Isolation and Morphological Characterization of Rice Blast Pathogen in Northern Ghana

P. Maaldu and S. O. Abebrese
Department of Biotechnology, University for Development Studies, Nyankpala
Council for Scientific Industrial Research - Savannah Agricultural Research Institute, Nyankpala

Corresponding Author: pmaaldu@gmail.com

Received: 23rd May 2019, Accepted: 16th August 2020

Abstract
Rice (Oryza sativa L.) is the second most important cereal food crop in Ghana. Domestic production of rice yields is low and Ghana produces only 30% of local demand. Rice blast has been the main constraint limiting local rice production in Ghana. The aim of this research was to isolate and morphologically characterize rice blast pathogen isolates obtained from Upper West, Upper East and Northern Regions of Ghana. In all, twenty-seven isolates were isolated and characterized morphologically on the basis of colony characters (colony color, surface texture, aerial mycelium, margin and size of the growing mycelium) and confirmed by microscopic examination of the conidia and conidiophores of the isolates. The colony color of the 27 isolates varied greatly from grey, light grey, black with thick cottony mass, grayish with white cottony mass, greenish, white, black and to creamy. The conidia of the isolates under 40x of the light microscope showed an oval shape, narrowed apex with 2 – 3 septate with long slender branched and unbranched conidiophore.

Keywords: Rice Blast, Rice, Potato Dextrose Agar, Rice Blast Isolates

Isolement et Caractérisation Morphologique de L'agent Pathogène de La Pyriculariose du Riz Au Nord du Ghana.

Résumé
Le riz (Oryza sativa L.) est la deuxième culture alimentaire céréalière en importance au Ghana. La production intérieure de riz est faible et le Ghana ne produit que 30% de la demande locale. La pyriculariose du riz a été la principale contrainte limitant la production locale de riz au Ghana. L'objectif de cette recherche était d'isoler et de caractériser morphologiquement les isolats de blastogènes du riz obtenus dans les régions du Haut-Ouest, du Haut-Est et du Nord du Ghana. Au total, vingt-sept isolats ont été isolés et caractérisés morphologiquement sur la base des caractères de colonie (couleur de colonie, texture de surface, mycélium aérien, marge et taille du mycélium en croissance) et confirmé par un examen microscopique des conidies et des conidiophores des isolats. La couleur des colonies des 27 isolats variait considérablement du gris, gris clair, noir avec une masse de cotonnier épaisse, grisâtre avec une masse de cotonnier blanc, verdâtre, blanc, noir et crémeux. Les conidies des isolats sous 40x du microscope à lumière présentaient une forme ovale, à apex rétréci avec 2 à 3 septates avec un long conidiophore ramifié et non ramifié.

Mots clés: La Pyriculariose du Riz, Riz, gélose dextrose de pomme de terre, isolats de pyriculariose du riz

Introduction

Rice (*Oryza sativa* L.) is an important staple food crop all over the world due to its vast consumption with Asia, Latin America and Africa as the largest consuming regions (Abed-Ashtiani *et al.,* 2012). Global rice production increased between 1961 and 2010, with a growth rate of 2.24% per year (http://ricepedia.org/rice-as-a-crop/rice-productivity). Most of the increase in rice production was due to higher yields, which increased at an annual average rate of 1.74%, compared with an annual average growth rate of 0.49% for area harvested.

Rice has become the second most important staple food after maize in Ghana. Ghana depends largely on imported rice to make up the deficit in domestic rice supply. On the average, annual rice import is about 400,000 tons (MoFA, 2009). Rice production in Ghana is faced with a number of challenges including biotic factors such as insect pests, diseases and weeds infestation with negative impact on rice production. Among diseases of rice in Ghana, the rice blast disease, mainly caused by *Pyricularia oryzae*, is the most devastating in the world including Ghana (Ou, 1985) since it is prevalent in most rice-growing regions and causes serious yield losses (Abed-Ashtiani *et al.,* 2012).

Rice blast presents serious concern in temperate areas as well as in tropical uplands, such as those found in West Africaand Iran (Mousanejad *et al.,* 2010). The disease results in yield loss as high as 70 – 80% when predisposition factors such as; high mean temperature, relative humidity higher than 85 – 89%, presence of dew, drought stress and excessive nitrogen fertilization favor epidemic development (Piotti *et al.,* 2005). According to (Wang and Valnet, 2009) India, Japan and Indonesia experienced the highest occurrence of blast more than 50%, 42.5% and 70% respectively. In Malaysia, it has been reported that almost 1,000 ha of rice plantation in Kedah state suffered rice blast disease annually (New Straits Times, 2012). *P. oryzae* was reported to destroy rice enough to feed an estimated 60 million people in the world in each year (Barman and Chattoo, 2005).

In West Africa sub-region, blast is recognized as a primary constraint to rice production causing 3.2 - 77% yield losses (Chipili *et al.,* 2003); particularly devastating in upland rice. It also causes serious damage in rainfed lowland and irrigated systems, mainly when farmers seek to intensify production by the use of improved varieties and fertilizers. Therefore, rice blast constitutes one of the main constraints militating against the intensification of rice production in Africa.

In Ghana, surveys conducted on more than 264 farmers' fields in all the major rice-growing areas across the country indicated that the incidence of blast varied considerably across sites, which have been grouped into low, moderate and high blast areas, with incidence levels of 1–10%, >10–50% and >50–100%, respectively (Nutsugah *et al.,* 2008). Heavy yield losses (up to 100%) largely due to blast infection were reported by the farmers. The survey results point out that Datano, Hohoe and Nyankpala are blast hot spots and key sites for varietal resistance screening, which correlates with the diversity of the blast pathogen populations in Ghana.

The major control measures currently include; the application of fungicides, good cultural practices and planting resistant varieties. Of these measures, the use of resistant varieties appears most effective. *P. oryzae* has been reported to have high pathogenic variation with respect to host range and variety specificity.

The level of pathogenic variation of *P. oryzae* isolates differs with rice varieties (Charles *et al.,* 2015). The structure and dynamics of the population of *P. oryzae* need to be understood thoroughly in the farmers' fields which would aid in identification of the most effective and durable blast resistance genes for incorporation into susceptible cultivars.

Jasmine and Agra rice varieties are the most important rice varieties in Northern Ghana. Farmers prefer to cultivate these varieties because they are high yielding, aromatic, have good cooking qualities and more importantly offer farmers high income as they are the consumers' preferred varieties. Currently, however, Jasmine and Agra varieties are highly susceptible to the disease resulting in a dip in rice production in Northern Ghana.

In order to release resistant Jasmine and Agra varieties in these regions, establishing a collection of existing strains of rice blast pathogen from blast epidemic regions would assist in identifying the most effective resistant genes for development of resistant varieties. Therefore, this study was aimed at isolating rice blast pathogen from the panicles and leaves of Jasmine and Agra varieties and morphologically characterize the isolates from the three Northern Regions of Ghana.

Materials and Methods

Source of Isolates

Lesions identified on leaves and panicles of two varieties of rice (Jasmine and Agra) were randomly sampled from rice growing farms in the three Northern regions (Upper West, Northern and Upper East). In each region, 10 panicles and leaves infected with lesions were randomly sampled from different rice growing farms. A total of 60 rice blast lesions samples were collected and placed in brown paper bags and sent to Savannah Agricultural Research Institute (SARI) - Plant Pathology laboratory for isolation of rice blast pathogen.

Isolation of Rice Blast Pathogen Isolates

For isolation, the infected portion of the panicles and leaves were diced into 5 mm^2 (small pieces) and surface sterilized with 1% Sodium hypochlorite for 30s and rinsed three times with sterile distilled water and transferred onto a sterilized Petri dishes containing freshly prepared potato dextrose agar (PDA) medium. Five pieces of diseased plant parts were placed in each Petri dish and incubated at 25 °C for 7 to 12 days to induce sporulation of the fungi (Aneja, 2005). The growing mycelium were purified and replicated on PDA. The isolates were characterized based on the morphological characteristics of the pathogen with the help of keys by (Barnett and Hunter, 1998).

Characterization of Rice Blast Pathogen Isolates on PDA Culture Medium

The mycelium color of each isolate on PDA medium, were examined daily from 5 to 12 days of incubation. The colony diameter of each isolate on Petri plates was measured in two directions with a ruler at two days intervals and the measurements were recorded in centimeter (cm). Type of margin, surface texture and aerial mycelium were also recorded according to (Barnett and Hunter, 1998) procedure.

Microscopic Observation of the Isolates Conidia/Conidiophores

The isolates were grown on PDA and incubated at 25˚C for 12 days. A small portion of the growing mycelium from each isolate was prepared on slides and observed under light microscope at 40X for confirmation of the rice blast pathogen isolates. The shapes of the conidia and conidiophore were determined as described by (Meena, 2005).

Results and Discussions

In all, 27 isolates were obtained and these isolates were grouped according to the three Northern regions of Ghana as shown in **Table 1a, 1b and 1c.** The colony color of the 27 isolates varied greatly from grey, light grey, black with thick cottony mass, grayish with white cottony mass, greenish, white, black to creamy. The color chart of (Rayner, 1970) was used to determine the colony color of the isolates. Most of the isolate's colonies surface texture appeared rough whiles others had smooth surface texture. Variation of the isolates colony color was as a result of different strains of the rice blast pathogen. The margins of isolates were clear for most isolates whiles other isolates had uneven or irregular margins. The results of the current research corroborate the findings of other authors. For example, (Mahboubeh *et al.,* 2017) isolated 13 *Pyricularia* isolates on the 10^{th} day on both PDA and Oat Meal Agar (OMA) media. The authors observed that, on PDA, the cultures showed a range of colors that varied from greyish brown to light cream. They reported that a typical *Pyricularia* was grayish brown in color. According to (Kariaga *et al.,* 2016), variation of colors among *P. oryzae* isolates ranged from white, light gray, dark brown to black. Their findings also revealed differences in terms of the colony textures which were smooth, rough and pits.

In the current study, the aerial mycelium of the 27 isolates varied from thick cottony, effuse to thin hairy mycelia (Fig. 1). (Meena, 2005) reported grayish black and raised mycelial growth of the rice blast pathogen isolates.

There was slight variation among isolates obtained from the Northern, Upper East and Upper West Regions. In the Northern region, seven isolates were obtained; three isolates appeared dark grey, two were light grey, one isolate was black and one isolate was creamy as shown in **Table 1a**. From the seven isolates obtained from the Northern region, five isolates were obtained from leaf samples while two were obtained from the panicles.

Table 1a: Morphological characterization of rice blast pathogen from the Northern Region

SN	Location	Variety	Plant Part	Colony Characteristics
1.	SARI A	Jasmine	Leaf	Dark grey, scanty mycelium, uneven margins and rough surface
2.	SARI B	Jasmine	Leaf	Light grey, scanty mycelium, uneven margin and rough surface
3.	Golinga A	Agra	Leaf	Black, scanty mycelium, uneven margins and rough surface
4.	Golinga A	Agra	Leaf	Dark grey, thick cottony mycelium, uneven margins and rough surface
5.	Golinga B	Agra	Leaf	Light grey, scanty mycelium, uneven margins
6.	Botanga A	Jasmine	Panicle	Dark grey, scanty mycelium, clear margins and rough surface
7.	Botanga B	Jasmine	Panicle	Creamy, scanty mycelium, clear margins and rough surface

Table 1b: Morphological characterization of rice blast pathogen from the Upper East Region

SN	Location	Variety	Plant Part	Colony Characteristics
1.	Gaani A	Agra	Leaf	Whitish, scanty mycelium, clear margins and rough surface
2.	Gaani B	Agra	Leaf	Light grey, thick cotton mycelium, uneven margins and rough surface
3.	Gaani E	Agra	Leaf	Light grey, thick cottony mycelium, uneven margins and rough surface
4.	Gaani B	Agra	Panicle	Dark grey, fluffy mycelium, uneven margins and rough surface
5.	Gaani D	Agra	Panicle	Light grey with whitish patches, scanty mycelium, uneven margins and rough surface
6.	Gaani F	Agra	Panicle	Dark grey, scanty mycelium, uneven margins and rough surface
7.	Latra 9 A	Agra	Leaf	Light grey, scanty mycelium, uneven margins and rough surface
8.	Latra 9 B	Agra	Panicle	Light grey, thick cottony mycelium, uneven margins and rough surface
9.	Kapalinia A	Agra	Leaf	Whitish, thick cottony mycelium, clear margins and rough surface
10.	Kapalinia A	Agra	Leaf	Light grey with whitish thick patches, scanty mycelium, uneven margins and rough surface
11.	Kapalinia B	Agra	Leaf	Light grey, scanty mycelium, uneven margins and rough surface
12.	Latra 6	Agra	Leaf	Black with scanted mycelium and smooth surface

The high incidence of leaf lesions could probably be due to the fact that, the rice blast pathogen was present on the seeds used for planting or the pathogen was present in the soil which infected seeds which affected the leaves of the rice crop in the Northern region under favorable conditions. Leaf blast incidence usually increases early in the season and declines late in the season https://www2.ipm.ucanr.edu/agriculture/rice/Rice-Blast/. High humidity, close to 100%, favors infection and spore formation when leaves are exposed to wet conditions for 6 – 8 hours http://www.pestnet.org/fact_sheets/rice_blast_252.htm.

Upper East region recorded the highest number of isolates 12; majority of the isolates seven in number from this region appeared light grey, two isolates looked dark grey, two others observed were white and one isolate appeared black as shown in **Table 1b.** The

Table 1c: Morphological characterization of rice blast pathogen from the Upper West Region

SN	Location	Variety	Plant Part	Colony Characteristics
1.	Sing-Bapong A	Agra	Leaf	Dark grey, thick cottony mycelium, uneven margins and rough surface
2.	Sing-Bapong A	Agra	Leaf	Light grey, thick cottony mycelium, uneven margins and rough surface
3.	Sing- Bapong B	Agra	Panicle	Black with scanted mycelium, uneven margin and smooth surface
4.	Sing- Bapong B	Agra	Panicle	White with scanted mycelium, uneven margins and smooth surface
5.	Goli A	Jasmine	Leaf	Dark grey, thick cottony mycelium, uneven margins and rough surface
6.	Goli D	Jasmine	Leaf	Dark grey, scanty mycelium, uneven margins and rough surface
7.	Goli B	Jasmine	Panicle	Dark grey, thick cottony mycelium, uneven margins and rough surface
8.	Goli C	Jasmine	Panicle	Creamy, scanty mycelium, uneven margins and rough surface

highest number of isolates from this region could be due to the ecological conditions which trigger the spread of the rice blast pathogen. Leaf lesion isolates accounted for eight whiles panicles isolates were four from the Upper East region. Similar to the Northern region, the high incidence of the leaf lesions isolates in the Upper East region, could also be due to the presence of rice blast pathogen on the seeds used for planting or the presence of the pathogen in the soil which infected the seeds and ultimately affected the leaves during the phenology of the plant.

In Upper West region, eight isolates were recorded; four isolates observed were dark grey in color, one isolate white, one isolate appeared black, one isolate looked creamy and one isolate appeared light grey. Four leaf lesion isolates and four panicle lesion isolates were obtained from this region. Similar reasons assigned for the development of lesions on leaves in the Northern and Upper East regions might be responsible for the situation in the Upper West region. The existence of slight variation among isolates from the three Northern regions with regards to the colony color was probably due to the different geographical distribution and ecological conditions which influence the development of the rice blast pathogen in the respective regions resulting in different colony development.

The conidium of the isolates under 40x of light microscope showed an oval shape, narrowed apex with 2 – 3 septate conidiophores which appeared long, slender, branched and unbranched under 40x of light microscope. (Kariaga *et al.,* 2016) isolated and described the conidia of *P. oryzae* isolates. The isolates conidia were typically pyriform with rounded base, apex narrowed, 2 - 3 septate, 2 - 4 celled, and middle cells were broader.

Fig. 1: Morphological variation of different rice blast pathogen isolates collected from the three Northern regions of Ghana

Conclusion

This research work points out morphological variation among the isolates collected from the Upper West, Upper East and Northern regions of Ghana. The findings of this work confirmed that rice grown in these areas were affected by different races of the rice blast pathogen. Management protocols must be established as a matter of urgency to enhance the productivity of rice in these regions. The development of blast resistant varieties appears a desirable option. It is recommended that further research work be carried out on the rice blast pathogen using molecular tools and the conduction of pathogenicity test would further confirm the identity of the pathogen.

Acknowledgements

The authors would like to thank the Council for Scientific and Industrial Research - Savannah Agricultural Research Institute (CSIR - SARI) for resources and facilities to carry out this research and the USAID Rice Seed Scaling Project for financial support. The authors would also like to thank Dr. Stephen K. Nutsugah (Director of CSIR – SARI), Dr. Wilson Dogbe (Head of Rice Research Program, CSIR – SARI) and Prof. Herbert Dei (Director of Academic Planning and Quality Assurance, UDS) for their immerse contributions towards this research work.

References

Abed-Ashtiani, F. Kadir, J., Selamat, A., Mohd-Hanif, A.H. & Nasehi, A. 2012.

Effect of foliar and root application of silicon against rice blast fungus in MR219 rice variety. *The Plant Pathology Journal*

28: 164–171.

Agriculture: Rice pest management guidelines. Available from: https://www2.ipm.ucanr.edu/agriculture/rice/Rice-Blast/. [October 2015].

Aneja, K.R. 2005. Experiments in Microbiology Plant Pathology and Biotechnology, New Age International Publishers, New Delhi.

Barnett, H. L., H.B.B. 1998. Illustrated Genera of Imperfecti Fungi, Burgess Publishing Company, West Virginia.

Barman, R.S. & Chattoo, B.B. 2005. Rice blast fungus sequenced. *Current Science* 89: 930-931.

Charles, J.C., Robert, B.M. & Mnyuku, S.O.W.R. 2015. Pathogenic variation and molecular characterization of *Pyricularia oryzae,* causal agent of rice blast disease in Tanzania. *Journal of Science and Research* 4(11):2319–7064.

Chipili, J., Sreenivasaprasad, S., Nutsugah, S. K., Twumasi, J.K. & Dartey, K. 2013. Rice blast management in Ghana: characterization of the pathogen diversity. *Journal of Ghana Science Association* 5: 20-25.

Kariaga, M.G., Wakhungu, J. & Hassan, K.W. 2016. Identification of rice blast pathogen (*Pyricularia grisea* cav.) races from Kenyan rice growing regions using cultural classical characterization. *Journal of Research in Agriculture and Animal Sciences* 4:16-22.

Mahboubeh, M., Ali, Y., Phuah, E.T., Zainal, A. M. A. & Wong, M. Y. 2017. Morphological and molecular characterization of rice blast fungus *Magnaporthe oryzae*. *Pakistan Journal of Agricultural Science* 54(4):765-772.

Mousanejad, S., Alizadeh, A. & Safaie, N. 2010. Assessment of yield loss due to rice blast disease in Iran. *Journal of Agricultural Science Technology* 12:357-364.

Ministry of Food and Agriculture: National rice development strategy draft. Available from: https://www.jica.go.jp/english/our_work/thematic_issues/agricultural/pdf/ghana_en.pdf. [February 2009].

Meena, B.S. 2005. Morphological and Molecular Variability of Rice Blast Pathogen *Pyricularia oryzae (Cooke) Sacc,* Dharwad University of Agricultural Sciences, Dharwad.

New Straits Times 2012, *Fungus causes padi farmers to lose RM150,000*. Available from: http://www.nst.com.my/fungus-causes-padi-farmers-to-lose-rm150-000-1.26624.

Nutsugah, S.K., Twumasi, J.K., Chipili, J., Sere, Y. & Sreenivasaprasad, S. 2008. Diversity of the blast pathogen populations in Ghana and strategies for resistance management. *Plant Pathology Journal* 7(1): 109–113.

Ou, S.H. 1985. Rice Disease. Commonwealth Agricultural Bureau International Mycological Institute, United Kingdom.

Pacific Pests and Pathogens – Facts Sheets. Available from: http://www.pestnet.org/fact_sheets/rice_blast_252.htm.

Piotti, E., Rigano, M.M., Rodino, D., Rodolfi, M., Castiglione, S., Picco, A.M. & Sala, F. 2005.Genetic structure of *Pyricularia grisea* (Cooke) Sacc. isolates from Italian paddy fields. *Journal of Phytopathology* 153: 80-86.

Ricepedia, The online authority on rice. Available from: http://ricepedia.org/rice-as-a-crop/rice-productivity.

Rayner, R.W. 1970. CMI and British Mycological Society, Amycological Color Chart, Kew, Surrey, England.

The importance of rice. Available from:

www.knowledgebank.irri.org/ericeproduction/importance_of_Rice.htm.
Wang, G.L. and Valent, B. 2009. Advances in genetics, genomics and control of rice blast disease. *Springer Verlag* 430-435.