**Title:** Physiological and Nutritional Studies on Growth and Sporulation of Pyricularia oryzae: the Blast Fungus.

**Introduction**

**Background to the study**

Rice is the staple food for over half of the world’s population, China and India alone account for about fifty percent (50%) of the rice grown and consumed (Jat et al., 2020). In Asian countries, >90% of the rice is produced and consumed. The other continents in which rice is grown are Africa (7.78% of the global area), South America (6.4%) and North America (1.4%) (Anonymous, 2018). The global cultivation of rice is now in 167 million ha with annual production of around 769 million tonnes (499.3 million tonnes, milled rice) of rough rice and average productivity of 4.6 tonnes/ha (3.62 t/ha, milled rice) of rough rice (Wallach, 2022). It is therefore becoming an important food staple in both Latin America and Africa.

Rice harvested area and yield have been on an increasing scale in Africa, however, the ratio between production and consumption (P/C ratio), which is an indicator for self-sufficiency, has been far below one for a considerable time, indicating that most countries in Africa are still far from being self-sufficient in rice. Meanwhile, the population and per-capita consumption are expected to continue to increase (Nasrin et al., 2015). In West Africa, the attention turned towards rice value chain because it is the most important calorie source according to Macauley & Ramadjita (2015) and this made domestic rice producers to increase their production after the rise of crisis which called for the need to address chronic hunger through macro-nutrients self-sufficiency where African policy makers developed targeted National Rice Development Strategies (NRDS) (CARD, 2019) but domestic rice value chain never managed to catch up with consumption leading to the current gap that is satisfied through imports (Soullier et al., 2020) and this imports make up to about 30% of the rice consumption (Food Security Information Network, 2020; FAO et al., 2019), nevertheless West African produces nearly two-thirds of Africa’s rice. In the case of Ghana, the country still import rice to meet its demand despite various programme initiated by the government to increase rice production (Tanko et al., 2019; Alidu, Tanko, & Iddrisu, 2016; Tanko & Alidu, 2016; MOFA, 2017). Africa is far from self-sufficient in rice and this situation is projected to worsen in the future (Van Ittersum et al., 2016; Van Oort et al., 2015)

Major issues of rice production underlined by researchers include both abiotic and biotic factors (Acharya et al., 2019); Drought, cold, acidity, salinity are abiotic factors (Onyango, 2014), emerging new diseases and insect-pests (Lohan et al., 2018), declining of soil health (Kakraliya, et al., 2018), climatic issues (Bhatt, 2013; Humphreys et al., 2010), poor income of farmers (Bhatt et al., 2016) etc. Among the biotic factors, fungal diseases alone are estimated to reduce annual rice production by 14% globally (Agrios, 2005), the major rice diseases caused by fungal pathogens are blast, sheath blight, bakanae, brown spot and dirty panicle (Rice Department, Ministry of Agriculture and Cooperatives of Thailand, 2014). Among the fungal diseases of rice, blast disease caused by a filamentous ascomycetes fungus known as Pyricularia oryzae and also know to be Magnaporthe oryzae in its ……… form is of significant economic important and can cause up to 70-80% yield loss of rice (Nasruddin & Amin, 2012; Miah et al., 2013). This study therefore attempts to document the nutritional and physiological conditions which suites the growth and sporulation of this pathogen.

**Statement of Problem**

The blast disease is considered as one of the largest obstructions to increase in rice production in not Ghana or west African alone but worldwide because the pathogen under its favorable condition can wipe an entire rice plant within 15-20 days of infection and cause severe yield loss (Musiime at al., 2005) and hence directly decreases rice yields and may indirectly also increase cost of production on the farmer’s side.

Pyricularia oryzae does not only affect rice by causing the blast disease, the same pathogen is believed to infect also a variety of gramineous hosts, causing gray leaf spot of turf grass (Milazzo et al., 2019) and also causing wheat blast (Ceresini et al., 2019) and according to Skamnioti & Gurr (2009), the fungus is part of the species complex that can cause blast diseases on about 50 grass (Poaceae) and sedge (Cyperaceae) species, including rice (Oryza sativa), barley (Hordeum vulgare), maize (Zea mays), oats (Avena sativa), rye (Secale cereale), finger millet (Eleusine corocana) and perennial ryegrass (Lolium perenne). The current threat of the fungus to rice can be traced back in China about 7000 years ago (Couch et al., 2005; Me, 2018) and now found in all rice-growing region in the world (Bhandari et al., 2017).

The blast disease can be controlled by various ways including cultivation techniques, planting resistant varieties and the use of fungicides. Using of resistant varieties is the most effective, economic and easy method however, the use of this technology is faced with faced with Pyricularia oryzae that have high genetic diversity, and can adapt and form new races is able to break the resistance of newly introduced varieties (Santoso et al., 2007; Fukuta et al., 2009; Lestari et al. 2011) and as a result, number of superior varieties which are targeted to control the blast disease on last for two to three seasons (Koizumi, 2009). The new trend in agriculture on disease control is to develop variety of approaches to manipulate the interaction to create a system in favor of the growth and development of host plants but suboptimum to the establishment, reproduction, and transmission of pathogens (Bisht, 2020), and among these approaches is biological control (Yang et al. 2019; Poveda et al., 2020) which calls for pathogen culture in-vitro for laboratory testing (Besset-Manzoni et al., 2019) and this makes it important to understand the nutritional and physiological requirement of pathogens.

**Justification of the Study**

Knowledge of the interaction of host and pathogen with environment factors has a practical significance because the environment could alter pathogen pathogenicity. Pathogen potential or its virulence highly depends on its nutrition, that is, the interaction of an invading fungal pathogen like Pyricularia oryzae with its host will depend on its nutrition (Gow & Brown, 2017; Pike et al., 2019) and environmental factors such as nutrition have effect on growth and sporulation of fungal pathogens (Gohel & Chauhan, 2015). The knowledge of nutritional and physiological studies on growth and sporulation of Pyricularia oryzae is needed to contribute to integrated disease management.

**Significance of the Study**

The information obtained from this study will help to understand how various nutrient and nutrient sources affect the performance of Pyricularia oryzae, and will hence used as a reference point for researchers on the nutrient requirements for the blast pathogen and how media components play a vital role in favouring growth and sporulation of blast fungus so as to study it in laboratories for disease control aspect and large-scale production of its spore suspension for large disease screening studies.

**Objective of the Study**

**Main objective**

The main objective of this studies is to compare the growth and sporulation of Pyricularia oryzae on both synthetic and natural medium.

**Specific Objectives**

The specific objectives of the study are;

1. Survey the blast disease in three districts in Ashanti Region of Ghana viz. Atwima Nwabiagya South, Atwima Nwabiagya North and Ahafo Ano South districts.
2. Compare the growth and sporulation of Pyricularia oryzae on different media.
3. Total Viable count on PDA plates using spore suspension.
4. Compare the growth and sporulation of Pyricularia oryzae under different temperature, light and pH.

**Scope of the Study**

The study will show the incidence and severity of blast disease and how famers control the pathogen and in the afore mentioned rice growing districts in Ashanti region of Ghana. The pathogen (Pyricularia oryzae) will be isolated from samples of infected rice plant during the minor growing season of 2022 and in-vitro testing of the pathogen on growth and sporulation will be done based on different conditions, this will add up to knowledge on good conditions for growth and sporulation of the pathogen and hence provide directions in other to manipulate the virulence of the pathogen.

**Limitations and Delimitations of the Study.**

**Limitation of the Study**

Most of the farmers in the study area are illiterates and may have little of no knowledge on what is know to be blast disease of rice, not even Pyricularia oryzae as its causal agent. This will require visual presentation of rice plant affected with the disease and vivid explanation of mechanism of the pathogen in causing the disease and its symptoms development in other to succeed in taking the data for the survey purpose. There are only two seasons for rainfed paddy rice production in the study area which include the minor season (November – February) and the major season (March – July), and based on the duration for this study, samples can only be taken during the minor season.

**Delimitation of the Study**

The samples for this study are set to be taken from and not outside Atwima Nwabiagya South because the study area is based on where potential rice blast disease is occurring and effects of the disease are reasonably foreseeable which will intern make the study technically feasible.

This study needs to be completed within one year and with a limited budget. The time required for the data to be collected, the requirement of labor in collection of samples and data collection and resources for the laboratory works compels me to choose only one study area, it is important to narrow the size of the study area instead of limiting the details and rigor of the analysis.

**Definition of key Terms**

**Materials And Methods**

Description of the Study area

The assessment of rice blast intensity will be carried out in rice cultivated fields in the Atwima Nwabiagya South zone in Ashanti region of Ghana during the minor cropping season from November, 2022 to February, 2023. The assessment of the disease intensity will be conducted at a total of six (6) localities; Amadum Adankwame and Asakraka, from Atwima Nwabiagya South district, Mfensi and Ntensere,from Atwima Nwabiagya North district and Mankranso and Adudwama, from Ahafo Ano district. The description of the study areas altitude, longitude, latitude, temperature and mean annual rainfall is given in table below:

**Assessment of the Blast Disease Intensity**

The survey will be conducted using simple random sampling method, within 1-2 km intervals on rice fields along the main and accessible road sides. The rice blast incidence and severity will be recorded along the two diagonal ‘X’ fashion of the fields at three (3) random spots using 1m2 quadrants and used to calculate the average values.

Totally, thirty (30) farmer’s rice fields will be will be surveyed at critical stage of the crop (tillering), at the stage the blast symptom is believed to have reached its maximum severity level. Ten (10) farmer’s field from each district (5 rice farmer’s field in each locality) will be selected.

The prevalence of the disease will be calculated using the number of fields affected by the disease divided by the total number of fields assessed and expressed in percentage and then the scoring scale of the of blast disease under field condition was rated according to the International Rice Research Institute (IRRI) scale of 1-9. Data on the geographical information (longitude, latitude and altitude) of each field will also be recorded using GPS (find GPS coordinates on Google Maps, 2021)

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| --- | --- |
| **Scale for Scoring Rice Blast Disease** | |
| **Scale** | **Symptom** |
| 0 | No lesions |
| 1 | Small brown speaks of pin points size or large brown speck without speculating center. |
| 2 | Small round dish to slightly elongated necrotic grey spots about 1-2 mm in diameter with distinct brown margin lesions are mostly found on lower leaves |
| 3 | Lesion type is the same as scale 2 but the significant number of lesions are found on upper leaves. |
| 4 | Typical susceptible blast lesion, 3 mm or longer infecting lesions on 2% of leaf area. |
| 5 | Typical blast lesion infecting 2-10% of the leaf area. |
| 6 | Typical blast lesion infecting 11-25% of the leaf area. |
| 7 | Typical blast lesion infecting 26-50% of the leaf area |
| 8 | Typical blast lesion infecting 51-75% of the leaf area. |
| 9 | More than 75% leaf area affected. |
| (IRRI, 2002) | |

**Diseased Plant Sample Collection**

Blast infected rice leaf samples at vegetative stage (tillering 3rd critical growth stage) will be collected from the fields at an altitude range between 500 and 800 m . Infected leaves will be cut from the mother plant and kept in well labelled envelopes; labels will include necessary information like;

1. District
2. Town
3. Cultivar
4. GPS data and
5. Date of collection.

Samples will then be kept in refrigerator at 4 0C until the surveys in all districts is finalized. After which the samples will be preserved in ice box and transported to Microbiology Laboratory, Crop Research Centre, Institute for Scientific and Industrial Research, Fomesua, Ghana for pathogen identification and characterization.

**Isolation, Purification and Identification of Rice Blast Isolates**

The Potato dextrose Agar (Ingredients here) will be used for the isolation of the blast pathogen. Diseased leaves of rice cultivars infected with pathogen will be cut into small pieces (less than 1.0 cm in size) around the area showing the blast lesion including the edges of the lesion and will be surface sterilized (with what?) for 1 minute (why?) followed by three (3) times washing with sterile distilled water. The cut leaves will then be placed in petri dishes with the media and lined with moist filter papers. The dishes will be incubated at 25 0C for 24 hours to encourage sporulation.

After incubation, these infected pieces will be examined under a stereo-dissecting microscope. The abundant pathogen growth and sporulation will be observed from in and around the lesions with grey, dense and bushy appearance. A sterile moistened needle will be used to pick out some conidia by brushing the needle across the sporulating lesion. The conidia will be placed on potato dextrose agar media plates. Plates will be incubated again at 25 0C for about 7-`10 days with 12 hours darkness and 12 hours light (why). The identification of Pyricularia oryzae will be verified by checking the conidia under light microscope (WARDA, 2004) and following the cultural and morphological characteristics described by Mew & Gonzale (2002). Isolates of the blast pathogen will be purified using single spore (mono conidial) technique

Water agar medium ([decription here](../PyriculariaOryzae/Asfaha(2015)AssessmentOfDiseaseIntensity......pdf)) will be used for the purpose of single conidial isolation. Mono-conidial cultures will be isolated from the field blast pathogen isolates (Gebremariam Asfaha et al., 2015), which will be derived by streaking a loopful of conidial suspension across water agar plates in a “W” pattern, thus spreading the conidia. Following 24 hours incubation at 25 0C, germinating conidia will then be picked and subcultured on the Potato Dextrose Agar with amendment (Gebremariam Asfaha et al., 2015)

**Evaluating Different Culture Media for Growth and Sporulation of Pyricularia oryzae Isolates.**

Four media viz. Potato dextrose agar, Rice flour agar, Nutrient agar and Dried rice leaf dextrose will be used to compare the growth rate of Pyricularia oryzae isolates after 10 days of inoculation (Meen, 2005).

From the ends or margins of actively growing Pyricularia oryzae isolates 7 mm diameter mycelia disc of 13 days old cultures of different P. oryzae isolates will be inoculated on the middle of the Petri plates and three replicates will be maintained for each media. The inoculated petri dish will be kept at 30 0C.

**Colony Diameter**

The colony diameter of growth of each isolate will be measured after the 10th day of the incubation period and the growth will be calculated in millimeters (mm) with the help of a scale.

**Microscopic Observations**

**Colony pigmentation**

The colony pigmentation will be determined for each isolate after the 10th day of the incubation period.

**Color of Mycelia**

The color of the mycelia will be determined in all the media under the microscopic observation.

**Surface texture of Mycelia**

The surface texture of the colony will be determined under the microscopic observation and classified ([Stalper, 1978](HandbooksForTheStudy/MyceliaTextures-%20Stalpers%20(1978).pdf) as cited in (Akhundova et al., 2019))

**Margin of Mycelia**

The margins of each mycelia on all the different media will be observed under the microscope and classified as done in Gebremariam Asfaha et al. (2015).

**Mycelia growth and Sporulation**

The growth and sporulation in each media will be observed and classified.

**Conidia Characteristics**

**Sizes of Conidia**

The sizes of conidia will be determined in each medium for all the different media.

**Shape of Conidia**

The shapes of conidia will be observed and classified in each medium for all the different media.

**Septation of Conidia**

The septation of conidia for all the different media will be observed and classified (Meena, 2005 as cited in Ashrafi et al. (2021)).

**Growth of Blast Isolates Under Different Temperature**

Selected isolates of P. oryzae will be grown on Potato dextrose agar media under different temperature levels. Mycelial disc of 10 days old culture of P. oryzae isolates (7 mm diameter disc) will be placed on the middle of PDA petri plates and incubated at five (6) different [temperature](../PyriculariaOryzae/Asfaha(2015)AssessmentOfDiseaseIntensity......pdf) levels; 10, 15, 20, 25, 30 and 35 0C.

The experiment will be laid out in a Completely randomized Design (CRD) with three replications. And after five (5) days of incubation, the colony diameter of each isolate will be measured in mm (Lule et al., 2014).

**Growth of Blast Isolates Under Different pH**

The growth of the pathogen will also be measured in terms of mycelial dry weight. Selected isolates of Pyricularia oryzae will be grown on Potato dextrose broth media and studied by the method of Meena (2005). The pH of the broth will be adjusted to 3.5, 4, 4.5, 5, and 5.5 with the help of digital pH meter using 0.1 N HCL and 0.1 NaOH. The reaction of the medium will be adjusted using di-hydrogen phosphate citric acid buffer as used in Vogel (1951).

The medium will then be sterilized in autoclave at 120 0C for 15 minutes. The 10 days old culture of P. oryzae (6 mm diameter disc) will be cut and inoculated into 30 ml basal medium in 250 ml flask and incubated at 30 0C. The experiment will be laid out in Completely Randomized Design with three replications. After five days of incubation at 30 0C, the dry weight of the mycelium of each isolate will be recorded. After the given incubation period, the mycelial mat of the pathogen will be removed and collected in pre-weighed what man’s filter paper No 42 and the filter papers with mycelial mat will be dried at 60 0C for 6 days in electric oven. After drying, the filter papers with mycelium will be re-weighed. The mycelial dry weight per culture will be determined by subtracting the weight of filter paper from the weight of filter paper + mycelial mat as used in Meena (2005).

[**Pathogenicity test for Pyricularia isolates**](../PyriculariaOryzae/Asfaha(2015)AssessmentOfDiseaseIntensity......pdf)**.**

[There is more information here](SimilarWorks/CulturalAndMorphologicalVariabilityInColletotrichum,Lindemuthianum%20-%20Narasimha%20%20(2022).pdf) on **Materials and Methods.**

**Media for the Study**

Different media; natural media and synthetic media viz. Nutrient media, Oat meal media, Potato dextrose agar, Rice straw dextrose and Dried rice leaf dextrose will be purchased and used for this study.