

Identification of Resistance-Breaking RYMV Isolates in Four Agro-Ecological Zones of Côte d'Ivoire

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Abstract: Rice is the third most produced cereal in the world after maize and wheat. In Côte d'Ivoire, rice is the main food of the population. However, despite its importance, rice cultivation is subject to biotic constraints, including rice yellow mottle virus (RYMV), which is the most damaging viral disease of rice native to Africa. RYMV is a highly variable virus. This study aims to assess the ability of RYMV isolates to overcome the resistance-breaking genes RYMV1, RYMV2 and RYMV3. Sixteen RYMV isolates from Côte d'Ivoire representative of the agro-ecological zones (AEZ) visited and the varieties Bouake 189, Gigante, Tog 5681, Tog 5674, Tog 7291 and Tog 5307, carrying respectively *rymv1-1*, *rymv1-2*, *rymv1-3*, *rymv1-5*, RYMV2 and RYMV3 alleles were used. Varieties were sown in trays filled with sterilized soil so as to keep six plants per variety, i.e. 36 plants per tray per isolate. Rice plants were inoculated mechanically 14 days after sowing. Leaves of infected plants were collected individually per variety 45 days after inoculation in order to determine the virus concentration by serological DAS-ELISA tests. Asymptomatic infections were also investigated. The results showed that 37.5% of the tested isolates broke only the *rymv1-2* gene with the presence of apparent symptoms. However, 12.5% of the isolates are infected both Gigante and Tog5674. None of the 16 isolates tested could induce symptoms in Tog5703, Tog5681 and Tog7291. Furthermore, only Tog7291 are showed the presence of the virus without apparent symptoms on rice leaves after inoculation. The identification of such isolates in rice-growing areas raises a serious threat to Ivorian rice production and calls for surveillance of this disease in Côte d'Ivoire.

Keywords: RYMV, Resistance-Breaking, Gigante, Tog 5674, Côte d'Ivoire

1. Introduction

Rice (*Oryza* sp.) is the third most important cereal produced in the world after maize and wheat, with global production in 2020 estimated at over 756 million tonnes [1]. In Côte d'Ivoire, rice cultivation plays a dual role in the fight

against food insecurity and poverty, and nevertheless, for more than thirty years, national production has been below the social need, estimated today at around 1,500,000 tonnes of rice [2]. This low production is partly due to biotic factors [3] notably diseases (bacterial, viral, and fungal), the most damaging of which is *Rice yellow mottle virus*. Discovered in

1966 in Kenya [4], rice yellow mottle is the most important viral disease of rice in African continent and is an obstacle to rice intensification efforts. The pathogen responsible for this virus is the rice yellow mottle virus (RYMV), member of the genus *sobemovirus* [5]. Yield losses due to RYMV are enormous and vary from 10 to 100% [6, 7] depending on the time of infection and the variety. Currently, seven strains have been identified [8] with specific geographical adaptations. RYMV is a highly variable virus. Diversity sources infections, vectors and viral isolates make it difficult to control Rice yellow mottle virus, which is why genetic control is considered the most effective way to resist this serious disease [9]. Furthermore, environmental instabilities influence the epidemiological components of the pathogens and consequently modify the structure and pathogenic properties of plant viruses [10]. As RYMV does not over ride this situation, the ability of RYMV viral isolates to adapt to resistant varieties is a major challenge with the aim of predicting the durability or otherwise of resistance. The search of resistance-breaking RYMV isolates is also used to develop control strategies by establishing a risk map of resistance-breaking. This map would be essential for the proper deployment of resistance in space and would take into

account the pathogenic profiles of the pathogen in a given environment. In the context of the control of damage caused by Rice yellow mottle virus, four independent resistance alleles was identified in both *O. sativa* (allele *rymv1-2*) and *O. glaberrima* species (allele *rymv1-3*, alleles *rymv1-4* and allele *rymv1-5*). In addition to *RYMV1*, *RYMV2* and *RYMV3* resistance genes have been described in accessions Tog 7291 and Tog 5307, respectively [11-13]. This study therefore aims to establish a distribution map of RYMV resistance-breaking isolates in Côte d'Ivoire.

2. Materials and Methods

2.1. Plant Materials

The highly resistant varieties Gigante, Tog 5681, Tog 5674, Tog 5307 and Tog 7291, carrying respectively *rymv1-2*, *rymv1-3*, *rymv1-5*, *RYMV3* and *RYMV2* alleles were used, while the Bouake 189 variety was used as a susceptible control (Table 1). Sixteen isolates (the most aggressive) representative of five agro-ecological zones (AEZ) of Côte d'Ivoire were used (Table 2).

Table 1. Rice varieties used.

Varieties	rice species	Origin	Resistance level	Resistance Gene	References
Bouake 189	<i>Oryza sativa</i> L.	AfricaRice Bouake (CI)	S	<i>rymv1-1</i>	-
Gigante	<i>Oryza sativa</i> L.	INERA Ouagadougou (BF)	HR	<i>rymv1-2</i>	[14]
Tog 5674	<i>O. glaberrima</i> Steud.	INERA Ouagadougou (BF)	HR	<i>rymv1-5</i>	[11]
Tog 5681	<i>O. glaberrima</i> Steud.	INERA Ouagadougou (BF)	HR	<i>rymv1-3</i>	[15]
Tog 5307	<i>O. glaberrima</i> Steud.	INERA Ouagadougou (BF)	HR	<i>RYMV3</i>	[12]
Tog 7291	<i>O. glaberrima</i> Steud.	INERA Ouagadougou (BF)	HR	<i>RYMV2</i>	[13]

S: Susceptible; HR: Highly Resistant; *rymv1-1* to *rymv1-5*: alleles of the *RYMV1* gene, CI: Côte d'Ivoire; BF: Burkina Faso
-: Missing data

Table 2. Isolates used and their agro ecological zone origin.

localities	Agro-ecological zone (AEZs)	Isolates
Gagnoa	AEZ 1	GB2CI
Gagnoa	AEZ 1	GB7CI
Divo	AEZ 1	GB48
Divo	AEZ 1	GB43
Divo	AEZ 1	GB27
Divo	AEZ 1	GB26
Mayo	AEZ 2	GB4
Daloa	AEZ 2	GB8
Oureyo	AEZ 2	GB32
Oureyo	AEZ 2	GB33
Yamoussoukro	AEZ 4	GB10
Yamoussoukro	AEZ 4	GB21
Bouake	AEZ 5	GB45
Bouake	AEZ 5	GB38
Korhogo	AEZ 6	GB62
Korhogo	AEZ 6	GB60

2.2. Conducting the Trial

The different varieties were sown in trays at a rate of five seeds per seed hole. A thinning of plants at one plant per seed hole was carried out seven days after sowing (7 DAS) in order to keep six plants per variety as described by Longue *et*

al. [16] i.e. 36 plants per tray and per isolate (Figure 1). Each isolate was regenerated on the susceptible variety Bouake 189 in order to have enough viral material for inoculation. Regenerated isolate was collected and ground with a mortar. Carborundum was added to the grind as an abrasive to facilitate penetration of the virus into the plants. Each isolates was inoculated into plants of each of the 6 rice varieties. The study was conducted in a greenhouse (greenhouse environment) and was repeated twice in 2018 and 2019.

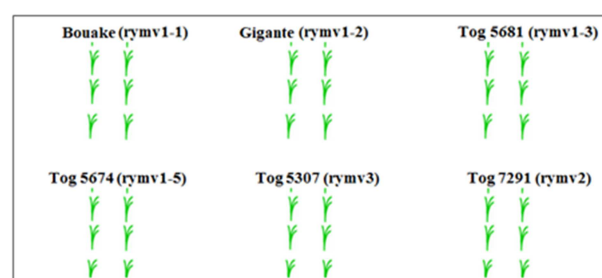


Figure 1. Design for pathogenesis testing.

2.3. Pathogenesis Assessment

Pathogenic diversity analysis of the isolates was carried

out using by the method of Traore *et al.* [17]. Thus, the inoculated plants were observed during 45 days. The dates and order of the plants/varieties that expressed symptoms during the experiment were recorded. Leaves of inoculated plants were collected individually by variety 45 days after inoculation (DAI) in order to determine their virus concentration by DAS-ELISA serological tests. The principle of the ELISA is to reveal the antigen-antibody interaction resulting from the labelling of immunoglobulins with enzymes by coloring [18]. This viral concentration was assessed from the optical density (OD) of the tested leaf extracts obtained at 405 nm. These ODs were obtained using an ELISA reader. Thus, two readings were taken. A first reading of the optical densities at 405 nm was carried out at 30 minutes after incubation and then a second, one hour later. An extract was considered positive if the absorbance's obtained are greater than the average of the absorbance of the negative control plus three times the standard deviation as done by Guinagui *et al.* [19]. The negative control was obtained from the absorbance of sterile water. In this study, the antibody, conjugate and positive control was provided by the International Mixed Laboratory PathoBios (LMI-PathoBios) in Ouagadougou (Burkina Faso). Leaves of asymptomatic plants were collected and pooled by variety to test for asymptomatic infections.

3. Results and Discussion

3.1. Resistance Breaking of Gigante and Tog 5674 Varieties

Isolates collected from the agro ecological zones visited were tested for their ability to overcome the resistance of five highly resistant varieties. The results show that symptom expression in the susceptible variety Bouake 189 was observed on 100% of plants for all isolates tested. These symptoms were observed between 6 and 8 days after inoculation (DAI). The highly resistant Gigante and Tog 5674 varieties could be infected with 6 RYMV isolates (table 3). These isolates are called symptomatic resistance breaking (SRB). Symptoms observed on Gigante and Tog 5674 were noted between 10 and 13 days after inoculation. These resistance-breaking isolates are originated from agro ecological zones 1, 2, 5 and 6. However, the incidence of the disease varied according to the varieties and isolates tested. For example, the incidence of RYMV varied from 20 to 100% in Gigante and from 80 to 100% in Tog 5674. Also, the infection was more severe in the Bouake 189 variety (scores 7-9) as opposed to the Gigante and Tog 5674 varieties (scores 3-5). RYMV isolates that did not induce symptoms are referred to as non-resistance breaking isolates (nRB). Out of the 16 isolates tested, two pathogenic profiles were distinguished. The first profile, with a proportion of 37.5% of the isolates tested, concerned isolates that had infected only the Gigante variety. The second profile consisted of 12.5% of isolates breaking both Gigante and Tog 5674 (Table 3). The frequency of isolates able to break only Gigante resistance would therefore be three times higher than that isolates able to overcome both Gigante and Tog 5674. This result shows that isolates from Côte

d'Ivoire tend to preferentially overcome the *rymv1-2* gene carried by the Gigante variety. Furthermore, none of the 16 isolates tested was able to induce symptoms in the varieties Tog 5703, Tog 5681 and Tog 7291, indicating that the Côte d'Ivoire isolates are particularly adaptable to the *rymv1-2* and *rymv1-5* genes (Figure 2).

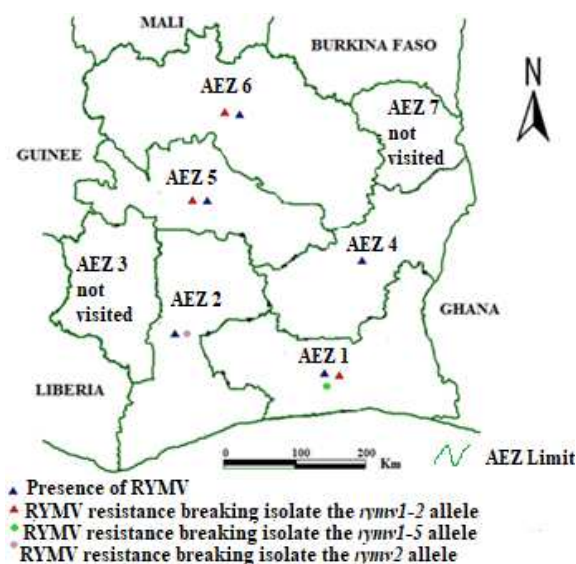


Figure 2. RYMV resistance-breaking isolates map.

3.2. Asymptomatic Infections

The determination of asymptomatic resistance breaking (ARB) was carried out by serology using the DAS-ELISA. In this study, only Tog 7291 showed the presence of the virus without apparent symptoms on rice leaves after inoculation. Absorbance at 405 nm reflecting virus concentration in diseased plants ranged from 1.5 to 2.1, with a threshold of positivity of 0.4. Extracts from diseased leaves of the Bouake 189 (susceptible control), Gigante and Tog 5674 varieties gave mean absorbance of 2.11, 1.58 and 1.82 respectively, while the asymptomatic leaf sample of the Tog 7291 variety produced a mean absorbance of 0.643. This absorbance is low in contrast to the absorbance recorded in the other varieties but is above the positivity threshold (Figure 3).

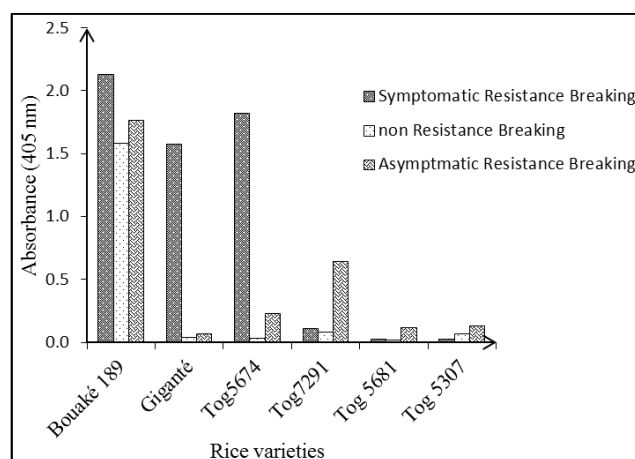


Figure 3. Absorbance of extracts from infected leaves of the tested varieties.

Table 3.1 Profile of Côte d'Ivoire isolates.

Agro-ecological zone	Isolates	Number of infected plants					
		Bouake 189	Gigante	Tog 5674	Tog 5307	Tog 5681	Tog 7291
AEZ 1	GB2C1	6/6	6/6	6/6	0/6	0/6	0/6
	GB7C1	6/6	6/6	4/6	0/6	0/6	0/6
	GB48	6/6	4/6	0/6	0/6	0/6	0/6
	GB43	6/6	2/6	0/6	0/6	0/6	0/6
	GB27	6/6	0/6	0/6	0/6	0/6	0/6
	GB26	6/6	0/6	0/6	0/6	0/6	0/6
AEZ 2	GB4	6/6	0/6	0/6	0/6	0/6	0/6
	GB8	6/6	0/6	0/6	0/6	0/6	0/6
	GB32	6/6	0/6	0/6	0/6	0/6	0/6
	GB33	6/6	0/6	0/6	0/6	0/6	0/6
AEZ 4	GB10	6/6	0/6	0/6	0/6	0/6	0/6
	GB21	6/6	0/6	0/6	0/6	0/6	0/6
AEZ 5	GB45	6/6	0/6	0/6	0/6	0/6	0/6
	GB38	6/6	6/6	0/6	0/6	0/6	0/6
AEZ 6	GB62	6/6	4/6	0/6	0/6	0/6	0/6
	GB60	6/6	0/6	0/6	0/6	0/6	0/6

Assessing the capacity of pathogens to adapt to plant resistance is a major challenge, the objective of which is to predict whether or not resistance will be durable. It also makes it possible to develop control strategies by establishing a map of the risk of circumvention in a given environment. This tool would be essential for the proper deployment of resistance in space and would take into account the pathogenic profiles of the pathogen in a given environment. In the context of rice yellow mottle disease control, the major sources of resistance governed by the *rymv1*, *rymv2* [14, 11, 13] and *rymv3* [20, 21] have been characterized in the Gigante variety (*Oryza. Sativa* L.) and in some African rice varieties (*Oryza. Glaberrima* Steud.), in this case accessions of the Tog series. On the basis of the incidence, severity and time to symptom expression after inoculation, the Gigante and Tog 5674 varieties showed variable responses to the isolates tested. Thus, the incidence of RYMV was higher in the Gigante variety while the disease was more severe in the Tog 5674 African rice. The low severity score observed in the Gigante variety, coupled with the early onset of symptoms, suggests a progressive adaptation (or evolution) of the virus populations to the genetic background responsible for the resistance in Gigante. This adaptation is said to be progressive because according to Pinel-Galzi *et al.* [22]. West African isolates would tend to adapt to the resistance of African rice varieties (*Oryza Glaberrima* Steud.), while East African isolates would preferentially bypass the resistance of Asian rice (*Oryza sativa* L.) such as the Gigante variety. The pathogenic profile of Côte d'Ivoire isolates as presented in this study differs from that reported by Amancho *et al.* [23]. Indeed, the work of these authors showed that the *rymv1-3* allele present in the Tog 5681 variety was broken contrary to our results which recorded no resistance-breaking of the Tog 5681 variety. Also, the virulent isolates identified in this study were collected in different localities from the one mentioned by Amancho *et al.* [23], thus demonstrating the pathogenic variability of isolates from Côte d'Ivoire. The identification of resistance-breaking isolates in agro-ecological zones 1, 2, 5 and 6 would raises a

serious threat to Ivorian rice production because, according to MINAGRI [2], these agro-ecological zones are part of the large and medium rice production areas in Côte d'Ivoire. The variety Tog 5674 showed symptoms with high severity, confirming the results of Pinel-Galzi *et al.* [22] which indicated that West African isolates preferentially break the resistance of *Oryza glaberrima* rice species. This variability observed at the level of the virus populations against the different resistant varieties would result from their capacity to adapt to their hosts and from mutations and genetic recombination in the virus populations. Indeed, resistance breakdown can occur when the resistant cultivar is subjected to a massive and repeated attack by the same virus population. The resistance-breaking would therefore be a phenomenon partly linked to agro-ecological conditions. Indeed, according to Jones and Martin [10], changes in the epidemiological components due to environmental instabilities would influence the structure and pathogenic properties of virus populations. Asymptomatic infection was noted in this study and was only observed in the Tog 7291 variety. The viral concentration of the asymptomatic infection was relatively lower compared to the symptomatic infections. This result would indicate a possible link between the virus load in RYMV-infected plants and the expression of symptoms in Tog 7291. However, virus loads in extracts from infected leaves of the resistant varieties Gigante and Tog 5674 were sometimes comparable to those of the susceptible variety Bouake 189. This result confirms that virus load alone cannot be used to characterize resistant accessions 45 days after inoculation [15, 24]. It is possible that at this age the virus load no longer differentiates these two types of varieties. The results showed that no isolate could overcome the resistance of Tog 5681 and Tog 5307. Under current conditions, the absence of such isolates in the agro-ecological zones of Côte d'Ivoire would mean that one of the *rymv 1-3* genes, *RYMV3*, could provide good protection of rice against rice yellow mottle disease in the country. These differences in pathogenic responses observed between genotypes would probably be related to the genetic background governing each

of the resistance alleles. Therefore, the concentration of virus particles in infected leaves would be a limiting factor in the expression of symptoms by the host [25], suggesting that the virulence observed in the isolates would depend on a higher level of virus replication, rather than on a specific property of these isolates. In order to investigate the genetic determinism of resistance-breaking, authors such as Poulicard *et al.* [26] have shown that the Threonine / Glutamic acid (T/E) amino acid polymorphism at position 49 of VPg modulates the ability of RYMV isolates to break the resistance conferred by the *rymv1-2* and *rymv1-3* alleles [26]. This variability observed at the level of the virus populations with respect to the different resistant varieties is thought to result from their capacity to adapt to their hosts [27].

4. Conclusion

The study of pathogenic diversity shows that RYMV isolates native from Côte d'Ivoire have been able to break the *rymv1-2* and *rymv1-5* genes identified respectively in Gigante and Tog 5674. The presence of such isolates in the Ivorian rice crop is a major concern, as they may compromise the use of these alleles in Côte d'Ivoire. The development of strategies for the deployment of resistant varieties adapted to the agro-ecological conditions of Côte d'Ivoire should be considered in order to reduce the risks of rice yellow mottle virus epidemics in Ivorian rice production. In addition, in order to better understand the mechanisms linked to the resistance-breaking of these isolates, the analysis of the viral protein genome (Vpg) of the isolates is proving to be a way of understanding the pathogenesis of the S2 strain in West Africa.

Declaration of Interest Statement

The authors declare that they have no competing interests.

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