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Prospecting and Identification of Fungi Associated with the Rice Leaf Scald Disease in Togo

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Abstract:- Leaf scald, caused by *Rhynchosporium oryzae*, is the third most damaging rice fungal disease worldwide after blast and helminthosporiosis. This study presents the first report of leaf scald on rice in Togo, based on a comprehensive survey of 93 rice plots across 28 out of 39 prefectures. The research aimed to identify, characterize, and test the pathogenicity of fungi associated with leaf scald symptoms, as well as determine the disease's distribution and severity in Togo. Samples were collected, processed in the laboratory, and cultured using blotting paper and potato dextrose agar media. Results revealed that 97,84% of surveyed plots exhibited symptoms related to leaf scald and severity of the disease record were most 5 except two plots which were recorded 9 on the scale of 1 to 9. Fungal isolates were confirmed as *R. oryzae* through macroscopic and microscopic analysis, adhering to criteria described in existing literature. Growth of mycelium where about 7,5 days in 90 mm petri dish from both paper blotting and PDA medium from initiative culture. Sporus where two or three septa and were obtained after 9 days. Koch's postulates were fulfilled by reproducing leaf scald symptoms using the isolated fungi on the variety IR 841 at 50 days stage. This study contributes valuable insights into the prevalence and characteristics of leaf scald in Togolese rice field.

Keywords:- *Rice, Disease, Prospection, Identification, Fungus.*

I. INTRODUCTION

Rice is a worldwide crop grown all over the world. This crop is the second most grown cereal in Togo after corn (DSID, 2022) covering 84 395 Ha in 2020 and producing 140 519 tons of rice paddy. It is cultivated in three ecologies including rainfed, lowlands and irrigated (DSID, 2022). Rice production in Togo as all crop faces several biotic and abiotic constraints. These constraints are essentially linked to the management of water, diseases, birds and salinity (MAPAH, 2020).

Several diseases have been reported infecting rice in Togo, notably rice blast, Bacterial leaf blight (Kassankogno, et al., 2016). Unfortunately, in recent years, in addition to these known diseases, for which management strategies are in development, rice production in Togo is facing new challenges such as new and unknown diseases that have been reported by rice growers. Symptoms of rice leaf burns have been reported in several rice fields in the central region of the country. At first, these attacks were confused with symptoms of rice blast (Amadou, 2020) but subsequent research has led to the association of the observed symptom to a new disease in Togo, the leaf scald disease (Amadou, 2023).

Leaf scald is a worldwide fungal disease of rice caused by *Rhynchosporium oryzae*. This disease appears late in the season or during the vegetative development of rice plants. It is reported to appear in India during the months of June and July (ICAR, 2021).

The pathogen is a seed born disease (Melville, 1984) and has been reported to cause up to 30% yield loss in rice (Filippi, et al., 2005). Leaf scald is ranked second or third fungal disease in certain geographic regions of the world such as India, Mali (ICAR, 2014; Nutsugah & Twumasi, 2001).

In Africa this disease has been firstly reported in west Africa in 1970 (Lamey & Williams, 1972). The disease was reported in Senegal where research was conducted to identify resistant varieties to the disease (Mbodj, 1989). A survey reported the disease in the 2000s in Ivory Coast and Sierra Leone (Turner & Black, 2001) as well as in Ghana where it was classified among the commonly found fungal infection of rice in the country with a significant severity (Nutsugah & Twumasi, 2001). The disease has been reported in many Asia countries in the same period (Shanmughom, et al., 1973; Ou, et al., 1978). For the period between 2000 and 2015, there has been less work on this disease. From 2015, some resistance methods have been studied (Tatagiba, et al., 2016; Almas & Kamrodi, 2019) and first report release in China (Yang, 2021)

To date, no scientific studies have been conducted on rice leaf scald disease in Togo. While this disease has been reported in various rice-growing ecologies worldwide, particularly in lowlands, its prevalence in Togolese rice fields, which are predominantly lowland, remains unknown.

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Given the potential severity of this disease under favorable environmental conditions, it is crucial to determine its distribution and incidence in Togo's rice cultivation areas. These initial surveys aim to understand the disease's distribution and assess its severity. Currently, the absence of information on rice leaf scald in Togo impedes the creation and implementation of specific management strategies.

Therefore, this study seeks to identify the disease's distribution across Togolese territory, determine the severity of leaf scald on rice plants at a national level, and collect samples from infected plants. This research will provide essential baseline data to inform future disease management efforts in Togolese rice production.

II. MATERIAL AND METHODS

A. Collection Site

The surveys were conducted in 28 out of the 39 prefectures of Togo. In each prefecture, the RICOWAS (Scaling up climate-resilient rice production in West Africa) project sites, a project aiming to increase rice production in west Africa, were prioritized, however, rice field encountered between two (2) RICOWAS project sites were also surveyed. The survey was conducted from July to August 2022 and October to November 2023.

B. Samples Collection

During the field visit, samples consisting in leaves and panicles were collected from the diseased plants by cutting fragments of at least 5 cm and placed directly in a paper envelope. The geographic coordinates of each site as well as information on the collection site (village, commune, prefecture), the variety, the type of rice ecology, and the incidence of the disease were collected.

The disease severity was scored from 1 to 9 according to the IRRI leaf scald rating scale (table 1). On the sites surveyed, only parts of the field with symptoms of Leaf scald were assessed.

Table 1: Standard Evaluation System (SES) Scale for Leaf Scald Disease of Rice

Scale	Affected leaf area
0	No incidence
1	Less than 1% (apical lesions)
3	1-5% (apical lesions)
5	6-25% (apical and some marginal lesions)
7	26-50% (apical marginal lesions)
9	51-100% (apical and marginal lesions

Source: (Murudkar, et al., 2022)

C. Pathogen Identification and Koch Postulate Fulfillment

The fungus was cultivated on medium, namely Potato Dextrose Agar (PDA) and on blotting paper (Parkinson, 1980; Mia, et al., 1985). PDA culture medium was prepared by adding streptomycin to inhibit bacteria growth.

The leaves were cut into fragments of 1 to 2 cm and 5 fragments were equidistantly spaced apart within the petri dishes. In both cases the leaves were surface sterilize in 70% alcohol for 10 seconds then for 2 minutes in 0.5% sodium hypochlorite and then rinsed well with sterilized distilled water and placed on the culture medium (Parkinson, 1980; Mia, et al., 1985; Yang, 2021).

The Petri dishes were then placed in a large transparent tray with a lid. This tray was placed in a culture chamber for 12 hours of light and 12 hours of dark at 22 ± 2°C.

Isolation was done 48 h after culture for both methods. The pure culture was carried out on the PDA medium. The incubation for the pure culture was 8 days regardless of the starting culture medium (Parkinson, et al., 1981).

On the 7^{th} or 8^{th} day of incubation, a piece of PDA containing mycelium or spores was taken and a suspension was made. This suspension served as an inoculum in the verification of Koch's postulate. The healthy plants used for this postulate were obtained by sowing surface sterilized seeds of IR841 variety treated with alcohol (70%) for 2 minutes and with bleach (0.5%) for 5 minutes before sowing. Five plants at 50 days by isolate were used for inoculation. Temperature in the green house was 25±4°C and relative humidity around 80%.

Information regarding the survey sites, site disease assessment, macroscopic and microscopic laboratory characteristics as well as the onset or no onset of disease during Koch's postulate were recorded a week after inoculation.

D. Data Analysis

The data collected was entered into Excel and analyzed using Statistical Software Package for Social Sciences (SPSS) version 23.0. Main analyses were descriptive statistics by performing crosstab method.

III. RESULTS

A. Location of Prospected Sites

In all, twenty-eight (28 prefectures) out of 39 in Togo have been explored for a total of 93 sites visited.

The samples were collected from plants showing symptoms of leaf scald and having a vegetation cycle between the tillering stage and the milky grain stage. The samples collected were leaves, panicles and seeds.

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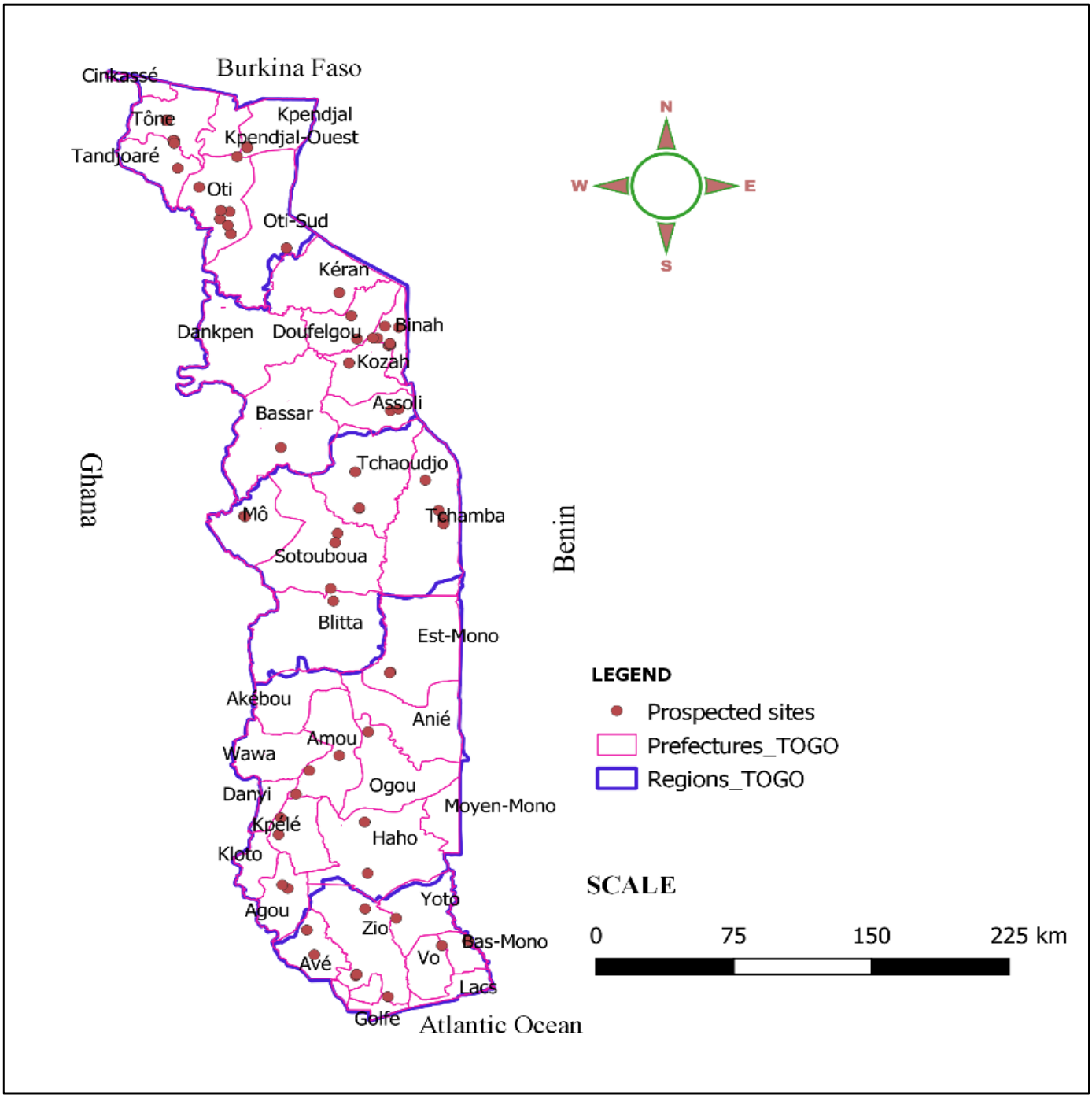

Fig 1: Map of Togo Showing the Prospection Sites
Source: Field data and (Akshay & Juhi, 2021)

The detection work was carried out at the ITRA Biosafety laboratory located in Lomé.

B. Description of the Prospected Sites

The most encountered variety was IR 841. This variety is cultivated in all regions of Togo and represents 58% of the cultivated varieties (table 1). Jasmine 85 and Yasmine varieties are the least cultivated, followed by Chapeau vert variety which was found in the maritime region only (table1).

The dominant ecology was the lowlands which represents 87%. This ecology was found in all regions of Togo (table 1). Irrigated ecologies were encountered in the savannah and maritime regions. On the other hand, the rainfed ecology which represents 2% of the sites visited was encountered in the savannah region (table 1). During the survey, the plants were in the milky stages at maturity for half of the sites (52%). These stages were most encountered in the Central, Kara and Savanes regions (table 1).

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Table 2: Characteristic of the Prospected Sites in Togo Focusses on Varieties, Ecology and Rice Stage

Varieties	Central	Kara	Maritime	Plateaux	Savanes	Line sum
varieties encountered at the collection sites						
Chapeau vert	0	0	3	1	0	4
IR 841	8	13	7	8	18	54
Jasmine 85	0	0	0	0	1	1
Not known	0	1	0	0	0	1
Local rice	11	5	2	5	8	31
Yasmine	2	0	0	0	0	2
rice ecology found during prospection						
Lowland	21	19	5	13	23	81
Irrigated	0	0	7	1	2	10
Rainfed	0	0	0	0	2	2
Rice stage found during prospection						
tillering	3	3	3	6	5	20
Stem elongation	3	4	1	0	5	13
Panicle initiation	5	5	5	0	9	24
Flowering stage	6	5	3	4	8	26
Milk stage	4	2	0	1	0	7
Dough stage	0	0	0	3	0	3
Total sites	21	19	12	14	27	93

Source: Field Data

C. Severity of Leaf Scald on the Surveyed Plots

Of the 93 sites visited, 91% presented symptoms of leaf scald ranging from 3 to 9. The severity of the disease for grades of 3 were the most encountered during the survey and represents almost half (48.38 %) of all sites visited (table 2). For major severities with destruction of plants (9) only 2% of sites were affected (figure 1). These sites were found in the Kara region (table 2).

In all regions, high disease severities were encountered except in the maritime region. The complete destruction of plants (severity 9) was noticed only in the Kara region. In the Savanes region, severities of 3 and 5 were the most encountered.

Table 3: Severity of Leaf Scald by Geographical Region of Togo

Notation	Centrale	Kara	Maritime	Plateaux	Savanes	Line sum
1	1.1	1.1	3.2	1.1	3.2	9.7
1-3	11.8	10.8	7.5	3.2	15.1	48.4
1-5	7.5	1.1	1.1	8.6	8.6	26.9
1-7	2.2	5.4	1.1	2.2	2.2	12.9
1-9	.0	2.2	.0	.0	.0	2.2

Source: Field Data

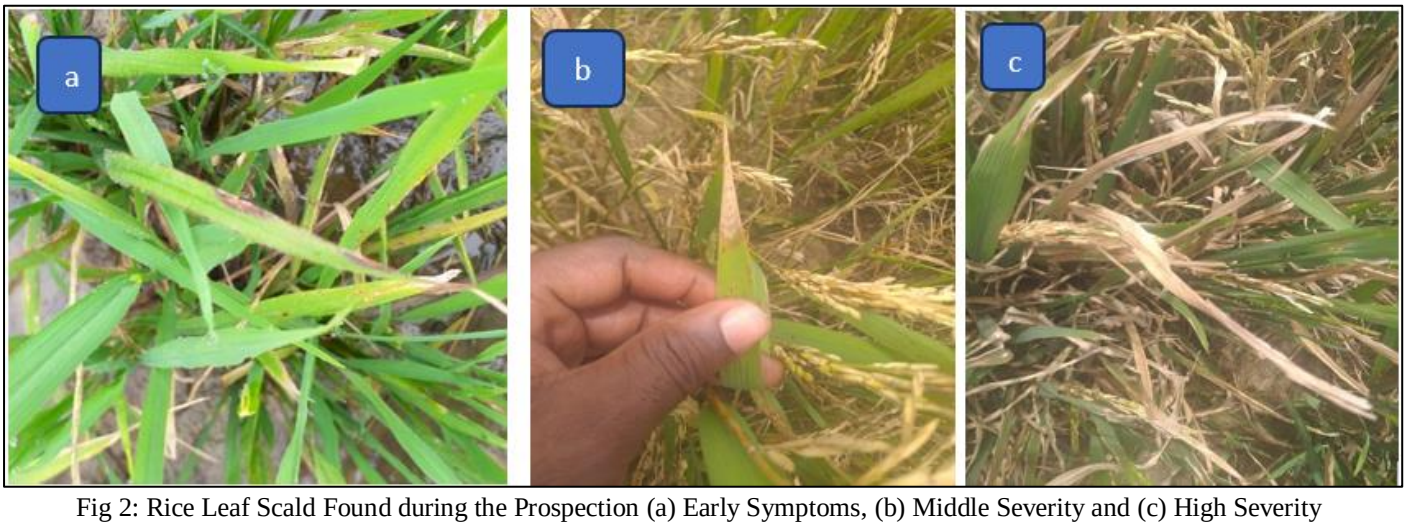

Fig 2: Rice Leaf Scald Found during the Prospection (a) Early Symptoms, (b) Middle Severity and (c) High Severity

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D. Identification of Rhynchosporium Oryzae

Culture on PDA medium and blotting paper made it possible to isolate the pathogen and put it in pure culture. In pure culture, the macroscopic and microscopic aspects have been described (figure 2).

On the macroscopic appearance the surface of the culture was pink in color with concentric bands made of alternating dark and faded. There is also a velvet or downy appearance to the surface. The underside has a brighter color tending towards black as the crop ages. Also, the lower part is made of concentric bands that alternate between bright and dark colors (figure 3 et 4).

In general, the coloring of the isolates varied independent on the site of origin of the sample. Thus, the colors were from milky pink to caramel pink. Coloration was not typical of a geographic region.

The hyphae were septate with globular shapes at the ends of the branches. Sporus were 1 to 3 septa. They had an arched shape like a crescent moon (figure 5)

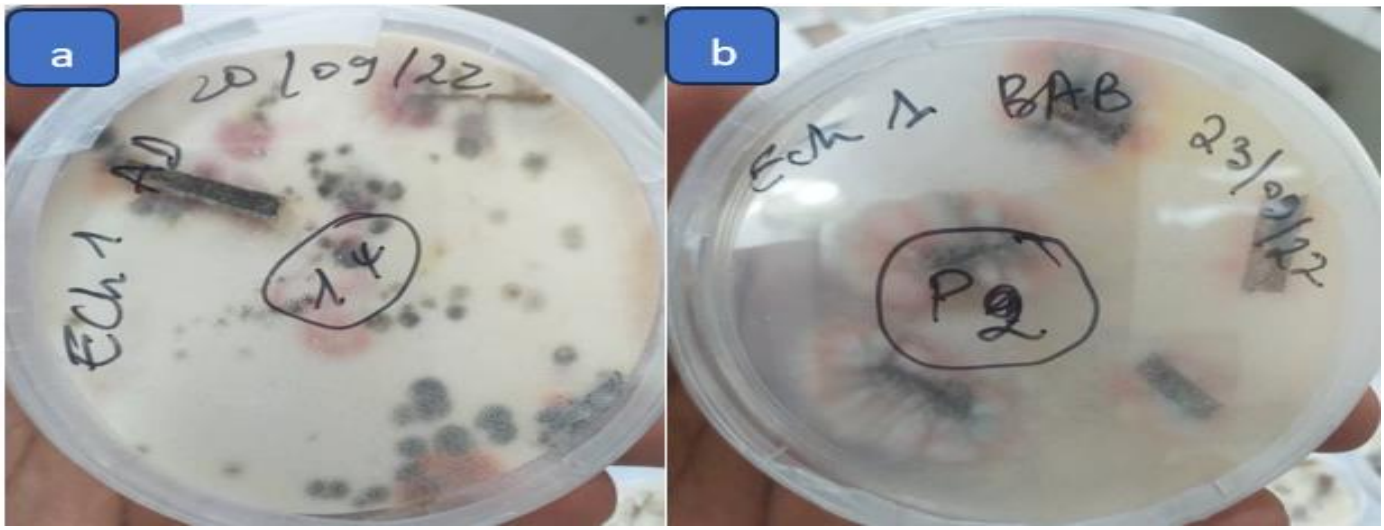

Fig 3: Culture of Leaves Samples on both Blotting Paper and PDA Medium after 2 Days: (a) Blotting Paper and (b) PDA Medium

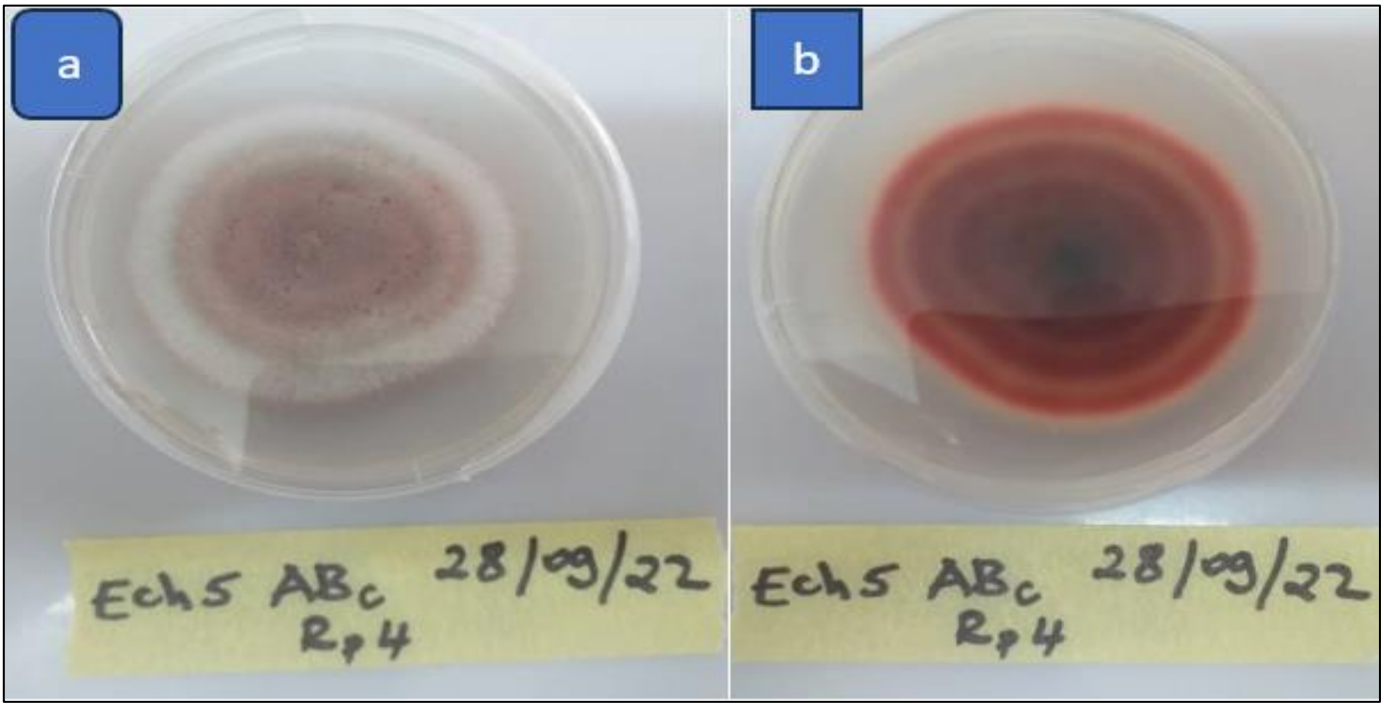

Fig 4: Macroscopic Aspect of *Rhynchosporium oryzae* Isolated on PDA Medium after 5 Days on PDA Medium: (a) Upper Side and (b) Underside, Culture Initiate on PDA

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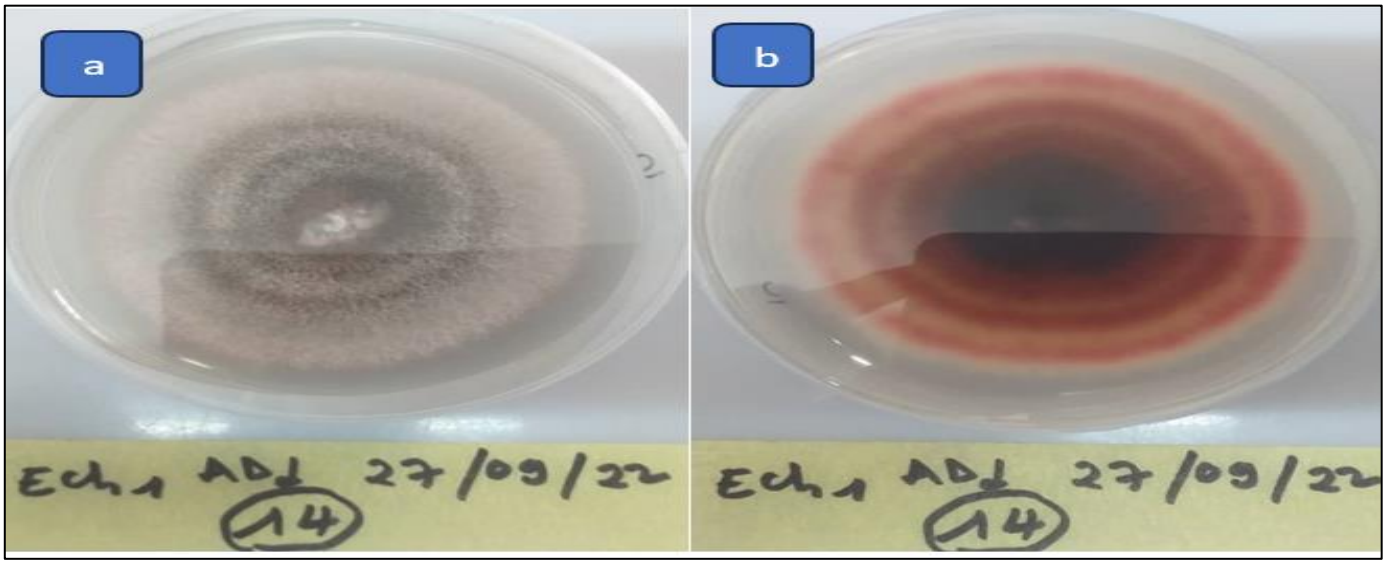

Fig 5: Macroscopic Aspect of *Rhynchosporium Oryzae* Isolated on PDA Medium after 5 Days on PDA Medium: (a) Upper Side and (b) Underside, Culture Initiate on Blotting Paper

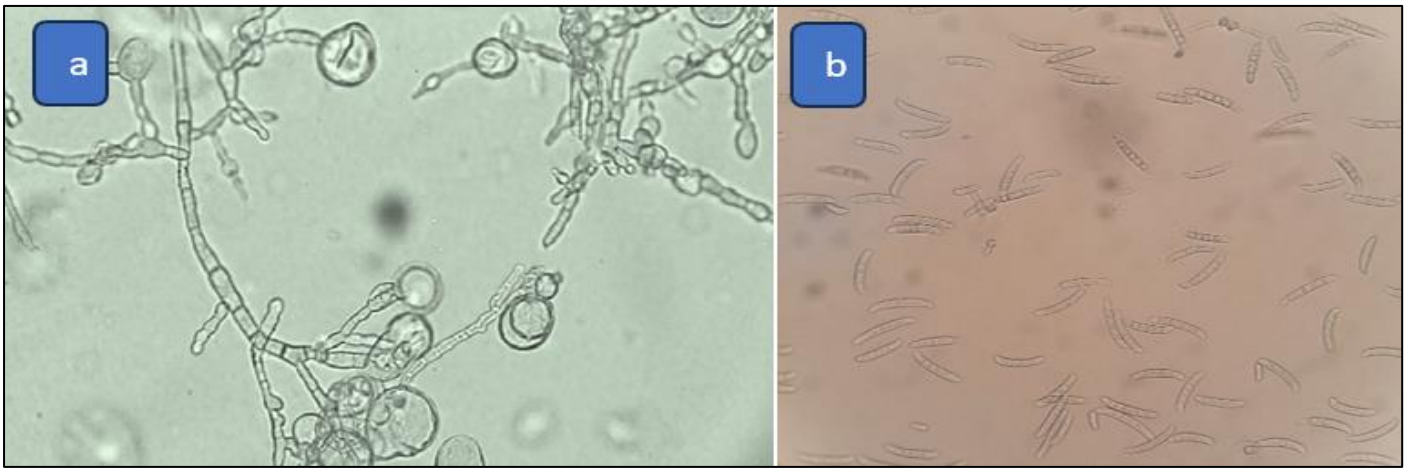

Fig 6: Microscopic Aspect of *Rhynchosporium oryzae* under Microscope Motic 2.0 (X 40): (a) Mycelium after 6 Days (b) Spores with 1 to 3 Septas after 10 Days

E. Determination of Mycelium Growth

The radial growth obtained from the observation of 27 isolates from the different regions did not evolve depending on the site of origin. Also, the initial cultivation method (PDA or blotting paper) did not influence the growth of the mycelium. The average diameter was 5,3 cm on average after 5 days of pure culture (figure 6). The petri dish was saturated for the majority of isolates after 7 days. A tiny portion of the cultured isolates occupied the total diameter of the dish 8 days after pure culture. The average daily growth of the mycelium was 1.1 cm (table 5).

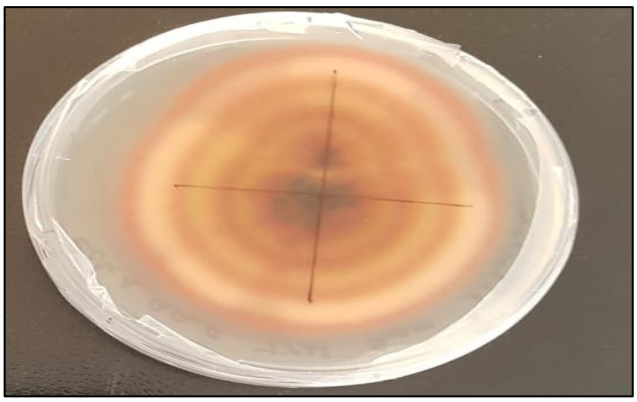

Fig 7: Mycelium Growth After 5 Days

Rhynchosporium mycelium *oryzae* in pure culture on PDA medium at a temperature of 22 ± 2°C

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Table 4: The Average Growth of Leaf Scald Mycelium on PDA at 22±2°C at 12 h Light and 12 h Dark Daily

Parameters	4th day	7th day	8th day
mycelium diameter (cm)	4.2	8.1	8.5
mode (cm)	4.2	8.5	8.5
growth per day (cm)	1.0	1.2	1.1

Source: Field Data

F. Koch Postulate Fulfillment

Disease symptoms began to appear 3 days after inoculation. All isolates tested caused disease on plants a week after infection (figure 7)

Fig 8: Onset Leaf Scald Disease during Koch Postulate Test: (a) Healthy Plants Before Test and (b) Plants Present Leaf Scald Symptoms After Test

IV. DISCUSSION

The prospecting made it possible to visit the three rice production ecologies in Togo. There was more lowland ecology found during the prospecting which reflects the characteristics of rice production in Togo made up of several lowland sites whose paddy production makes up more than 50% of national production. despite their low yield (DSID, 2022; MAPAH, 2020)

The disease was present in almost all sites visited. This presence makes leaf scald one of the fungal diseases frequently encountered in Togo in the same proportion with blast (Kassankogno, et al., 2016). Leaf scald has been noticed to be a frequently common disease in the rice fields of Ghana (Nutsugah & Twumasi, 2001). The areas covered by leaf scald in the fields visited were larger in the lowlands than in the irrigated areas. Also, the higher severity was found in lowlands were environmental conditions such drought, higher humidity and higher nitrogen use were observed (Srinivasan, 1981; MONDAL, et al., 1986).

The severity of the disease varied up to 9 on lowland sites leading to the total loss of production on 2 sites in the Kara region. In this case, yield loss was higher than 30% (Filippi, et al., 2005) and approach to 100% loss. That effectively proves that when environmental conditions are met, leaf scald can be dangerous for rice crops (Srinivasan, 1981).

During laboratory identification, the macroscopic and microscopic characteristics found were similar to the description in 1978, 2000 and 2001 (Ou, et al., 1978; Parkinson, et al., 1981; Turner & Black, 2001; Gams & Muller, 1980) with the particularity that a very bright coloring of the cultures especially on the underside of the petri dishes was noticed for some isolates.

Identification could be done on blotting paper for the first culture. The blotting paper method had the advantage of allowing samples to be taken over at least 7 days as part of the isolation, unlike the PDA culture medium where after 4 days invaded and completely filled with opportunistic fungi which covered Rhynchosporium mycelia in the petri dish.

The radial growth of the mycelium under the temperature of 22 ± 2°C was 7 to 8 days. Sporulation was obtained after 8 to 10 days similarly to previous results ((Parkinson, 1980; Manandhar, 1998).

The Koch postulate applied made it possible to re-isolate the fungi tested with identical characteristics.

V. CONCLUSION

The survey over the entire territory made it possible to map the rice leaf scald disease. Leaf scald has been found on several sites throughout Togo. The severity of the disease was higher in the lowlands up to 9 and especially in the dry lowlands. The Maritime region, having more irrigated sites, presented less severity and less damage of leaf scald.

Laboratory characterization made it possible to confirm the disease by highlighting the macroscopic aspects of the petri dish containing the fungus as well as the microscopic aspects of the fungus with the shapes of the mycelia and spores. Spores in this study were 1 to 3 septate.

This survey collection made it possible for the first time to identify the disease of rice leaf scald in Togo.

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REFERENCES

[1]. Akshay, U. & Juhi, A., 2021. *iGISMAP*. [Online] Available at: https://www.igismap.com/download-togo-administrative-boundary-gis-data-for-national-regions-prefecture-and-more/ [Accessed 12 juin 2024].

[2]. Almas, D. E. & Kamrodi, A. R., 2019. Defense responses of rice plant to Monographella albescens attack. *Journal of Plant Protection Research*, 59(4), p. 535–543.

[3]. Amadou, S., 2020. *Rapport attaque des plants de riz sur le site de la FAMETA*, Sokodé: ITRA.

[4]. Amadou, S., 2023. *prospection, collecte et identification de la rhynchosporiose du riz dans la région centrale du Togo*. Kara, Colloque des université de Kara et Parakou.

[5]. DSID, 2022. *Production national des cultures céréalières au Togo*, Lomé: MAEDR.

[6]. Elazegui, F. & Islam, Z., 2003. *Diagnosis of Common Diseases of Rice*. Training Center éd. Los Banos: International Rice Research Institute.

[7]. Filippi, M. C., Prabhu, A. S. & da Silva, G. B., 2005. Rice leaf scald and its control. *Embrapa Rice and Beans, Brazil, EMBRAPA-CNPAF, Circular Técnica*.

[8]. Filippi, M., Prabhu, A. & Silva, G., 2005. Escaldadura Do Arroz E Seu Controle. *Goiânia, Goiás, Brazil, EMBRAPA-CNPAF, Circular Técnica*.

[9]. Gams, W. & Muller, E., 1980. Conidiogenesis of Fusarium nivale and Rhynchosporium oryzae and its taxonomic implications. *Neth. J. Pl. Path.*, Volume 86, pp. 45-53.

[10]. ICAR, R. C. f. N. R., 2014. *Annual Report 2013-14*, Meghalaya: Umiam 793 103.

[11]. ICAR, R. C. f. N. R., 2021. *Annual Report 2020*, Umiam 793 103.: Meghalaya.

[12]. IRRI, 2021. *Rice knowledge Bank*. [Online] Available at: www.knowledgebank.irri.org [Accessed 15 mars 2021].

[13]. Kassankogno, A. I. et al., 2016. Analyse de la diversité génétique des isolats de Magnaporthe oryzae du Burkina Faso et du Togo par les marqueurs microsatellites (SSRs). *International Journal of Biological ans Chemical Sciences*, 10(5), pp. 2259-2267.

[14]. Lamey, H. A. & Williams, R. J., 1972. Leaf scald of rice in west Africa. *Plant diease*, Volume 56, pp. 106-107.

[15]. Manandhar, J. B., 1998. Effect of Light, Temperature, and Water Potential on Growth and Sporulation of Microdochium oryzae. *Mycologia*, 90(6), pp. 995-1000.

[16]. MAPAH, 2020. *STRATEGIE NATIONALE DE DEVELOPPEMENT DE LA RIZICULTURE 2019-2030 (SNDR 2) Togo*. s.l.:s.n.

[17]. Mbodj, Y., 1989. La luute intégrée contre les maladies du riz en Casamance, Sénégal. *Thèse de doctorat, Université Catholique de Louvain, Facultés des sciences agronomiques*, 18 avril, p. 217.

[18]. Melville, D. T., 1984. Dry-Season Survival of Rhynchosporium oryzae in Rice Leaves and Stored Seeds. *Mycologia*, 76(6), pp. 1111-1113.

[19]. Mia, M. A. T., Mathur, S. B. & Neergaard, P., 1985. Gerlachia oryzae in rice seed. *Translations of the British Mycological Society*, 84(2), pp. 337-338.

[20]. MONDAL, A. H., AHMED, H. U. & MIAH, S. A., 1986. Effect of n itrogenon the developpment of leaf scald disease of rice. *Bangladesh Journal of Botanic*, 15(2), pp. 213-2015.

[21]. Murudkar, A. C. et al., 2022. Management of leaf scald disease of rice caused by Microdochium oryzae with selective Botanicals, Bio-agents and Fungicides. *The Pharma Innovation 2022*, 11(9), pp. 3014-3016.

[22]. Nutsugah, S. K. & Twumasi, J. K., 2001. FUNGAL DISEASES OF RICE IN GHANA: KNOWLEDGE AND RESEARCH NEEDS. In: S. S. a. R. J. (eds.), ed. *Major Fungal diseases of Rice Recent Advances*. s.l.:s.n., pp. 321-329.

[23]. Ou, S. H., Nuque, F. L. & Vergel de Dios, T. I., 1978. Perfect stage of rhynchosporium oryzae and the symptomsof rice leaf scald. *Plant diseases*, 62(6), pp. 524-528.

[24]. Parkinson, V. O., 1980. Culture characteristics of the rice scald fungus, Rhynchosporium orizae. *trans. Br. mycol. Soc.*, 74(3), pp. 509-514.

[25]. Parkinson, V. O., Sivanesan, A. & Booth, C., 1981. The perfect state of the rice leaf scald fungus and the taxonomy of both the perfect and imperfect states. *Translations of the British Mycological Society*, 79(1), pp. 59-69.

[26]. Shanmughom, S. N., Gopalan, N., George, K. M. & Gopalakrishnan, R., 1973. LEAF SCALD OF RICE (RHYNCHOSPORIUM ORYZAE HASHIOKA AND YOKOGI). *Current Science*, Vol. 42(No. 16), pp. pp. 582-583.

[27]. Srinivasan, S., 1981. Yield losses due to leaf scald disease. *International Rice Research Newsleller 6*, p. 15.

[28]. Tatagiba, S. D., DaMatta, F. M. & Rodrigues, F., 2016. Magnesium decreases leaf scald symptoms on rice leaves and preserves their photosynthetic performance. *Plant Physiology and Biochemistry*, 108(2016), pp. 49-56.

[29]. Turner, H. & Black, R., 2001. Rice leaf scald : pathogen biology and diversity. In: S. Sreenivasaprasad & R. Johnson, eds. *Major Fungal diseases of Rice Recent Advances*. s.l.:Kluwer Academic Publishers, pp. 307-319.

[30]. Yang, Z., 2021. Short Note On Leaf Scald. *Rice Resaerch*, 9(7), p. 253.