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Impact of eight isolates of Rice Yellow Mottle Virus (RYMV) from Gagnoa (Côte d'Ivoire) on rice (*Oryza sp*) cultivars production

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ABSTRACT

Rice yellow mottle virus, of the genus Sobemovirus, causes a major disease in Africa especially in the lowland and irrigated rice ecologies. Yield losses due to the virus were estimated between 5-100% and depend on genotype. This study, conducted in a screen house, aimed at assessing the impact of eight RYMV isolates from a restrictive environment Gagnoa (Côte d'Ivoire) on 10 differential rice varieties. The plants were inoculated manually by rubbing the leaves from the leaf base to the tip with fingers moistened with inoculum. Virus content and percentage yield reduction of different rice cultivars have been assessed. Result showed that an important variability was observed in the reaction of different rice genotypes infected by different isolates of RYMV. In susceptible cultivars Bouaké 189, PNA647F4-56 and H232-44-1-1 virus content and yield reduction varied respectively from 0.08 to 0.264 and 20 to 95%. In resistant cultivars, Gigante, Lac 23, Morobérékan and Faro 11, virus content and yield reduction varied respectively from 0.03 to 0.188 and 5 to 17%. The lowest virus content and yield reduction was observed with the isolate 7 from upland rice. The significant difference in the interaction observed between the different genotypes of rice and isolates of RYMV suggested the existence of different strains of RYMV within a restrictive environment.

Keywords: AfricaRice, Sobemovirus, aggressive, virus content, yield reduction, virulence.

INTRODUCTION

Rice has long been an important staple food for many traditional rice growing communities. To reduce malnutrition in developing country, intensification of rice crop using good production varieties is necessary. Unfortunately, the varieties are very susceptible to many pathogens such of virus, fungal and bacterial. Among these diseases, Rice yellow mottle virus, of the genus Sobemovirus, causes a major disease in Africa (Kouassi et al., 2005; Sere et al., 2008a; Ochola and Tusiime, 2011a) especially in the lowland and irrigated rice ecologies

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(Banwo et al., 2004). Yield loss due to the virus was estimated between 17-100% and depends on genotype (Onwughalu et al., 2011) and time of infection (Onwughalu et al., 2010). The virus has a narrow host range restricted to the cultivated rice species, *Oryza sativa* and *O. Glaberrima*, and a few grasses (Allarangaye et al., 2007). A few rice cultivars exhibit a high resistance to RYMV characterized by an absence of symptoms and no viral detection by ELISA (Ndjiondjop et al., 2001).

The RYMV is mechanically transmissible by insects, genus Chrysomelidea (Abo et al., 2000, Nwilene et al., 2009). These insects play a major role in the virus transmission. Traore et al. (2008) found an abiotic transmission by soil, seedbed, and cultural. RYMV disease is characterized by mottle and yellowing symptoms of varying intensity depending on genotype and time after infection (Soko et al., 2010 ; Onwughalu et al., 2010, 2011). Others symptoms observed on infected plants are stunted, reduced tillering, pour panicle exertion and grain discoloration (Gnanamanickam, 2009). Natural and transgenic sources of resistance were reported (Sorho et al., 2005). Many studies reported the existence of different strains of the virus (Sorho et al., 2005; Amancho et al., 2009 ; Onasanya et al., 2012; Issaka et al., 2011). Many resistance genes were described and a major gene of resistance against RYMV was identified in the *O. sativa* resistance variety Gigante (Albar et al., 2006). A new resistance gene was recently reported as RYMV2 in Tog5672 and Tog5691 (Tiemele et al., 2010). Different serotypes (Sere et al., 2007) and pathotypes of RYMV isolates are known to exist (Onasanya et al., 2004 ; Ochola and Tsiime, 2011a).

The study was undertaken to investigate the impact of eight isolates of rice yellow mottle virus from Gagnoa, Côte d'Ivoire (west-Africa) on Africa Rice differential rice varieties production.

MATERIALS AND METHODS

Materials

Ten AfricaRice differential rice cultivars were used for this study (Table 1).

Virus isolates: eight isolates of rice yellow mottle virus used for this study were originated from different rice crops in Gagnoa (Côte d'Ivoire) (Table 2). They were obtained from plant pathology unit, AfricaRice.

Experimental design

The ten rice cultivars were potted and raised in a screen house at AfricaRice in a split plot design with three replicates, in which the main plot was attributed to RYMV. Each variety is transplanted on six poquets among which two being control are not inoculated. Seedlings were thinned to three plants per pot. The space between replication is 50 cm, and 40 cm between plot and 10 cm between varieties on the line. The trial was fertilized with NPK (10-18-18) at the rate of 120 kgN/ha (50 kgN/ha were brought at tillering and 50 kgN/ha at panicle initiation) with watering and weeding carried when necessary.

Inoculum preparation and plant infection

Each isolate was first propagated in the susceptible rice variety Bouake 189 by mechanical inoculation of 21days old plant in screen house. Three weeks after, a mixture of 60 g of infected leaves from each isolate were harvested and ground, using sterile mortars and pestles in 10 ml of double distilled water. The inoculum was then rubbed onto leaves of the ten differential rice cultivars from the leaf-base to the tip with finger moistened with prepared inoculum after 14 days post-emergence (most plant had achieved a 3 leaf stage).

Virus content assessment

Virus content was assessed using different sample of infected leaves 28 days after inoculation with IACP (Indirect antigene coated plate) - ELISA method (Clark et Adams 1977). Absorbance at wavelength 405 nm was performed after 1 h of substrate incubation

Grain yield evaluation

Rice was harvested after five months and yield component of healthy plant and infected plant was determined after drying to constant weight. Yield loss was evaluated using the following formula:

PYR = [(GYI-GYH) x100] /GYI

PYR: Percentage of grain yield reduction
GYH: Grain yield production with healthy plant
GYI: Grain yield production with infected plant

Statistical analysis

Experiments were performed using a split plot design. Viral content (optical density) and percentage yield reduction were subjected to analysis of variance (ANOVA) using IRRISTAT version 5.1 statistical software and differences between means were compared using Duncan multiple range test (DMRT). Differences at 5% were considered as significant.

RESULTS

Varietal reaction to RYMV isolates accumulation

Different levels of viral accumulation are presented in Table 3. Considerable diversity was observed in the reaction of the ten rice cultivars to 8 isolates in terms of virus content. Virus accumulation varies with varieties and isolates. Analysis of variance showed significant ($p<0.01$) effect of varieties, isolates and an interaction between varieties and isolates (Table 3). These results showed that RYMV isolates used in this study differ by their virulence and the varieties by their vertical resistant. ELISA test showed high viral content in susceptible rice cultivars Bouaké 189, PNA647F4-56, H234-18-1-1-1 and H232-44-1-1. Virus content observed in the cultivar Bouaké 189 ranged from 0.148 for isolate 5 to 0.310 for isolate 4. Considering PNA647F4-56, isolate 4 showed the lowest virus content. Virus content observed in the susceptible rice cultivars H232-44-1-1 ranged from 0.177 for isolate 7 to 0.333 for isolate 8 (Table 4). These results showed that virus content depends on rice cultivars genotype. The highest level of virus content was observed when the cultivar H232-44-1-1 was infected by isolate 8 from Odienné, considered as a control (Table 4). The rice cultivars Faro11, Morobérékan and IR47686-15-1-1 and Lac 23 showed the lowest content of RYMV isolates. The rice cultivar Faro 11 could not be infected by isolate 2 and 7 and Morobérékan is only infected by isolate 1, 2 and 8 with a virus content equal to the threshold of positivity (0.08). The cultivars IR47686-15-1-1 is only infected by isolates 1 and 6 with a viral content mean of 0.155. The cultivar Gigante has no virus content and is considered as immune (DO<0.05) and highly resistant to all isolates. Considering all isolates, isolate 8 has the highest virus content and affects 80% of all varieties and isolate 7 presents the lowest viral content. This isolate infects only 50% of all the varieties.

Percentage yield reduction due to RYMV

Considerable diversity was observed in the reaction of the ten rice cultivars to 8 isolates in terms of yield reduction. Analysis of variance for the percentage of yield reduction showed significant ($p<0.01$) effect of varieties, isolates and an interaction between varieties and isolates (Table 3). These results showed that RYMV isolates used in this study differ by their virulence and the varieties by their vertical resistant. Globally, the percentage of yield reduction of each rice cultivars varied considerably with the RYMV isolate (5 to 95%) (Table 5). The highest yield losses were observed in susceptible varieties Bouaké 189, PNA647F4-56, H234-18-1-1-1 and H232-44-1-1 varying from 20% to 95%) when infected by all isolates. The highest yield losses were observed in susceptible Bouaké 189 (94.40%) when infected by isolate 6. The lowest yield losses were obtained with isolate 7 in all rice cultivars and were estimated to (27.90%) suggesting that this isolate is less aggressiveness. Globally, the isolates 1 and 8 were more aggressive. The rice cultivars Lac 23, ITA235, FARO11 (OS6), MOROBEREKAN, IR47686-15-1-1, are characterized par low yield reduction when infected by all isolates. Yield reduction varied from 5 to 16%.

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

Varieties	Varietal reaction	Origin
IR47686-15-1-1	Resistant	IRRI
H234-18-1-1-1	Positive of Elisa	Argentina
ITA235	Resistant/susceptibility	IITA/Nigeria
PNA647F4-56	Resistant/susceptibility	Peru
BOUAKE189	susceptibility	Côte d'Ivoire
H232-44-1-1	susceptibility	Argentina
FARO 11	Resistant/susceptibility	Nigeria/DRC
GIGANTE (tete)	Resistant	Asia
Lac 23	Resistant/susceptibility	Liberia
MOROBEREKAN	Resistant/susceptibility	Côte d'Ivoire

DRC: Democratic Republic of Congo.

Table 2: Characteristic of RYMV isolates and their naturel host.

Code	Isolates	Samples locality	Host
SP	ISO1	*Site clé prolongement*	*Sacciolepis africana*
RM	ISO2	*Route Mahiboua*	Rice
SE	ISO3	*Site clé extention*	Rice
RA	ISO4	*Route Abidjan*	Rice
CI	ISO5	CRNA ex Idessa	Rice
AF	ISO6	Afridougou	Rice
GP	ISO7	Gagnoa Plateau	Rice
OD	ISO8	Odienné	Rice

Table 3: Analysis of variance for RYMV accumulation and percentage yield reduction.

Source	Viral accumulation				% Yield reduction		
of variation	DF	SS	MS	F	SS	MS	F
Rep	2	0.00038	0.00019	1.41ns	16.20	8.10	1.20ns
Varieties (V)	9	0.97	0.11	423**	137789	15309	2274**
Error (a)	18	0.0047	0.00026		121.4	6.74	
Isolates (I)	7	0.084	0.012	85.70**	7772	1110.3	165**
VXI	63	0.047	0.00075	5.35**	11562	183.5	27.3**
Error (b)	140	0.0189	0.00014		942.3	673	
Total	239						

** = significant at 1% level; ns = not significant; DF: degree of freedom, SS: sum of square; MS: mean square; Rep: replicate.

Table 4: Analysis of mean comparison for RYMV accumulation in varieties.

Varieties	RYMV isolates								V. Mean
	ISO1	ISO2	ISO3	ISO4	ISO5	ISO6	ISO7	ISO8	
BOUAKE189	0.264**a**	0.239**b**	0.210**b**	0.310**a**	0.148**d**	0.218**a**	0.089**b**	0.281**b**	0.219
PNA647F4-56	0.182**d**	0.274**a**	0.183**c**	0.130**c**	0.203**b**	0.217**a**	0.189**a**	0.225**c**	0.200
H232-44-1-1	0.204**c**	0.273**a**	0.258**a**	0.195**b**	0.226**a**	0.208**a**	0.177**a**	0.333**a**	0.234
H234-18-1-1-1	0.228**b**	0.147**c**	0.153**d**	0.139**c**	0.136**d**	0.185**b**	0.073**c**	0.192**d**	0.157
LAC23	0.044**f**	0.066**f**	0.060**e**	0.109**d**	0.09**e**	0.038**e**	0.06**c**	0.049**g**	0.064
ITA235	0.180**d**	0.084**e**	0.094**e**	0.188**b**	0.094**e**	0.191**b**	0.094**b**	0.170**e**	0.137
FARO11(OS6)	0.161**e**	0.063**f**	0.080**e**	0.126**cd**	0.180**c**	0.108**d**	0.071**c**	0.155**e**	0.118
MOROBEREKAN	0.086**f**	0.109**d**	0.062**e**	0.054**f**	0.073**e**	0.042**e**	0.046**d**	0.080**f**	0.069
IR47686-15-1-1	0.160**e**	0.086**e**	0.147**d**	0.091**e**	0.099**e**	0.150**c**	0.086**b**	0.068**f**	0.111
GIGANTE (tete)	0.031**f**	0.039**g**	0.043**f**	0.050**f**	0.036**f**	0.039**e**	0.049**d**	0.041**g**	0.042
Mean (isolate)	0.154	0.138	0.139	0.140	0.128	0.139	0.093	0.160	

ISO : isolate , ISO1= SP (*site clé prolongement*), ISO2 = RM (*Route mahiboua*), ISO3 = SE (*Site clé extension*), ISO4 = RA (*Route abidjan*), ISO5 = CI (CNRA ex idessa), ISO6 = AF (Afridougou), ISO7 = GP (Gagnoa plateau), ISO8 = OD (Odienné) ; In a column, means followed by the same letter are not significantly different at 5 % (test of DMRT); DMRT: Duncan multiple range test; positive control = 0.350 ; negative control = 0.04 ; level of positivity = 0.08.

Table 5: Analysis of means comparison for percentage yield reduction due to RYMV.

Varieties	RYMV isolates								V. Mean
	ISO1	ISO2	ISO3	ISO4	ISO5	ISO6	ISO7	ISO8	
BOUAKE189	91.80**a**	78.4**a**	79.40**a**	72.33**a**	76,27**a**	94.40**a**	27.90**a**	88.17**a**	76.08
PNA647F4-56	66.70**b**	63.13**b**	63.00**b**	59.77**b**	64,50**b**	58.17**b**	23.30**b**	71.93**b**	58.81
H232-44-1-1	51.33**c**	37.50**d**	31.50**c**	34.60**c**	42,63**c**	56.93**b**	19.63**b**	49.87**c**	40.50
H234-18-1-1-1	51.13**c**	42.90**c**	34.17**c**	31.63**c**	28,83**d**	51.07**c**	30.70**a**	44.47**d**	39.36
LAC23	16.13**d**	11.47e	15.80**d**	9.63**d**	13,87**e**	14.73**d**	6.73**c**	13.40**e**	12.72
ITA235	9.27**e**	10.93**e**	10.67**e**	12.83**d**	12,87**e**	9.97**e**	6.73**c**	11.57**e**	10.60
FARO11(OS6)	10.93**e**	9.93**e**	7.30**e**	7.07**e**	8,33**f**	9.23**e**	4.93**c**	10.03**ef**	8.52
MOROBEREKAN	7.03**e**	4.47**e**	7.63**e**	5.37**e**	6.37**f**	8.47**e**	5.13**c**	7.07**f**	6.57
IR47686-15-1-1	9.33**e**	7.70**e**	6.87**e**	7.13**e**	8,37**f**	10.47**e**	5.47**c**	10.87**ef**	8.27
GIGANTE (tete)	8.40**e**	6.17**e**	7.93**e**	6.13**e**	5,70**f**	7.63**e**	5.47**c**	7.97**ef**	6.92
Mean (isolate)	32.21	27.36	26.47	24.65	26,77	32.11	13.60	31.53	

ISO : isolate, ISO1= SP (*Site clé prolongement*), ISO2 = RM (*Route mahiboua*), ISO3 = SE (*Site clé extension*), ISO4 = RA (*Route Abidjan*), ISO5 = CI (CNRA ex idessa), ISO6 = AF (Afridougou), ISO7 = GP (Gagnoa plateau), ISO8 = OD (Odienné) ; In a column, means followed by the same letter are not significantly different at 5 % (test of DMRT); DMRT: Duncan multiple range test.

DISCUSSION

The reaction of different isolates of rice yellow mottle virus (RYMV) was studied and the results showed variable reactions on rice cultivars. According to Astiers et al. (2001), different methods were used to know the biological properties of virus, but pathogenicity of the virus is the most important criteria used. Viral replication is the formation of biological viruses during the infection process in the host cell. Virus must first get into the cell before viral replication can occur. In this study, indirect antigen coated plate enzyme linked immunosorbent assay (IACP-ELISA) was performed on infected leaves to determine virus content or virus accumulation. Analysis of variance (ANOVA) showed a significant ($p<0.01$) interaction between varieties and isolates, for virus content and percentage yield reduction at 28 days after inoculation. The variable reactions of varieties indicated that the RYMV isolates from Gagnoa (Côte d'Ivoire) used in this study were made up of different strains of RYMV. The significant interaction ($p<0.01$) between varieties and isolates, for virus content and percentage yield reduction indicated that RYMV isolates differ by their virulence, and the varieties by their vertical resistance (Adugna, 2004). Considerable diversity observed in the reaction of the ten rice cultivars to 8 RYMV isolates accumulation in different rice cultivars showed that viral replication depends on the genotype of the rice cultivars and virus isolates involved in these interactions. Similar results were obtained by Onasanya et al. (2004), Sorho et al. (2005) and Issaka et al. (2012). The variability in the response of rice cultivars showed that the RYMV isolates had various levels of pathogenicity. In susceptible varieties, virus content was correlated to yield loss, but few varieties had high virus content meanwhile, yield was not affected. The cultivar Bouaké 189 was susceptible to all isolates used in this study with an important yield loss except isolate 7. This isolate is less aggressive and virulent than the others isolates. This isolate was collected on upland rice ecology in Gagnoa. Yield loss was evaluated to 27.9% and it can only infect 50% of all the cultivars. These results showed that pathogenicity has two components: virulence and aggressiveness. Virulence is defined as the capacity of the pathogen to induce disease. Virulence is also the capacity to overcome resistance by pathogens. This component of pathogenicity favors epidemic progress in the field.

Therefore, the aggressiveness expresses the severity of the diseases (Astier et al., 2001). In the present study, the isolates 1, 3, 4 and 5 have infected more rice cultivars, but isolates 1 and 2 are responsible for the highest yield reduction.

Resistance breaking observed in the field is related to the emergence of new strains of RYMV isolates (Sorho et al., 2005; Traore et al., 2010 ; Poulicard et al., 2010). Transformation during viral replication can lead to virulent isolates. Thus, virulent isolates were obtained experimentally by amino acid substitution in Vpg protein (Poulicard et al., 2010). Onasanya et al. (2006, 2012), studying RYMV variability in different localities in Côte d'Ivoire, showed the existence of several pathotypes of RYMV. Most interactions observed between isolates of RYMV and varieties was significant using ANOVA test suggesting that the RYMV isolates in most rice localities differ by their virulence (Adugna, 2004). The RYMV isolates differ also by their aggressiveness and were responsible of high yield losses. Sorho et al. (2005) showed that the properties of virulence and aggressiveness of the virus were not linked. Aggressive isolates have a negative impact on plant growth by reducing photosynthesis activity (Soko et al., 2010). In this study the rice cultivars Lac 23, ITA235, FARO11 (OS6), MOROBEREKAN, IR47686-15-1-1 could be described as possessing both stable and acceptance levels of resistance to RYMV. Under different rice ecologies in Gagnoa, these cultivars possessed heterogenous viral resistance making them to be more stable and more resistant to stress induced by different isolates of RYMV. The cultivars Gigante did not show compatibility with all isolates while the cultivars Bouaké 189, PNA647F4-56, H234-18-1-1-1 and H232-44-1-1 present high compatibility. The resistance genes *rymv1-2* of the cultivars Gigante was useful against the different isolates of RYMV (Albar et al., 2006). Isolate 1 from an alternative host *Sacciolepis* *africana*, was very virulent and aggressive. Therefore, we could say that this alternative host plays an important role in RYMV transmission.

Intensive rice cultivation using the same productive cultivars would be the causes of virulent and aggressive isolates emergence in Gagnoa. Sorho et al. (2005) showed that several propagations of RYMV on the susceptible rice cultivar Bouake 189, favors emergence of virulent isolates. Onasanya et al. (2004) indicated the existence of different pathotypes among the isolates collected from different rice ecosystem in Côte d'Ivoire. The durability of the rice cultivars resistance could be compromised by the existence of different strains of the virus in Gagnoa.

Conclusion

The interactions in the reaction of the rice genotypes to RYMV isolates suggest the existence of different strains in a restrictive environment. These different strains of RYMV are responsible for yield losses in rice ecosystem of Gagnoa. This information could be useful in the rice breeding program aiming at deployment of RYMV resistant genotypes to different rice ecologies in Gagnoa.

COMPETING INTEREST

Authors have declared that no competing interests exist.

AUTHOR'S CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors SDF, AK designed the study, performed the experiments and wrote the manuscript. Author SDF, KTH and KNBC were responsible for data interpretation, statistical analysis and literature searches. Authors TDC, SY and AS carried out the experimental process, helped in experiments and managed the analyses of the study. All the authors red and approved the final manuscript.

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