are more extended than for those of S2, but the phylogeographic reconstructions suggested that these RYMV

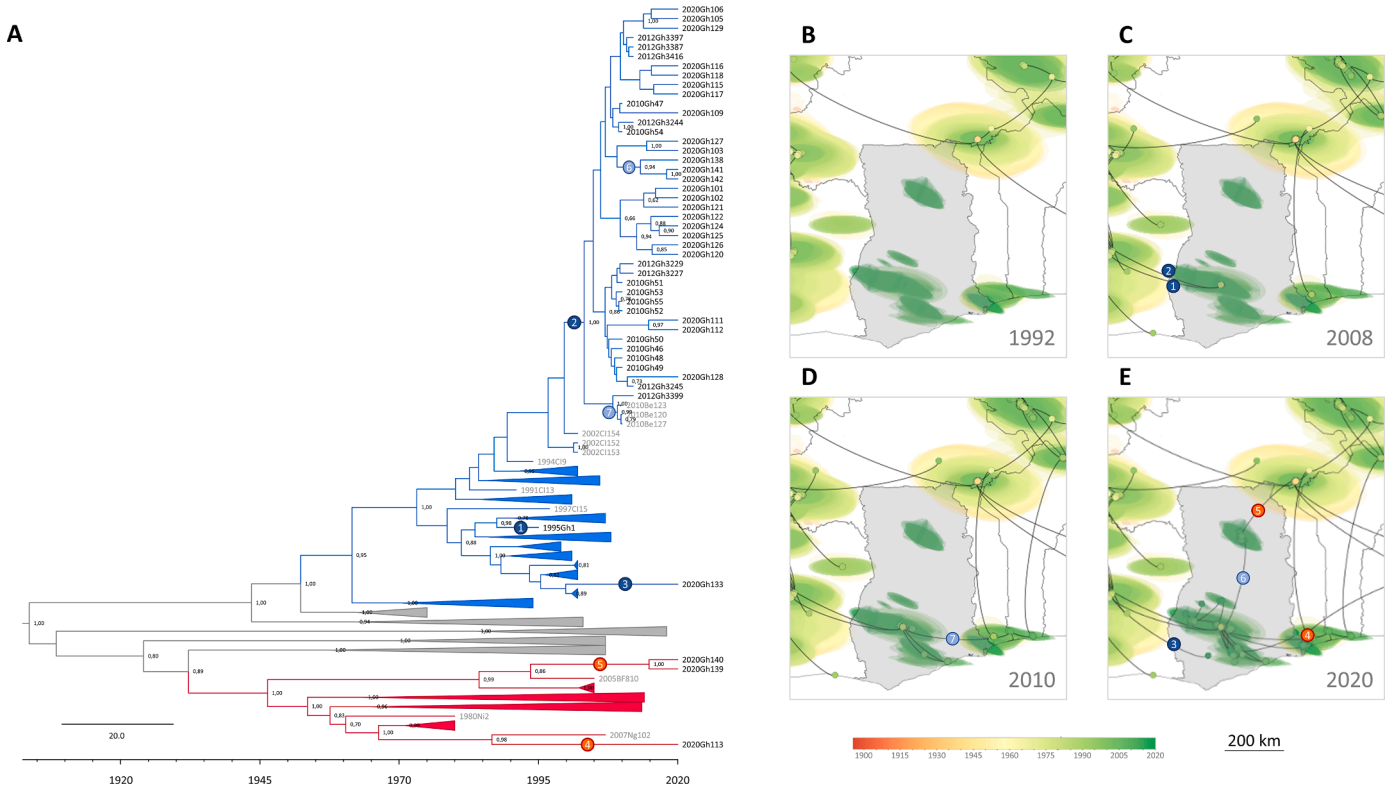

Fig. 3. Phylogeographic reconstructions of RYMV epidemics in Ghana. (A) Time-calibrated maximum clade credibility (MCC) tree reconstructed by continuous Bayesian evolutionary inference with the 307 coat protein gene sequence dataset (WA307). Branches are colored according to the RYMV strains of West Africa (grey: S1wa, S3, Sa, Sg; red: S1ca; blue: S2). The values on the nodes correspond to their posterior probabilities (only values above 0.60 are indicated). The bottom axis gives the timeframe (by date) of the RYMV diversification. Seven branches of interest are labeled on the MCC tree (from 1 to 7) and their relative nodes (1, 2a, 2b, 3, 4, 5a, 5b, 6a, 6b, 7a, 7b) were annotated with their time to the most common ancestor and their 95% highest probability density (HPD) intervals (Table 2). (B to D) Reconstruction of the continuous spatio-temporal dispersion of RYMV in West Africa with snapshots in 1992 (B), 2008 (C) and 2020 (D) that capture introductions and release of RYMV in/out Ghana (delimited by the gray area). Black lines show a spatial projection of the MCC tree and colored areas represent statistical uncertainty in the dated estimated locations of RYMV internal nodes (95% HPD; from red to green to cover the time period from 1900 to 2020). The seven branches of interest in Fig. 3A are indicated.

Table 2
Dating estimates of nodes of the seven branches of interest identified in the maximum clade credibility (MCC) tree (Fig. 3A).

Time before present (2020.6)				
n° node	Time	Time 95%HPD	Date	Date 95%HPD
1	32.8	[37,3 - 28,2]	1987.8	[1983,3 - 1992,5]
2a	21.4	[27,5 - 18,1]	1999.2	[1993,1 - 2002,5]
2b	17.1	[21,6 - 13,3]	2003.5	[1999,0 - 2007,3]
3	20.8	[25,6 - 18,2]	1999.8	[1995,0 - 2002,4]
4	33.7	[50,0 - 16,4]	1986.9	[1970,7 - 2004,2]
5a	27.7	[41,1 - 16,0]	1992.9	[1979,5 - 2004,6]
5b	5.6	[9,8 - 1,3]	2015.0	[2010,8 - 2019,3]
6a	10.7	[13,3 - 8,0]	2009.9	[2007,3 - 2012,6]
6b	6.8	[10,7 - 3,8]	2013.8	[2009,9 - 2016,8]
7a	11.8	[13,5 - 10,4]	2008.8	[2007,1 - 2010,2]
7b	11.0	[12,0 - 10,1]	2009.6	[2008,6 - 2010,5]

The estimates of the time to the most common ancestor (in years before July 2020, *i.e.* 2020.6), the dates and the 95% highest probability density (HPD) intervals are mentioned.

movements occurred after the 1990′s. A first S1ca introduction to Ghana was identified as the 2020Gh113 isolate (branch 4, interval between the node 4 and the date of collection of the 2020Gh113 isolate, *i.e.* 1986.9–2020.6 [1970.7–2020.6]; Fig. 3A–E; Table 2). This isolate clustered with isolates from Nigeria and Niger, suggesting an RYMV way of transmission from these countries (or other intermediate ones as Benin or Togo) to the South-eastern part of Ghana. In addition, an independent S1ca introduction occurred in the Northern regions of Ghana at the same period (1992.89–2015.02 [1979.46–2019.32]; Fig. 3A–D, Table 2), probably from isolates genetically related to the ones reported in South-East of Burkina Faso.

4. Discussion

The observation of rice plants with RYMV characteristic symptoms and the confirmation of the infection status of these plants by DAS-ELISA and CP gene or complete genome sequencing demonstrated that the virus has been widely distributed in Ghana since 1995, the date of collection of the first RYMV isolate in this country. Actually, RYMV has been detected in nine regions covering five ecological zones as Guinea savannah (Northern region), forest savannah transition (Bono East), semi-deciduous rainforest (Ahafo, Ashanti, Central and Eastern regions), high rainforest (Western region) and coastal savannah (Greater Accra and Volta regions) (Fig. 1C-D). In addition, RYMV was detected in all improved and local rice varieties surveyed during this study (Table S1), suggesting that the resistance to RYMV is not yet a “usual selection” criteria for the industrial rice producers or the small farm holders. Note that some samples from 2020 presented symptoms but DAS-ELISA assays did not detect RYMV infection (Table S1). These samples were probably collected because of symptom confusion between RYMV and other biotic (or abiotic) factors, especially viruses infecting rice in West Africa as the rice stripe necrosis virus (RSNV; Bagayoko et al., 2021) or other virus species that have still to be identified.

Field surveys between 1995 and 2012 only collected RYMV isolates from S2 strain in Ghana. The survey in 2020 covered a wider

geographical scale and larger agro-environmental conditions (from 2 to 11 regions) and confirmed that RYMV isolates circulating in this country belong mainly to the S2 strain (Fig. 2 and Fig. S2). Surprisingly, the genetic diversity detected with the CP gene sequences of the S2 isolates in Ghana is high, which is similar to that reported for the whole S2 strain dataset (96 isolates) from Ivory Coast, Guinea, Mali, Benin and Burkina Faso (Table 1). This observation suggests that the RYMV isolates collected in Ghana are representative of the genetic diversity of the S2 strain in West Africa, which reflect the complex epidemiological history of RYMV in Ghana. In addition, the field survey in 2020 identified three isolates (out of 47, *i.e. ca.* 6%) from the Northern and the Eastern regions clustering within the S1ca strain. This suggests that Ghana has faced dynamic RYMV epidemics in the last decades. Although both RYMV strains circulate within the same agro-environment (Fig. 1C, Table S1), the analysis of complete RYMV genomes of isolates from Ghana did not reveal any recombination event (data not shown).

Based on 307 CP gene sequences of RYMV isolates collected in 15 West African countries since 1975, including 49 isolates from Ghana sampled from 1995 to 2020, the phylogeographic reconstructions showed that RYMV was first introduced in Ghana in the 1990′s (Fig. 3C). This introduction date is remarkably later than that in other countries from West and Central Africa, including neighboring countries of Ghana. Actually, spatio-temporal modeling revealed that RYMV disseminated efficiently from Mali to Burkina Faso and Ivory Coast during the 1950′s, to Nigeria during the 1960′s, to Gambia, Sierra Leone and Cameroon during the 1970′s, and then to Guinea, Niger and Chad during the 1980′s (Fig. S5; Dellicour et al., 2018; Issaka et al., 2021; Trovão et al., 2015). We identified at least five independent introductions of RYMV into Ghana (Fig. 3). An important way of S2 strain transmission was identified between Ivory Coast and the Southern Ghana (probably Ahafo, Ashanti and Western regions). In addition to similar environmental conditions on both sides of this border (high rainforest), these reiterated RYMV introductions could be favored by agronomical interactions between Ivory Coast and Ghana that could drastically increase the probabilities of RYMV dispersion by movement of infected biological material. The most recent introduction in Ghana was apparent through the isolate 2020Gh133 only (branch 3, Fig. 3A–E) collected in 2020 in Western region, *i.e.* at the border with Ivory Coast, illustrating the continuous introduction of RYMV isolates from this country since the 1990′s. Interestingly, these introductions are paralleled to the regular increase in rice cultivation areas in Ghana from the 1990′s and more intensively from 2008 (FAOSTAT 2021, Fig. S1; https://data.un.org/Data.aspx?*d*=FAO&*f*=itemCode%3A27). Note that the number of RYMV introductions in Ghana is certainly underestimated by these epidemiological models as we have a limited number in time and space of RYMV samples and not all introductions lead to successful epidemics. For instance, our study shows that no RYMV isolates genetically related to the 1995Gh1 isolate (branch 1, Fig. 3A) were sampled during the 2010, 2012 and 2020 surveys, which suggest that this RYMV lineage went extinct. Nevertheless, although the risk of sampling biases is permanent in phylogeographic analyses (Layan et al., 2023), we were able to reconstruct the main dispersion process of RYMV in Ghana which was consistent with the distribution of RYMV strains in neighboring countries, the phylogeography of RYMV in West Africa and the history of rice in Ghana.

While RYMV isolates belonging to the S2 strain were present in Ghana since the 1990′s, the virus spread very recently to the Northern regions (2010–2017; Table 2, Fig. 3E). The delay of RYMV dispersion to the North could be due to agronomical and topological factors. The discontinuity of the rice cultivation landscape in Ghana, and especially with less rice cultivation area in the Central regions (Fig. 1C), could make a barrier against the RYMV expansion between the Southern and the Northern regions. Actually, the rice connectivity landscape has been suggested (Trovão et al., 2015), although not statistically confirmed (Dellicour et al., 2018), to be the major determinant of RYMV spread. The barrier to RYMV dispersion at the North could also be due to biogeographical factors. As Northern Ghana is characterized by drier conditions, the agricultural and environmental conditions could be less favorable to RYMV epidemics in this region (different cultural practices, less alternative hosts, rice landscape more fragmented; Traore et al., 2009). However, the initial limitation of RYMV to spread from the South to the North certainly lessened in the last decades in parallel to rice intensification and extensification in Ghana (Demont, 2013; FAOSTAT 2021, Fig. S1; https://data.un.org/Data.aspx?*d*=FAO&*f*=itemCode%3A27). Similarly, the late introduction of S1ca could also demonstrate the tendency of RYMV to circulate more easily since the 1990′s. Actually, while the phylogeographic reconstructions estimate that RYMV is present in South-East of Burkina Faso since the 1950′s (Fig. 3B) and later on in Niger and Nigeria, we identified two RYMV movements from these countries to the Northern and the South-eastern regions of Ghana only after the 1990′s (branches 4 and 5 in Fig. 3A–E; Table 2). Altogether, because of the recent introductions of RYMV to the Northern regions of Ghana (both S2 and S1ca strains) and the agro-environmental factors that could be unfavorable to virus transmission or dispersion, RYMV has probably not reached an epidemic status in the North of Ghana. This could explain why no RYMV infected plant has been detected in the Upper East region (Fig. 1D).

Surprisingly, although S1wa strain is also present in Ivory Coast (Pinel-Galzi et al., 2015), our results show that only the S2 strain was introduced several times in Ghana from Ivory Coast. This suggests that S2 strain has advantageous biological or genetic properties to disperse in time and space compared to S1wa strain. Contrary to the lineage genetically related to Gh1 that probably got extinct (branch 2, Fig. 3A), we observed that one RYMV lineage (branch 2, Fig. 3A) has been detected during the 2010, 2012 and 2020 surveys and showed an efficient expansion to several Southern and Northern regions of Ghana, and also to Benin (branches 6 and 7, respectively; Fig. 3A–E). These observations suggest that, in addition to agronomical and anthropological factors, some intrinsic genetic factors could favor the epidemic success of specific RYMV lineages or strains in West Africa. Earlier studies showed that isolates from S2 strain dominated over those from S1wa strain in experimental conditions (N'Guessan et al., 2000).

The phylogeographic reconstructions established only one release of RYMV from Ghana. One route of RYMV dispersion was identified from Ghana to Benin (branch 7 in Fig. 3A–D) with a date estimated between 2008 and 2010 (Table 2, interval between nodes 7a and 7b). In West Africa RYMV history, this movement is only the second dispersion event of RYMV to pass across the Ghana/Burkina Faso axis, *i.e.* dispersion from West Africa to Central Africa. Actually, a first expansion of RYMV to Central Africa occurred during the 1950′s through Burkina Faso and led to the genetic differentiation and the emergence of the S1ca strain (Dellicour et al., 2018; Issaka et al., 2021; Trovão et al., 2015). Sixty years later, our results show that RYMV dispersed for a second time to Central Africa through Ghana, which resulted in the introduction of the S2 strain in West-Central Africa. Altogether, while the dispersion of RYMV from West Africa to Central Africa across the Burkina Faso/Ghana axis has been very rare during the RYMV history, this study show that Ghana corresponds to a junction area of RYMV populations that have circulated independently in West and Central Africa until the 1990′s. Such movements will probably occur more frequently in the next decades because of the drastic intensification and the extensification of rice cultivation and in the increase of commercial and agronomical exchanges in all this region (Demont, 2013).

5. Conclusion

The generalization of the RYMV movements in West and Central Africa will definitely favor the coexistence of several strains in fields. As each RYMV strain is characterized by specific biological and pathogenic properties (as fitness in susceptible plants and ability to overcome different source of rice resistance; Bonnamy et al., 2022; Hébrard et al., 2018; Pidon et al., 2020; Pinel-Galzi et al., 2007; Poulicard et al., 2012;

Traore et al., 2010), the overlapping of the distribution area of RYMV strains could, in the future, lead to difficulties by controlling RYMV through the local deployment of rice varieties resistant to the virus. Thus, our study underlines the importance of both epidemiological surveillance and sanitary regulation at the local, regional, and international levels to limit the RYMV strain circulation in West Africa to avoid the co-circulation of RYMV genetic diversity that could make more difficult the design of sustainable control strategies against the virus.

CRediT authorship contribution statement

Emmanuel Gilbert Omiat: Resources, Investigation, Data curation, Writing – original draft. **Maxwell Darko Asante:** Conceptualization, Supervision, Resources, Investigation, Data curation, Writing – review & editing. **Valentin Stanislas Edgar Traoré:** Resources, Investigation, Writing – review & editing. **Allen Oppong:** Validation, Investigation, Writing – review & editing. **Beatrice Elohor Ifie:** Validation, Investigation. **Kirpal Agyemang Ofosu:** Validation, Investigation, Writing – review & editing. **Jamel Aribi:** Methodology, Investigation. **Agnès Pinel-Galzi:** Methodology, Investigation. **Aurore Comte:** Software, Data curation, Visualization. **Denis Fargette:** Conceptualization, Software, Writing – review & editing, Supervision. **Eugénie Hébrard:** Writing – review & editing, Supervision, Funding acquisition. **Oumar Traoré:** Supervision, Project administration, Funding acquisition. **Samuel Kwame Offei:** Conceptualization, Supervision, Project administration, Funding acquisition. **Eric Yirenkyi Danquah:** Conceptualization, Supervision, Project administration, Funding acquisition. **Nils Poulicard:** Conceptualization, Software, Data curation, Writing – original draft, Visualization, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Genetic data obtained during this study are publicly available (accession numbers indicated in the text and tables).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2023.199106.

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