

Full Length Research Paper

Inhibition of root growth as mode of action of two rice yellow mottle virus pathotypes isolated in Mali

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In Mali, the economy is mainly based on agriculture. Rice contributes about 5% of the GDP and constitutes a strategic sector with enormous potential. Despite its strategic importance, rice cultivation faces multiple biotic constraints among which rice yellow mottle virus disease is the most important one with an incidence varying from 60 to 100%. To reduce yield losses due to this disease, we determined the mode of action of two rice yellow Mottle virus (RYMV) pathotypes previously isolated in rice fields in Mali, and identified by Reverse transcription polymerase chain reaction (RT-PCR). Both types significantly decrease rice height with a more pronounced effect with the type A, which showed more aggressivity even on the SK20-28 genotype recognized as RYMV disease-tolerant variety. Root dry weight, grain production and tiller number were the most affected by the disease. Except for tiller number, the virus type A was always more virulent than the type B. Significant positive correlation was found between disease severity and % reduction in tiller number, percentage reduction in plant height, and grain production. The data presented root inhibition as a mode of action of the two RYMV pathotypes isolated in Mali. This information should be used in order to improve the management strategies for these pathogens on field rice in Mali.

Key words: Rice yellow mottle virus, root inhibition, rice, Mali.

INTRODUCTION

Rice yellow mottle virus is a disease of rice causing high losses to rice production (Onwughalu et al., 2010; Traoré et al., 2009). Rice plants affected by this virus show yellow or orange leaves at the early stage of the crop,

and leaves roll up and dry in severe cases (Kouassi et al., 2005; Odedera et al., 2016). Stunting, reduced tillering and poor panicle filling are other possible symptoms (Onwughalu et al., 2010; Suvi et al., 2018). The effects of

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the virus on rice plants result in low or no seed production if affected at booting stage and poor grain quality (Onwughalu et al., 2010). Rice yield losses caused by field infestation by rice yellow Mottle virus (RYMV) were estimated between 17 and 100% (Onwughalu et al., 2011) with significant socio-economic impacts on smallholder farmers (Abo et al., 1998; Onwughalu et al., 2010; Asante et al., 2013; Sereme et al., 2016).

Characterization of rice varieties using the lack of expression of symptoms and the un-detectability of the virus content in plant tissues by ELISA test as resistance factor has made it possible to identify rice (*Oryza sativa*) varieties (Gigante and Bekarosaka) as well as *Oryza glaberrima* species (Tog5681, Tog5672, Tog5674 and Tog7291) as very resistant to RYMV (Oludare et al., 2016). For that, the most widely strategy currently used for disease control is genetic resistance, through the use of resistant varieties against pathogens (Samaké et al., 2018; Suvi et al., 2018).

However, the ability of pathogens to overcome resistant genes in crops compromised the durability of these resistances (Ochola and Tusiime, 2011). West and Central Africa have a high pathogenic diversity (Abo et al., 1998; N'Guessan et al., 2001; Oludare et al., 2016; Odedara et al., 2016) and this genetic diversity occurring among RYMV populations seems to present a big challenge for breeding rice for durable resistance to plant virus (Munganyinka et al., 2016). An hypervirulent pathotype of Rice Yellow Mottle Virus was identified in West and Central Africa (Hebrad et al., 2018; Pidon et al., 2017). This pathotype named pathotype T, is able to overcome all type known sources of high resistance and appears to be more abundant than suspected (Hebrad et al., 2018).

Despite the production losses, socio-economic impacts are caused by the disease across Africa, and the ever-increasing resistance breakdown (Sereme et al., 2016); research activities continue mainly in the search for resistant varieties (Ndjiondjop et al., 1999; Albar et al., 2006; Thiémélé et al., 2010; Suvi et al., 2018), and few studies have been conducted to study how the virus affects the growth of the different parts of the plant to impact its production (Opalka et al., 1998; Abebrese et al., 2019).

The studies carried out in this direction have shown that partial digestion of pit membranes resulting from programmed cell death may permit virus migration through them, concomitant with autolysis (Opalka et al., 1998). In addition, the displacement of the Ca^{2+} from pit membranes to virus particles may contribute to the disruption of the pit membranes and facilitate systemic virus transport (Opalka et al., 1998). It was also shown that the virus negatively impacts plant water and mineral nutrition by initiating an imbalanced growth between roots and leaves (Guinagui et al., 2018). In Mali, these modes of action were not detected by the works of the researcher. This suggests that the rice yellow mottle virus

genotypes affecting rice varieties in Mali use different modes of action. To be able to efficiently control the RYMV disease in Mali, we must know how the virus cause damages to rice plants. In this study, we firstly identified the type of RYMV in rice fields in Mali (Ohasanya et al., 2006). Secondly, we determined the mode of action of the different types of the identified virus using different rice varieties cultivated in Mali (Mogga et al., 2012).

MATERIALS AND METHODS

Study area

To determine the pathogenicity of two types of Rice Yellow Mottle Virus newly isolated in Mali, an experiment was conducted in a greenhouse under artificial conditions at the Laboratory of Research in Microbiology and Microbial Biotechnology (LaboREM-Biotech), Faculty of Sciences and Techniques; University of Sciences, Techniques and Technologies of Bamako in Mali. This experiment was conducted between June and October 2017.

Rice varieties

In this study, nine rice varieties most cultivated in Mali (Table 1) were used. These rice varieties were obtained from the Institute of Rural Economy of Mali (IER).

Isolates of rice yellow mottle virus

Two types of the RYMV isolated from leaves of infected rice plants collected from farmers' field at Niono, Baguineda and Selingué (Samaké et al., 2018) were used in this study. These two isolates were identified by molecular typing following the method described by Uke et al. (2016), and propagated in greenhouse using the highly susceptible cultivar Kogoni 91-1 by mechanical inoculation of two-week-old seedlings using finger-rub technique (Samake et al., 2018). Four weeks after inoculation, rice leaves showing typical yellow mottle symptoms were harvested to prepare the inoculum used for inoculating rice genotypes in the greenhouse experiment.

Inoculum preparation

The inoculum used was prepared according to the method by Munganyinka et al. (2016). Briefly, one gram of infected leaf tissue was first crushed in a drop of doubly-distilled water using sterile mortars and pestles until 80% of the leaf tissue material was macerated. The resultant leaf extract was diluted 10 times by adding 10 ml of doubly-distilled water and kept in a refrigerator.

Experimental design and treatments

In this experiment, we used a complete randomized bloc design (CBRD). The RYMV isolates type A and type B, and the non-inoculated represent the three blocs studied. Rice varieties represent the twenty-one treatments studied. Each treatment was replicated four times. Five seeds of each tested rice variety were seeded directly in a plastic pot filled with 10 kg of soil. After one week the seedlings were thinned to two plants per pot. The cultivar Kogoni 91-1 was used as the highly susceptible check while Gigante was the highly resistant check. Each pot was constantly

Table 1. Analysis of variance for rice tiller number, plant height, grain weight and dry root weight.

SOV	df	Tiller number	Plant height	Grain weight	Dry root weight
Rice varieties	8	96.67 ^{***}	1707.04 ^{***}	3193.25 ^{***}	4000.27 ^{***}
Repetitions	2	0.48 ^{NS}	0.65 ^{NS}	34.26 ^{NS}	23.83 ^{NS}
Rice varieties*Repetitions	18	0.37 ^{NS}	1.10 ^{NS}	32.03 ^{NS}	9.60 ^{NS}
Treatments (Virus + control)	2	1009.91 ^{***}	13817.97 ^{***}	507.33 ^{***}	14872.73 ^{***}
Treatments*Varieties	18	16.57 ^{***}	409.67 ^{***}	272.19 ^{***}	165.54 ^{***}
Treatments*Repetitions	4	0.04 ^{NS}	1.43 ^{NS}	28.89 ^{NS}	15.68 ^{NS}
Error	16	0.34	0.89	30.79	9.67

*** Significant at $P < 0.001$ and NS: Not significant at $P < 0.05$.

supplied with tap water in the morning. To avoid confusion between the yellowing associated with disease development and that due to malnutrition, 2 g of NPK fertilizer was applied to the plants at 28 days after planting followed by application of 2 g urea at 45 days after planting and at early flowering stage of the plants (Onwughalu et al., 2010).

Inoculation of rice varieties

The inoculation which started 14 days after transplanting using a mixture of carborundum powder to aid the infection, was performed by rubbing the mixture onto the leaves from the base to the top using pieces of cotton wool (Munganyinka et al., 2016). Care was taken to ensure maximum wetting and formation of mild bruises that acted as infection passageways, according to Ochola and Tusiime (2011).

Data collection

Disease severity

Appearance of symptoms and disease progress were monitored for each inoculated genotype from 7 to 32 days post inoculation. Disease severity was scored with a version of the scale developed by John and Thottapilly (1987) and modified by Ochala et al. (2011), where 0 was equivalent to non- infection and 9 was 100% infection.

Components of the yield

Data on important agronomic traits were collected according to Munganyinka et al. (2016). The data include the plant height of inoculated and uninoculated plants (measured from the soil surface to the tip of the shoot in cm); root architecture and length were determined and measured after carefully washing the roots; the number of tillers per plant was recorded for each hill, and the seed weight was determined. These data were used to assess the impact of the disease on the growth of the rice (Munganyinka et al., 2016). In each case, the impact of the disease was assessed using the following formula:

$$\text{Impact (\%)} = (Ni - I) \times 100 / Ni \text{ (Zouzou et al., 2008)}$$

Where: Ni = mean values on the seedlings not inoculated and I= mean values on the seedlings inoculated.

Data analysis

Analysis of variance was performed for each parameter (Rice genotypes and Virus types) according to the General Linear Model (GLM) procedures using SAS software (version 9.2). Whenever the Fisher test indicates a significant effect at a probability of 0.05, Fisher's Protected Least Significant Difference (LSD) test was used to compare the means.

RESULTS AND DISCUSSION

Effect of different types of RYMV on rice aerial and root growth

Effect of two RYMV pathotypes isolated in Mali on rice aerial growth

The effect of two types RYMV on the growth of the aerial part of the rice is presented in Figure 1. No significant effect was observed on the growth of the aerial part of the two rice genotypes after inoculation with water in the absence or presence of carborundum (Figure 1). However, inoculation with RYMV shows a decrease in plant size with a stronger effect of the two types of RYMV in Kogoni 91-1 (Figure 1). Indeed, both types of RYMV significantly decrease the hard rice size with a more pronounced effect with the RYMV type A on Kogoni 91-1. RYMV type A was more aggressive even on the SK20-28 genotype, which is recognized as disease-tolerant, than type B (Figure 1).

Effect of RYMV on rice root growth

The effect of two RYMV types on rice root growth is shown in Figure 2. No significant effects of inoculation with water were observed on root growth. However, inoculation with RYMV showed a decrease in root size in the Kogoni91-1 and BG 90-2 genotypes with a strong effect of both RYMV types in Kogoni 91-1 (Figure 2). The more root growth is inhibited, the less the aerial part of the rice grows (Figures 1 and 2).

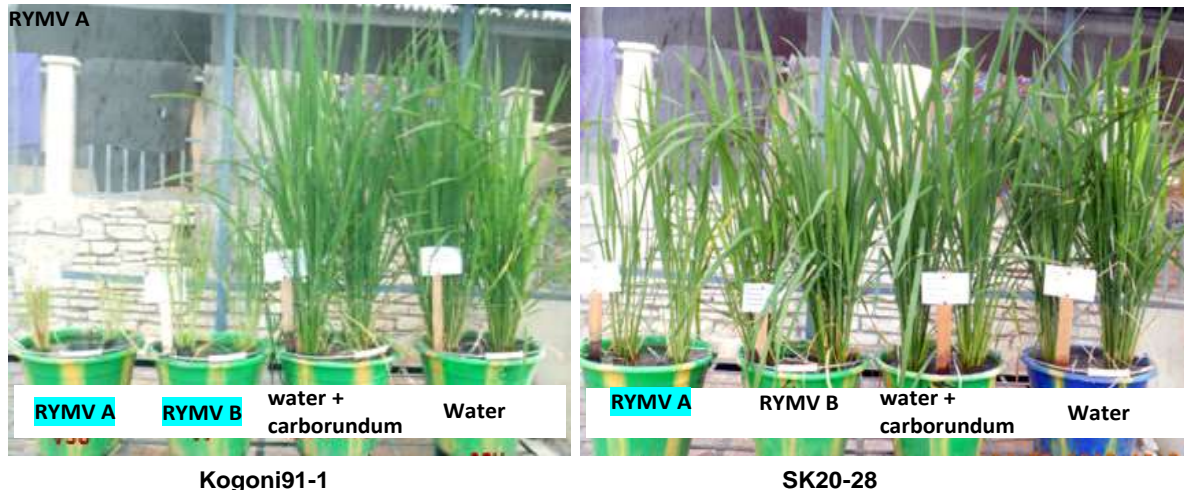


Figure 1. Effect of two RYMV pathotypes (RYMV A and RYMV B) isolated in Mali on the aerial growth of the rice genotypes Kogoni 91-1 and SK20-28.

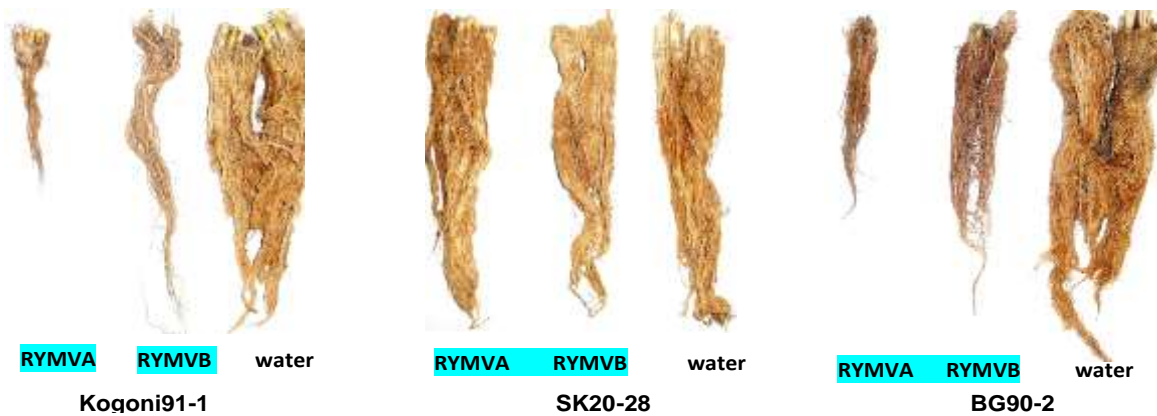


Figure 2. Effect of two RYMV types isolated in Mali on the root growth of the rice genotypes Kogoni91-1, SK20-28 and BG90-2.

Table 2. Effect of inoculation of two RYMV genotypes on tiller number, plant height, grain weight and root dry weight of 9 rice (*O. sativa* L.) genotypes.

Virus type	Tiller number	Plant height (cm)	Grain production	Root dry weight (g)
Control	7.10 ^a	17.29 ^a	13.20 ^a	14.43 ^a
Type A	4.57 ^b	11.02 ^b	4.02 ^b	3.13 ^b
Type B	3.42 ^b	15.03 ^a	5.59 ^a	4.67 ^c

Effect of the different types of RYMV isolated in Mali on rice yield components

The mean squares for the effect of RYMV disease on rice yield components are presented in Table 1. Rice varieties, treatments (virus pathotypes and an uninoculated control) and their interactions significantly ($P \leq 0.001$) affected rice tiller number, plant height, grain weight and dry root weight (Table 1). Contrariwise, no

significant effect was observed with repetitions and their interaction with rice varieties or treatments.

Inoculation with RYMV reduced the yield parameters studied as compared to the non-inoculated treatments (Table 2). Root dry weight, grain production and tiller number were the most affected by the disease. Except for tiller number, the virus type A was always more virulent than the type B (Table 2). Results presented in Table 3 show the disease effect on yield components.

Table 3. Effect of RYMV disease on the components of the yield for 9 rice genotypes after artificial infection under greenhouse condition during the season 2016-2017.

Rice genotype	Disease severity scores	Impact			
		Tiller reduction (%)	Height reduction (%)	Grain production reduction (%)	Root dry weight reduction (%)
Kogoni 91-1	4	37.5	34.88	53.09	51.23
BG90-2	6	15.04	22.95	21	21.48
Adny11	5	23.23	25.86	23.43	29.1
Fan	5	31.68	30.86	31.04	40.61
NERICA1	7	13.11	7.22	13.1	19.4
NERICA2	7	15.02	9.18	12.13	18.78
Wassa	4	44.25	41.95	56.99	58.93
SK20-28	8	6.39	2.88	4.2	8.91
Sim2-Simadel	7	12.8	11.88	9.52	16.11

Table 4. Mean squares from analysis of variance for regression of reduction in plant number of tillers, plant height and grain weight on RYMV disease severity.

SOV	df	Tiler number reduction	Plant height reduction	Grain production reduction
Regression	1	1325.95***	1336.89***	2761.24***
Residual	7	8.97	160.21	85.61
Total	8			
"b"		0.016	-1.34	-6.94
R ²		0.99	0.89	0.97
S.E.		1.13	4.78	3.50

*** Significant at 0.001 probability level.

Results showed that RMYV infection reduced rice production between 4.2 to 56.99% depending on the genotypes whereas reduction in tiller number per plant varied from 6.39 to 44.25%, plant height from 2.88 to 41.95% and plant root dry weight from 8.91 to 51.23% (Table 3). The rice genotype Sim2-Simadel showed a small reduction in grain production compared to the tolerant check SK20-28. The susceptible check varieties, Kogogni 91-1 lost 53.09% of its production. The variety Wassa which appears to be more susceptible than Kogoni 91-1, lost 56.99% of its production in contrast to NERICA 2, NERICA 1 and BG90-2, which lost 12.13, 13.1 and 21% respectively (Table 3). Significant positive correlation was found between disease severity and % reduction in tiller number ($R^2=0.99$), percentage reduction in plant height ($R^2=0.89$), and grain production ($R^2=0.97$) (Table 4).

DISCUSSION

Root growth and plant height of rice genotypes Kogoni 91-1 and BG90-2 (*Oryza sativa* L.) were strongly inhibited by the two types of RYMV isolated from Mali. These

results confirmed that the intraspecific lines (*Indica* species) were highly susceptible (Munganyinka et al., 2016). Guinagui et al. (2018) showed that RYMV infected leaves contain less minerals (P, K, Ca and Mg) and chlorophyll than those of the non-infected rice plants. The early inhibition of rice root growth may affect water and mineral absorption and can explain the reduction in grain yield in susceptible rice varieties, mainly in *O. sativa* L. intraspecific lines. Contrary, to susceptible rice lines, in the presence of these two types of RYMV the rice genotypes SK20-28 and Sim-simadel showed no significant inhibition in root growth. Abebrese et al. (2019) indicated that some rice hybrids decrease the incidence of the RYMV disease and improve rice yield. So, they recommended the introduction of these rice hybrids.

In this work the effect of RYMV disease on yield components differed among varieties and the type of virus. Results were confirmed by a highly significant regression of disease score on % reduction in tiller number, plant height, grain production and dry root yield. Results from this study confirm the findings of Munganyinka et al. (2016) in which they showed that RMYV infection reduced plant height between 2.0 to 20.3% depending on genotype; whereas tiller number per

plant varied from 4.84 to 30.6%. Onwughalu et al. (2010) stated that the behavior of the rice varieties they tested varies by variety and the parameter selected, and revealed negative corrections with RYMV infection.

Likewise, Guinagui et al. (2018) obtained a high yield loss of 94.4% in Bouake 189 at seedling and booting infection stages. These results establish the fact that yield losses to RYMV are strongly influenced by host cultivars as well as time of virus infection, and revealed that the period from seedling and booting represents the most vulnerable phase to RYMV infection in rice growth stages (Onwughalu et al., 2010).

Conclusion

The rice varieties Kogoni91.1 and BG90-2 were very susceptible to rice yellow mottle virus. These rice varieties were the most appreciated by consumers and smallholder farmers in Mali. Two RYMVs were identified in rice fields in Mali. It appeared through this research results that: these two RYMV genotypes impact negatively the tested rice varieties' growth and yield, by strongly inhibiting root sizes (length, width and volume). Thus, improving rice root growth in the presence of RYMV disease could improve growth and yield of susceptible rice varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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