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RESEARCH ARTICLE
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Genetic and Pathogenic Diversity within RYMV Pathotypes S2 and S1 Isolated in Côte d'Ivoire

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ABSTRACT

Rice yellow mottle (RYMV) is the most well-known viral disease of rice fields that causes significant economic loss. However, the high resistant rice genotypes that are available can be circumvented by emergent virulent pathotypes. In order to develop sustainable rice breeding strategies against RYMV in Côte d'Ivoire, both resistance spectra and intraspecific viral pathogenecity have to be taken into account. For this purpose, RYMV 95 strains were sampled from infected leaves within the 7 rice growing regions in Côte d'Ivoire and were confirmed serologically as S2 (98%) and S1 (2%). The phylogeny of the Cp protein amplified by RT-PCR revealed that while the S1 pathotype represented one clade, an intrapathotype diversity was observed within S2 in Côte d'Ivoire. An extended resistance spectrum analysis of 22 strains representing S1 and S2 pathotypes was carried out using the promising resistant rice varieties Gigante, TOG 5674, TOG 5672, TOG 5681 that carry four different alleles rymv 1-2, rymv 1.5, rymv 1-4, rymv 1-3 and the sensitive check Bouaké 189. Whithin the S2 pathotypes, a group of viruses could circumvent the resistance by giving symptoms (SRB) while the second group could bypass resistance without giving symptoms (nSRB). While the resistance genes Rymv1-5, Rymv 1-3 and Rymv 1-4/Rymv2 could be bypassed by 81%, 9% and 22.7% of S2 pathotype, no S2 pathotype bypassed the gene Rymv1-Moreover, the two S1 that were genetically identical showed a pathogenic variation and a divergence within the Vp region.

Keywords: RYMV, Rice, Resistance, Isolates, Symptoms, Circumvention.

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INTRODUCTION

RYMV (rice yellow mottle virus) is an endemic virus in Africa and represents a major biotic constraint for rice production ecosystems (Kouassi et al., 2005; Séré et al., 2013; Savary et al., 2019; Omiat et al., 2023). Since its first appearance in East Africa (Bakker, 1974) and West Africa (Fauquet and Thouvenel, 1977), the RYMV has been described by its genetic diversity (Pinel-Galzi et al., 2015; Omiat et al., 2023) and spatial structuring (Fargette et al., 2004; Pinel-Galzi et al., 2015). Six pathogenic strains including S1ca, S1wa, S2, S3, Sa and Sg have been specifically identified in West Africa (Hébrard et al., 2020; Billard et al., 2023). Rice is important in terms of food security in Africa, there is an increasing interest in sustainable management for this disease. So far, the development of rice varieties resistant to RYMV has remained the most practical and sustainable approach for long-term management of the disease. Advances in genetics and molecular biology have led to the identification and mapping of RYMV resistance genes and quantitative trait loci (QTLs) from both *O. sativa* and *O. glaberrima* (Ndjiondjop et al., 1999; Ioannidou et al., 2003; Albar et al., 2006; Rakotomalala et al., 2008; Thiémélé et al., 2010; Pidon et al., 2017). However, Traoré et al. (2006) and Fargette et al. (2008) have underscored the challenges in managing RYMV resistance. As the virus evolves quickly, resistance-breaking variants have been reported throughout Sub-Saharan Africa (Traoré et al., 2006;

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Fargette et al., 2008). Two types of resistance to RYMV comprising a polygenically controlled partial resistance (Thottappilly and Rossel, 1993; Albar, 1998) and a strong resistance governed by a single recessive RYMV1 gene located on chromosome 4 (Ndjiondjop et al., 1999; Albar et al., 2003) have been described. The study of the diver's resistance reveals four different alleles including rymv1-2, rymv1-3, rymv1-4 and rymv1-5 in the varieties Gigante (*O. sativa*), Tog5681 (*O. glaberrima*), Tog5672 (*O. glaberrima*) and TOG5674 respectively (*O. glaberrima*) (Thiémélé et al. 2010; Agnoun et al., 2019). The development of resistant cultivars that can disrupt this interaction is the most effective strategy for managing the disease. The presence of the RYMV2 resistance gene, which is characterized by two distinct alleles, has been confirmed in the *O. glaberrima* varieties Tog 7291 and Tog 5307 (Omiat et al., 2023). A third resistance gene, RYMV3, was recently identified in Tog 5307 (Pidon et al., 2017). Current research has facilitated the widespread adoption of certain resistant rice lines in agricultural settings. However, some RYMV virus pathotypes (E/T/T') are capable of overcoming one or more of these resistance sources (Hébrard et al., 2010). Given the high genetic diversity and high adaptive capacity of RYMV, it is important to assess and anticipate the risks of resistance circumvention to optimize the deployment and sustainability of improved varieties. In Côte d'Ivoire, this virus is responsible for rice yellow mottle observed mainly in rice crops and causes yield reduction ranging from 20 to 100% (Abo et al., 1997; Kouassi et al., 2005). Previous studies have already highlighted the serological diversity of RYMV in Côte d'Ivoire that evidenced the presence of 02 serotypes (S1 and S2) of the RYMV virus with probable competition between them (N'guessan 1999). In addition, it was shown that the distribution of these strains depending on the climate in the country with the S2 strain is predominant in the forest zone, while the S1 strain is more present in the north in the Guinean savannah zone (Dossou et al., 2023). Furthermore, phylogeographic studies on the scale of the African continent have highlighted the main characteristics of the spatio-temporal dispersal process of the virus (Pinel et al., 2000; Fargette et al., 2004; Traore et al., 2005, Omiat et al., 2023). However, the genetic diversity of the serological pathotypes S1 and S2 isolated in Côte d'Ivoire has not been locally addressed. Furthermore, information regarding the resistance spectrum of RYMV is limited. Indeed, a preliminary study revealed the presence of a pathotype in the southern half of Côte d'Ivoire capable of dodging the resistance of the rymv1-2 and rymv1-4 alleles (Amancho et al., 2009). It is then necessary to assess the current dynamics of the virus and the different pathotypes in Côte d'Ivoire in order to develop effective methods of control RYMV. The objectives of this work were i) to determine the extent of the genetic diversity study of RYMV S1 and pathotypes collected in different agro-ecological zones of rice cultivation in Côte d'Ivoire, and ii) evaluate the extended resistance spectrum of these isolates.

MATERIALS & METHODS

RYMV Isolate Sampling

Surveys of rice showing typical yellowing and mottling symptoms were collected in Côte d'Ivoire in August and September 2016 on rice-growing sites affected by RYMV (Fig. 1). The collected diseased leaf samples were conserved in bags, and hands were disinfected with alcohol (70%) after each sampling. These Nighty five (95) samples were then set in codified envelopes and stored in the icebox and transferred to the laboratory in a freezer at -20°C.

Serological Detection and Molecular Identification

ELISA test

Serological typing was performed on all strains using polyclonal anti-RYMV Mg IgG antibody purified at 1/1000 (Mab). A panel of monoclonal antibodies (D and C) was used according to the EAVir protocol modified by Pinel et al. (2000). S1 isolates react with Mabs D, while S2 type isolates react with Mabs C (N'Guessan et al., 2000). These MAbs were used in a triple antibody-sandwich (TAS)–ELISA.

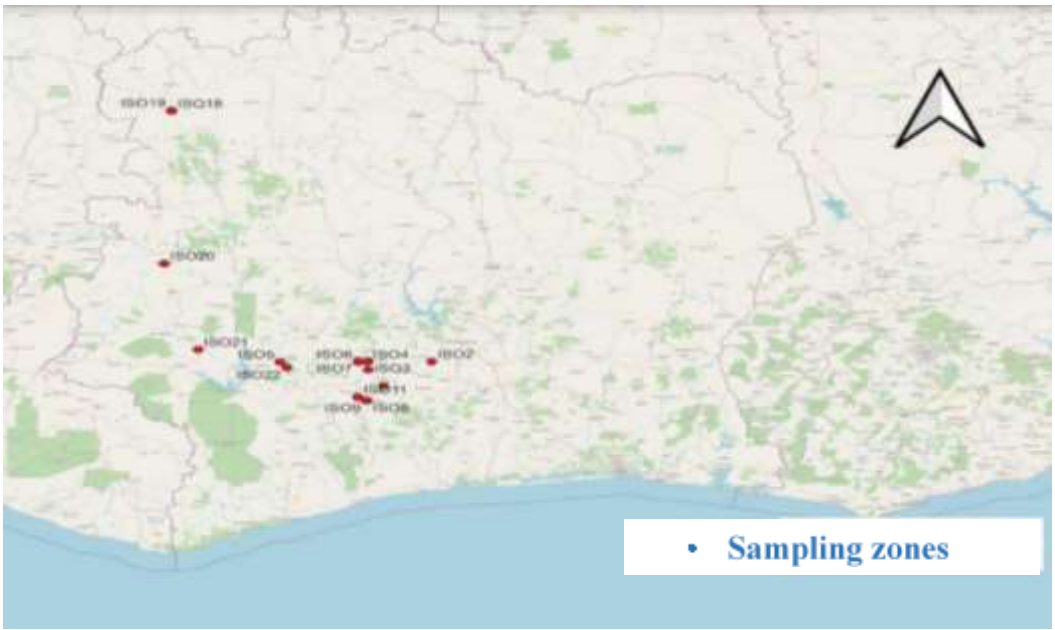

Fig. 1: Map of Côte d'Ivoire with the sampling areas of all 22 RYMV viruses in the test; Zone d'échantillonnage

Amplification and Sequencing of the Capsid Protein Cp and Movement Protein Vpg

Isolates representative of all the rice-growing areas in Côte d'Ivoire were selected among the collected samples. Total RNA was extracted from 0.05g of infected rice leaves using the Thermo scientific Gene Jet Plant RNA Purification Mini Kit according to the supplier's instructions. RNA elution was done with 50µL of pure RNAse free water. Reverse transcription and polymerase chain reaction (RT-PCR) of the viral capsid (CP) and the Vpg genes were carried out according to the protocol modified by Pinel et al. (2000), using the primers RYMVII/RYMVIII and F1SNP/R14bis (Issaka et al., 2012). The fragments obtained were then sequenced. Phylogenetic trees were constructed using Mega 7 software and SeaView4.

Evaluation of the Resistance Spectrum

Plant resistant genotypes included Gigante, TOG 5672, TOG 5674 and TOG 5681 with resistance genes as indicated in Table 1. The experiment was conducted in a greenhouse at the CNRA Adiopodoumé station. Rice seeds were pre-germinated in an oven at 37°C for 3 days before planting in 2L pots. Three seeds were sown per mound, with a total of three mounds per pot. Twelve days after germination, one plant per mound was removed. The experimental design followed a split-plot layout, with varieties as the main plots and RYMV isolates as the subplots, replicated three times. A base fertilizer, consisting of 2g of NPK per pot, was applied during sowing. The plants were regularly watered to ensure proper nutrition. For the pathogenicity test, leaf samples from the studied varieties were artificially infected with each of the selected RYMV isolates. Each isolate was ground separately in an inoculation buffer (phosphate buffer, pH 7, containing 0.05% Between 20 and 2% PVP 24 kD), using 10mL of buffer per gram of leaf. The inoculate were manually rubbed onto the leaves of 21-day-old rice plants using carborundum (320 GRIT) as an abrasive, following the method described by Pinel et al. (2018). Disease symptoms were monitored weekly until the plants reached the flowering stage. RYMV severity was assessed based on discoloration and mottling of the rice leaves at 14, 28 and 42 days after inoculation (DAI), according to the standard evaluation system for rice (IRRI, 2002). The quantity of virus was measured using the DAS-ELISA test, with absorbance readings taken at 405 nm.

Table 1: Characteristics of different varieties of rice used in this study.

Variety/ Accession	Species	Genes/Alleles	Resistance level
Gigante	*Oryza sativa*	Rymv1-2	Resistant
Tog5681	*Oryza glaberrima*	Rymv1-3	Resistant
Tog5672	*Oryza glaberrima*	Rymv1-4 & Rymv 2	Resistant
Tog5674	*Oryza glaberrima*	Rymv1-5	Resistant
Bouaké 189	*Oryza sativa*		Susceptible

Statistical Analysis

Excel software was used for data analysis. The raw data was coded and dendrograms and ACP analysis were produced to identify the reaction groups of the virus according to their symptoms and ODs. All these statistical analyses were performed using STATISTICA 7.1 software. The data of severity and OD was analyzed by using a principal component analysis to summarize and visualize the information described by multiple inter-correlated quantitative variables. Therefore, the data was standardized before analysis and visualized using the FactorMineR (Lê et al., 2008) and factoextra (https://rpkgs.datanovia.com/factoextra) R packages.

RESULTS

The RYMV Pathotype S2 were Genetically Diverse

The 95 collected isolates were observed only in irrigated rice plantations (lowland cultivation) (Table 2). The rainfed rice plantations near the affected irrigated rice plantations had no infected plants. Of the 95 samples collected, 86 were confirmed with RYMV virus after DAS-ELISA testing. The Mab-ELISA tests confirmed the presence of pathotype S1 in the north while pathotypes S2 were found in the south (Table 2). Twenty-two (22) isolates representative of all the rice-growing areas in Côte d'Ivoire selected among the 86 RYMV strains were inferred using the coat protein gene (Fig. 2). It was shown that all the isolates from the center west (Gagnoa, Bayota, Issia) and west (Biankouma) of Côte d'Ivoire were found in the same pathotype S2 clade, while the only 02 isolates collected in the northwest were in a S1 pathotype clade.

Table 2: Origin and serotype of the 86 RYMV as identified by the DAS-ELISA test

Sampling localities	Number of samples collected	Serotype
BAYOTA SR-CNRA	29	S2
GAGNOA	25	
GUESSHIO	26	
BIANKOUMA	1	
DUEKOUE	1	
ISSIA	2	
ODIENNE	2	S1

Fig. 2: Phylogenetic tree using the distance method: Neighbor Joining with MEGA 7 software on CP sequences of Côte d'Ivoire RYMV isolates obtained during this study. Sample Tz11 was used as an out-group.

Pathogenic Diversity within S2 and S1 Pathotypes

22 RYMV isolates representative pathotypes S1 and S2 were used to inoculate in controlled conditions the rice varieties TOG 5674, TOG 5681, TOG 5672 and GIGANTE that harbored the resistance alleles rymv1-2, rymv1-3, rymv1-4 and rymv1-5. The viral adaptability and resistance spectra of all the S2 and S1 pathotypes used in this study were evaluated. The principal component analysis of the response of the RYMV viruses showed two groups of viruses. One group included viruses that had a causative relationship on one hand with symptom severity at days 28 and 42 and on the other the virus presence within rice varieties TOG 5674, TOG 5681, TOG 5672 and GIGANTE. The other group of viruses had no impact on the measured parameters. Both groups included some S2 and one S1 serotype (Fig. 3).

Rice Genotypes Exhibited a Highly Contrasted Resistance Breaking Abilities

The resistant rice genotype TOG 5674 and the Bouake 189 rice variety which constituted the sensitive control during this study were found to be sensitive to all RYMV isolates (Fig. 4).

All plants of the resistant variety Gigante remained symptom-free, and viral multiplication was not detected by DAS-ELISA (Table 3). In contrast, typical RYMV symptoms appeared in inoculated plants of the Tog 5674 variety 42 days after inoculation. Resistance-breaking was also confirmed in the TOG 5681 and TOG 5672 varieties, where RYMV was detected in asymptomatic plants by DAS-ELISA. The resistant rice accessions and RYMV isolates used in this study exhibited a variable resistance spectrum and resistance-breaking (RB) capacities (Table 3 and Fig. 5).

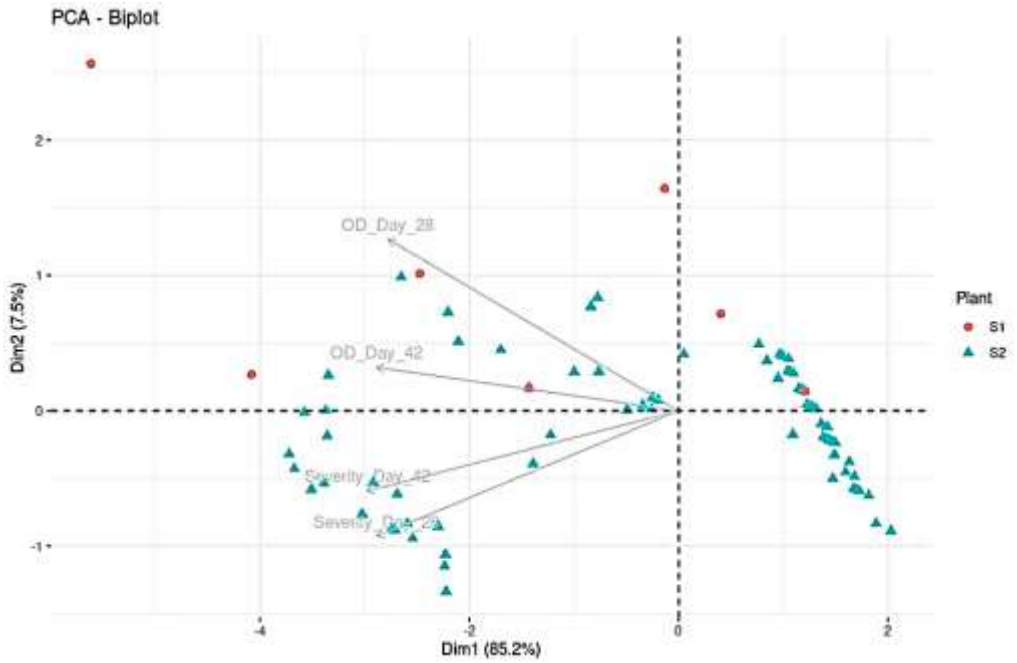

Fig. 3: Causative relationship between rice varieties TOG 5674, TOG 5681, TOG 5672 and GIGANTE, symptom and viral load at 28 days and 42 days after inoculation with pathotypes S2 and S1.

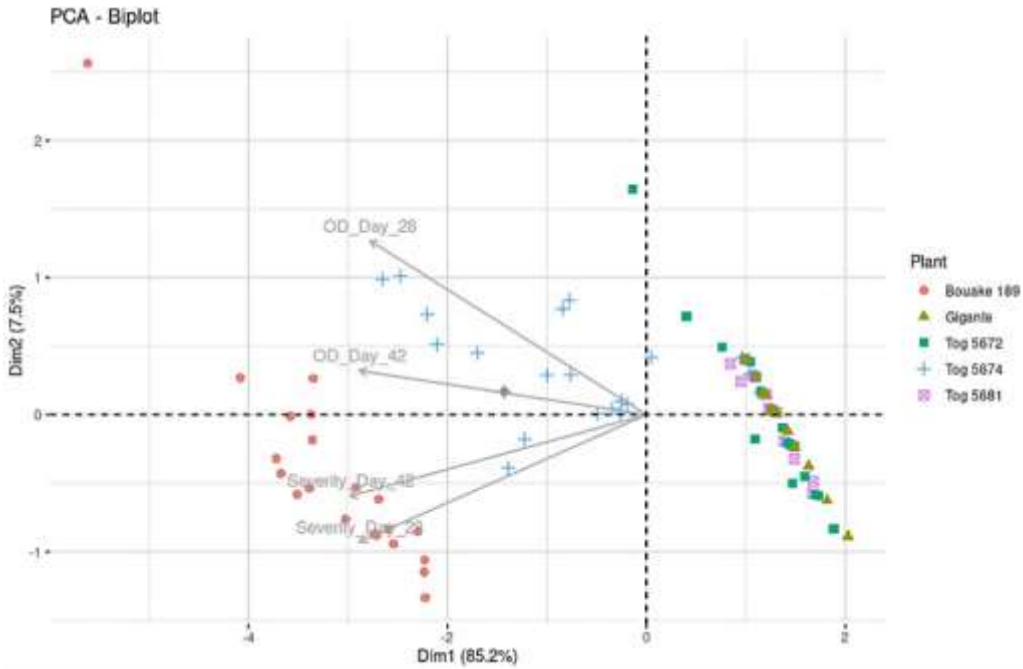

Fig. 4: Behavior of rice varieties TOG 5674, TOG 5681, TOG 5672 and GIGANTE at 28 days and 42 days after inoculation.

Table 3: Spectrum of isolate responses on analyzed genotype

Isolat	*TOG 5674 Rymv1-5*	*TOG 5681 Rymv1-3*	*TOG 5672 Rymv1-4 & Rymv2*	*GIGANTE Rymv 1-2*
ISO 1	SRB	-	-	-
ISO 2	-	-	-	-
ISO 3	nSRB	-	-	-
ISO 4	SRB	-	-	-
ISO 5	SRB	-	-	-
ISO 6	-	-	-	-
ISO 7	SRB	-	-	-
ISO 8	SRB	-	-	-
ISO 9	SRB	-	-	-
ISO 10	-	-	-	-
ISO 11	SRB	-	-	-
ISO 12	SRB	nSRB	nSRB	-
ISO 13	SRB	-	nSRB	-
ISO 14	SRB	-	-	-
ISO 15	SRB	-	-	-
ISO 16	SRB	nSRB	nSRB	
ISO 17	SRB	-	-	-
ISO 18	SRB	-	nSRB	-
ISO 19	SRB	-	nSRB	
ISO 20	SRB	-	-	-
ISO 21	SRB	-	-	-
ISO 22	-	-	-	-

No resistance-breaking; nSRB: Non-Symptoms Resistance-breaking RYMV (RYMV symptom-free); SRB: Symptoms Resistance-breaking (with RYMV symptom)

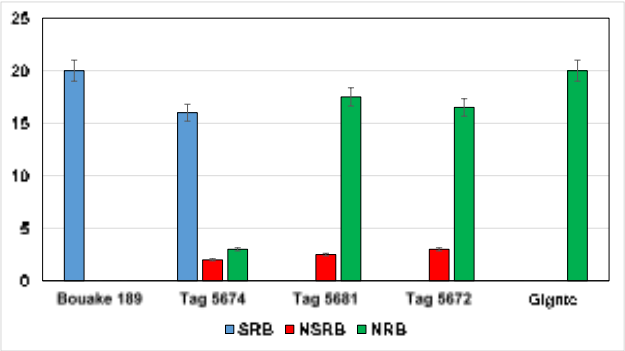

Fig. 5: Distribution of RYMV S2 population resistance breaking spectrum on 05 rice varieties; 22 RYMV isolates were tested on varieties Bouaké 189, Tog 5674, Tog 5681, Tog 5672 and Gigante. For each variety, 03 plants were used. Values represent number of RYMV pathotype S2, with the characteristic: **SRB**: Symptoms causing resistance breaking; **NSRB**: No Symptoms causing resistance breaking; **NRB**: No resistance breaking.

Among the analyzed RYMV strains, 77% were able to bypass the resistance of the Rymv1-5 gene, leading to symptom development (Symptoms Resistance Breaking = SRB). In contrast, 4.5% managed to bypass the same gene without causing visible symptoms (Non-Resistance Breaking = nSRB). Resistance genes Rymv1-3 in Tog 5681 and Rymv1-4/Rymv2 in Tog 5672 were also circumvented, with 9% and 22.7% of the analyzed viruses, respectively, achieving this without inducing symptoms. Particularly virulent isolates, ISO12 and ISO16 from the S2 pathotype, caused symptoms in the TOG 5681 and TOG 5672 varieties, which contain the Rymv1-3 and Rymv1-4/Rymv2 resistant genes. These isolates also bypassed the Rymv1-5 gene in the Tog 5674 variety, resulting in symptomatic infection.

The Vp Region was Different within the S1 Pathotypes

The nucleotide sequences of the viral protein VPg linked to the Virulence domain of the RYMV genome of isolates ISO18 and ISO19 presenting serotype S1 after ELISA tests were compared. Each sequence had at position 49 a Threonine (T) and a glutamic acid (E) respectively for isolates ISO 19 and ISO 18 (Fig. 6).

DISCUSSION

A total of 95 leaf samples, all showing symptoms of RYMV disease across various lowland and irrigated fields in 7 localities in Côte d'Ivoire, were collected. Despite the presence of disease symptoms in all surveyed areas, DAS-ELISA testing confirmed RYMV infection in only 86 of these samples. The discrepancy could be attributed to the fact that RYMV symptoms can be easily confused with symptoms of other stresses, such as nitrogen deficiency (Oludare et al., 2016). This result indicates that the RYMV was present in all the rice-growing areas surveyed as already reported (Amancho, 2009; Abrokwah et al., 2024). The TAS-ELISA test allowed the detection of the S2 pathotype that was dominantly distributed in the surveyed areas and the S1 pathotype in the North. Previous studies using serological tests revealed that RYMV in Côte d'Ivoire almost exclusively belonged to the strain S2 generally reported in West Africa and the strain S1 (N'guessan et al., 2000; Odongo et al., 2021). The sequencing and phylogeny of the coat protein gene also confirmed the same structure as recently evidenced in Ghana (Omiat et al., 2023). Indeed, the genetic diversity detected within the S2 isolates using the CP gene phylogeny was high as already reported generally in West Africa (N'guessan et al., 2000; Issaka et al., 2021).

In order to evaluate RYMV threat in Côte d'Ivoire, an extended resistance spectrum was realized using resistance varieties GIGANTE, TOG 5672, TOG 5674 and TOG 5681. Pathogenicity tests carried out in the presence of the 22 isolates showed a diversity in the response of the varieties according to the isolates. Indeed, the S2 pathotypes, a group of viruses could avoid the resistance by giving symptoms (SRB) while the second group could bypass resistance without giving symptoms (nSRB). The genes Rymv1-5, Rymv 1-3 and Rymv 1-4/Rymv2 harbored by *O. gblaberrima* were bypassed with Rymv1 - 5 the most

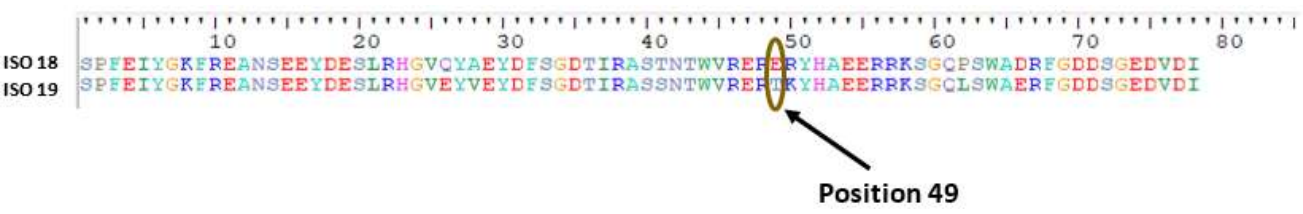

Fig. 6: Comparative profile of the VPg sequences of isolates ISO 18 and C ISO 19; Position 49 in surrounded corresponds to the mutation site involved in the virulence of the isolates.

bypassed by 81% of S2 pathotype. It was reported that virus lineages located in West Africa (strain S1–S3) had a higher propensity to adapt to *O. glaberrima* resistant accessions regardless of the resistance genes and alleles they carried (Pinel-Galzi et al., 2007, Traoré et al., 2010 Pinel-Galzi et al., 2016, Pidon et al., 2017, Bonnamy et al., 2023). Moreover, the two S1 that were genetically identical showed a pathogenic variation. According to Adugna (2004), a variability could exist among isolates concerning their aggressiveness and virulence. Virulence would be linked to host spectra while aggressiveness could reflect the intensity of the damage caused by the pathogen on its host. The variety GIGANTE and TOG5681 were characterized by an absence of the virus inside their plants (OD negative) for all isolates. This demonstrated the tolerance of these two varieties. The appearance of symptoms is a complex phenomenon, in which the phenotype is intimately dependent on the host and the pathogen (Casadevall and Piroski, 2001). Besides the variability within isolates, rice varieties also had a different resistance level to isolates. None of the tested strains were able to infect the accessions TOG 5681 (rymv1-3) and Gigante (rymv1-2), which carried the known resistance alleles of the RYMV1 gene. Similar results were reported by Onasanya et al. (2011), who found that rymv1-2 and rymv1-3 provided protection against the S2 and S1 strains. These findings were corroborated by DAS-ELISA testing. The results confirmed that all leaf samples from the GIGANTE variety were RYMV free showing this variety's efficacy as a resistance donor in various varietal improvement programs (Soko et al., 2015, Bouet et al., 2013; Ramathani et al., 2023). This resistance could be due to the existence of the resistant gene (Ndjiondjopal, 1999; Bagayoko et al., 2021). Later, a major resistance gene, called RYMV1, was identified in this rice variety (Albar et al., 2003; Pidon et al., 2020); then a second major gene, RYMV2, was identified (Thiémélé et al., 2010). Concerning the rice varieties TOG 5672, TOG 5674 recognized as resistant to RYMV (Thottappilly, 1993), they were either resistant or sensitive to the virus isolates under our experimental conditions. The TOG 5672 rice variety was steered clear by 5 isolates including the ISO12 and ISO13 isolates from the CNRA Gagnoa station, the ISO18 and ISO19 isolates from Odienné and finally the ISO16 isolate from Guessiho. The rice variety TOG 5681 was also circumvented by 2 isolates which are ISO 12 and ISO 16 respectively collected at the CNRA station in Gagnoa and Guessiho. This result showed that there was a relationship between viral load and symptoms. Plants may appear healthy but be infected. Similar results were found elsewhere (Coulibaly, 1999; Abbas et al., 2024). After inoculation of the TOG 5672 and TOG 5681 varieties with various RYMV isolates, the plants did not harbor any symptoms but were infected by the isolates. The low multiplication of the virus observed in certain hosts might have led to the existence of relay hosts without symptoms as reported early (Osterbaan et al., 2019). Such plants could constitute RYMV reservoirs and sources of contamination when grown on the same plot with susceptible varieties. Thus, the varieties, TOG 5681 and TOG 5672 once infected, do not have symptoms. Consequently, they constituted dangerous hosts because they can appear healthy and contribute to the increase in the natural inoculum. This study revealed a diversity of response within even individuals of the same S2 serotype. All these data showed the importance of a more in-depth study in the RYMV population in Côte d'Ivoire for a better analysis of the resistance spectra and virus adaptability.

Conclusion

The study highlights the genetic and pathogenic diversity within RYMV pathotypes S2 and S1 in Côte d'Ivoire, emphasizing significant variability in their ability to overcome rice resistance genes. Findings reveal that while S1 strains are genetically uniform, they exhibit pathogenic divergence, whereas S2 strains display both genetic and functional diversity. These insights are critical for developing durable rice breeding strategies by targeting resistance genes like Rymv1-2, which remain effective against S2 strains. Overall, the research underscores the need for integrating pathogen diversity into rice resistance management in Côte d'Ivoire.

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Data Availability: If you have presented all the data in the article, then write: All the data is available in the article. Or if you cannot present all data and have more data to show, write "Data will be available at the request."

Author's Contribution: Conceptualization: writing-original draft preparation: Kouadjo Claude Ghislaine; Methodology: M'BRA Kofi Hermann, KOFFI Kan Ghislain, Coulibaly Sidonie; Validation: Noumouha Epa Ghislain; Formal analysis: Kouadjo Claude Ghislaine; Noumouha Epa Ghislain.

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