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Research Article
Development of Rice Yellow Mottle Disease Depending on Topographic Positions of the Lowland in Côte D'Ivoire

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Abstract
Background and Objective: The development of rice cultivation in Côte d'Ivoire is based on the naturally more productive flooded system. The objective of this study was to examine the development of rice yellow mottle virus (RYMV) according to topographic positions. **Materials and Methods:** The rice varieties Nerica L19, Orylux 6 and Wita 9 were used during this work. The development of rice yellow mottle rice was assessed according to a stratified random sampling per topographic position with two factors. RT-PCR enabled detection of RYMV in the collected rice leaf samples. **Results:** The results revealed a downstream disease development gradient with an incidence of 63.33% compared to the upstream one which was 36.85% at the longitudinal position of the lowland. The interaction between the positions of the lowland indicated incidences of 64.00 and 62.67%, respectively in Downstream/Middle and Downstream/Border. The study also found a positive correlation of 93.6% between the depth of runoff and the incidence of the disease. **Conclusion:** One of the means for controlling RYMV might be based on a good leveling and drainage during crop cycles in the rainy season.

Key words: Rice cultivation, rice yellow mottle virus, longitudinal position, transverse position, lowland, rice leaf

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice (*Oryza* spp.) is the third crop produced in the world. According to FAO statistical data in 2016, the world production was estimated at about 741 million t. This production is largely provided by south-east Asian countries[1]. In Africa, particularly in west Africa, rice consumption has risen sharply in recent years to reach[1] just over 17 million t. In Côte d'Ivoire, national rice production has increased from 140,000 t in 1961 to reach about over 1.768 million t in 2016 of paddy rice[2]. This production, estimated at 700,000 t of milled rice[3] covers only about 50% of the national requirements[4]. To fill the production gap, the country uses massive imports which cost 235 billion CFA francs in 2009 for just over 900,000 t of milled rice[5]. This dependence on imports, due to low rice yield, has led the country to develop a national strategy for the development of the rice sector which ranks among the priority directions, the development of irrigated and flooded rice or lowland rice cultivation, more yielding than the rainfed system[6]. However, several constraints hinder rice yield in Côte d'Ivoire; these include biotic constraints, the most prominent of which in Africa is rice yellow mottle virus. As a disease afflicting the African continent[7], this disease represents, according to the AfricaRice Center, a major constraint for rice yield and intensification in irrigated lowland systems[8]. Discovered in 1966 in Kenya[7], the disease then spread to the whole African continent. The pathogen responsible for this virus is the rice yellow mottle virus (RYMV), member of the genus *sobemovirus*[9,10]. The organization of the genome of *sobemovirus* has five open reading frames[11]. Yield losses due to RYMV are enormous and vary[12,13] from 10-100% depending on the time of infection and the variety. Currently, seven strains have been identified[14] with specific geographical adaptations. In Côte d'Ivoire, heavy pressure from RYMV has been observed in several rice-growing areas[15]. Among these localities, that of Bouaké, precisely the Mbé area cannot disregard it. Given the importance of the damage, extensive research has been conducted on yellow mottle in Côte d'Ivoire, but those relating to the conditions of establishment of the rice yellow mottle epidemic (RYMV) in lowlands are unknown. The aim of this study was to assess the development of rice yellow mottle disease according to topographic positions in lowland.

MATERIALS AND METHODS

Geographical location of the study: The study was carried out from June to December, 2015 in the Mbé II valley of the Gbèkê region (7°55N, 5°03 W, 246 m) in the Centre of Côte d'Ivoire an area of 1.5 ha in natural infestation on the plot of a producer. The temperature, the rainfall and the average annual relative humidity are 28°C, 1200 mm and 85%, respectively. This zone is characterized by rainforest small islands in a dominant savannah[16]. Three rice varieties (Table 1) from the Africa Rice Center catalog, commonly used by farmers, made up the plant material. RYMV viral isolates collected at the study site were used as viral material. The study was carried out under natural infestation a condition on the plot of farmers. Land preparation was done manually with the racks were not hovered. A nursery of the three varieties was planted in the plot. Plant transplanting was carried out 21 days after sowing (Das), three plants were transplanted in a spacing of 20×20 cm. The fertilizer NPK (12 24 18) was applied as a bottom fertilizer at a rate of 200 kg ha^{-1}. Urea (46% N) was fed at the rate of 100 kg ha^{-1}. The chemical weeding was carried out with a selective herbicide (Propanil 360 g L^{-1}+Trichopy 72 g L^{-1}). Insecticide protection was provided by applying lambdacyhalothrin at a dose of 30 g L^{-1}. Irrigation was carried out by an opening in the bunds for a water supply from one rack to another from the main canal. Rainfall records of study area were graciously made available by AfricaRice.

Prospecting design: The device of the survey is a stratified random sampling by topographic position with three factors. First, the variety with three modalities (Nerica L19, Wita 9 and Orylux 6) then, the longitudinal position of the lowland with three modalities (the upstream, the middle and the downstream) and finally the transversal position with two modalities (border and middle of the lowland). During this study each variety constituted a sub-parcel of 0.5 ha. Each sub-parcel was sub-divided into 6 compartments and in each rack, 6 yields square of 2 m^2 of surface area were set up (Fig. 1).

Collection of samples: The collection of samples was stratified according to the positions defined in the lowland. The collection of samples was carried out in each bin based on the characteristic symptoms of yellow mottle.

Table 1: Plant material

Varieties	Average yields (t ha^{-1})	Genetic origin	Nature of resistance to RYMV
Nerica L19	6	WAS 122-IDSA-1×WAS-6-1	Moderately susceptible
Orylux 6	6	Wita 1×Pusa Basmati	Moderately susceptible
Wita 9	6	IR 2042-178-1×CT 19	Moderately susceptible

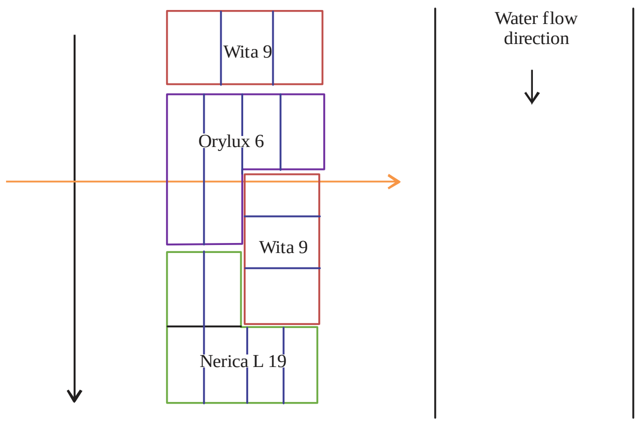

Fig. 1: Prospecting design showing the longitudinal positions (upstream, middle and downstream) and the transverse positions (border and middle)

Incidence and severity of RYMV in the lowland: The severity of the disease was assessed on an individual plant basis in each bin using IRRI rice yellow mottle virus scale[17]. The incidence in the lowland was determined by averaging the incidences obtained in the different bins of each sub-plot. It was assessed according to the relationship described by Sereme *et al.*[18]:

$$I\ (\%) = \frac{Npi}{Npt} \times 100$$

where, I is the disease incidence, Npi is the number of infected plants (one plant was considered infected when symptoms were immediately observed) and Npt is the total number of plants.

Assessment of agromorphological parameters of infected plant: In this study, plant height, number of tillers, number of panicles and yield grain of infected plant of the different varieties were assessed. The height of each collected plant sample was measured using a tape measure. This measurement was made from the base of the tillers to the top of the highest leaf. The number of tillers and panicles produced by the infected plants was counted in each bin according to the positions of the lowland. The yield grain was determined according to the relation of Gaya *et al.*[19] as:

$$Yg\ (t\ ha^{-1}) = \frac{P \times (100 - H)}{2\ m^2 \times (100 - 14\%)}$$

where, H is the moisture content (%), Yg (kg ha^{-1}) is the yield grain and P is the average weight in kg of paddy harvested in 2 m^2.

Measuring the depth of runoff in the lowland: The depth of runoff was measured according to the positions of the lowland. This measurement was made using a tape measure. Thus, before collection of the isolate, the tape measure was immersed vertically in the water near the sample to be collected until reaching the surface of the soil. The immersed part of the tape measure represented the value of the depth of runoff.

Detection of the presence of RYMV in samples rice leaves

Renovation of collected isolates: The collected isolates were renovated under greenhouse so as to obtain the necessary material for molecular characterization. Isolates were propagated by mechanical inoculation on the susceptible variety cultivar Bouaké 189 to increase the viral concentration and to get sufficient material for RNA extraction. Symptomatic leaves were collected 2 weeks after inoculation and used for molecular typing.

Extraction of total RNAs: Total RNA was extracted from infected rice leaves using Trizol reagent (Eurogentec, Belgium) according to the manufacturer's instruction. The mains steps were as follows: The mortars were first sterilized for 2 h at 120°C and stored overnight at -80°C. The frozen leaves were ground to a fine powder and transferred into sterile 2 mL Eppendorf tubes. The powder was mixed with TRIzol reagent (about 1 mL) and the mixture was vortexed. Then, chloroform was added and the tubes were placed on ice for 5 min to separate RNA into cellular proteins and DNA. After centrifugation 12,000 rpm for 15 min at 4°C, the aqueous phase was transferred into sterile 1.5 mL Eppendorf tubes. To precipitate total RNAs, 550 µL of isopropanol were added and left for 30 min at -20°C. After centrifugation at 12,000 rpm for 10 min, total RNAs (pellet) were then washed with 500 µL of alcohol 75%. The resulting total RNAs were air dried, resuspended in 30 µL of sterile water and stored at -20°C.

Amplification of the capsid protein gene by RT-PCR: In RYMV, the gene encoding the capsid protein (ORF4) is a marker of diversity[20].The first strand of the CP gene cDNA was synthesized using M-MLV reverse transcriptase (Promega corp, Belgium) according to the method described by Pinel *et al.*[21]. A volume of 5 µL of total RNAs was denatured in presence of 10 µM of reverse primer RYMV II or P052 (5'CTCCCCCACCCATCCCGAGAATT3') for 5 min at 70°C. The RT reaction mixture contained 5×RT buffer, dNTP (10 mM), M-MLV-RT (200 U), RNase inhibitor (40 U) and sterile water (8 µL) to a total volume of 25 µL and followed by incubation at 42EC for 1 h. The PCR amplification was performed as described earlier[13]. Specific primers P013 5'CAAAGAT

GGCCAGGAA3' (forward) and P0525'CTCCCCCACCCATCC CGAGAATT3' (reverse) were used to cover the full length of the CP gene. The thermal conditions were as follows: Initial denaturation at 94°C for 5 min and 30 cycles of denaturation at 94°C for 1 min, hybridization at 55°C for 30 sec, extension at 72°C for 1 min, followed by final extension 10 min at 72°C and then held at 15°C. All RT-PCR reactions were conducted in Applied Biosystems 2720 Thermal Cycler (Life Technologies, France). The amplified fragments were subjected to electrophoresis in 1% (p/v) agarose gels containing ethidium bromide and were visualized using a UV transilluminator in order to check the size of the amplified fragments according to the covering region.

Analysis method: The averages of the different parameters were compared by ANOVA 2 with STATISTICA version 7.1 software. The Newman-Keuls and Fisher test at 5% threshold were used to classify the averages. The Pearson correlation test demonstrated the relationship between the incidence of RYMV and the level of the depth of runoff in the lowland.

RESULTS

Observations in the lowland: Observations in the lowland showed yellowing of few rice plants. Those plants showing yellowing were located in a rack of the Orylux 6 variety sub-plot. In that zone, the producer observed a stagnation of water during the establishment of the crop. In the lowland, in varieties Orylux 6 and Nerica L19, rice plants showing RYMV symptoms were randomly distributed in the bins. In contrast, with variety Wita 9, the diseased plants were more observed along the bunds.

Rainfall records: Rainfall records during the study period showed an increasing trend in rainfall amounts during the month of September. This evolution reached a peak of 122 mm of rain (Fig. 2). Two weeks after this heavy rain, there was an invasion of insects in the plot.

Molecular analysis: Molecular analysis consisted of amplification of the capsid protein (CP) gene, used as a diversity marker in RYMV. Amplification helped to obtain a strip of about 1000 base pairs (Fig. 3).

RYMV incidence and severity depending on lowland positions: The results consigned in the Table 2 and 3 showed that the incidence of the RYMV varied significantly according to longitudinal and transverse positions (TP) of the lowland.

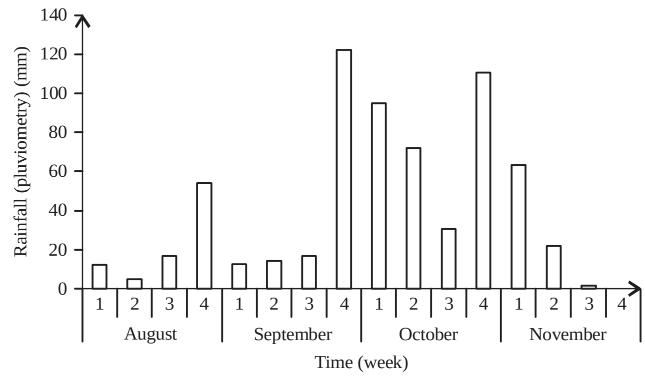

Fig. 2: Weekly rainfall records of the study area during the study period

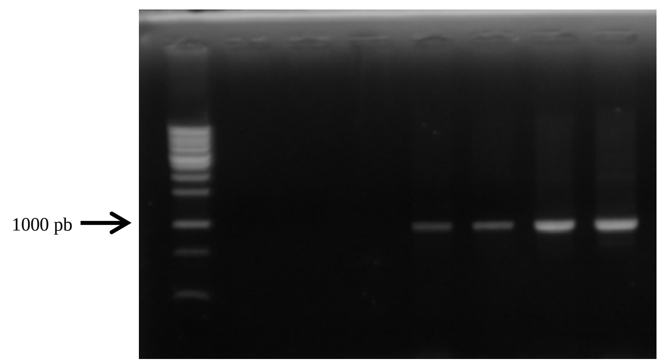

Fig. 3: Electrophoresis of coat protein gene amplification products on 1% agarose gel

M: 1 kb DNA ladder size marker (invitrogen), 1: Negative control using water in place of RNA, 2: Negative control using healthy leaf RNA, 3: Negative sample of Wita 9 leaf RNA, 4: Positive sample of Wita 9 leaf RNA, 5: Positive sample of Orylux 6 leaf RNA, 6: Positive sample of Nerica L19 leaf RNA, 7: Positive control using infected leaf RNA

Table 2: Disease severity and incidence depending on the longitudinal position of the lowland

Longitudinal position (LP)	Severity	Incidence
Downstream	5.83[a]	63.33[a]
Middle	4.52[b]	43.24[b]
Upstream	3.57[c]	36.86[c]
Average	4.64	47.81
p-value	<0.0001	<0.0001
Coefficient of variation (%)	26.29	16.62

Values followed by the same letter in the same column are not significantly different according to the Newman-Keuls test at 5% threshold

Table 3: Disease severity and incidence depending on the transverse position of the lowland

Transverse position (TP)	Severity	Incidence
Middle	4.42[a]	42.47[b]
Border	4.45[a]	46.97[a]
Average	4.44	44.72
p-value	0.57	0.015
Coefficient of variation (%)	33.10	32.35

Values followed by the same letter in the same column are not significantly different according to the fisher test at 5% threshold

According to the longitudinal position (LP), the disease induced an incidence going from 36.86-63.33% whereas this incidence varied from 42.47-46.97% in the transverse direction. According to longitudinal position, the incidence of the disease was more intense downstream (63.33%) while the upstream (36.86%) of the lowland presented the weakest incidence. As for the middle of lowland, it was noted an average incidence of 43.24%. In the transverse direction, the impact of the disease was pronounced more on the border (46.97%) than in the middle (42.47%) of the lowland. A significant effect of the longitudinal position on the severity of the RYMV was observed. Thus, the severity of the virus disease was more intense downstream with an average note of 5.83 when the attacks of RYMV were less severe upstream with an average score of 3.57 (Table 2). Contrary to the longitudinal position, no significant difference was noted on the severity of the RYMV according to the transverse direction (Table 3). However a significant effect of interaction (LP×TP) was noted as well on the incidence and severity of the disease, so the incidence ranged from 32.22-64.00% while the severity increased from 3.40-6.00 (Table 4).

Depth of runoff according to lowland positions: The depth of runoff in the lowland increased from 3.29-5.13 cm. The results of the analysis showed a highly significant effect depth of runoff for the longitudinal position of the lowland (Table 5).

Pearson correlation between the depth of runoff and disease incidence: The data in Fig. 4 demonstrated that there was a positive correlation of 93.6% (R = 0.936, R^2 = 0.876) between RYMV incidence and the depth of runoff. This interaction showed that the depth of runoff and RYMV incidence evolve in the same direction.

Evaluation of the agromorphological parameters of infected plants according to lowland positions

Number of tillers of infected plants depending on position of the lowland: A significant effect of the longitudinal position of lowland on the number of tiller of the infected plant by the RYMV was noted (Table 5). Tillers produced by the infected plant varied from 7.58-12.19 tillers.

Number of panicles of infected plants depending on position of the lowland: The number of panicles produced by the infected plant highlighted a significant difference between the longitudinal positions of the lowland, the panicles produced by the infected plant ranged from 7.58-12.38 panicles per plant (Table 5).

Height of infected plants depending on position of the lowland: The variance analysis of the data relating to the height of the infected plant by the virus of RYMV did not reveal any significant difference between the positions of the lowland (Table 5, 6).

Grain yield of infected plants depending on position of the lowland: The results consigned in the Table 5 and 6 showed that no significant difference between the positions of the

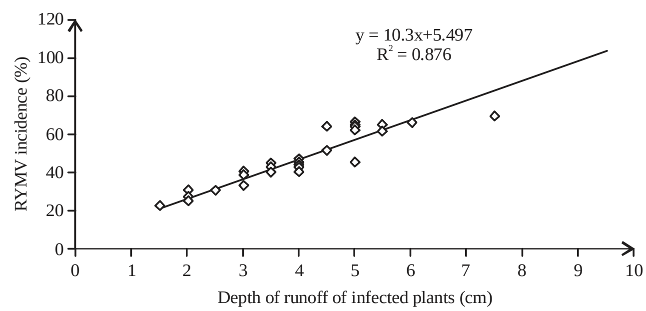

Fig. 4: Relationship between RYMV incidence and the depth of runoff in the lowland

Table 4: Interactions between longitudinal and transverse position of the severity and incidence of disease

Interactions between TP and LP	Severity	Incidence
Downstream×middle	6.00[a]	64.00[a]
Downstream×border	5.67[a]	62.67[a]
Middle×middle	3.67[b]	32.22[c]
Border×middle	5.17[a]	51.50[b]
Upstream×middle	4.00[b]	36.33[c]
Upstream×border	3.40[b]	37.07[c]
Average	4.65	47.29
p-value	0.01	0.001
Coefficient of variation (%)	24.94	13.68

Values followed by the same letter in the same column are not significantly different according to the Newman-Keul test at 5% threshold. TP: Transverse position, LP: Longitudinal position

Table 5: Agromorphological parameters of infected plants and depth of runoff according to longitudinal positions

Longitudinal positions (LP)	Grain yield (t ha^{-1})	Height (cm)	Tillers	Panicles	Depth of runoff (cm)
Downstream	1.64[a]	92.33[a]	7.58[c]	7.58[c]	5.13[a]
Middle	1.95[a]	97.11[a]	9.24[b]	9.33[b]	3.76[b]
Upstream	2.21[a]	96.71[a]	12.19[a]	12.38[a]	3.29[b]
Average	1.93	95.38	9.67	9.77	4.05
p-value	0.053	0.46	<0.0001	<0.0001	<0.0001
Coefficient of variation (%)	33.16	11.40	25.64	25.51	23.70

Values followed by the same letter in the same column are not significantly different according to the Fisher test at 5% threshold

Table 6: Agromorphological parameters of infected plants and depth of runoff according to transverse position

Transverse positions (TP)	Grain yield (t ha^{-1})	Height (cm)	Tillers	Panicles	Depth of runoff (cm)
Middle	1.96[a]	95.71[a]	9.38[a]	9.48[a]	3.60[a]
Border	1.99[a]	96.01[a]	10.42[a]	10.55[a]	4.06[a]
Average	1.98	95.86	9.90	10.01	1.30
p-value	0.61	0.99	0.58	0.059	0.07
Coefficient of variation (%)	34.51	11.41	30.90	31.06	34.03

Values followed by the same letter in the same column are not significantly different according to the fisher test at 5% threshold

Table 7: Interactions between longitudinal and transverse position of the agromorphological parameters and of the depth of runoff of infected plants

Interactions between TP and LP	Grain yield (t ha^{-1})	Height (cm)	Tillers	Panicles	Depth of runoff (cm)
Downstream×middle	1.21[b]	92.88[a]	4.67[d]	4.67[d]	5.33[a]
Downstream×border	2.08[a]	91.76[a]	10.50[bc]	10.50[bc]	4.92[a]
Middle×middle	2.36[a]	99.11[a]	10.22[bc]	10.33[bc]	2.72[b]
Border×middle	1.64[ab]	95.62[a]	8.50[c]	8.58[c]	4.54[a]
Upstream×middle	2.13[a]	93.45[a]	12.83[a]	13.00[a]	3.17[b]
Upstream×border	2.24[a]	98.02[a]	11.93[ab]	12.13[ab]	3.33[b]
Average	1.94	95.14	9.77	9.86	4.00
p-value	0.001	0.53	<0.0001	<0.0001	0.005
Coefficient of variation (%)	27.83	11.47	18.01	17.64	20.25

Values followed by the same letter in the same column are not significantly different according to the Newman-Keul test at 5% threshold. TP: Transverse position, LP: Longitudinal position

lowland was noted. In addition a significant effect of interaction (LP×TP) was observed between the positions of the lowland for the yield grains, number of tillers, the number of panicles and the depth of runoff (Table 7).

DISCUSSION

The molecular analysis helped detect the presence of rice yellow mottle virus (RYMV) in leaf samples stemming from the three varieties studied (Wita 9, Orylux 6 and Nerica L19). Infection of these three varieties by RYMV once again demonstrates that rice yellow mottle is a major threat to rice cultivation in Côte d'Ivoire. Examination of the results for the occurrence of the disease showed that the first characteristic symptoms of the disease were observed on the study site on variety Orylux 6. The occurrence of the epidemic in the plot might be justified by the abundance of insects, observed after the heavy rain. These results were consistent with the works of Abo *et al.*[22,23], who reported that RYMV was transmitted by insects. The observation of RYMV symptoms on rice plants along grassed bunds would justify the external source of the disease. Thus, Bakker[7] reported that in eastern Africa, diseased rice plants have been systematically found at the borders of rice plots along irrigation bunds. Similarly, in Côte d'Ivoire, Amancho *et al.*[24] showed that rice yellow mottle disease was most often observed as isolated foci along the borders of rice plots, suggesting that virus transmission by insect vectors is from external sources. The attribution of rice yellow mottle virus symptoms to a deficit of mineral fertilizers testifies to the lack of training of producers on the recognition of this disease. The analysis of the quality of drainage and smoothing in the occurrence of RYMV epidemic showed that the depth of runoff was higher downstream contrary to the upstream of the lowland. The positive correlation observed between the depth of runoff and the incidence of the disease confirms that the deeper the runoff, the higher the incidence of the disease. This result is consistent with those of Poulicard *et al.*[25], who showed that irrigation increases the prevalence of rice yellow mottle virus. In fact, a quasi-permanent presence of moisture in the lowland favors the establishment of a microclimate conducive to the spawning of RYMV vector insects; such conditions would also allow the development of weeds, which could be natural host reservoirs of RYMV disease. The farmer noted that few days after a heavy rain in the last week of September, the invasion of the field by insects was observed and this was followed by plant yellowing. This yellowing might be due to the outbreak of those insects. Moisture would be a favorable parameter for the spawning of these RYMV vector insects. Observations in the lowlands showed an increase in plant yellowing during weeding. The disease started in a bin of Orylux 6 variety and was spread throughout the plot. This is achieved through cultural practices such as weeding by the farmer during this period[23,26]. The distribution of the disease depending on the topographic position showed the existence of a gradient of evolution of the disease downstream with an incidence of 63.33% compared to the upstream which was 36.86% as for the longitudinal position of the lowland. Contrary to the downstream of the lowland, watercourses descend from the upstream of the lowland, which implies that little water accumulates upstream hence the weak incidence of the disease in that area of the lowland. The differences in yield grain observed between the positions of the lowland might be explained by the difference in incidence of the RYMV which would have impacted the yield parameters of the varieties. The disease affects the yield of susceptible, moderately resistant and resistant varieties[27]. The period of infection and the physiological stage of the plant during infection is an important factor in the occurrence and development of RYMV disease[28]. Thus, the differences observed between the number of tillers of healthy plants and that of infected plants showed that the infection might have occurred before tillering. Young plants are more vulnerable to biotic attacks, unlike older plants. In a young plant the necessary natural defenses that may allow it to partially fight against the disease are not sufficiently developed, unlike a mature plant[29]. From a pathogenic point of view, this difference can be explained in yield by an unequal aggressiveness of isolates. Aggression expresses the intensity of symptoms in a variety in which the infection can develop. For example, a strain will be more or less aggressive depending on whether it will induce more or less strong mosaic symptoms[29]. The criteria for assessing the aggressiveness of a virus include viral accumulation rate, leaf symptoms and the impact on yield and plant growth. Thus, in an infected plant, the more the virus is aggressive, the greater the severity of symptoms on the plant. Similarly, multiple infections or coinfections might justify such variation in yield[29] between healthy plants and infected plants. A multiple infection or coinfection is an infection of one or more viral strains. The multiple infections might be due to a preference of certain insect vectors for certain varieties of rice.

CONCLUSION

This study in the Mbé lowland (Centre of Côte d'Ivoire), conducted on the occurrence of RYMV disease in a lowland agrosystem revealed some elements of Rice yellow mottle virus epidemiology. The results of this work showed that, in a stratified lowland, longitudinally, if the disease is present, it will be more significant downstream than upstream. At the transverse level, no evolution gradient of rice yellow mottle has been noted. These results highlighted a few epidemiological parameters of rice yellow mottle virus (RYMV) in lowlands in Côte d'Ivoire.

SIGNIFICANCE STATEMENT

This study discovered that, when Rice yellow mottle disease is present in stratified lowland, its incidence are preferentially intense downstream of the lowland. The information generated in this article will help the researchers and farmers in the management of the epidemic of the RYMV in the lowland. The better planing of the ground is an approach which helps to prevent the water stagnations so appearance of the insects and can constitute an exploitable advantage by the small producers in the prevention and managements of the epidemics of the RYMV in the lowland.

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