Original Research Article

Effect of Rice yellow mottle virus, Sobemovirus on the N P K Ca and Mg content in infected rice leaves.

aims: The objective of this study is to evaluate the effect of the RYMV on the N content P K Ca and Mg in the rice sheets.
Design study: device in Fisher block
Place and Duration of Study: The study was carried out in condition controlled with the scientific pole of Felix Houphouët-Boigny University in Bingerville from June to December 2018.
Methodology:The variety Bouaké 189 was sown in pots filled with ground analyzed before and.The 14 days old seedlings were inoculated mechanically with an isolate of RYMV. manure NPK 12 24 18 and the urea (46 % N) was applied respectively to the amount of 200 and 100 kg/ha. The N content, P, K, Ca, Mg and out of protein in the sheets was given. The content chlorophyll was given using Spad-505.The severity and the AUSPC of the RYMV were evaluated. The presence of the RYMV in the infected sheets was confirmed by serology. The averages of the various parameters were compared by ANOVA 2 with software STATISTICA version 7.1
Results: The study revealed a significant difference ($p < 0.001$) between the seedlings for the N content, P, K, Ca, Mg and out of proteins. The content P, K, Ca and Mg in the healthy sheets was respectively 0.40;1.10;1.49 and 0.39 % whereas that relating to the infected seedlings was of 0.39;0.75;0.67 and 0.24.The N content was 2.41 % for the inoculated plants and 1.75 % for the healthy plants. The content of protein was 10.95 % for the healthy witnesses and 15.04 % for the infected plants. The content chlorophyll of the healthy seedlings was 37.37 whereas that of the inoculated seedlings was 21.23.The severity of the RYMV passed from 6.22 to 3.88 under the action of the mineral fertilization.
Conclusion: The infected sheets have a food value in rise. An increase in the amount of NPK can reduce the losses of production due to the RYMV..

Keywords**:** RYMV, N, P, K, Ca, Mg, proteins, fertilization.

1. INTRODUCTION

Rice (Oryza spp) is the third cereal produced in the world. According to FAO statistical data in 2016, world production is estimated at about 741 million tons. This production is largely provided by the countries of Southeast Asia [**1**]. In Côte d'Ivoire, domestic production is estimated at 700 000 tons of milled rice [**2**] and only covers about 50% of national requirements [**3**], a deficit that is being filled by imports. These imports cost 235 billion CFA francs in 2009 for just over 900 000 tons of milled rice [**4**].In this context, mineral fertilization, more accessible, appears as an ideal cultural practice to increase production. Indeed, the mineral fertilization is carried out according to the objectives of yield which one is fixed. This practice consists in providing the plants with reserves of assimilable mineral elements and known to give a good yield to the rice plant [**5**]. It is essential for the growth and development of plants. Among these mineral elements, nitrogen, phosphorus, potassium, calcium and magnesium are the major elements controlling the growth of plants and the proper development of them. However, the low production is partly due to biotic constraints, the most important in Africa being the rice yellow mottle virus (RYMV). Discovered in 1966 in Kenya [**6**], the disease then spread to the entire African continent. Infection with RYMV also causes cellular disruption, with mainly changes in the nucleus, increased vacuole and reduced size of chloroplasts and mitochondria [**7**]. Infected plants show pale yellow variegation on leaves, stunting, reduced tillage, asynchronous flower formation, poor panicle exsertion, leaf discoloration and spikelet sterility [**8**]. Yield losses due to RYMV are enormous and vary from 10 to 100% [**9**;**10**] depending on the time of infection and the variety. Studies have reported physiological and biochemical disorders due to pathological changes in plants. Thus, the protein content of bean leaves increases with *Pseudomonas syringae* infection. Cucumber mosaic virus (CMV) infected leaves show high enzymatic activity of chlorophyll and drop in chlorophyll content

[11]. Infection with (CMV) decreases sugar content, while free amino acids and protein levels are significantly elevated in infected cucumber leaves [**12**;**13**;**14**] Infection with mosaic virus Watermelon (WMV) increases the protein content, but decreases other physiological and biochemical activities such as respiration rate and reduces the starch, sugar and total nitrogen content of diseased plants compared to healthy plants [**13**]. The aim of this study is to evaluate the effect of RYMV on the N P K Ca and Mg content in rice leaves infected with RYMV..

2. MATERIEL AND METHODE

The study was conducted under controlled conditions at the scientific division of the Félix Houphouët-Boigny University in Bingerville. The relative humidity and mean temperature of the greenhouse during the test period were 83.5% and 27.4°C, respectively. RYMV-sensitive Bouake 189 was used in this study. The RYMV isolate collected under natural infestation conditions in the Gagnoa region (06°07N, 05°54W, 213m) in southwestern Côte d'Ivoire was used to inoculate rice plants.

2.1 Propagation of the isolate

The isolate was grown under glass to obtain fresh material needed for the study. The multiplication consisted in mechanical inoculation of the isolate on the Bouaké 189 sensitive rice variety. Leaves of young plants exhibiting the characteristic symptoms of RYMV were then collected and analyzed by the Double Antibody Sandwich Enzyme Linked Immuno- Sorbent Assay (DAS ELISA)

2.2 Sampling and physicochemical composition of the soil

The soil was taken at Bingerville. After drying in the open air, it was ground in a mortar. The root debris has been entirely removed. The assembly was mixed so as to obtain a homogeneous support. This mixture was sterilized in an oven at 150 ° C. for 3 hours before determining the physicochemical composition.

2.3 Experimental design

The experiment was conducted according to a randomized complete block device with 3 repetitions. Six rice grains of the Bouaké 189 variety were sown at a depth of 2 cm in 5 kg of soil initially placed in plastic pots of 5 L volume and 24 cm diameter at the opening. Mating at 3 plants per pot was performed 7 days after sowing (das).The inoculated plants were separated from the 1 cm controls in each block. The NPK chemical fertilizer (12 24 18) was applied as a manure at the rate of 200kg / ha. Nitrogen was supplied as urea (46% N) at the rate of 100 kg / ha, a third of which was added to tillering, and the remaining two-thirds were applied to panicle initiation. The plants were watered regularly for the duration of the test

2.4 Inoculum production and inoculation

The infected leaf samples collected from the multiplication were crushed in a mortar at a rate of 10 g of leaves per 100 ml of phosphate buffer (KH 2 PO 4 and K 2 HPO 4 at 0.1 M, adjusted to pH = 7.2). Carborundum (600 mesh, 5 mg / ml) was added to the crude extract to promote virus infiltration [**15**]. The tested rice plants were mechanically inoculated by manual friction. During inoculation, the gloved fingers were soaked in the inoculum, then the leaves of the rice plants were rubbed slightly from bottom to top. The inoculated plants were then rinsed with distilled water three minutes after inoculation. Negative controls (healthy plants) were rubbed with distilled water. Inoculation of the plants was carried out 15 days after sowing (das).

2.5 Data collection

2.5.1 Chlorophyll content

Leaf chlorophyll levels were measured at 28 and 60 days after inoculation (JHA) using a SPAD-502 chlorophyll meter [**16**; **17**]. SPAD-502 determines the relative amount of chlorophyll present in the plant by measuring the absorbance of the leaf at two wavelengths (about 650 and 950 nm).

2.5.2 Area Under the Symptom Progression Curve (AUSPC)

The area under the symptom progression curve (AUSPC) of infected plants was evaluated according to the method of [**18**].

$$AUSPC = \Sigma [(Y_{i+1} + Y_i) / 2] [X_{i+1} - X_i]$$

With Yi: Severity at the time of observation, Xi: Time (days) at the time of observation.

2.5.3 Serological analysis

The presence of RYMV in infected rice leaf samples was confirmed using the DAS-ELISA technique as described by [19]. A first reading of the optical densities at 405 nm was carried out at 30 minutes after incubation and then a second, 1 hour later. An extract was considered positive if the absorbances obtained are greater than the average of the absorbances of the negative control plus three times the standard deviation [20]

2.5.4 Average height of plants at tillering and flowering

The height of healthy and inoculated plants was measured 28 and 60 days after inoculation (JAI) at tillering and flowering, using a tape measure, starting from the neck to the end of the leaf. Higher.

2.5.5 N, P, K, Ca, and Mg content in leaf samples

Rice leaves (young and old, mixed) with RYMV symptoms and healthy controls were used for the analyzes. The samples were dried in an oven (Memmert brand, maximum capacity 260 ° C) at 60 ° C for 48 hours until a constant weight was reached. The dried samples were ground with a mortar and pestle and stored in polyethylene bottles. The nitrogen content of the samples was determined according to the Kjeldahl method [21]. Phosphorus, potassium, calcium, and magnesium content in plant tissues of leaf samples was obtained according to [22].

2.5.6 Protein content

The total protein content of the infected plants and that of the healthy controls was determined by multiplying the amount of nitrogen by the factor 6.25 [21]

2.5.7 Grain production

Grain from the harvest of infected plants and healthy plant controls were weighed and corrected to 14% moisture content.

2.6 Statistical Analysis

The averages of the different parameters were compared by ANOVA 2 with the STATISTICA version 7.1 software. The Fischer and Newman-Keuls test at the 5% threshold was used to rank the averages.

3. RESULTATS

3.1 Physical and chemical composition of experimental soil

Table 1 presents the physical and chemical composition of the soil used for the realization of the study

Table 1. Physical and chemical composition of experimental soil

Chemical physico composition	Content
Clay (%)	32
Sand (%)	41
Silt (%)	3
C (%)	7.72
NT (%)	0.82
P (mg/Kg)	24.48
Ca (cmolkg $^{-1}$)	1.432
Mg (cmolkg $^{-1}$)	1.343
K (mg/Kg)	315.07
Na (cmolkg $^{-1}$)	0.135
CEC (cmolkg $^{-1}$)	6.56
Conductivity (µS)	278
pH	6.67

3. 2 Effect of the RYMV on the chlorophyll content

Analysis of the results showed a significant effect of RYMV on the chlorophyll content of the rice plants (Table 2). Thus, based on the RYMV data, healthy plants reported at 28 days post-inoculation (dpi) a mean leaf spot intensity of 28.48 while chlorophyll levels observed in infected plants were low (19.72). Similarly, at 60 dpi, chlorophyll content was higher in healthy plants (37.37) than inoculated plants (21.23).

3.3 Effect of the RYMV on the rice plants height

The general finding is that the height of healthy plants is higher than that of infected plants. The height of the plants varied significantly according to the status of the plants.

Thus, the average heights of healthy plants recorded at 28 and 60 JAI were 50.33 and 78.61 cm, respectively, while those observed in the inoculated plants obtained mean values of 27.94 and 46.94 cm (Table 2).

Table 2. Effect of the RYMV on the chlorophyll content and on the rice plants height

	chlorophyll content		Hauteur (cm)	
Status of plants	28 dpi	60 dpi	28 dpi I	60 dpi
Infected plants	19.72 b	21.23 b	27.94 a	46.94 b
Healthy plants	28.48 a	37.37 a	50.33 a	78.61 a
Average	24.10	29.3	39.14	62.77
P-value	< 0.0001	< 0.001	< 0.001	< 0.0001
Coefficient of variation (%)	22.49	36.79	34.83	32.72

3.4 Effect of the mineral fertilization on the chlorophyll content

A significant difference in mineral fertilization on the chlorophyll content of rice plants was noted from the analysis of variance. The application of NPK and urea induced a high chlorophyll content in the fertilized plants when the non-fertilizer controls had the lowest chlorophyll content. Observations recorded 42 and 75 days after planting (DAS) in fertilized plants had a higher average chlorophyll content than that observed in control plants lacking mineral fertilizers (Table 3).

3.5 Effect of the mineral fertilization on the rice plants height

Mineral fertilization has shown a significant effect on height growth of rice plants. Indeed, the height of the plants without fertilizer, recorded at 42 and 75 days after sowing (JAS) was respectively 32.89 and 50.28cm while the size of the plants resulting from the fertilizer supply presented respective average values. 45.39 and 75.28 cm (Table 3)

Table 3. Effect of the mineral fertilization on the chlorophyll content and on the rice plants height

	Chlorophyll content		Height (cm)	
Fertilizers	28 dpi	60 dpi	28 dpi	60 dpi
With NPK addition	27.04 a	36.04 a	45.39 a	75.28 a
Without NPK addition	21.15 b	22.56 b	32.89 b	50.28 b
Average	24.1	29.3	39.14	62.77
P-value	< 0.0001	< 0.001	< 0.001	< 0.0001
Coefficient of variation (%)	22.49	36.79	34.83	32.72

3.6 Effect of the RYMV on N. P. K. Ca and Mg content in the rice leaves

The amounts of NPK Ca and Mg in inoculated rice leaf samples and in healthy controls are shown in Table 4. The protein content was 10.95 for healthy plants and 15.04 for plants infected with RYMV, respectively. P,.K, Ca and Mg levels in healthy leaves were higher, while those for the amount of N in infected leaves were higher (**Table 4**).

Table 4. Nutrients content in infected with RYMV and healthy rice plants

		Nutrients				
RYMV	Protéins	Nt	P	K	Ca	Mg
Infected plants	15.04 a	2.41 a	0.39 b	0.75 b	0.67 b	0.24 b
Healthy plants	10.95 b	1.75 b	0.40 a	1.10 a	1.49 a	0.39 a
Average	12.99	2.07	0.39	0.92	1.08	0.31
P-value	< 0.001	< 0.001	< 0.0001	< 0.001	< 0.001	< 0.0001
Coefficient de variation (%)	26.02	26.8	2.5	31.81	53.7	25.8

3.7 Effect of the RYMV and the fertilization on the grain production

Figure 1 shows the effect of RYMV disease on grain yield of seedlings. Thus grain production varied significantly depending on the status of the plants. the infected plants were less productive with an average of 24.85 g unlike the healthy controls which have an average production of 33.95 g. As for fertilization, it induced a high average production in plants that received mineral fertilizer (37.32 g) while untreated controls showed low average production (21.48 g)

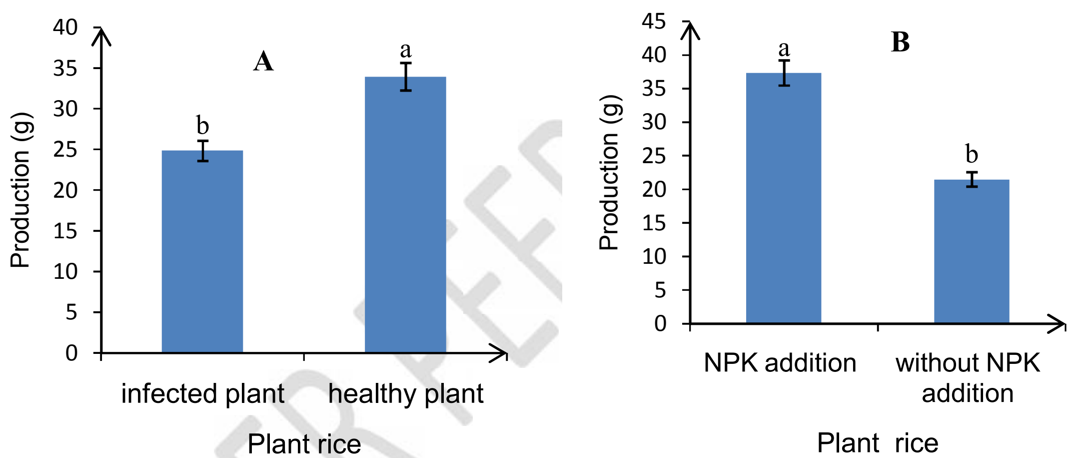

Fig. 1. Effect of the RYMV (A) and the fertilization (B) on the grain production

3.8 Effect of the mineral fertilization on RYMV severity

The severity and area under the RYMV symptom progression curve (AUSPC) varied significantly with mineral fertilization. The average severity of seedlings inoculated with a mineral fertilizer was 6.22 while recorded in seedlings with mineral fertilizer averaged 3.88. The mean AUSPC observed in plants inoculated with NPK (276.77), which was observed in the absence of fertilizer (Table 5)

Table 5. Effect of the mineral fertilization on RYMV severity

Mineral **fertilization**	RYMV severity	AUSPC
Inoculated + NPK	3.88 b	138.38 b
Inoculated without NPK addition	6.22 a	276.77 a
Average	5.05	207.58
P-value	< 0.0001	< 0.0001
Coefficient of variation (%)	30.49	44.48

3.9 Effect of the studied factors (RYMV and Fertilization) on the mineral content

The results of the analysis of variance recorded in Table 6 show that the interactions identified had a significant effect on the mineral content in the leaf samples analyzed. In the RYMV-Fertilizer interaction plants inoculated with NPK showed higher Ca and Mg levels than plants inoculated without NPK. However, the Nt and P contents were lower in the inoculated plants without fertilizer (Table 6). However, the content of N. P. K. Ca and Mg recorded in uninfected plants with application of NPK fertilizer was higher than that observed in healthy plants without NPK input.

Table 6. RYMV-Fertilization Interaction on the N P K Ca and Mg content in Rice Leaves infected

	Mineral				
RYMV-Fertilisant	Total N	P	K	Ca	Mg
Inoculated+ NPK addition	2.32 b	0.39 c	0.88 b	0.82 c	0.26 c
Inoculated without apport NPK	2.48 a	0.40 b	0.62 d	0.52 d	0.21 d
not inoculated+ NPK addition	2.31 c	0.41 a	1.38 a	2.00 a	0.43 a
not inoculated without NPK addition	1.18 d	0.39 c	0.80 c	0.97 b	0.35 b
Averagee	2.070	0.390	0.920	1.080	0.310
P-value	< 0.001	< 0.0001	< 0.001	< 0.001	< 0.0001
Coefficient of variation (%)	26.8	2.5	31.81	53.7	25.8

3.10 Effect of the factors studied (RYMV and Fertilization) on the mineral elements content

A highly significant effect of the RYMV-Fertilizer interaction was noted on height grain yield and chlorophyll content of the plants (Table 7).

Table 7. RYMV-Fertilization interaction on the N P K Ca and Mg content in the rice leaves

RYMV- Fertilizer	Chlorophyll content	Height (cm)	Grain production (g)
Infected plant + NPK addition	26.16 c	60.66 c	32.33 b
Infected plant without NPK addition	16.29 d	33.22 d	17.37 d
Healthy plant + NPK addition	45.91 a	89.88 a	42.31 a
Healthy plant without NPK addition	28.82 b	67.33 b	25.60 c
Average	29.3	62.77	29.4
P-value	< 0.001	< 0.001	< 0.0001
Coefficient of variation (%)	36.79	32.72	31.56

4. DISCUSSION

Evaluation of the effect of Rice yellow mottle virus on N. P. K. Ca and Mg content in infected rice leaves revealed variation in mineral content. chlorophyll content. the height and grain production of rice plants. The results obtained showed that the protein content of healthy plants was 10.95% whereas the protein content observed in the infected plants was 15.04%. P.Ka and Mg levels in healthy leaves were higher than those observed in infected plants. The amount of total N recorded in the infected leaves was 2.41% when that of healthy leaves was 1.75%. The results obtained during this study are in agreement with those of [14 Indeed, these authors showed that the P.Ka and Mg content in healthy leaves was higher than that observed in infected leaves. these authors also meant that the levels of N and protein recorded in the infected leaves were higher than the amounts of N and protein observed in healthy leaves. These results could be due to possible adverse effects and alterations in plant metabolism and cell integrity induced by viral infections. N. P and K are mobile elements in plants and are actively transported to young tissues when needed Disease resistance and plant growth can be negatively affected by their deficiency. Calcium is a non-mobile element. it makes it possible to create links between the cell walls. It therefore maintains the structure between the cells by cementing them to each other. A lack of calcium implies a loss of cohesion between the cells; young leaves and fruits are therefore more sensitive to calcium deficiency. Mg deficiency during growth reduces the energy production required for defense functions and the inactivation of the pathogen metabolites. Indeed, plants have preformed physical and chemical defenses [23; 24] and active defenses produced after infection [25] that require both energy and photosynthetic substrates involving magnesium (Mg) as a component of the chlorophyll molecule or as a cofactor for the different physiological processes.Previous studies. showed that plants infected with the virus contained more nitrogen than healthy controls. According to analytical calculations. However. [26] observed. in diseased plants. a decrease in their own protein level. but an increase in the specific protein of the virus. Therefore. the high total protein content was probably due to the increased viral protein content in the plant. Authors have confirmed a general reduction in photosynthesis of infected plants by a pathogen; and a positive correlation between these effects and the chlorophyll content was noted. The reduction of chlorophyll content due to microbial pathogens is almost universal in most plants. However, [27] reported that the structure and chlorophyll content in annual and perennial foliar-infected crops depend on the type of disease and the organism in question. However. [28] reported photosynthetic capacity as a function of chlorophyll content in rice leaves. Similarly. [29] found a positive correlation between leaf chlorophyll content and rice yield. Ninety percent of the grain yield of rice comes from the photosynthetic activity of the leaves [30]. As is. reducing the rate of photosynthesis would have a negative effect on rice productivity. resulting in significant yield losses. The results of this study confirm the conclusions of [31]. who reported a reduction in chlorophyll levels in rust-infected beans. angular leaf spot and anthracnose. This observation is consistent with the results of [32]. who reported decreased photosynthesis in coconut palms infected with lethal yellowing due to reduced chlorophyll in the leaves. Similarly. [33] showed that potato virus Y (PVY) negatively affected photosynthesis in leaves of Nicotiana tabacum L. attributing this decrease to a reduction in chlorophyll content. When the effects of NPK fertilizer were on RYMV-induced leaf chlorosis. analysis of variance showed a significant effect on leaf chlorosis intensity after the application of the second urea fraction. This regression of symptoms suggested the implementation of a resistance mechanism induced by the application of urea. When the rice variety Bouake 189 was infected with RYMV. [34]. showed that the viral load was high in the first days of infection, then dropped to a lower value at 42 dpi. This level of viral load. associated with nitrogen intake. would explain the suppression of the leaf symptom. Whatever the level of N brought. the regression of the effects of RYMV on leaf chlorosis was observed. Our work supports the effectiveness of nitrogen on leaf symptoms. caused by the RYMV. which would depend on the period of application. This period would be the limit at which nitrogen input increases the resistance of RYMV susceptible cultivars.

5.CONCLUSION

The study showed that rice leaves infected with RYVM have high levels of nitrogen and protein, unlike phosphorus. to potassium. calcium and magnesium levels are higher in healthy plants. The application of NPK causes RYMV-induced leaf chlorosis to drop in infected plants and increases the grain yield of healthy plants. Therefore. rice leaves infected with RYMV used in animal feed are not dangerous. As well. the nutritional value of the infected leaves is even improved because of the increased protein content. An increase in the dose of NPK can significantly reduce production losses due to RYMV.

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