Response of 20 Rice Genotypes to the Rice yellow mottle virus (RYMV) under Natural Infection Conditions in the M'bé Valley of Côte d'Ivoire

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Abstract: One of the major constraints on rice production in Africa is the yellow mottle virus (RYMV) disease caused by Rice yellow mottle virus. In the context of the complex nature of this disease and the rapid development of its epidemics, only an integrated approach within a controlled ecological framework can lead to the development of an effective and sustainable control strategy. In order to search for varieties able to resist this virosis, a screening was carried out under natural infection conditions to assess the resistance capacity of 20 rice genotypes, including ten F1 hybrids and ten locally grown varieties. The study was carried out in Bouaké using a split plot system with three replicates. NPK fertilizers (12 24 18) and urea (46% N) were applied at 150 and 100 kg/ha respectively. The incidence, AUSPC and progression rate of RYMV disease were evaluated in the different varieties tested. Serological analysis confirmed the presence of RYMV in the collected leaf samples. The results showed that Palawan (0.13), Wita 9 (0.11), AR051H (0.19) and AR034H (0.09) had the lowest disease progression rates compared to Kpatè (2.3); Soungrouba (1.26); Marigbè (1.26); AR638H (1.56); Kouiklonlé (0.88) and GT11 (0.89) in which the highest rates of disease progression were observed. Similarly, the incidence of RYMV recorded by stage of genotype development was lower in Palawan, Wita 9, AR051H and AR034H. The Palawan, Wita 9, AR051H and AR034H varieties can be deployed without great risk in central Côte d'Ivoire. However, an evaluation under semi-controlled conditions should be considered in order to confirm the behaviours of the different genotypes. These varieties could be exploited pending the large-scale deployment of improved, naturally resistant RYMV genotypes.

Keywords: Resistance, Incidence, AUSPC, Rice yellow mottle virus, hybrid, RYMV

Introduction

Agriculture contributes about 20% of Côte d'Ivoire’s gross domestic product (GDP) (MINAGRI, 2012) and remains the basis of the economy. In this context, rice farming plays a dual role, both in the fight against food insecurity and poverty. However, for more than thirty years, local rice production has remained below the social need currently estimated at 1500,000 tonnes of milled rice. The country therefore relies on massive imports to fill the production gap. These imports cost about 235 billion CFA francs in 2009 for just over 900,000 tons of milled rice (Bouet et al., 2013). This low production is due to several factors, including the lack of productive and tolerant/resistant varieties to abiotic and biotic factors (Sido et al., 2013). Abiotic (drought, flood) and biotic (rice diseases) constraints are the main causes of low rice productivity. Thus, for this last constraint, several rice diseases (fungal, bacterial and fungal viral) hinder efforts to intensify rice cultivation, including rice yellow mottle virus. Discovered in 1966 in Kenya (Bakker, 1974), rice yellow mottle virus is the main viral disease of rice inferred from the African continent.

The pathogen responsible for this virus is the Rice yellow mottle virus (RYMV), a member of the genus Sobemovirus (Fauquet, 1987). Yield losses due to RYMV are enormous and vary from 10 to 100% (Kouassi et al., 2005; Amancho et al., 2009) depending on the time of infection and the variety. Currently, seven strains have been identified.
(Ndikumana et al., 2017) with specific geographical adaptations. The diversity of infection sources, vectors and viral isolates makes it difficult to control yellow mottle virus, making genetic control the most effective way to resist this major disease (Leung et al., 2003). Two types of resistance against RYMV have been reported, high resistance and partial resistance (Albar et al., 2006; Thiémélé et al., 2010). Despite the strengthening of some resistance traits by crossbreeding or transgenesis in popular rice varieties, RYMV continues to wreak havoc. In response to this situation, new high-yielding rice varieties that are resistant/tolerant to RYMV are required. This study aims to screen twenty rice genotypes for RYMV resistance under natural infection conditions.

3 Materials and methods

3.1 Material

Twenty rice varieties, including ten F1 hybrids and ten local varieties (Table I) were used. The local varieties (VL) were obtained following a collection of local rice cultivars from producers in different regions of Côte d'Ivoire. This collection was carried out with the aim of preserving rice biodiversity. The hybrid varieties (VA) have been made available free of charge by AfricaRice. According to Sorho's 2011 studies on the phylogeography of Rice yellow mottle virus in Côte d'Ivoire, it is the S2 strains that are found in central Côte d'Ivoire with different levels of pathogenicity.

3.2 Methods

3.2.1 Trial management and experimental design

The study was carried out in the Mbé II valley of the Gbéké region (7E55N, 5E03 W, 246 m) in central Côte d'Ivoire over an area of 1.5 ha under natural infestation conditions (Figure 1). The average temperature, rainfall and relative humidity were 28°C, 1200 mm and 85% respectively during the study period. The experimental site is a semi-drained lowland drained by a canal (Akassimadou & Yao-kouamé, 2014). The lowlands are Sudanian and Sudano-Guinean type (Albergel et al., 1993). The plot was first set up by clearing an area of 1909 m², the plot was ploughed using a power cultivator (model DTE serial number 18801339 of June 2013) for sludge disposal operations. Boards were then set up in the field to create nurseries for the rice varieties used. The nurseries were preceded by pre-sprouting of the seeds. The plot was then subdivided into separate 3 m subplots in which 15 m² (3 m × 5 m) microplots spaced 1 m apart by stakes in a split-plot design. The plot was managed by adding NPK fertilizer (12 24 18), which was applied as bottom fertilizer at a rate of 200 kg/ha during transplanting. Urea was applied at a rate of 100 kg/ha, one third of which was applied to the tillering and the remainder to the head. Irrigation of the plot was carried out by supplying water from one trap to another from a drainage channel located near the plot. Chemical weeding after seeding was done with a selective herbicide (Propanil 360g/l+Trichopy 72g/l). Insecticidal protection was provided by the application of Decis (lambdacyclonothrin) at a dose of 30g/l. Manual weeding was carried out where necessary. To trigger and homogenize the epidemic at the study site, infestation strips, planted with the susceptible variety Bouaké 189, were set up around the plot. (Figure 1).

Tableau I. List of varieties used and their characteristics
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3.2.2 Data collection
Rainfall and temperature data of the area during the test period were made available free of charge by AfricaRice.

**Disease incidence**
The incidence of RYMV was assessed at different stages of plant development in each microplot according to Sérémé *et al.* (2016) formula.

\[ I = \frac{N_{pm}}{N_{pt}} \times 100 \]

I: incidence, Npm: number of diseased plants, Npt: total number of plants

**Disease progression in the field**
The pattern of rice genotypes against the rice yellow mottle virus was studied using two variables: the disease progression rate (R) and the area under the symptom progression curve (AUSPC). Data were recorded at tillering, booting and heading stage

**a. Area under the Symptom Progression Curve (AUSPC)**
The area under the Symptom Progress Curve (AUSPC) is assessed according to the visual severity scales of the rice yellow mottle virus (IRRI, 2013) at different stages of development of rice plants. This index represents the accumulation of leaf symptoms of the disease during the observation period. It allows to judge the reaction of genotypes to RYMV disease at the study site. Scores are assigned to diseased plants and expressed as a percentage of diseased leaf area according to the Shaner and Finney relationship (1977)

\[ AUSPC = \sum \frac{(S_i + S(i+1)) \times (T(i+1) - T_i)}{2} \]

Where Si and Si+1 are the disease scores expressed as a percentage of leaf area showing symptoms of the disease according to the IRRI visual severity scale, (2013), and observed respectively at times Ti and Ti+1 (Ti+1 - Ti) represents the time interval in days between two observations.

**b. The disease progression rate**
The rate of progression of the epidemic (R) expressed in units per day is assessed with *R* Rapilly (1991) formula.

\[ R = \left( \log \left( \frac{1}{1 - X_2} \right) - \log \left( \frac{1}{1 - X_1} \right) \right) \times \frac{1}{(T_2 - T_1)} \]

Where: X1 and X2 denote the incidence of the disease expressed as a percentage (t2 - t1) : the time interval (days) between two observations.

3.2.3 Serological analysis
In order to ensure that the symptoms observed are due to the *Rice yellow mottle virus*, a DAS ELISA test was performed with anti-RYMV polyclonal antibodies on three symptomatic leaf samples collected by micro plots.

3.2.4 Statistical analysis
The percentage data were transformed into Arcsin (William *et al.*, 1990) before being submitted to variance analysis by ANOVA main effects using the Xlstat software version 2016. The Newman-Keuls test at the 5% threshold was used to compare the means in case of significant differences between the genotypes studied.

4 Results
4.1 Climate data
Climate data recorded during the study period indicate that the test area was particularly watered. The highest rainfall amount was observed in September with an average of 133.2 mm while the lowest amount of rainfall was observed in July (41 mm). As for the temperature recorded during the study period, it was lower in August with an average

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of 24.6 °C while the highest average was observed in November (Figure 2).

Figure 2 : Rainfall and thermal surveys of the Mbé area from June to November 2016

4.2 Rice yellow mottle disease incidence

Data Analysis for Rice yellow mottle disease incidence reveals a significant difference between rice genotypes at all stages of development. Table II shows that infection of the disease started at tillering with low incidences in the varieties Kouiklonlé (0.55%), Soungrouba (0.67%), Marigbé (1%), Kpatè (0.77%) and AR597H (0.76%). This infection subsequently spread to all genotypes at the time of the booting stage. The Newman-Keuls test identified three genotype groups at booting stage. First, group (a), characterized by the varieties Kpatè (8.19%) and AR638H (7.89%) in which the highest incidences of yellow mottle were observed. Then the varieties Palawan (0.6%), Djoukemé (0.74%), GT11 (1.16%), Danané (2.24%), Demamba (2.24%), Kouiklonlé (3.35%), Soungrouba (4.14%), Marigbé (3.51%), AR624H (1.25%), AR593H (0.99%), AR034H (1.42%), AR051H (0.75), AR630H (2.36%), AR043H (2.87%), AR601H (2.06%), AR629H (3.56) and AR597H (4.56%) that experienced relatively low incidences constituted the intermediate group (ab). Finally, the lowest incidence represented by group (b) was observed in Wita 9 with an average of 0.08%. At heading stage, an increase in the incidence of RYMV was generally noted in genotypes (Table II). However, examination of the variety types revealed a decrease in the incidence of yellow mottle in hybrids. Thus, three variety groups were defined at the end of the mean comparison test. The group with an average of 10.99% was the highest incidence and was recorded in the Kpatè variety while the GT11, Kouiklonlé, and Soungrouba varieties with respective incidences of 5.03%, 8.40%, 5.67% constituted the intermediate group ab (Table II).

| Code | Genotypes | Tillering | Booting | Heading |
|------|-----------|-----------|---------|---------|
| VL1  | Palawan   | 0         | 0.6 ab  | 0.98 a  |
| VL2  | Djoukemé  | 0         | 0.74 ab | 1.20 a  |
| VL3  | GT11      | 0         | 1.16 ab | 5.03 ab |
| VL4  | Danané    | 0         | 2.24 ab | 3.3 b   |
| VL5  | Demamba   | 0         | 2.24 ab | 1.65 b  |
| VL6  | Kouiklonlé| 0.55 a    | 3.45 ab | 8.40 ab |
| VL7  | Soungrouba| 0.67 a    | 4.14 ab | 5.67 ab |
| VL8  | Marigbé   | 0         | 3.51 ab | 4.21 b  |
| VL9  | Wita 9    | 0         | 0.08 b  | 0.74 b  |
| VL10 | Kpatè     | 0.77 a    | 8.19 a  | 10.99 a |
| VA1  | AR624H    | 0         | 1.25 ab | 1.58 b  |
| VA2  | AR593H    | 0         | 0.99 ab | 0.58 b  |
| VA3  | AR034H    | 0         | 1.42 ab | 1.25 b  |
| VA4  | AR051H    | 0         | 0.75 ab | 0.5 b   |
| VA5  | AR630H    | 0         | 2.36 ab | 2.46 b  |
| VA6  | AR043H    | 0         | 2.87 ab | 1.43 b  |
| VA7  | AR601H    | 0         | 2.06 ab | 0.74 b  |
| VA8  | AR638H    | 0         | 7.89 a  | 2.60 b  |
| VA9  | AR629H    | 0         | 3.56 ab | 2.55 b  |
| VA10 | AR597H    | 0.76 a    | 4.56 ab | 4.32 b  |

MG: 0.18  Pr (>F): 0.48  Significance: ns
Values followed by the same letter in the same column are not significantly different according to the Newman-Keuls Test at the 5% threshold.

4.3 Area under the Symptom Progression Curve (AUSPC)

The AUSPC values obtained were 0 to 72, 6 to 1230 and 6 to 2655 for tillering, booting and heading stages respectively (Table III). AUSPC varied significantly from one variety to another regardless of the phenological stage of the plants (Table III). On tillering, the Marigbé (72), Kpatè (54.66),

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Soungrouba (48), AR597H (18) and Kouiklonlé (36) varieties had the highest AUSPCs, unlike the other varieties which did not show symptoms of RYMV. The disease was more severe in the Kpatè variety with AUSPCs of 1230 and 2655 respectively recorded at the booting and heading stages respectively, while for the same stages of development, the Djoukèmè variety had the lowest AUSPC with an average of 6%.

**Tableau III : RYMV AUSPCs by stage of development**

| Codes | Genotype   | Tillering | Booting         | Heading         |
|-------|------------|-----------|-----------------|-----------------|
| VL10  | Kpatè      | 54.66 b   | 1230.00 a       | 2655.00 a       |
| VL7   | Soungrouba | 48.00 c   | 644.00 c        | 1288.00 b       |
| VL8   | Marigbè    | 72.00 a   | 572.00 e        | 1096.00 c       |
| VA10  | AR597H     | 18.00 e   | 624.00 d        | 692.00 f        |
| VL6   | Kouiklonlé | 36.00 d   | 204.00 j        | 852.00 e        |
| VA8   | AR638H     | 0.00 f    | 916.00 b        | 428.00 h        |
| VA5   | AR630H     | 0.00 f    | 574.00 e        | 352.00 i        |
| VA9   | AR629H     | 0.00 f    | 543.33 f        | 460.00 g        |
| VL3   | GT11       | 0.00 f    | 158.00 k        | 952.00 d        |
| VA1   | AR624H     | 0.00 f    | 282.00 g        | 272.00 j        |
| VA6   | AR043H     | 0.00 f    | 270.00 h        | 228.00 i        |
| VA3   | AR034H     | 0.00 f    | 138.00 l        | 240.00 k        |
| VA7   | AR601H     | 0.00 f    | 226.00 j        | 144.00 p        |
| VL4   | Dananè     | 0.00 f    | 108.00 n        | 216.00 m        |
| VL5   | Demamba    | 0.00 f    | 116.00 m        | 180.00 o        |
| VL1   | Palawan    | 0.00 f    | 60.00 p         | 184.00 n        |
| VA4   | AR051H     | 0.00 f    | 80.00 o         | 112.00 r        |
| VL9   | Wita 9     | 0.00 f    | 6,000 q         | 128.00 q        |
| VA2   | AR593H     | 0.00 f    | 58.00 p         | 96.00 s         |
| VL2   | Djoukèmè   | 0.00 f    | 6,000 q         | 6,000 t         |
| Average|           | 11,433    | 340.76          | 529.05          |
| Pr > F | <0.0001    | <0.0001   | <0.0001         |

*The values followed by the same letter in the same column are not significantly different according to the Newman-Keuls Test at the 5% threshold.*

### 4.4 RYMV symptom progression rate

Data analysis of RYMV disease progression rate in infected varieties revealed a highly significant difference between genotypes at all stages of development (Table IV). For example, the rate of progression of RYMV disease ranged from 0 to 1.6 at the tillering stage.

At booting stage, disease progression ranged from 0.03 to 4.1. The Kpatè variety in which the disease infection was intense recorded the highest progression of the disease as opposed to the Wita 9 variety in which the lowest progression was observed. Data in Table IV show a rapid progression of the disease at the booting stage.

At heading, a decrease in the progression rate was observed with most genotypes; GT11 (2.1) and Kpatè (1.6) varieties showed the highest disease progression, in contrast to AR034H hybrid, which had the lowest rate of RYMV progression (Table IV).

When considering the average rate of disease progression over the entire observation period, three groups can be defined:

- **Group I**, consisting of genotypes with low disease progression (R<0.2). This group refers to the varieties Palawan, Wita 9, AR034H, AR051H.

Then group II represented by the varieties in which the progression is relatively low (0.2<R<0.9). These are the genotypes Djoukèmè, GT11, Dananè, Demamba, AR624H, AR593H, AR043H, AR630H, AR601H, AR597H. Finally, group III with R > 0.9; which includes genotypes that have shown rapid disease progression, were considered susceptible to RYMV. This group is composed of varieties Kouiklonlé, Soungrouba, Marigbè, Kpatè, AR638H and AR629H (Figure 3).
The incidence of Rice yellow mottle virus (RYMV) under natural infection conditions in the M’bé Valley of Côte d’Ivoire. However, the response of varieties to RYMV varies according to the genotypes and the stage of development of the plants. When examining the results of the disease occurrence, it was found that the first characteristic symptoms of yellow mottle were observed at tillering. The development of the disease is believed to be due to the presence of insect vectors of RYMV (Abo et al, 2004) at the study site. The population dynamics of RYMV insects vector have been shown to fluctuate over time (Henrisch, 2001), so the massive flow of insects during this rainy period could explain the sudden appearance of the disease (Guinagui 2018).

The rapid spread of the epidemic, characterized by an increase in the incidence of the disease observed from tillering to run-up in hybrids, is believed to be due to the lack of adaptation of hybrids to the study environment. This result reflects the fact that, in the absence of effective protection, modern high yield varieties are more exposed than older populations because of the suppression of protective adaptations during domestication, as well as the homogeneity of varieties, which facilitates pathogens adaptation. Intensifying cultivation would be conducive to the spread of pests. This result could also be explained by cultural practices such as weeding, which had been carried out at that time of the study because, according to Wopereis et al (2008), simply touching infected injured plants and then healthy plants can cause the disease to progress, under these circumstances weeding by hand or machete could cause the disease progression.

### Table IV : RYMV progression rate by stage of genotype development

| Code | Genotypes | Tillering | Booting | Headingn | Rate of progression |
|------|-----------|-----------|---------|----------|---------------------|
| VL10 | Kpatè     | 1.2 b     | 4.1 a   | 1.6 b    | 2.3 a               |
| VL7  | Soungrouba| 1.1 b     | 1.8 c   | 0.9 c    | 1.267 c             |
| VL8  | Marigbé   | 1.6 a     | 1.8 c   | 0.4 cde  | 1.267 c             |
| VA9  | AR629H    | 0.00 e    | 2.8 b   | 0.8 cd   | 1.200 c             |
| VA10 | AR597H    | 0.4 d     | 1.6 cd  | 0.43 cde | 0.810 d             |
| VA8  | AR638H    | 0.00 e    | 4.4 a   | 0.3 cde  | 1.567 b             |
| VL6  | Kouiklonlé| 0.84 c    | 1.5 cde | 0.3 cde  | 0.880 d             |
| VL3  | GT11      | 0.00 e    | 0.57 f  | 2.1 a    | 0.890 d             |
| VA6  | AR043H    | 0.00 e    | 1.5 cde | 0.7 cde  | 0.733 d             |
| VA7  | AR601H    | 0.00 e    | 1.1 cde | 0.9 e    | 0.667 de            |
| VA4  | Danané    | 0.00 e    | 1.1 cde | 0.9 c    | 0.667 de            |
| VA1  | AR624H    | 0.00 e    | 1.4 cde | 0.6 cde  | 0.667 d             |
| VA5  | Demamba   | 0.00 e    | 1.2 de  | 0.3 cde  | 0.500 e             |
| VA2  | Dpokéné   | 0.00 e    | 0.4 fg  | 0.4 cde  | 0.267 f             |
| VA2  | AR593H    | 0.00 e    | 0.5 fg  | 0.23 de  | 0.243 f             |
| VA5  | AR630H    | 0.00 e    | 0.33 fg | 0.270 de | 0.201 f             |
| VA4  | AR051H    | 0.00 e    | 0.4 fg  | 0.17 e   | 0.190 f             |
| VA9  | Wita 9    | 0.00 e    | 0.3 g   | 0.3 cde  | 0.110 f             |
| VA1  | Palawan   | 0.00 e    | 0.2 fg  | 0.21 de  | 0.137 f             |
| VA3  | AR034H    | 0.00 e    | 0.2 fg  | 0.09 e   | 0.097 f             |

The values followed by the same letter in the same column are not significantly different according to the Newman-Keuls Test at the 5% threshold.

#### Figure 3: Curve of the progression rate for the different groups of varieties by stage of development.

**5 Discussion**

This study aimed to identify natural resistance sources to RYMV in 20 rice varieties adapted to lowland conditions. The different rice varieties were assessed on the basis of incidence, AUSPC and disease progression rate at different stages of development (Tillering, booting and heading). An ELISA confirm the presence of RYMV in infected plants, in addition to visual symptoms.

Analysis of the foliar incidence of RYMV showed that all varieties tested in this study developed foliar symptoms of Rice yellow mottle disease. The presence of the typical symptoms of the disease in the different varieties shows that *Rice yellow mottle virus* continues to spread in the rice growing areas of Côte d’Ivoire.
At heading, there was a general decrease in the infection rate among hybrid varieties. The second nitrogen application at heading, combined with insecticide protection, would have reduced the yellowing characteristic of RYMV. These results are in line with the work carried out by Sokol, 2013; Guinagui, 2019 which stipulates that treatment with mineral fertilization of plants infected with RYMV can reduce leaf chlorosis. The treatments have restored the plant's vigour. As a result of the action of mineral fertilization, a resistance mechanism of the plant would have been established. In the case where the Bouaké 189 rice variety was infected with RYMV, Sorho et al (2005) showed that the viral load was high in the first days of infection, then decreased to a lower value at 42 days after inoculation. This decrease in viral load, combined with nitrogen intake, would explain the regression of the foliar symptom.

The relative difference in the evolution of symptoms in the different varieties could be due to a very diversified genetic basis (QTLs) involved in the expression of disease resistance in the plant material studied (Amancho et al., 2009). These different results could also be explained by the presence of isolates with different aggressivities on one hand and by multiple infections on the other hand. Indeed, aggressiveness expresses the intensity of symptoms in a plant in which infection can develop (Fargette et al., 2004). These authors pointed out that the criteria for assessing the aggressiveness of a virus are the rate of viral accumulation, leaf symptoms and impact on plant production and growth. Thus, in an infected plant, the severity of symptoms may be a function of the virus strain, the more aggressive the virus, the more severe the symptoms on the plant. In addition, multiple infections or co-infections (Astier et al., 2001) could explain these results. Multiple infection is thought to be due to a preference of certain insect vectors for certain rice varieties.

Analysis of the rate of progression of the epidemic during the study period revealed a significant difference in accession patterns. When considering the average rate of disease progression over the entire observation period varieties could be classified into different groups. This diversity of genotype responses could reflect two scenarios. In the first case, it would be a resistance of a specific type or vertical resistance that delays the onset of the disease, this ability of the plant to react depends on a gene that is most often dominant. In the second case, the progression of the disease was only delayed. This reaction of rice genotypes therefore makes it possible to consider the presence of virulence genes in the pathogen population. The result is a non-specific resistance or horizontal or polygenic resistance reaction, which manifests itself in variable but partial inhibition of infection by different strains of the disease (VAN Der Plank, 1984). Disease rate of progression shows a peak at the booting. This period according to Wopereis et al (2008) corresponds to the maximum tillering and early booting stages when rice plants are highly exposed to foliar diseases during their development.

6 Conclusion

Field trials have shown that local varieties Djoukémé, Palawan, hybrids AR034H, AR051H have shown a low incidence and progression rate of RYMV disease after infection. These varieties can be an alternative to the RYMV-sensitive Bouaké 189 variety currently in use by producers. The local varieties Djoukémé, Palawan and the hybrids AR034H, AR051H can be deployed in central Côte d'Ivoire. Nevertheless, trials in a semi-controlled environment should be considered with isolates representative of Côte d'Ivoire in order to confirm the resistance of these varieties for better deployment throughout the national territory. The results of this study provide some varieties that can replace sensitive varieties before the large-scale deployment of natural resistance genes in farmers' rice growing systems.

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