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Report of High Resistance-Breaking Isolates of *Rice yellow mottle virus* in Côte d'Ivoire

Nicaise A. Amancho[1] • Nazaire K. Kouassi[1] • Hortense A. Diallo[2*] • Alphonse Bouet[1] • Abdourahamane Sangaré[1] • Justin Y. Kouadio[2]

[1] CNRA, Laboratoire Central de Biotechnologie, 01 BP 1740, Abidjan, Côte d'Ivoire
[2] Université d'Abobo-Adjamé, UFR-SN, Laboratoire de Biologie et Amélioration des Productions Végétales, 02 BP 801 Abidjan 02, Côte d'Ivoire
Corresponding author: * **hortensediallo@live.fr**

ABSTRACT

In order to assess *Rice yellow mottle virus* (RYMV) epidemiological risks for a sustainable management of high resistance in rice in Côte d'Ivoire, the relationships between virus isolates and rice varieties were studied under artificial selection pressure. The results of the inoculation tests of the highly resistant varieties Gigante and Tog5681 with 120 isolates of RYMV obtained from the National Agricultural Research Centre (CNRA) indicated the existence of resistance-breaking virus isolates within 10 localities in the southern part of Côte d'Ivoire. Two pathogenic groups were found. The first one infected only Tog5681 and had a disease prevalence of 19.48% while the second group, showing a prevalence of 3.8%, overcame the resistance present in the two genotypes. Depending on the observed resistance phenotype, resistance-breaking pathotypes were divided into asymptomatic (detected by ELISA) and symptomatic (inducing symptoms) sub-groups. The differential interaction between isolates and plant genotypes are highlighted, although no close relationship was observed between the foliage colour of the infected varieties and the virus titer. The presence of such pathotypes in Côte d'Ivoire could undermine all successful sustainable management of yellow mottle based on the use of highly resistant varieties.

Keywords: allele, diversity, pathotypes

INTRODUCTION

Rice yellow mottle is an endemic rice viral disease in Africa. The disease was first reported in Kenya in 1970 (Bakker 1970) and is now widespread to all rice-producing areas in Africa. Rice yellow mottle and rice blast disease are the major causes of yield losses in rice production in Africa. Symptoms vary according to rice variety, virus strain, plant development stage and the environment (Raymundo and Buddenhaggen 1976; Awoderu 1991; N'Guessan 2001). In Côte d'Ivoire, the disease was first reported in 1977 in the locality of Dabou (Fauquet and Thouvenel 1977) then became widespread to all irrigated rice-growing areas. Crop loss was mostly substantial in Gagnoa and Daloa regions, resulting in farmers giving up their rice fields (Bouet *et al.* 2001). The disease is characterized by a yellow mottling on the infected leaves and plant stunting (**Fig. 1**). The yellowing can turn orange or even become necrotic in some rice varieties. Diseased plants are stunted and show development impairments, sprout reduction or proliferation, malformation, exerted panicles and spikelet sterility (Bakker 1974; Awoderu 1991).

The disease is caused by the *Rice yellow mottle virus* (RYMV). The primary disease transmission is carried out by a biological complex involving insect vectors mainly beetles of Chrysomelidae family and Orthoptera such as *Conocephalus* spp. (Bakker 1975; Abo 1998; Abo *et al.* 2000; Banwo *et al*. 2001).

The virus is disseminated by wind through leaf contact between healthy and diseased plants and other mechanical mechanisms such as farming equipments used for harvest, and also by contaminated hands (Sara *et al.* 2004). The dissemination of the virus can also be done by cows, donkeys and some rats species (Sara *et al.* 2003) and by burying infected residues in the field and planting right away or 5 days later (Tsuboi *et al.* 2001).

Due to the diversity of infection sources, managing the

Fig. 1 Rice yellow mottle symptoms on infected leaves.

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disease using resistant varieties appears to be the most adapted method for farmers. Two types of resistance are known within the *Oryza* genus: partial resistance (Thottappilly and Rossel 1993) with polygenic genetic determinism (Albar 1998) and high resistance (Thottappilly and Rossel 1993) controlled by a single gene (Ndjiondjop *et al*. 1999). This gene called *Rymv*1, is recessive and localized on chromosome 4 (Albar *et al*. 2003). RYMV resistance gene diversity studies revealed three different alleles: *Rymv*1-2, *Rymv*1-3 and *Rymv*1-4 of the gene *Rymv*1, respectively in rice varieties Gigante (*O. sativa*), Tog5681 (*O. glaberrima*) and Tog5672 (*O. glaberrima*) (Thiémélé *et al*. 2008). The first option in genetic control strategies consisted in the exploitation of the partial resistance with regard to its stability *vis-à-vis* the available pathotypes. Despite genetic progress made in the creation of partially resistant varieties, RYMV is still causing problems in rice fields. The second option guided by high resistance and even immunity noticed with some virus isolates in the rice varieties Tog5681 (*O. glaberrima*) and Gigante (*O. sativa*), was about introgressing the gene *Rymv*1 into high-yielding rice varieties. While promising rice lines having this high resistant gene developed by some research institutes are being experimented, the existence in nature of resistance-breaking RYMV isolates was reported in some Soudano-sahelian regions (Traoré *et al*. 2006). Similarly, high resistance-breaking isolates have been obtained through serial inoculation experiments (Fargette *et al*. 2002; Sorho *et al*. 2005). The presence of such virus variants in the field before the deployment of this resistance represents a serious threat to a successful approach of the genetic control strategy.

In Côte d'Ivoire, few studies have been devoted to researching high resistance-breaking pathotypes. Moreover, no information related to such isolates is available. The aim of this study was to determine the occurrence of high resistance-breaking RYMV pathotypes in Côte d'Ivoire and also to specify the relationships between these pathotypes and the various alleles of the resistance genes.

MATERIALS AND METHODS

Plant and viruses sources

The plant varieties used were the high RYMV-resistant Gigante and Tog5681. Bouaké 189, a RYMV-susceptible variety (**Table 1**), was used as the control check. For the virus, 120 RYMV isolates were obtained from the "Centre National de Recherche Agronomique" (CNRA). These isolates were collected from 2004 to 2006 in farmers' fields in 28 localities in the southern part of Côte d'Ivoire. Each RYMV isolate was artificial inoculation into variety Bouaké 189 to build fresh virus isolates, 21 days post inoculation (DPI). The polyclonal antibodies IgG-Mg directed towards the Madagascar isolate of RYMV (RYMV-Mg1, accession #AJ608211) and obtained from a commercial company (DSMZ, Germany), were used in the ELISA test.

Table 1 Origin of rice varieties/ accessions.

Rice varieties	Origin	Alleles of resistance gene
Gigante	WARDA	*Rymv*1-2
Tog5681	WARDA	*Rymv*1-3
Bouaké 189	CNRA	-

-: no resistance gene
WARDA: West African Rice Development Association
CNRA: Centre National de Recherche Agronomique

Planting

The rice varieties were sown in 2-liter pots, with six plants per variety and per pot.

Experimental design

The experimental design was a non-replicated split plot having the virus as the main factor and the variety as the secondary factor.

Inoculation

The leaf samples of Bouaké 189 variety artificially infected with each of the 120 isolates of RYMV were collected and ground separately in the inoculation buffer (phosphate buffer pH 7, 0.05% Tween 20 and 2% PVP 24 kD). For 1 g of leaf, 10 mL of inoculation buffer were used. Each of the 120 inocula was manually rubbed on leaves of 21 day-old rice seedlings as described by Thouvenel and Fauquet (1977). Carborundum (320 GRIT) was used as an abrasive. Disease symptoms were monitored once a week until the flowering stage of the plants.

Serological test

The double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as described by Clark and Adams (1977) and modified by Ghesquière *et al.* (1997) was used to test for the presence of the virus. Rice leaves (0.5 g) were ground in liquid nitrogen, homogenised in 10 mL PBS-Tween and then centrifuged at 5000 rpm for 5 min. A 1/1000 dilution in the coating buffer (15 mM Na_2CO_3, 34 mM $NaHCO_3$, pH 9.6) was made for the IgG and added to each well of the certified Nunc-Immuno plates MaxiSorb F96 (Nunc, Roskilde, Denmark). After an incubation of 2 h at 37°C, the plates were washed with PBS-Tween and filled with the supernatant previously collected and incubated overnight at 4°C. After washing, each well of the plates were filled with the anti RYMV-Mg antibodies conjugated with Alkaline phosphatase diluted 1/1000 in PBS-Tween. The plates were incubated for 2 h at 37°C and washed with PBS-Tween. The substrate buffer (1 M diethanolamine, pH 9.8) containing *p*-nitro-phenyl phosphate (1 mg/mL) was added to the wells. The plates were incubated in the dark for 1 h. The absorbance reading determined by the intensity of the colouring, was done at 405 nm with the ELISA plate reader (Multiskan EX Labsystems, Helsinki, Finland) 1 h after incubation of the plates.

Geographical distribution of resistant-breaking isolates of RYMV

The geographical distribution study was based on the presence or absence of high resistance-breaking virus pathotypes in the visited rice production areas.

Relationship between virus titer and resistance phenotype

Plants of Tog5681 rice variety infected with each of the 13 RYMV isolates were observed for symptom development at 42 DPI. For each of the 13 isolates, six plants were tested. The corresponding virus titer was determined for each sample (bulked leaves of the six plants) by the absorbance value. For that, two leaf extracts were made and used separately for the ELISA test. The relationship between virus titer and the resistance phenotype (no symptom observed) was then established.

Interaction virus-varieties

The virus-varieties interaction was studied from the responses collected with 38 RYMV isolates selected among the 120 tested. The choice of virus isolates was based on their ability to infect (cause symptoms or not but with virus accumulation) at least a high RYMV-resistant host. For this study, the differential action test defined by Van der Plank (1968) was used. According to the test, there is no sign of differential interaction if randomly selected varieties and used separately, is sufficient to classify the pathotypes correctly.

Measures and observations

1. Disease symptoms development

Plants of each rice variety were observed for symptom development from 14 to 42 DPI. This time period was selected based on preliminary results.

2. RYMV pathotypes prevalence rate

The RYMV pathotypes prevalence rate was determined according to the formula used by Traoré *et al*. (2006):
P_i (%) = n_i / N x 100
P_i (%): percentage of pathotype i; n_i: number of pathotype i; N: total number of pathotypes

3. Virus titer

The virus titer was determined by the absorbance value measured by the reader ELISA at 405 nm.

Statistical analysis

Statistical analyses were carried out to determine the relationship between rice varieties and virus isolates and also the relationship between the virus titer and the resistance phenotype. An analysis of variance was carried out. The LSD test was used for mean classification at P = 0.05. The software used was XLSTAT 2007.6.

RESULTS

Impact of vertical resistance on the disease symptom expression's time period

All artificially inoculated varieties did not show RYMV symptoms at 42 DPI. On the Gigante rice variety carrying the *Rymv*1-2 allele of the high resistant gene, no disease symptom was observed even when infected by some isolates. However, variety Tog5681 having the *Rymv*1-3 allele of the same gene and the susceptible check Bouaké 189 (**Table 1**) showed symptoms (**Fig. 2**) with a difference in disease outbreak period. With Bouaké 189, virus symptoms were observed at the second symptom rating date (14 DPI). With Tog5681, the virus symptoms were observed at 35 DPI (**Fig. 3**). These observations indicate that the RYMV symptom onset period was between 7 and 14 DPI for the partly resistant variety and between 28 and 35 DPI for the highly resistant variety.

Inventory and prevalence of virus pathotypes under selection pressure

At the end of the test on plant-virus interactions, symptomatic and asymptomatic plants were observed. The DAS-ELISA test revealed the presence of virus in all leaf samples showing the virus symptoms and in some asymptomatic samples for the two highly resistant varieties. RYMV detection rate in the samples was 3.85% for Gigante and 13.33% for Tog5681. On the basis of the isolates' ability to infect the two high-resistant genotypes, two isolate groups were found in the RYMV population: the first group comprised isolates infecting high-resistant varieties and represented the high resistance-breaking (RB) isolates group or virulent isolates. The second group consisted of isolates unable to infect varieties and constituted the non resistance-breaking isolates or avirulent (nRB). A high presence of nRB pathotypes regardless of the plant genotype was observed (**Fig. 4**). Within RB isolates, two pathogenic variants characterized by their ability to cause the disease symptom were observed. They were symptom-inducing isolates or symptomatic isolates (SRB) and isolates unable to cause visible symptoms or no symptom-causing isolates (nSRB). Pathotypes breaking the resistance of the *Rymv*1-2 allele only were not identified in this study. Prevalence rates of these pathogenic variants depended on the allele of the resistance gene present in the rice genotypes (**Fig. 4**). So, for the pathotypes breaking the resistance with no symptom (nS), the disease prevalence rates were 5 and 17% with Gigante and Tog5681, respectively. For the S pathotypes, the rates were 0% with Gigante and 8% with Tog5681.

Fig. 2 Variety Tog5681 (left) and the susceptible check Bouaké 189 (right) both showing symptoms of RYMV.

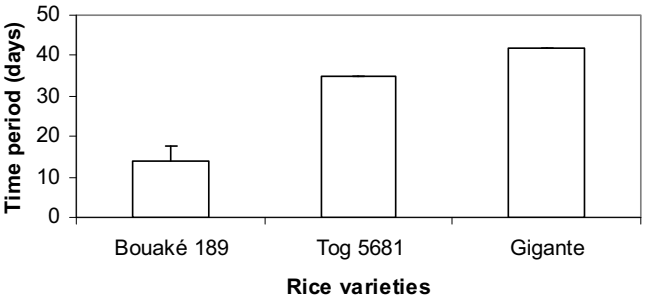

Fig. 3 Effect of high resistance on symptoms expression time period. Values represent the average time period for disease appearance. Bars are standard errors. No variation in time period was observed on Tog5681 and Gigante. For each of the 120 RYMW isolates tested, six plants were used per variety.

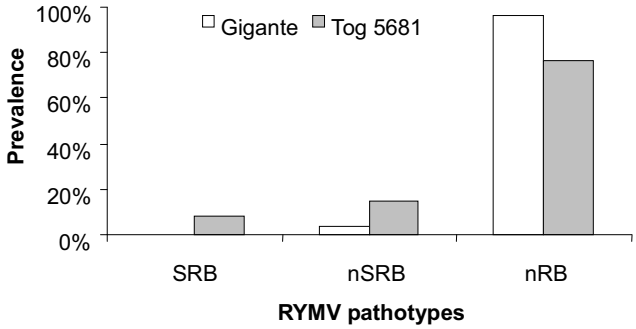

Fig. 4 Distribution of RYMV population under selection due to high resistance rice varieties. 120 RYMW isolates were tested on varieties Gigante and Tog681. For each variety, six plants were used. Values represent percentage of RYMV pathotypes. SRB: symptom-causing and resistance-breaking; nSRB: no symptom-causing and resistance-breaking; nRB: no resistance-breaking.

Geographical distribution of resistant-breaking isolates

High RB isolates derived from *Rymv*1-2 and *Rymv*1-3 alleles respectively present in varieties Gigante and Tog5681 were not encountered in any of the visited localities. Over the 28 localities visited, RB pathotypes were found in 10 localities. In general, the distribution range of the two observed virus pathotypes was not similar. The *Rymv*1-3 alleles of RB pathotypes present in Tog5681 were encountered in the West (Guibéroua, Diégonéfla, Issia and Lakota), Center (Bongounou and Agnibilékro) and East of Côte d'Ivoire (**Table 2**). However, isolates breaking the resistance conferred by the two alleles of the resistance gene were exclusively observed in the West, including Sassandra

Table 2 Distribution of high resistant RYMV isolates in visited localities.

Localities	Isolates	High resistant gene alleles	
		*Rymv*1-2	*Rymv*1-3
Abengourou	CI139; CI141	-	+
Adzopé	CI131; CI132	-	-
Agboville	CI 133	-	-
Agnibilékro	CI89; CI87	-	+
Akoupé	CI127; CI130	-	-
Bassam	CI20, CI19	-	-
Bongouanou	CI175	-	-
Bouaflé	CI1; CI13	-	-
Dabou	CI33	-	-
Daloa	CI40	-	-
Diégonéfla	CI147; CI151	+	+
Divo	CI66; CI67	-	-
Gagnoa	CI166; CI157; CI5	-	+
Grand-Lahou	CI193	-	-
Guibéroua	CI149; CI115	+	+
Issia	CI10	+	+
Lakota	CI43; CI44	-	-
M'Batto	CI73	-	-
San pédro	CI49; CI48	-	-
Sassandra	CI194	-	+
Sinfra	CI16; CI190	-	-
Soubré	CI148; CI3	-	-
Tiassalé	CI23; CI24	-	-
Toumodi	CI101; CI96; CI113	-	+

These are isolates encountered in the visited production areas
+ Compatibility between RYMV isolates and high resistant gene alleles
- Incompatibility between RYMV isolates and high resistant gene alleles

(**Fig. 5**). Additionally, in the western part of the country, both pathotypes were found in the same fields in Guibéroua and Diégonéfla.

Interactions between alleles of the vertical resistance gene and the RYMV virulent pathotypes

Diverse responses were observed on the varieties tested after virus inoculation. In Tog5681, a compatibility reaction was recorded with a predominance of nSRB type. The *Rymv*1-2 and *Rymv*1-3 alleles of the resistance gene present in Gigante and Tog5681 respectively, compatible with isolates CI94, CI10, CI5 and CI149, classified them in the same nSRB group (**Table 3**). However, isolate CI151, although showing a compatibility reaction with the two alleles of the resistance gene, was nSRB as regards to *Rymv*1-2 allele and SRB regarding *Rymv*1-3 allele. For the other isolates tested, a nRB was observed with *Rymv*1-2 whereas SRB or nSRB was observed with *Rymv*1-3. The differential action test was therefore positive. Statistical analysis related to the virus titer revealed an isolate effect and a highly significant variety effect (**Table 4**). The isolates-varieties interaction effect was also highly significant. The LSD test indicates a difference in both varietal groups (**Table 5**).

Relationship between virus tier and resistance phenotype

The absorbance values recorded varied between 0.294 and 0.527 with an average of 0.399 for nS and between 0.372 to 0.802, with an average of 0.565 for S (**Fig. 6**). The statistical analysis pointed out a significant difference between isolates ($P = 0.012$). The classification made from the LSD test showed five isolate classes of different size. The different classes did not show the same phenotypes. If three classes, characterized by a high virus titer, were made of S phenotypes exclusively, on the contrary, one class consisted of nS and S phenotypes and had a relatively low virus titer. Some RYMV isolates with low virus titer in Tog5681 induced disease symptoms. Similarly, the presence of virus symptoms on this rice accession was not compulsorily associated with a high virus titer. The isolates CI169 and CI147 belonging to the same phenotypical class (nS), differed in their recorded virus titer. The measured absorbance values were 0.527 and 0.294, respectively for isolates CI169 and CI147.

Table 3 Differential virulent isolate-resistant genotype interaction expressed by observed resistance phenotype.

Isolates	Origins	Gigante	Tog 5681
CI194	Sassandra	SRB	nSRB
CI10	Issia	nSRB	nSRB
CI5	Gagnoa	nSRB	nSRB
CI149	Guibéroua	nSRB	nSRB
CI151	Diegonefla	nSRB	SRB
CI175	Bongouanou	nRB	nSRB
CI96	Toumodi	nRB	nSRB
CI4	Gagnoa	nRB	SRB
CI141	Abengourou	nRB	nSRB
CI100	Toumodi	nRB	SRB
CI40	Daloa	nRB	SRB
CI33	Dabou	nRB	SRB
CI153	Diégonéfla	nRB	SRB
CI144	Abengourou	nRB	SRB
CI166	Gagnoa	nRB	nSRB
CI157	Gagnoa	nRB	SRB
CI147	Diégnofla	nRB	nSRB
CI69	Bongouanou	nRB	nSRB
CI183	Daloa	nRB	SRB
CI146	Gagnoa	nRB	SRB
CI113	Toumodi	nRB	nSRB
CI115	Guibéroua	nRB	nSRB
CI121	Gagnoa	nRB	nSRB
CI101	Toumodi	nRB	nSRB
CI107	Toumodi	nRB	nSRB
CI139	Abengourou	nRB	nSRB
CI125	Gagnoa	nRB	nSRB
CI148	Soubré	nRB	nSRB
CI89	Agnibilékro	nRB	nSRB

nRB: No resistance-breaking, translating a non host relationship
nS: Resistance-breaking RYMV symptom-free, compatibility relationship
S: Resistance-breaking with RYMV symptom, compatibility relationship

Table 4 Effects of isolates, varieties, and isolate-varieties interaction obtained through variance analysis.

Source	DOF	Sum of squares	Mean square	F	Pr > F
Isolates	28	1.073	0.038	8.644	< 0.0001
Varieties	1	3.469	3.469	782.149	< 0.0001
Isolates*Varieties	28	0.883	0.032	7.110	< 0.0001

DOF: degree of freedom; F: F value of Fisher; Pr: probability

Table 5 Distribution of varieties according to measured viral load.

Modality	Estimated mean	Groups
Gigante	0.156	A
Tog5681	0.502	B

DISCUSSION

Impact of vertical resistance on the expression of disease symptom over time

All tested rice varieties showed different reaction profiles regarding RYMV. The overall recorded responses in relation to the period of virus symptoms appearance indicate that this criterion was determinant in distinguishing resistance rice types. Thus, varieties with vertical resistant can show a delay in disease appearance after inoculation compared to RYMV-susceptible varieties. In this study, the period of the appearance of RYMV symptoms ranged from 7 to 14 DPI for susceptible varieties such Bouaké 189 and from 28 to 35 DPI for varieties with vertical resistance although no symptoms were observed on Gigante during the study. Similar results were reported by Traoré *et al*. (2006). In their work, the statistical analysis realized did not show any difference between Gigante and Tog5681 ($P > 0.33$).

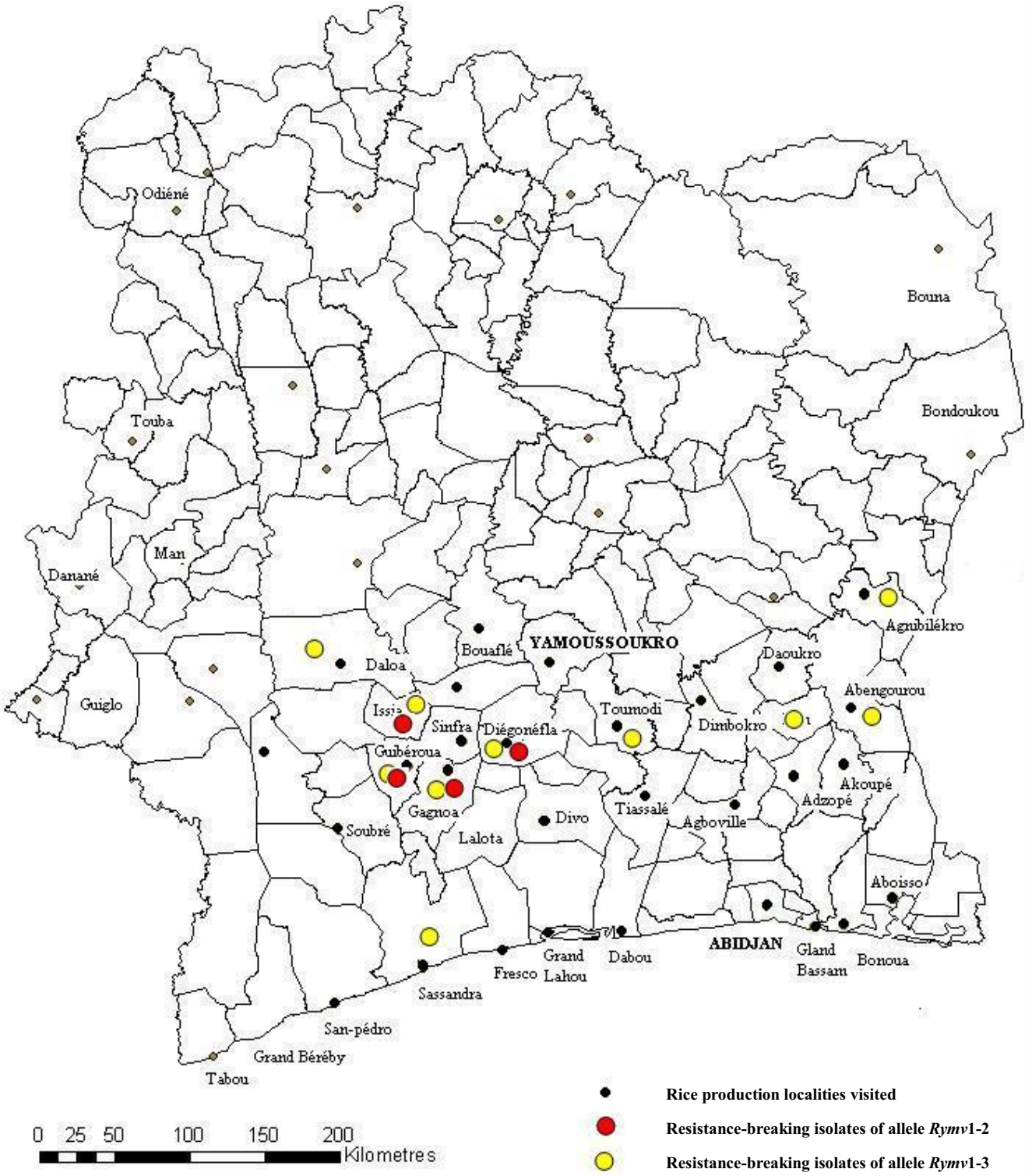

Fig. 5 Geographical distribution of high resistance-breaking isolates of RYMV in Côte d'Ivoire.

Serological tests indicated the presence or absence of virus on tested leaf samples. These tests were positive for some leave samples of Gigante even though no symptoms were observed. That is evidence that this variety is not immune to RYMV.

Classification of RYMV isolates and geographical distribution of resistance-breaking isolates

Based on foliar reaction (symptoms or not) and the virus presence in the leaf samples tested, three virus pathotypes were observed with a predominance of the non resistance-breaking types (nRB). This situation seems normal since the genotypes deployed in the field still show horizontal resistance. Indeed, irrigated rice growing in Côte d'Ivoire is marked by a predominance of rice varieties Bouaké 189, susceptible to RYMV and WITA 9, tolerant to RYMV (Amancho *et al.* 2008). By growing RYMV-susceptible and/or partially resistant varieties, selection would be made in favour of pathotypes lacking unnecessary virulence factors as indicated by the selective law reported by Van der Planck (1968). According to this law, when a vertical resistance gene is present in a host plant, in order to survive, the pathogen races should have high virulence to counterbalance it. But when the gene is missing, to counterbalance it, the virulence becomes unnecessary and the stabilizing selection operates in favour of pathogen races lacking unnecessary virulence. The presence of resistance-breaking pathotypes (RB) in low proportion (detection rate of 3.85 and 13.33%, respectively for Gigante and Tog5681) in this

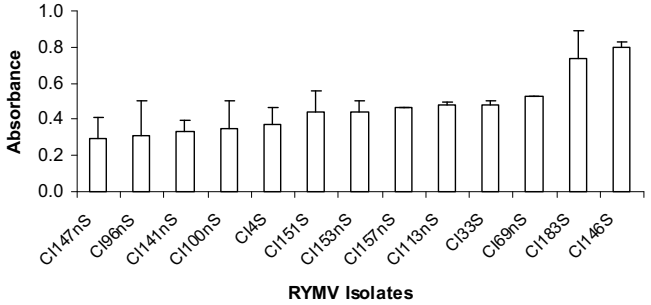

Fig. 6 Relationship between phenotype and virus titer in relation with tested isolates. Two leaf extracts obtained from bulked leaves of the six plants were tested by ELISA. Values represent the mean absorbance for each of the 13 isolates tested on six plants. Bars indicate standard errors.

study could be explained by this selective law. In the absence of this selection pressure (host plants with vertical resistance), the presence of high resistance-breaking isolates indicate that they were counter-selected. However, this counter-selection could not be due to a long period of growing susceptible and partially resistant varieties.

The results related to the prevalence of identified pathotypes confirm the previous work conducted by Traoré *et al*. (2006) for nSRB. The recorded prevalence rates on Tog5681 in both studies are not different (17% in our study vs. 13.9% in the previous work). In Gigante, values were 5% in our study vs 4.6% in theirs. However, regarding the symptomatic isolates (SRB), the results are opposite. The recorded prevalence values on Tog5681 and Gigante were 8 and 0%, respectively in our work vs 1.1 and 12.9%, respectively in the previous study. These results could be due to the fact that the samples tested came from different agro-ecological zones on the one hand, and on the other hand, by the sample size used along with the surface covered in these two works. Indeed, the study conducted by Traoré *et al*. (2006) covered some West African countries (Burkina Faso, Mali and Togo) and part of Central Africa (Cameroon and Chad), while our study were conducted in only the Southern part of Côte d'Ivoire. The relatively high detection rate of pathotypes with the *Rymv*1-3 allele resistance-breaking in Tog5681 could be due to the fact that this variety is a *glaberrima* (*Oryza glaberrima*) which has it centre of origin in Africa. Therefore, the presence of resistance-breaking virus variants could stem from a long period of co-evolution between the ancestor of Tog5681 and RYMV isolates. This *O. glaberrima* ancestor would have been domesticated in the Delta region in Niger, in West Africa (Porteres 1950). The low presence of virus isolates having the ability to break the high resistance conferred by both alleles in this study could be associated with an unnecessary excess virulence (at least two virulence factors). This situation could make them less competent regarding genotypes lacking vertical resistance as mentioned previously. Consequently, these isolates can be presumed less fit to survive.

The existence of resistance-breaking isolates highlighted in the Ivorian rice fields in this study can be a major threat to a sustainable management of rice disease using highly resistant varieties. Experiments with RYMV showed that an avirulent isolate can become virulent after several inoculations to highly resistant host by mutation (Sorho *et al*. 2005). In this case, the deployment of improved rice varieties with the highly resistant gene could put selection pressure in favour of virulent pathotypes (SRB and nSRB) to counterbalance this resistance. This threat can become a reality if other epidemic factors are involved concomitantly (Cowling 1976; Thresh 1982) namely the virus inoculum concentration, a key resistance-breaking factor (Sorho 2006) which is modulated, under natural conditions, by the dynamic of the vector population. However, cases of durable resistance against several plant viruses have been reported despite the identification of resistance-breaking isolates. Taking into account the environment in view of using improved varieties through introgression of the vertical resistance gene for a sustainable management of this resistance is a necessity. Knowledge of the distribution area of these pathotypes shall also be taken into consideration. Thus, the localities of Lakota, Gagnoa, Issia, Toumodi, Bongouanou, Abengourou, Agnibilékro, Diégonéfla, Guibéroua and Sassandra could be areas at risk for growing rice varieties having resistant genes in Côte d'Ivoire. According to the number of virulence factors present in pathotypes with resistance-breaking ability, all conditions being equal, epidemic risks could be higher in the localities of Gagnoa, Issia, Diégonéfla and Guibéroua. The highlighted pathotypes in these localities which cumulated at least two virulent factors are potentially more likely to infect varieties Gigante and Tog5681 used as sources of high resistance.

Interactions between virus isolates and plant genotypes

The study of the interactions between the alleles of the high resistance gene and pathotypes revealed diverse responses corresponding to different resistance phenotypes (nRB, nS and S). The differential activity test conducted was positive, indicating a differential interaction between isolates and genotypes. The results of the statistical analysis (high significant positive variety and isolate-variety interaction effects) not only confirm the results of differential action test but also and more specifically indicate that *Rymv*1-2 and *Rymv*1-3, alleles of the high resistance gene *Rymv*1 present respectively in varieties Gigante and Tog5681, are different. Results obtained by Ndjiondjop *et al.* (1999) were therefore confirmed in this study. Moreover, among the factors likely to influence the resistance phenotype, the virus titer was tested in accession Tog5681. The recorded responses seem to indicate that the resistance phenotype is not closely linked to the virus titer. The absorbance values were also as low in some nSRB as in SRB. These two parameters could be independent. However, a correlation study taking into account several accessions of the Tog series and variety Gigante is necessary for confirmation.

CONCLUSION

The work gives an epidemiological description of *Rice yellow mottle virus* in Southern Côte d'Ivoire, simulated by an artificial selection pressure due to vertical resistance. With the different isolate-genotype interactions, the presence of resistance-breaking pathotypes derived from *Rymv*1-2 and *Rymv*1-3 was highlighted in some rice fields in South Côte d'Ivoire in the absence of highly resistant *Oryza* hosts. Under artificial infection, high resistance was characterized by a delay in virus appearance from 28 to 35 DPI as compared to the susceptible check (7 to 14 DPI) used. Three pathotypes were identified with a predominance of no resistance-breaking isolates (nRB). Among the resistance-breaking isolates, asymptomatic (nSRB) and symptomatic (SRB) pathotypes were identified at different rates. The observed resistance phenotype depended on the *Rymv*1-2 and *Rymv*1-3 alleles thus showing the existence of differential isolate-genotype interactions. With variety Tog5681, this study did not establish a close link between the virus titer and the foliar reaction although an independent relationship between these two parameters could actually exist.

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