Assessing the Impact and Causes of Rice Panicle Necrosis in Côte d'Ivoire".

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ABSTRACT

Rice is a staple food for millions of people around the world, particularly in Côte d'Ivoire. Its production is subject to numerous biotic constraints, including rice panicle necrosis. Rice panicle necrosis, previously neglected, has taken on worrying proportions in Côte d'Ivoire's rice-growing basins over the past half decade. The aim of this study is to diagnose rice panicle necrosis in Côte d'Ivoire. A phytosanitary survey was conducted in rice-growing basins across three agroecological zones of Côte d'Ivoire in order to describe the symptoms of rice panicle necrosis and assess its importance. The fungi responsible for rice panicle necrosis were identified. Rice panicle necrosis is characterized by the development of grain necrosis and the development of thin, empty grains on the panicles. Rice panicle necrosis was found in all agroecological zones, with the highest prevalence (58.49 to 72.21%), severity index (72.32 to 85.27%), and production loss (48.70 to 54.75%) in the Divo and Gagnoa rice-growing basins. Three species of *Fusarium*, including *Fusarium fujikuroi*, *Fusarium verticillioides*, and *Fusarium proliferatum*, are responsible for rice panicle necrosis in Côte d'Ivoire. It is important to assess the impact of these fungi on rice production in order to design a program to manage rice panicle necrosis.

Keywords: Côte d'Ivoire, Fusarium Isolates, Panicle necrosis, Rice-growing basins

1. INTRODUCTION

Rice is the second most important cereal in the world behind wheat [1]. Côte d'Ivoire is the 6th largest rice producer in Africa. Rice has become the population's main food, with consumption estimated at 1 300 000 tonnes of milled rice per year, or around 58 kg per year per capita. Rice has become part of people's dietary habits, especially in urban areas, moving from a cultural staple in the West and Central-West regions to a daily diet for Ivorians as a result of growing urbanization [2].

Despite this importance, during its development cycle, rice is subject to the harmful effects of abiotic factors (flooding, drought, unfavorable temperatures) and biotic factors (insects, birds, animals, weeds). These constraints increase production costs through control measures [3]. It also has to contend with diseases caused mainly by fungi, bacteria, viruses, and nematodes. Helminthosporiosis, blast, and cercosporiosis, among others, dominate fungal diseases and significantly reduce production [4].

However, diseases such as false smut, Bakanae, sheath rot, and grain discoloration, which were previously less important, are now emerging as a serious threat to rice cultivation. Fusarium fujikuroi Nirenberg, the cause of Bakanae disease, has become a major rice disease in Asia and other rice-producing countries worldwide This disease causes necrosis of rice grains [5], reduces yield, and also produces mycotoxins hazardous to human and animal health [6][7]. Several rice fields in Côte d'Ivoire's rice-producing agroecological zones exhibit similar symptoms. Producers, left to fend for themselves, have little knowledge of this disease. The general aim of this study is to diagnose rice panicle necrosis. Specifically, the aim is to: 1) determine the disease's symptomatology; 2) assess its importance in production areas; and 3) identify the agent responsible for the disease

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2. MATERIAL AND METHODS

2.1- Study sites

Three agroecological zones (Figure 1) served as the study's sites. Zone 1 (Agboville, Tiassalé, N'douci, Divo, and Gagnoa), with its tropical climate, is characterized by a rainy season throughout the year, with the exception of the period from December to February, which is marked by a short dry season. The average temperature is 27 °C, with average rainfall reaching 1 584 mm. The vegetation is transitional forest. Zone 2 (Sinfra) has a tropical climate. The rainy season dominates the entire year, except for the dry season from November to February. The average temperature is 27.8 °C, with average rainfall reaching 1 572 mm. The vegetation is open forest. Zone 4 (Yamoussoukro) has a tropical climate, with a year-round rainy season, except for the dry season from December to February. The average temperature is 28 °C, with average rainfall reaching 1 041 mm. The vegetation is composed of pre-forest savannah.

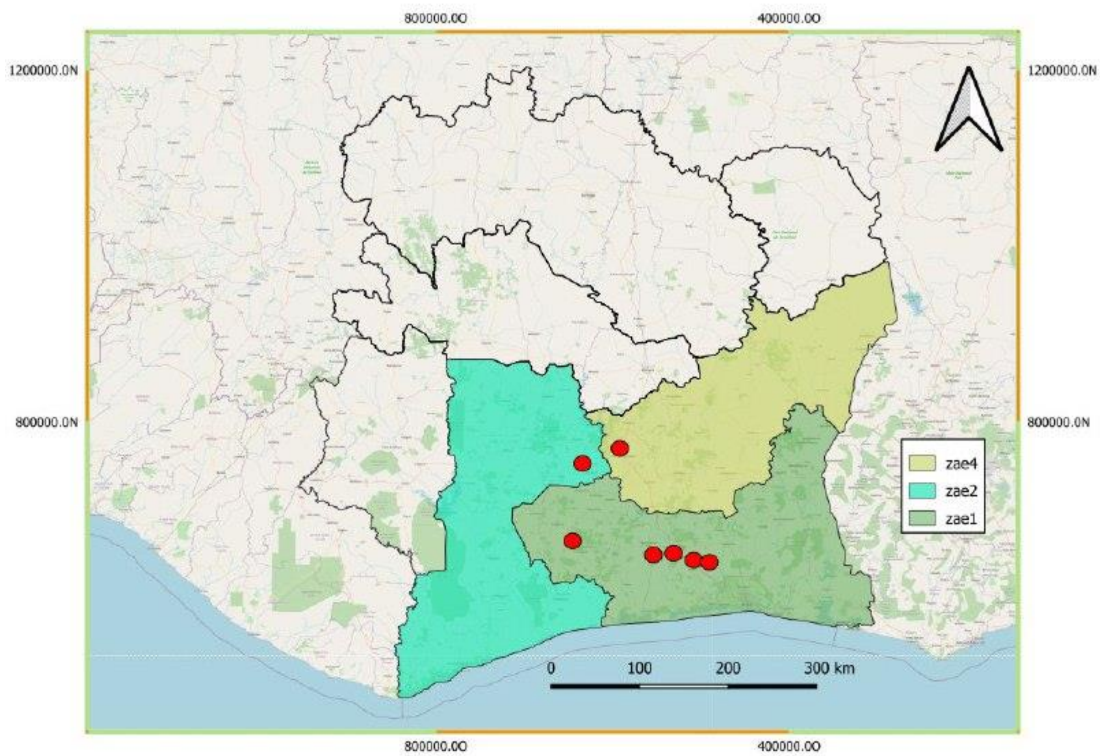

Figure 1: Study areas in Côte d'Ivoire

Zae1: Agroecological zone1; Zae2: Agroecological zone2; Zae 4: Agroecological zone 4

2.2- Phytosanitary status of rice fields

2.2.1. <u>Description of disease symptoms</u>

A phytosanitary survey was conducted in seven rice-growing basins in Côte d'Ivoire. Three rice fields were inspected in each locality. Infected rice panicles and their grains were observed and described.

2.2.2. <u>Evaluation of the importance of the disease</u>

Ninety rice panicles were randomly collected from each field. They were used to calculate the prevalence of disease. The ratio between the number of infected panicles and the total number of panicles in a square (1 m2) was calculated using the formula of [8] to obtain the prevalence of the disease in each rice field

$$\mathrm{PM}(\%) = \frac{1}{N} \times \frac{NPi}{NPt} \times 100$$

PM: Prevalence of the disease; N: number of prospected fields; Npi: number of infected panicles in each plot, Npt: total number of panicles in each plot.

The infection status of rice grains per panicle and the number of collected samples per plot determined the severity. The scale of [9] was used to calculate the severity of symptoms formula [10] is used to calculate the severity index (SI) of symptoms.

$$\mathrm{IS}\ (\%) = \frac{\Sigma\ (\mathrm{ni} \times \mathrm{Ni}) \times 100}{\mathrm{N} \times \mathrm{Ne}}$$

IS: symptom severity index; ni: severity score assigned to panicles i on the rice plant, Ni: number of panicles to which score ni has been assigned; Ne: total number of rice panicles used × highest score; N: number of plots

2.2.3. <u>Evaluation of production loss</u>

The evaluation of production loss was carried out using the production loss rate parameter. In each delimited square, 30 infected panicles were collected and dehulled to weight their corresponding grains. Thirty healthy panicles were also collected and dehulled to weight their grains. There were used as a control. The production loss rate due to the panicle necrosis was calculated using the following formula.

$$\mathrm{TP}\ (\%) = \left(\frac{\mathrm{MGPs} - \mathrm{MGPi}}{\mathrm{MGPs}} \right) \times 100$$

TP (%): Rice production loss rate; MGPs: weight of grains from healthy rice panicles; MGPi weight of grains from infected panicles

2.3- Identification of fungal isolates associated with rice panicle necrosis

Infected rice grains collected in the study areas were rinsed with tap water and dried on blotting paper in a laminar flow cabinet. These infected grains were disinfected with sodium hypochlorite and inoculated onto PDA medium in Petri dishes (four gains per Petri dish). Cultures were incubated in the dark at room temperature (27 ± 2 °C) for 2 days. Fungal colonies growing around the infected grains were individualized on PDA medium. Fungal colonies were identified on the basis of morphological characters using the key of [11][12][13].

2.4-Identification of fungal isolates responsible for rice panicle necrosis

A pathogenicity test was carried out to identify the fungal isolates responsible for the rice panicle necrosis. A potted trial was conducted in a greenhouse at the Université Nangui ABROGOUA. The spacing of 40 cm between rows and 20 cm between pots was established. Each pot was filled halfway with sterilized soil and supplemented with manure. Four grains of rice of cultivar C26 (with a production cycle of 3.5 months) were sown per pot. Fusarium isolates were inoculated into the rice plants by soft inoculation. A first batch of plants was inoculated before the milky stage of grains. A second batch of plants was inoculated after the milky stage of grains, i.e., during the ripening stage. A suspension of 106 spores per mL was sprayed one week after panicle emergence. Inoculated plants were observed daily until symptoms appeared. Only the Fusarium isolates that caused rice panicle necrosis were identified at species level using molecular biology tools.

2.5- Identification of *Fusarium* species responsible for rice panicle necrosis

DNA from Fusarium isolates responsible for rice panicle necrosis was extracted using the modified method of [14]. A polymerase chain reaction of fungal DNA was performed with the universal primers ITS1 (5' TCCGTAGGTGAACCTGCGC 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') according to the method of [15]. PCR products were migrated by electrophoresis on 1.5% agarose gel. Amplicons deposited at 600 bp were sent to Eurofins Genomic in France for sequencing. DNA sequences were compared to their homologous sequences in the GenBank database using the BLAST method to identify the fungal species responsible for rice panicle necrosis.

2.6- Statistical analysis

The data were analyzed using Statistica 7.1 software. Before analyzing, the parametric test conditions (independence of variables, normal distribution, and homoscedasticity) were checked [16]. Analysis of variance was performed to compare i) symptom-level disease prevalences, ii) average symptom severity indices, and iii) average production loss rate based on the localities. If there was a significant difference at the 5% level, Fisher's LSD test was performed to determine the homogeneous groups.

3. RESULTS

3.1 Characteristic symptoms of rice panicle necrosis.

Some rice panicles showed partial grain necrosis (Figure 2a), while others showed complete grain necrosis (Figure 2b). In addition, empty, unfilled panicles with aborted, thin, empty grains (Figure 2c) or empty, filled panicles with necrotic empty grains (Figure 2d) were observed. Meanwhile, rice panicles without necrotic grains were also noted (Figure 2e).

Figure 2: Different states of necrosis of rice panicles

a: panicles with partially necrotic grains; b: panicle with totally necrotic grains; c: unfilled panicles with empty grains; d: open panicles with empty grains; e: young control panicles (1) and mature control panicles (2).

3.2- Prevalence of rice panicle necrosis in the field

The prevalence of rice panicle necrosis varied from 20 to 72.21% depending on the production locality (Table 1). There was a significant difference in disease prevalence between localities (P = 0.0432). The rice fields of Sinfra had the lowest prevalence (20%) of rice panicle necrosis. The rice fields of Divo recorded the highest prevalence of rice panicle necrosis at 72.21%. In addition, the prevalence of rice panicle necrosis was above 50% in the largest rice-producing areas, such as Divo and Gagnoa. In contrast, the prevalence of rice panicle necrosis ranged from 20 to 42.22% in smaller production areas such as Sinfra, Tiassalé, N'Douci, Agboville, and Yamoussoukro. Rice panicle necrosis is more prevalent in the larger rice-growing areas of Côte d'Ivoire.

Table 1: Prevalence of rice panicle necrosis in surveyed localities

Localities	**Prevalence**
Agboville	42.22 ± 9.45^{abc}
Divo	72.21 ± 8.01^{a}
Gagnoa	58.49 ± 9.45^{ab}
N'Douci	36.55 ± 7.04^{bc}
Sinfra	20.00 ± 3.33^{c}
Tiassalé	42.22 ± 2.93^{abc}
Yamoussoukro	27.66 ± 8.57^{bc}
H	*12.99*
P	*0.0432*

3.3- Severity index of rice panicle necrosis

The average severity of rice panicle necrosis varied between 56.77 and 85.27% according to the rice-growing locality (Table 2). The rice fields of Yamoussoukro recorded the lowest severity of rice panicle necrosis at 56.77%, while those of Divo recorded the highest severity at 85.27%). However, there was no significant difference between them (*P* = 0.140). Rice panicle necrosis was therefore severe in rice fields in all the localities surveyed.

Rice grains on panicles with necrosis showed varying levels of necrosis, from 1 to 100%. Rice grains with fully necrotic panicles had a necrosis rate of between 50% and 100%. However, on partially necrotic panicles, the majority of rice grains had less than 50% necrosis. Panicles with level 3 necrosis contained rice grains with more than 50% necrosis. The state of panicle necrosis corresponds to the level of necrosis in the rice grains (Figure 3)

Table 2: Severity of rice panicle infection by locality

Localities	Severity scores	Severity index (%)
Agboville	4	65.27 ± 8.28^{a}
Divo	3	85.27 ± 1.00^{a}
Gagnoa	3	72.32 ± 0.92^{a}
N'Douci	3	69.55 ± 7.97^{a}
Sinfra	3	58.05 ± 8.19^{a}
Tiassalé	3	64.44 ± 8.83^{a}
Yamoussoukro	3	56.77 ± 8.5^{a}
H		*9.15*
P		*0.140*

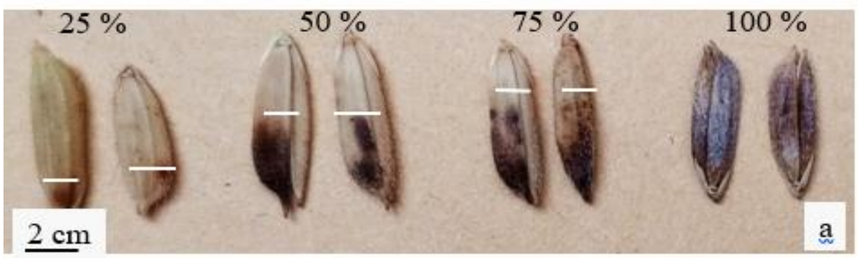

Figure 3: Different states of necrosis in rice grains

3.4- Rice production loss rate

The rate of rice production loss ranged from 35.9% to 54.75%, depending on the rice-growing locality (Table 3). The rice fields of Yamoussoukro recorded the lowest rate of production loss, estimated at 35.9%. The Divo rice fields recorded the highest production loss rate, 54.75%. Despite this variation, no significant difference was revealed in production loss rates between localities (*P* = 0.206). However, the major rice-growing areas of Côte d'Ivoire noted the greatest losses in rice production due to rice panicle necrosis.

Table 3: Production loss rates due to panicle infection of necrotic rice

Localities	Production loss rates (%)
Agboville	42.30 ± 2.90^{a}
Divo	54.75 ± 7.00^{a}
Gagnoa	48.70 ± 0.82^{a}
N'Douci	42.45 ± 2.64^{a}
Sinfra	38.67 ± 4.56^{a}
Tiassalé	43.50 ± 4.33^{a}
Yamoussoukro	35.90 ± 5.85^{a}

H	*8.45*
P	*0.206*

3.5- Diversity of fungi associated with rice panicle necrosis

Various fungal isolates, mainly *Fusarium*, were associated with rice panicle necrosis (Figure 4). Macroscopically, mycelial colonies of *Fusarium* spp. showed a range of colors, from white to beige, burgundy red, and purple (Fig. 4). The appearance of the fungal colonies also varied, from cottony to flaky. Microscopically, fusiform microconidia and macroconidia with one to seven septa were observed (Figure 5). All these macroscopic and microscopic characteristics enabled us to distinguish 11 morphotypes of Fusarium sp. associated with rice panicle necrosis in Côte d'Ivoire.

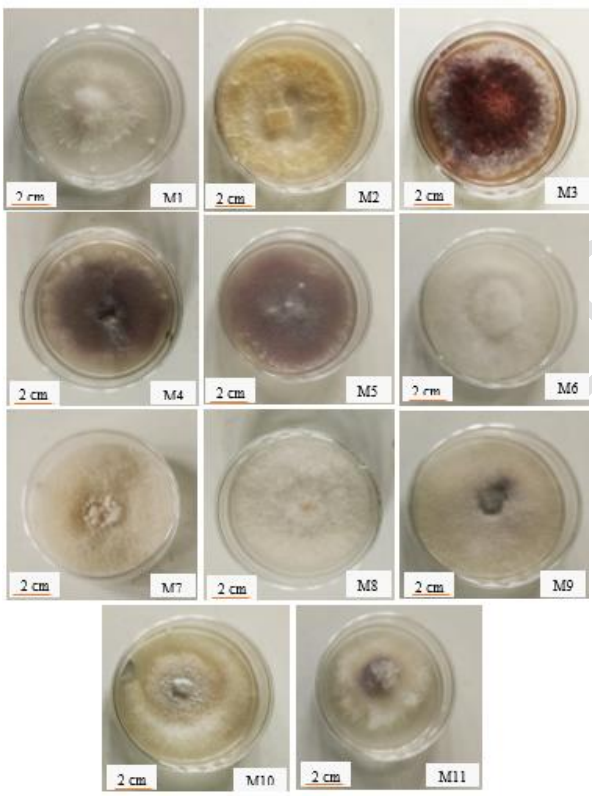

Figure 4: Morphology of Fusarium sp.isolates associated with rice panicle necrosis
(Isolates: M1; M2; M3; M4; M5; M6; M7; M8; M9; M10; M11)

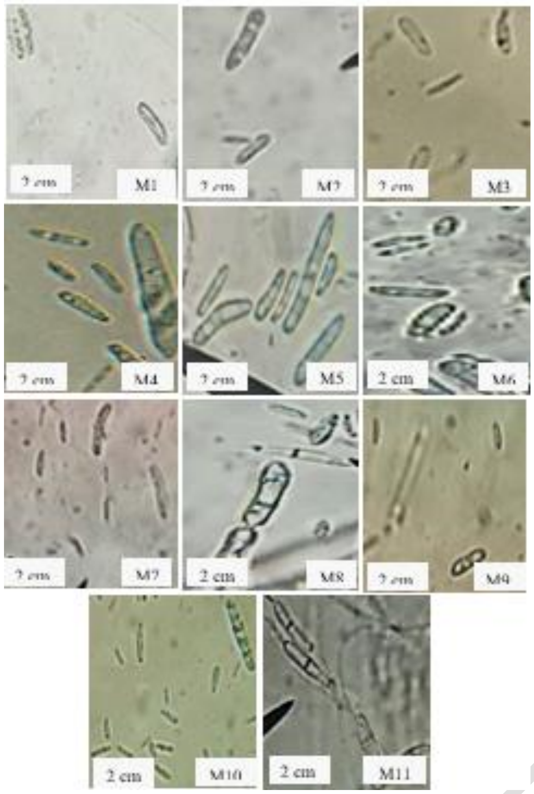

Figure 5: Microscopic characteristics
of Fusarium isolates associated to rice panicle necrosis (G×200)

3.6- Fungi responsible for rice panicle necrosis

The results of the pathogenicity test showed that panicles inoculated with *Fusarium* spp. isolates developed the symptoms observed in the field (Figure 6). Partially necrotic non-expanded panicles (Figure 6a), partially necrotic expanded panicles (Figure 6b), and completely necrotic and empty panicles (Figure 6c) were observed in rice plants inoculated with all *Fusarium* morphotypes. Meanwhile, control rice plants showed apparently healthy panicles with no grain necrosis (Figure 6d).

The difference in necrosis observed on the rice grains may be linked to the period of attack by the fungus (Figure 7). If the attack occurs before and during the milky stage, all the grains remain thin and empty, resulting in empty panicles (Figure 7a). On the other hand, if the attack occurs during the grain maturity stage, the grains become necrotic (Figure 7b). If there is no fungal infection of the rice plants or panicles, the rice grains remain healthy (Figure 7c).
In addition, the *Fusarium* isolates found to be associated with panicle necrosis in rice plants in the pathogenicity test exhibited morphological characteristics similar to those of *Fusarium* isolates inoculated into rice plants. Thus, rice panicle necrosis is caused by several *Fusarium* isolates.

Figure 6: Symptoms observed on rice panicles under greenhouse conditions

a: unfolded panicle with partially necrotic grains; b open panicle with necrotic grains;

c- necrotic and empty panicle; d- apparently healthy control panicles

Figure 7: Comparison between a healthy grain (control) and grains that have undergone necrosis at different stages
(a: necrotic rice grains before and during the milky stage, b: necrotic grains during the ripening stage; c: apparently healthy control rice grain)

3.7- *Fusarium* species responsible for rice panicle necrosis

The fungal DNAs were successfully amplified. The PCR carried out with the ITS1/ITS4 primers generated a single fragment of approximately 600 base pairs (Figure 8). The *Fusarium* species sequences compared with those available in the NCBI GenBank database through BLAST revealed a 100% percentage identity. Three *Fusarium* species were identified using molecular biology techniques based on the seven morphotypes. These are *Fusarium fujikuroi, Fusarium verticillioides*, and *Fusarium proliferatum* (Table 4).

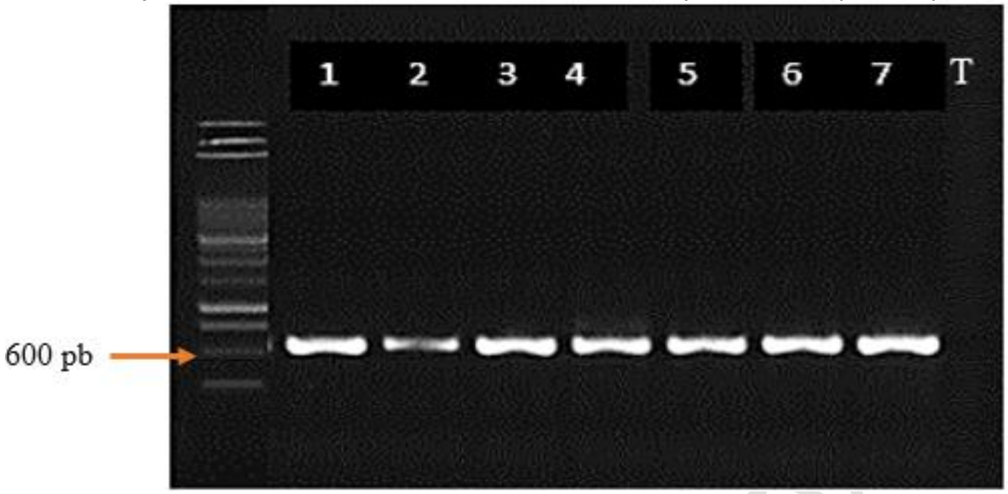

Figure 8: Electrophoretic profile of fungal DNA amplification products on 1.5% agarose gel
M: molecular weight marker (600 bp); T: negative control; 1 to 7: fungal DNA samples

Table 4 Homology rates between DNA nucleotide sequences of *Fusarium* rice panicle necrosis isolates and those of the GenBank database (NCBI)

Sample	Morphotypes	Homology (%)	Species	Accession
1	M1	100	*Fusarium proliferatum*	ON573411.1
2	M2	100	*Fusarium proliferatum*	KT581408.1
3	M4	100	*Fusarium fujikuroi*	MT603299.1
4	M5	100	*Fusarium verticillioides*	MT594370.1
5	M6	100	*Fusarium fujikuroi*	MT603295.1
6	M8	100	*Fusarium fujikuroi*	MT603294.1
7	M10	100	*Fusarium proliferatum*	ON573410.1

4. DISCUSSION

Assessment of the phytosanitary status of rice fields in the Agboville, N'Douci, Tiassalé, Divo, Gagnoa, Sinfra, and Yamoussoukro areas reveals a general infection. This infection manifests itself in a variety of complex symptoms of organ deterioration and immaturity. Producers' varieties are highly susceptible to the agents responsible for infection. These agents find in these varieties the nutrients they need to develop and grow. Indeed, rice, which is very rich in starch, is a source of carbon for many phytopathogenic agents, of which fungi are at the forefront. Fungi are generally unable to photosynthesize carbon dioxide from the air because they lack assimilating pigments and require organic food as a carbon source. This forces them to take advantage of other organisms, either through parasitism (in our study), symbiosis, or by utilizing waste or manufactured products (in saprophytism). This result is similar to [17], who states that fungi are heterotrophs for carbon, so they use organic carbon as a carbon source for their development. [18] added that fungi hydrolyze starch easily and rapidly.

Based on the symptoms, pathogenic fungi could be the cause of the rice panicle necrosis in Côte d'Ivoire. A variety of spores carried by the wind into the field without other plant barriers could end up on the panicle and infect it through the susceptibility of the rice variety grown. In this case, the spores could initially attach themselves to the rice grain via the hairs covering the epicarp. Under favorable conditions, these spores germinate by emitting a germ tube, which enters the rice grain through the natural opening at the apical end of the grain on the panicle. The rice grain, thus infected becomes necrotic. This result aligns with the findings of [19], who demonstrated that ascospores in the air, produced during crop flowering, or conidia contaminating the grains during grain harvest, infect the plants after germination. In 2017, [20] goes on to say that once the spores are in contact with the plant, they germinate and then penetrate the tissues, either directly through the epidermis, via natural orifices such as stomata, or through wounds caused, for example, by another pest, an insect, or a tool. At this stage of infection, no symptoms are yet visible on the plant. [21] have also shown that *Fusarium* fungi cause several types of symptoms in rice, namely: seed rot, stunted seedlings, chlorosis, and leaf elongation in rice fields. The susceptibility of the varieties grown by producers, diversity, and climatic conditions favorable to phytopathogenic fungi are factors that could account for the aggressiveness and severity of rice panicle necrosis disease. The results of the present study show that the disease is more prevalent in the major rice-growing areas of Côte d'Ivoire. These areas bring together the right conditions for the development of rice panicle necrosis: high hygrometry (73 to 87 % humidity), favorable temperatures (25 to 35 °C), and high severity. This translates into the highest loss rates in this zone. [20] produced a similar result, indicating that high hygrometry (70 to 90% humidity in the air) and suitable temperatures generally favor the development of phytopathogenic fungi.

Crop residues could harbor fungi that develop as saprophytes throughout the period when the soil remains uncultivated. [22] reported that infected crop residues from previous harvests allowed perithecia and ascospores to develop while the field was at rest, thereby contaminating subsequent crops. This would contaminate subsequent crops. [23] also reported this, demonstrating that the pathogen can survive under challenging conditions as spores on the seed coat and as macroconidia, or thick-walled hyphae, in plant debris. Soil is also a source of inoculum, harboring the *Fusarium* fungus. [24] added that residues of previous crops in the soil are one of the determining factors that constitute the major source of primary inoculum likely to infect the following crop. There are 11 *Fusarium* isolates associated with rice panicle necrosis. They are named M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, and M11. This diversity of *Fusarium* isolates could be due to climatic conditions, which strongly influence the distribution of Fusarium species and could therefore play a role in the differences in species diversity observed. In addition to the trophic mode of the agent responsible for infection, rice growing conditions in Côte d'Ivoire could be a factor favoring the development of infection. In Côte d'Ivoire, rice is cultivated in low-lying areas that are constantly wet and sometimes sunny. This would increase the temperature of the water, followed by evaporation, and would therefore be favorable to fungus development. This result is similar to that of [20], mentioned above. All this contributes to an increase in atmospheric humidity, creating a microclimate favorable to phytopathogenic fungi. This result is similar to that of [25], whose work showed that climatic conditions strongly influence the spatial distribution of *Fusarium* species. [26] asserts that the host plant, the region, and climatic conditions (temperature, humidity, duration and intensity of sunshine, photoperiod, atmospheric pressure, etc.) determine the distribution and predominance of these pathogenic species. As a result of global climate change, new species may appear in regions where they were not previously present. This is the principle of population dynamics. This is similar to [26], who showed that global climate change can allow species to emerge in previously uninhabited areas.

The fungal species *Fusarium fujikuroi, Fusarium verticillioides,* and *Fusarium proliferatum* were identified as responsible for rice panicle necrosis. Therefore, *Fusarium* disease infects the rice fields surveyed in this study. This finding aligns with [27] observation that people commonly refer to *Fusarium* infections as "fusariosis." This small-grain cereal disease is present all over the world. These three fungi are thought to be responsible for Bakanae disease, or fusariosis. This result aligns with the findings of [28] who showed that *Fusarium fujikuroi, Fusarium verticillioides*, and *Fusarium proliferatum*, were associated with Bakanae disease of rice in India. [29] agreed that one or more species of *Fusarium* cause Bakanae disease, with *Fusarium fujikuroi* being the most virulent. These fungi can attack plants at all stages of rice development. So, when the attack occurs during and before the milky stage, the grains abort or remain thin; in this case, the panicle remains empty. When the attack takes place during the ripening stage, the grains become filled and undergo necrotic changes. This result is similar to that of [30], who showed that infection can occur at different developmental stages of the host plant. This mode of infection could explain the presence of empty panicles in infected fields. In fact, infection of rice plants at an early stage could prevent normal panicle development and growth as a result of physiological dysfunction in the infected rice plant. Therefore, a stoppage in the plant's synthesis of reserve rice grains could explain the empty panicles. As a result, this would be the cause of the observed production losses. This result is similar to that of [31][32] who showed that the pathogen was associated with grains of various genotypes having a huge impact on rice grain quality. Desjardins et al, (2000) (7) noted that Bakanae disease is known to produce various types of symptoms ranging from seedling rot before emergence to infection of mature grains, seedling blight, crown rot, root rot, plant stunting, excessive or abnormal plant elongation or hypertrophy, grain sterility and discolouration, production of empty panicles under different climates and favourable conditions around the world. [6] have noted that Bakanae disease is known to produce various types of symptoms, ranging from seedling rot before emergence to infection of mature grains, seedling blight, crown rot, root rot, stunted plants, excessive or abnormal plant elongation or hypertrophy, sterility and discoloration of grains, and the production of empty panicles under different climates and favorable conditions. Fungi of the *Fusarium* genus also produce mycotoxins that threaten human and animal health, with dramatic consequences if not treated promptly [7]. The present study reports on the first infection of rice in Côte d'Ivoire by Bakanae disease, a Fusarium head blight.

5. CONCLUSION

The present study reports for the first time the existence of Bakanae disease of rice (a fusariosis) in Côte d'Ivoire. This disease is present in both large and small rice-growing areas, but it is more prevalent in larger production zones. It causes partially or completely necrotic panicles, as well as panicles with aborted, thin, empty grains. *Fusarium fujikuroi*, *Fusarium proliferatum*, and *Fusarium verticillioides* are some of the fungi that cause Bakanae disease. Ivorian rice fields are a favorable place for them to grow and spread. This emerging disease poses a significant threat to rice cultivation because of the significant losses it inflicts. In managing rice fungal diseases, the rice industry must consider this new disease to ensure sustainable rice food security

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