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RESEARCH ARTICLE

ASSESSMENT OF YIELD LOSSES DUE TO RICE YELLOW MOTTLE VIRUS UNDER FIELD CONDITIONS IN BURKINA FASO

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A B S T R A C T

Rice yellow mottle virus (RYMV) is one of the most devastating plant viruses and belongs to the Genus *Sobemovirus*. In West and Central Africa, three RYMV strains (S1, S2 and S3) have been identified based on their molecular typing (coat protein sequences). In 2013, and 2014, a field experiment was conducted, under rainfed lowland conditions in order to evaluate the effect of RYMV on yield and yield components of resistant, tolerant, susceptible and popular cultivars. Yield components and agronomic parameters were evaluated in relation with grain yield and disease severity. In each experiment, ELISA was performed to confirm symptomatic observation. Based on disease rating scale of RYMV, mean incidence and severity of disease were higher in 2013 than in 2014. Except for the 1000-grain weight, all agronomic parameters considered in this study differed significantly between potential and disease-affected conditions ($P \leq 0.01$). Experiments on the effect of the virus on yield indicated that mean losses of 33.23% occurred due to RYMV infection. The degree of negative effects as measured by grain yield loss (GYL) varied widely between genotypes, ranging from 0.64% to 51.28%. RYMV-susceptible genotype IR64 was the most affected by RYMV infection (51.28% GYL) followed by the two popular genotypes FKR19 and TS2 with 30.09 and 36.91% GYL respectively. The resistant genotype Gigante recorded 0.64% GYL and while the tolerant genotype showed 21.38% GYL. Our results confirmed the importance of RYMV in rice production and emphasize the need to control the disease. Furthermore, more research is needed to elucidate the role of RYMV in yield loss for current and newly released rice cultivars. The outcome of the study could be used as guidance for further display of rice genotypes in West Africa.

INTRODUCTION

Rice (*Oryza sativa L.*) is one of the most important crops for world food security. Rapid population growth requires increased food production (Zhang 2007), and a rice yield increase of more than 1.2% per year will be required in the next decade (Normile 2008). Therefore, investments in the rice sector in Africa should be designed to alleviate poverty and meet the food demands of still growing and increasingly urbanized-populations. Unfortunately, at the same time improvement in the yield potential of rice is limited by many diseases reducing both the quality and quantity of the production. In Burkina Faso, the most economically important rice pathogens are *Xanthomonas oryzae pv oryzae*, *Magnaporthe oryzae Cav*. and *Rice yellow mottle virus* (RYMV) (Balasubramanian *et al*., 2007).

First reported in Western Kenya in 1966 (Bakker, 1974), RYMV is an emergent virus in Africa and also one of the most damaging and widespread pathogen throughout West Africa (Kouassi *et al*., 2005). Its genome is a single-stranded RNA species of the genus Sobemovirus with five open reading frames (ORF) (Ling *et al*., 2013).

RYMV has a narrow host range restricted to the cultivated rice species *Oryza sativa* and *O. glaberrima*, the wild rice species *O. longistaminata* and *O. barthii*, and a few other wild Poaceae species (Bakker, 1974). Infected plants show characteristic mottling and yellowing of the leaves that are associated with stunting, partial emergence of the panicles and sterility. Under natural conditions, RYMV is transmitted by beetle species from the family of Chrysomelidae. Transmission by rats, donkeys, cows and wind was also reported (Sarra and Peters, 2003, Sarra *et al*., 2004). More recently, transmission via soil and water has been reported (Uke *et al*., 2014). Biological tests indicated that RYMV is not transmitted through rice seeds or through seeds of wild host species (Bakker, 1974; Konaté *et al*., 2001, Allarangaye *et al*., 2006). Most rice cultivars, especially those of the *Oryza sativa indica* species, are susceptible to RYMV. Previous research has shown that RYMV by itself can cause significant yield losses fluctuating between 10% and 100%, depending on plant age prior to infection, the rice genotype, and various environmental factors (Abo *et al* 1998, Konaté *et al*., 1997). Recently, Traoré *et al*. (2015) found that incidence of RYMV-infected plants in three rice cultivars namely FKR56N, FKR62N, and TS2 was associated with a decrease in grain yields. These authors reported average yield loss of up to 84%, 79% and 75% respectively for these varieties. However, the impacts of incidence of the virus on yield components were not investigated. Although the incidence of RYMV on yield and agronomic parameters was assessed under greenhouse conditions in Nigeria (Salaudeen, 2014), there is a real need to conduct this experiment under field conditions in order to establish more realistic data. Indeed, experimental conditions (greenhouses, growth chambers, pots …) can significantly differ from the agricultural growing environment.

Quantitative information on yield losses due to diseases is pre-requisite in order to develop policies, set research priorities, assess the progress made in protecting crops, and develop efficient integrated pest management schemes (Zadoks and Schein, 1979). Variations in cropping practices have a strong influence on pathogens profiles and, therefore, on the importance of diseases making information on yield losses are more critical. Thus, the risks associated with such changes can be assessed from a plant protection viewpoint.

Burkina Faso harbours the most aggressive and most of the high resistance-breaking isolates of RYMV. The aim of the study was to investigate on the effect of RYMV on yield and yield components of resistant, tolerant, susceptible and popular cultivars, under field conditions over two growing seasons in Burkina Faso. The outcome of the study could be used as guidance for future rice production in West Africa.

MATERIALS AND METHODS

Biological material

Five rice genotypes, comprising *O. japonica and O. sativa* species, were evaluated for the effect of RYMV on yield and yield components (Table 1). Two (2) improved and popular rice varieties (FKR19, TS2) were selected based on their organoleptic and agronomic features and three (3) reference rice varieties IR64, Azucena, and Gigante, were used as controls in the present study.

Table 1 Background information of rice genotypes used in the experiment

Genotypes	Pedigree	Origin	RYMV status	Species or sub-species*
IR64	IR5657-33-2-1/IR2061-465-1-5-5	IRRI-Philippines	Susceptible	*Indica*
Azucena	Traditional Landrace	Philippines	Tolerant	*Japonica*
Gigante	Traditional Landrace	Mozambique	High resistant	*Indica*
FKR 19	TOX 728-1 (Local-Nigeria)	WARDA	Susceptible	*Sativa*
TS2	-	Taïwan	Susceptible	*Sativa*

*interspecific rice genotypes were obtained from crosses between Oryza indica x O.glaberrima species

IR64 is considered as a high yielding cultivar; but susceptible to RYMV in contrast to Gigante which is highly resistant to RYMV. Azucena is used as a RYMV tolerant genotype.

Field Plot Preparation and Experimental Design

A field experiment was conducted, under rainfed lowland conditions, in Banfora (N: 10°63067 W: 004°77846) located in West Burkina Faso during the fall season in 2013 and repeated in 2014. This site is considered as a hot spot for RYMV. The average temperatures ranged between 25 and 31 °C and a mean annual rainfall of 1,000 mm. Treatments were arranged in randomized complete block design with four replicates. In both years, field plots were 4m × 3m. The same planting procedures and treatments were evaluated in the two (2) growing seasons. The seedbed was installed on June 15, each year. Fifteen-days old seedlings were manually transplanted at a spacing of 20 cm × 20 cm with one seedling per hill. Agronomic practices followed the standard production guide for rice in Burkina Faso. However, no insecticides were applied during the growing season. Fertilizer was applied at the rate of 200 kg ha^{-1} of NPK14-23-14 (basal), 35 kg urea ha^{-1} at first weeding, 15 days after sowing (DAS) and also 65 Kg at heading. Weeds were controlled manually. Harvesting was done by hand. The fields were left under natural infection.

Rice yellow mottle virus disease identification

To assess damage caused by RYMV, number of RYMV-infected plants in each genotypes and disease scoring scale were considered together.

In a first time, the number of plants infected by RYMV in each genotype was presented in percentage using the following formula:

$I(\%) = \frac{PA \times 100}{PT}$ Where I: disease incidence; PA: number of infected or dead plants (a plant was considered as infected as soon as a visible symptom was observed); PT: total number of plants in the observed sample.

Secondly, disease was score at 45 days after sowing based on a scale used for RYMV resistance assessment in international testing nurseries (IRRI, 2002). This scale ranged from 1 to 9 and is based on the degree of foliar discoloration and on the alteration of plant development. A score of 1 corresponds to healthy plants (asymptomatic), thus were considered highly resistant (HR). Moderately resistant (MR) plants were score as 3 and has green leaves with sparse dots or streaks. Plants scored 7 are yellow, mottled and stunted and, thus are considered susceptible (S); while score 9 is highly susceptible (HS) plants that showed yellowing, mottling, stunting followed by death.

Serological tests. In order to detect RYMV in asymptomatic leaf samples and to confirm visual observations, leaf samples were collected 28 days after sowing from diseased and asymptomatic plants in each experimental plot and submitted to RYMV identification. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to detect RYMV in leaf samples as described by Clark and Adams (1977). A polyclonal antibody that reacts strongly and similarly with all the RYMV isolates of West and Central Africa was used as coating antibody (N'Guessan *et al*., 2000). The same antibody was coupled to alkaline phosphatase and used as conjugate. Between steps, microplates were washed three times with 0.15 M phosphate buffer (pH 7.2) containing 0.05% Tween (PBS-T). Wells were saturated with milk solution (3 g of powdered milk per 100 ml of PBS-T) and then incubated 1 h at 37°C. Analyses were done on extracts obtained after grinding the leaves (1 g in 10 ml of buffer) and centrifugate at 8,000 × g for 10 min. The mean absorbance value (A_{405} nm) from healthy controls plus three times the standard deviation was taken as the negative-positive threshold.

Agro-morphological traits and yield measurements

Data on four agro-morphological descriptors including number of tillers (T_{60}), plant height (H_{60}), 1000-grain weight (P_{1000}), number of panicle per m^2 (P_m) and yield (kg $ha^{-}1$) were collected at the appropriate growth stage according to the evaluation scale described by Bioversity International – IRRI-AfricaRice (2007) and the Standard Evaluation System for rice (INGER–IRRI, 1996). Tillering and plant height were evaluated 60 DAS. To evaluate grain yields, each plot was harvested 1 m far from the plot edges to minimize border effects. Grain yield (Kg) was obtained at maturity by converting plot yield to a per hectare basis (kg ha^{-1}). Seed weight was measured by weighing a subsample of 1000 seeds from each plot at 14% moisture content.

Assessment of yield losses attributed to RYMV

Environmental conditions especially favoured the development of RYMV in 2013. Damage was evident in late July (max tillering stage) and worsened during the following months. In order to evaluate grain yield losses associated with RYMV, grain yields of the same cultivars were evaluated in the following season (2014) and potential yield was used as a reference. Materials in the second season were planted in the same experimental station and using a similar design and agricultural practices; the presence of RYMV was negligible. Grain yield loss (GYL %) was calculated as following:

GYL (%) = (GY 2014 − GY 2013) / GY 2014 × 100, where GY 2014 represents the potential grain yield obtained in the 2014 season when RYMV was not present, and GY 2013 corresponds to grain yield in the presence of RYMV. Finally, in order to confirm the causal relationship between the presence of the disease and the grain yield losses - ignoring possible water stress effects - grain yields from the 2014 growing season (not affected by RYMV) in rain-fed conditions only, hereafter considered as sub-optimal yield conditions, were compared to grain yields of the 2013 season (affected by RYMV)

Data Analysis

Statistical analysis was done on agronomic traits measured. An analysis of variance (ANOVA) was performed across the tested years, with year as random effects and genotype as a fixed effect. Mean separation was done where there are significant differences using Duncan Multiple Range Test. Significance was accepted at $p < 0.05$. Data were analysed with Statistica (version 11). Figures were constructed using Microsoft Excel 2016.

RESULTS

Monitoring Virus Incidence and severity

The response of the five rice genotypes to RYMV infection was observed under field conditions in 2013 and 2014. In each experiment, ELISA was done to confirm symptomatic observation (Table 2). No virus was detected by ELISA in asymptomatic samples collected in each plot. Based on disease rating scale of RYMV, mean severity of disease was higher in 2013 than in 2014 (Table 2).

As previously described, field conditions especially favoured the spreading of RYMV in 2013. The first symptoms appeared earlier in the susceptible genotypes IR64, TS2 and FKR19. On IR64, typical leaf mottling began at 20 days after sowing corresponding to six days after transplanting. The severity of infection was relatively low in the tolerant genotypes Azucena on which a score of 5 was recorded. On TS2 and FKR19, the most frequently grown genotypes, the incidence of disease was 49.83 and 42.83% respectively. However, the resistant genotype Gigante recorded 0.00% of RYMV infection in 2013 as well in 2014.

RYMV incidence and severity were low during the second year (2014), ranging from 0.00 to 0.75% and from score 1 to 3 respectively. No symptom was noted on the tolerant and resistant genotypes. Subsequently, leaves turned yellow in the susceptible cultivars and the intensity of infection was very conspicuous. Thus, data collected that year were used as reference i.e. yield potential conditions to calculate yield loss and agro-morphological traits reduction due to RYMV infection.

Table 2 Symptom scoring and virus assessment in ELISA test of the response of rice genotypes under field condition in two seasons

Genotypes	2014			2013		
	Severity	Incidence (I%)	ELISA	Severity	Incidence (I%)	ELISA
IR64	3 (23)*	1.8	+	9 (20)*	69.8	+
Azucena	1 (0)	0.00	-	5 (24)	37.74	+
Gigante	1 (0)	0.00	-	1 (0)	0.00	-
FKR19	1 (26)	0.00	-	7 (22)	42.83	+
TS2	3 (24)	1.38	+	9 (22)	49.83	+

*in bracket indicates days after sowing

Grain yield and grain yield loss

Grain traits in term of grain yield and grain yield loss of different rice variety were recorded and shown in Table 3. Genotypic differences were found in grain yield in the presence of RYMV, in potential yield conditions and also in grain yield loss (Table 3). In the season affected by RYMV (2013), there was a significant difference intra-genotype compare to uninfected season (2014).

However, under potential conditions, the difference within genotype was of 1.6 t ha^{-1} for the susceptible genotype (IR64) and only 0.016 t ha^{-1} for the resistant one (Gigante) which was not significant with Pr > F = 0.9722 (Table 3).

Mean grain yield losses for all genotypes exceeded 1.177 t ha^{-1}, or was on average about 33.23 % of the losses in grain yield. Averaged yield across year 2014 (potential conditions) showed that FKR19 had the highest grain yield (4525.38 kg ha^{-1}) and Azucena the lowest (2680.75 kg ha^{-1}).

The degree of estimated negative effects as measured by GYL varied widely between genotypes, ranging from 0.64% to 51.28%. IR64 (51.28) was the most affected by RYMV followed by the two popular genotypes FKR19 and TS2

Table 3. Means and standard deviation of grain yield (kg ha^{-1}) in disease conditions and potential conditions and grain yield loss (GYL) of five rice varieties

Genotype	GY disease conditions*	GY potential conditions	Pr > F	GYL (kg ha-1)	GYL (%)
IR 64	1520.34±349.5a	3120.77±349.5b	0.0192	1600.43	51.28
Azucena	2107.51±114.82a	2680.75±114.82a	0.0124	573.24	21.38
Gigante	2915.07±318.02a	2931.39±318.02a	0.9722	16.32	0.64
FKR 19	3391.79±276.39a	4852.34±276.39b	0.0097	1460.55	30.09
TS2	2289.32±718.95a	4525.38±718.95a	0.0134	2236.06	36.91
Mean	2364.806	3542.126		1177.32	33.23

Different letters indicate significant differences within line according to Duncan's test (P < 0.05).
*: Standard deviation

Effect of RYMV on agronomic traits

Except for the 1000-grain weight, all agronomic parameters, considered in this study, differed significantly between potential yield and disease-affected conditions (P ≤ 0.01) (Table 4). The numbers of tillers (T60) and plant height (H60) were significantly reduced in disease-affected condition compared to potential yield conditions (Fig 1). However, for 1000-grain weight, there was no significant difference for each genotype under disease-affected and potential yield conditions. The reduction of grain weight due to RYMV was very low for each genotype. The difference of 1000-grain weight means varied between 0.31 g for the resistant genotype Gigante and 3.5 g recorded on the popular genotype TS2.

For all the genotypes, uninfected plants produced more tillers than the RYMV-inoculated plants.

In 2014, under potential yield conditions, susceptible IR64 plants produced a mean of 13 tillers per plant which differed from the 9 tillers obtained under disease-affected regime. The resistant genotype was not affected by RYMV with respect to tiller production. The highest reduction of tillers due to RYMV infection was recorded on the popular genotypes FKR19 and TS2 with percentage reduction of 40 and 40.79 respectively.

Table 4. Mean Tillering (T_{60}) plant height (H_{60}), number of panicle per m^2 (P_m), 1000-grains weight (P_{1000}) disease (2013) and potential yield conditions (2014)

Genotypes		T_{60}	H_{60}	P_m	P_{1000}
IR64	**2014**	13.00[a]	84.750[a]	222.25	18.00[a]
	2013	9.19[b]	54.68[b]	131.5	15.23[a]
	R²	0.58	0.76	0.63	0.26
	Pr > F	0.02	0.004	0.01	0.19
Azucena	**2014**	11.75[a]	107.50[a]	232.5	18.25[a]
	2013	9.11[b]	87.75[b]	154.75	16.93[a]
	R²	0.50	0.89	0.79	0.25
	Pr > F	0.049	0.0004	0.002	0.201
Gigante	**2014**	10.69[a]	111.36[a]	202.5	22.31[a]
	2013	11.75[a]	112.75[a]	190.5	22.00[a]
	R²	0.057	0.044	0.03	0.009
	Pr > F	0.567	0.617	0.67	0.817
FKR19	**2014**	14.25[a]	88.25[a]	262.5	23.25[a]
	2013	8.55[b]	69.19[b]	171.0	21.01[a]
	R²	0.537	0.922	0.83	0.132
	Pr > F	0.0386	0.0002	0.001	0.3758
TS2	**2014**	15.25[a]	107.00[a]	272.5	23.75[a]
	2013	9.03[b]	80.96[b]	174.25	20.25[a]
	R²	0.523	0.883	0.83	0.331
	Pr > F	0.0425	0.0005	0.001	0.1356

Different letters indicate significant differences within column according to Duncan's test (P < 0.05).

For plant height, the two extreme values came from the highly susceptible genotype IR64 and the resistant control Gigante, respectively. Gigante recorded an increase of their height (-1.25%) under disease conditions, however this rise was not significant (P = 0.617). When the height of the disease-affected plants was compared with their healthy control (potential yield conditions), height reductions varied between 1.25 and 35.48%. Furthermore, height reduction in the tolerant genotype Azucena (22.57%) was similar to the 21.60 and 24.34% values recorded in the popular cv. FKR19 and TS2 respectively.

Panicle number per square meter was significantly affected by RYMV.

Except for the resistant genotype Gigante, significant variation within genotype was recorded with respect to panicle number with r^2 ranging from 0.63 to 0.83 (Table 4). The lowest mean reduction of panicle number was found in cv. Gigante (5.93%) whereas the highest reduction occurred in the susceptible cv. IR64 (40.83%).

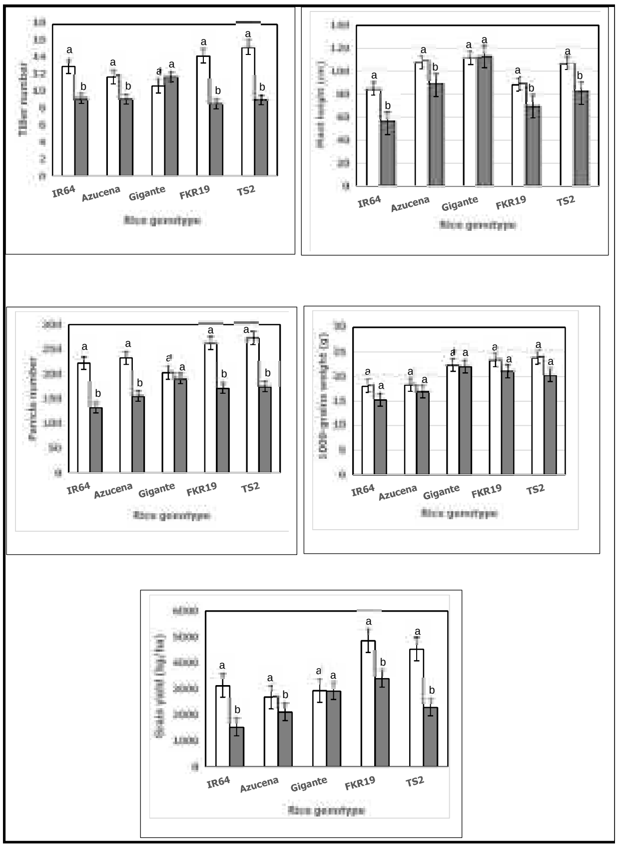

Figure 1 Tiller number, plant height, panicle number, 1000-grain weight, and yield of five rice genotypes from field experiments. White poles represent disease conditions (2013), and striped poles represent potential conditions (2014). Bars in the figure show the standard error; different letters in the panel denote significant differences according to Duncan's test (P < 0.05).

DISCUSSION

The aim of the study was to evaluate yield loss induced by RYMV on resistant, tolerant, susceptible and popular rice cultivars under field conditions in Burkina Faso. To the best of our knowledge, the current study is one of the few that present quantitative data on this topic in Burkina Faso.

In 2014, RYMV severity and incidence were low as compared to 2013 where the infection was severe. The absence of any detectable virus multiplication by ELISA in asymptomatic sample indicates the non-occurrence of RYMV asymptomatic isolates in the experiment site in 2013 and 2014. This result showed that the inoculum pressure that season was not high or this year was not favourable to the insect vector populations. Similar result was reported by others authors who pinpointed that the pathogens incidence is dependent on the population dynamics of the vector (Heinrichs *et al*., 1997; Sisterson, 2009). Consequently, for most genotypes, grain yield was significantly higher in 2014 than in 2013. This response was probably the result of lower virus incidence resulting from field not being infected in 2014.

In our study, plant height, tiller number, panicle number of all genotype were significantly reduced under infected conditions than their uninfected counterparts. Similar results were obtained by those authors who reported the negative effect of RYMV infection on yield components and agronomical characteristics in rice (Kanyeka, 2006; N'Guessan *et al*., 2001). More tillers were produced by plants under yield potential conditions as compared to their infected counterparts indicating the negative significance of RYMV infection in rice productivity. Tiller production is of great importance in rice because of its direct relationship with yield. So, according to Miller *et al*. (1991) grain yields were dependent on final tiller density rather than plant population. However, results by Kanyeka (2006) reported the opposite. This author found that in some rice varieties, the number of tillers of the infected plants were significantly higher than the uninfected plants suggesting that RYMV infection promoted tillering in these cultivars. This high number of tillers under disease conditions could be explained by the favourable growing conditions during tillering and before disease spread which enabled good establishment of tillers. Many authors have reported similar finding when working on another crop like wheat (Richards *et al*., 2001; Duggan *et al*., 2005; Elhani *et al*., 2007).

The highest height reduction found in cv. IR64 and the popular genotypes TS2 and FKR19 can be attributed to its poor resistance to RYMV. This is in agreement with previous studies by Abo *et al*. (2002) and Traoré *et al*. (2015). Others authors have reported that the reduction in plant height of susceptible local rice cultivars due to RYMV infection was as high as 100% (Kihupi *et al*., 2000). Thus, because of these genetic differences among genotypes, the level of plant height reduction was the lowest in the resistant Gigante cultivar followed by the tolerant Azucena.

No significant difference was observed for 1000-grain weight in all genotypes. This finding suggests that 1000-grains weight is less affected by RYMV infection compared to other yield components. This could be explained by the fact that the size of rice grain is physically restricted by the size of the hull and its weight under most conditions appears to be a very stable varietal characteristic as reported by Yoshida since 1981.

Tillering ability in rice is an important agronomic trait for panicle number per unit land area as well as grain production (Moldenhauer *et al*., 2003). Thus, the significant differences observed for tillers and panicle number probably indicate that a large tiller number was associated with panicle production among rice genotypes suggesting that panicle number is an important component of yield. These results are consistent with those from Gravois and Helms (1992) who found that panicle per square meter had the largest positive effect on grain yield, while the effects of filled grain per panicle and grain weight were of secondary and/or tertiary importance.

Under field conditions as in the present study, favourable environmental conditions are necessary for RYMV infection occurrence. Such favoured conditions are likely to increase RYMV incidence on susceptible genotypes. In the current study, the most severe symptoms (yellowing and stunting) were observed on the susceptible genotype IR64 under disease conditions (2013). This may explain the drastic yield loss in this cultivar. Interestingly, despite such high inoculum pressure in the field, there was a nonsignificant decrease in yield on Gigante in both years. This result confirms the high resistance status of Gigante to RYMV under field conditions and indicates the lack of high resistance-breaking isolates in the experiment areas these years. These results are consistent with those from greenhouse studies, under mechanical inoculation (Kam *et al*., 2013; Traoré *et al*., 2015).

Altogether, the contribution of each agro-morphological parameter to variation in grain yield showed clear differences depending on the experimental conditions. Thus, our results suggest that the reduction in grain yield in disease conditions was probably due, firstly, to decreased grains per square meter shown by a reduction in tiller and panicle number, and secondly, a reduction in plant growth (Gaunt, 1995), explaining the reduction in plant height. Indeed, plant height is related to the productivity and growth rate of a plant. According to Sritarapipat *et al*. (2014), plants tend to grow to a certain height in each of its growth state. Nevertheless, it is important to mention that all the factors contributing to this, reducing yield in all genotypes in 2013 are not known, but they may involve other rice pathogens not considered in this study, fluctuations in temperature, humidity, and timing of rainfall. In other words, favourable environmental conditions are essential not only for disease expression but also for high performance of crops against diseases.

The results of these experiments highlighted the importance of RYMV in rice production and emphasizes the need to control it. This study demonstrated the effects of RYMV on RYMV-susceptible, resistant, tolerant and popular cultivars under field conditions. More research is needed to elucidate the role of RYMV in yield loss in current and newly released rice cultivars.

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