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Screening of upland-rice landraces for resistance to rice blast disease (*Magnaporthe oryzae*)

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Abstract

Rice blast disease (*Magnaporthe oryzae*) is one of the major biotic factors limiting rice production. An experiment was conducted in four locations to screen and identify upland rice accessions that are resistant to *Magnaporthe oryzae*. 25 upland rice landraces were collected and screened against *Magnaporthe oryzae* by artificial inoculum under the field condition in four locations for two cropping seasons. The experiments were laid in a Randomized Complete Block Design with three replications across the locations. Data were collected on grain yield, the weight of 1000 seeds, numbers of tiller per plant, panicle weight, grains per panicle, and plant height. Disease scoring was done using the Standard Evaluation System of the International Rice Research Institute and analyzed with IRRI STAR software. The screening result revealed that none of the 25 prominent upland rice landrace accessions is highly resistant or resistant. Five accessions; Acc. 1, Acc. 2, Acc. 7, Acc. 10, and Acc. 22 were moderately resistant, 11 accessions were moderately susceptible while eight accessions were susceptible. None of the accessions screened is highly susceptible to the pathogen. All moderately resistant accessions that gave high grain yield could be used as a gene pool for *Magnaporthe oryzae* resistance breeding for cultivar improvement except accession 22 that recorded a low grain yield. The use of the identified moderately resistant accessions with high yield by farmers is encouraged pending the time commercial *Magnaporthe oryzae* resistant cultivars will be readily available and accessible in rainforest and derived guinea savanna agro-ecological zones of Nigeria.

Keywords: Accession, Inoculum, *Magnaporthe oryzae*, Resistance, Rice grain

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Introduction

Rice (*Oryza sativa* L.) is a cereal crop that is commonly cultivated across the world (Shahriar et al., 2020). About 50% of the world's human population relies on rice grain as their source of staple food in different processed forms (Ghimire et al., 2019; Cavite et al., 2021). Rice is widely grown in all the agro-ecological zones of Nigeria (Idowu et al., 2013). Almost a billion households depend on rice production, processing, distribution, or marketing for their livelihood across the world (Asante et al., 2019). Rice grains are processed domestically and industrially for human consumption and the by-products are used as livestock feeds and energy sources. Some leguminous grain such as cowpea, African yam bean, pigeon pea, lima bean is cooked with rice or maize to enhance the protein content and taste. According to Thirze (2016), the annual shortage of rice grain production will continue to rise globally from 400,000 tons in 2016 to 800,000 tons by 2030 if appropriate measures in controlling this shortage were not taken. In Nigeria, rice grain production is lesser compare to its demand (Agbowuro et al., 2020a). Rice grains production rate was estimated to be four million metric tons in Nigeria in the year 2018 while its consumption rate was seven million metric tons, thereby rice grains importation becomes unavoidably necessary to fill the demand and supply gap (Kamai et al., 2020; Agbowuro et al., 2021). The high cost of import duty on rice grain resulted in to increase in the price of the commodity thereby making the commodity not easily affordable for the poor citizens. According to Agbowuro et al. (2020b) rice is no longer affordable to the low-class citizens in Nigeria because of the high cost. Increasing domestic rice production for food security enhancement, factors affecting its production and processing stages should be pointed out and sustainable solutions should be provided.

Different biotic and abiotic factors were responsible for the low rice grain yield globally especially in developing nations where modern farming techniques is not available. Poor harvesting, processing, and storage, and pests, insects and rodents attack on the crop on the field and in the store also contributed to rice shortage in Nigeria. Miah et al. (2013) reported that rice blast disease caused by *Magnaporthe oryzae* is the most dangerous and destructive among the biotic factors limiting rice grain production. *Magnaporthe oryzae* could cause about 70% or more rice grain losses, however farmer could experience a total yield loss during an epidemic growing season (Prasad et al., 2006; Dean et al., 2012). *Magnaporthe oryzae* infect the aerial plant parts such as leaves, internodes, nodes, neck, panicle, and seeds (Idowu et al., 2013). Leaves are the rice plant part that is mostly affected by blast disease but the disease becomes more destructive when it attacks rice plant nodes, internodes, and neck. Whenever rice leaves are infected with rice blast pathogen (*Magnaporthe oryzae*), the leaf area that should available for photosynthesis become reduce, hence carbohydrate assimilation is limited. Thus, the quantity of the grain yield per plant produced will be reduced (Agbowuro et al., 2020a). Blast pathogen (*Magnaporthe oryzae)* disorganize infected plant tissues and disrupts the movement of nutrients and water within the plant system (Zhu et al., 2005). Ram et al. (2007) explained that the attack of rice blast rice plant nodes, internodes, and neck may result in partial or complete sterility. Severely infected rice plant internodes or neck becomes weak that could not support the rice panicle, hence the rice panicle will fall off. Whenever the rice pedicles are infected with the rice blast pathogen, the development of the grains may fail. Most farmers don't know the extent of damage caused by rice blast disease on their rice field because the effect is indirect, thus they are less concern in controlling the disease most especially leaf and nodes blast.

Rice blast diseases are mostly managed by the use of fungicides that are not only expensive but not eco-friendly by the rice farmers. These fungicides are detrimental to human and livestock health. Cultivation of cultivars that are resistant to the fungus, adoption of good agronomic practices, the use of biological control agents, biotechnological approaches, and nutritional management or combination of any of these methods are good measures in combatting the disease (Agbowuro et al., 2020a; Ribot et al., 2008). The cost of controlling the disease increases the cost of rice production thereby increasing the cost of rice grain in the market. Growing blast disease-resistant rice cultivars is safe, economical, and ecofriendly (Akos et al., 2021). The objective of this research work is to screen upland rice landrace accessions morphologically and identify the accession(s) that are resistant to rice blast disease (*Magnarporthe oryzae*) in rainforest and derived guinea savanna agro-ecological zones of Nigeria to be used for further breeding work for crop improvement.

Materials and Methods

The study locations

This research work was carried out at the Department of Microbiology Laboratory, Elizade University, Ilara-Mokin, Nigeria, and four field locations; Biological Garden of Elizade University, Ilara Mokin, Ekiti State University Teaching and Research Farm, Ado-Ekiti, Oke-Ako/Irele Farm Settlement, Oke-Ako Ekiti, Royal Farm, Oro all in Nigeria in the early cropping season of 2019 and 2020. Ilara-Mokin and Ado-Ekiti lies in rainforest agro-ecological zone, while Oke Ako-Ekiti and Oro falls in derived guinea savanna. Soil samples were randomly taken with a soil auger at the depth of 0-15cm from each of the research locations for the two years. The soil samples were packed separately and well labeled to avoid mixed up for analysis. The soil samples were analyzed at the laboratory of the Department of Environmental and Toxicology Management of Elizade University, Ilara-Mokin, Nigeria.

Experimental materials

The experimental materials used for this research work comprised 25 upland rice landrace accessions collected in some agro-ecological zones of Nigeria (Table 1). The pathogen, *Magnaporthe oryzae* was isolated from infected rice plant leaves and nodes showing typical symptoms of the fungus from the rice field of Ekiti State Ministry of Agriculture and Rural Development at Oke-Ako Farm Settlement, Oke-Ako Ekiti, Nigeria.

Isolation, purification of the *Magnaporthe oryzae* inoculum and its pathogenicity test

Infected rice plant leaves and nodes showing typical symptoms of *Magnaporthe oryzae* collected from the rice field of Ekiti State Ministry of Agriculture and Rural Development at Oke-Ako Farm Settlement, Oke-Ako Ekiti were chopped into small pieces (0.4-1cm) with a sterilized knife and surface sterilized with 0.1 percent mercuric chloride for 30 seconds and later washed with sterilized water. The water in the sliced and sterilized *Magnaporthe oryzae* infected rice leaves and nodes were allow to drained and transferred into potato dextrose agar (PDA) medium in a Petri dishes aseptically. Streptomycin (40 μgl^{-1}) was added to the Petri dishes to avoid contamination with bacteria before pouring the potato dextrose agar. The PDA Petri dishes were incubated for five days at 28 ± 2 ºC. After three days of inoculation, radiating mycelia growth was seen at the edges of the infected bits and the edge of the fungal colonies were carefully transferred to PDA medium slants in a refrigerator at 10ºC and were periodical sub-culturing for the studies. The pathogen was identified as *Magnaporte oryzae* based on its morphological and cultural characteristics i.e pyriform to oblong conidia which are hyaline in colour and biseptate measuring 19-27 X 8-10 µm in size (Tuite, 1969; Namrata et. al., 2019). Koch's postulates were used to establish the pathogenicity of the isolates. The agar slants and Petri plates containing *Magnaporte oryzae* inoculum were stored at 5^0C for further use (Harlapur et al., 2007).

Field Experimental design and cultivation condition

The experiment work was laid out in a Randomized Complete Block Design (RCBD) with three replicates in four locations, Biological Garden of Elizade University, Ilara Mokin, Ekiti State University Teaching and Research Farm, Ado-Ekiti, Oke-Ako/Irele Farm Settlement, Oke-Ako Ekiti, and Royal

Farm,Oro. The seeds collected from different sources were put to germination test to confirm their viability. None of the rice accession has lesser than 90% germination percentage. The rice seeds were planted in-situ across the four locations for the two cropping seasons on a well-tilled and leveled field when the rain had become steady. The spacing of 15 cm by 20 cm was adopted at three stands per hill and later thinned to two stands per hill two weeks after planting (Azgar et al., 2018). Each of the accessions was raised in $8m^2$ plots. Weeding was carried at 2, 6, 10, 14 weeks after planting manually. The crop was netted at flowering stage to prevent birds. The experimental sites surrounding was made clean to scare grass cutters.

Table 1 List of 25 upland rice landrace accessions screened for resistance to rice blast disease and their source of collection

Accession Code	Source of collection	Accession Code	Source
Acc. 1	Abakaliki, Ebonyi State	Acc. 14	Minna, Niger State
Acc. 2	Abeotuta, Ogun State	Acc. 15	Mokua, Niger State
Acc. 3	Alero, Kebbi State	Acc. 16	Okenne, Kogi State
Acc. 4	Ayingba, Kogi State	Acc. 17	Oktupo, Benue State
Acc. 5	Dangi, Plateau State	Acc. 18	Omu-Aran, Kwara State
Acc. 6	Gunki, Nasarawa State	Acc. 19	Owo, Ondo State
Acc. 7	Igbemo, Ekiti State	Acc. 20	Patiji, Niger State
Acc. 8	Igbokoda, Ondo State	Acc. 21	Sokoto, Sokoto State
Acc. 9	Ijube ode, Ogun State	Acc. 22	Uromi, Edo State
Acc. 10	Ikole-Ekiti, Ekiti State	Acc. 23	Zango, Katsina State
Acc. 11	Jalingo, Taraba State	Acc. 24	Zaria, Kaduna State
Acc. 12	Kaduna, Kaduna State	Acc. 25	Zongo, Bauchi state
Acc. 13	Markurdi, Benue State		

Inoculum preparation and inoculation

The isolated and purified *Magnaporthe oryzae* inoculum stored at 5^0C was re-cultured in PDA medium. Miura et al (2005) procedure was adopted in the preparation of conidial suspension. The inoculated plates were incubated at 26 ± 10 °C for 12 to 14 days in the dark. For the inducement of heavy sporulation, the culture was scraped aseptically with a sterilized toothbrush and the plates were exposed to near-ultraviolet light at 25±10C for 10 days. Dislodging of conidia was achieved by gently rubbing a small-sized sterilized toothbrush from the incubated plates to sterilized distilled water. The conidial suspension was welled filtered through layers of gauze mesh (aperture 300 lm) and concentration was adjusted to a final concentration of 1 x 106 spores per ml using a haemocytometer. Tween 20 was added to gelatin (0.02% Tween 20 in 0.25% gelatin) to the prepared suspension to enhance a proper adherence of conidia to the rice aerial parts (Jia et al, 2003).

The rice plant leaves were inoculated 20 days after planting by spraying the prepared 1 x 10^6 spores per ml of conidial suspension containing 0.02% Tween 20 in 0.25% gelatin per plot using a knapsack sprayer. Spraying was done slowly and carefully to achieve uniformity on the plant aerial parts until runoff. The inoculum was sprayed around 18:00 hours of the day and ensured that the entire rice plant surface became wet with conidial suspension and left overnight. To maintain high humidity in the rice field after inoculation, water was sprayed with knapsack sprayer on the rice plants six times at three hours intervals after 15 hours of inoculation.

Disease assessment, data collection and analysis

Disease scoring of the inoculated rice plants was done 10 days after inoculation (Challagulla et al., 2015). The severity of the disease was estimated and recorded by using the disease rating scale of the Standard Evaluation System of the International Rice Research Institute, Philippine based on the level of severity of the infection on each entry (International Rice Research Institute [IRRI], 2013). Based on leaf blast scores assessment, the accession was categorized as highly resistant (0), Resistance (1), moderately resistant (2-3), moderately susceptible (4-5), susceptible (6-7), and highly susceptible (8-9) (Standard Evaluation System of IRRI, 2013) as shown in Table 2. Data were collected on grain yield per plant (g), the weight of 1000 seeds (g), number of tiller per plant, panicle weight per plant (g), filled grain per panicle, and plant height (cm). Data were collected from 25 randomly selected rice plants on a plot basis for disease incidence and disease severity. The 25 rice plants were tagged and well labelled to avoid mixed up and confusion. The data collected was analyzed using IRRI STAR software. Means were separated by Duncan's multiple range test (DMRT) (P = 0.05).

Table 2 Disease rating scale 0-9 by International Rice Research Institute, Phillipines (IRRI, 2013)

Grade	Disease severity	Host response
0	No lesion observed	Highly Resistant
1	Small brown specks of pin point size	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves	Moderately Resistant
3	Lesion type same as in 2, but significant number of lesions on the upper leaves	Moderately Resistant
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area	Moderately Susceptible
5	Typical susceptible blast lesions of 3mm or longer infecting 4-10% of the leaf area	Moderately Susceptible
6	Typical susceptible blast lesions of 3 mm or longer infecting 11-25% of the leaf area	Susceptible
7	Typical susceptible blast lesions of 3 mm or longer infecting 26-50% of the leaf area	Susceptible
8	Typical susceptible blast lesions of 3 mm or longer infecting 51-75% of the leaf area many leaves are dead	Highly Susceptible
9	Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected	Highly Susceptible

Results and Discussion

Soil analysis

The soil physical and chemical properties of the experimental sites are shown in Table 3. The soils in all the locations were slightly different from one another. The soil pH value at each of the experimental sites ranges from 5.00- 5.90 across the four locations for the two year. The pH values recorded in the research sites shown that the soils were slightly acidic and soil-plant nutrients will still be readily available for plant roots uptake (Golla, 2019). The soil organic matter, carbon content, and nitrogen were presented in percentage in the result. The soil organic matter in the soils ranges from 1.30-1.88, carbon content ranges from1.20-1.50 while the nitrogen level ranges from 1.04-1.58 across the locations. Soil organic matter is very important in plant response to soil nutrient uptake particularly nitrogen and phosphorus (Olugbemi et al., 2018). The result revealed that the soils across the locations were sandy loam.

Table 3 Physical and chemical characteristics of the soils in the experimental sites

Properties	Locations Year I				Locations Year II			
	Oke-Ako-Ekiti	Ilara-Mokin	Ado-Ekiti	Oro	Oke-Ako-Ekiti	Ilara-Mokin	Ado-Ekiti	Oro
Sand (%)	60	62	56	59.5	62	64	60	61
Clay (%)	19	18	23	18	18	19	20	19
Percentage silt	21	20	21	22.5	20	17	20	20
Textural class	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
pH	5.80	5.90	5.55	5.78	5.10	5.00	5.04	5.81
Carbon (%)	1.50	1.20	1.29	1.47	1.20	1.00	1.49	1.52
Organic matter (%)	1.88	1.60	1.64	1.76	1.58	1.30	1.58	1.83
Nitrogen (%)	1.53	1.24	1.46	1.34	1.23	1.04	1.56	1.45
Phosphorus (mg kg $^{-1}$)	10.62	9.83	12.54	11.6	10.12	9.23	13.00	11.9
Ca^{2+} (C mol kg^{-1})	1.70	1.45	1.92	1.65	1.40	1.15	1.20	1.89
Mg^{2+}(C mol kg^{-1})	0.70	0.68	0.84	0.81	0.60	0.61	0.93	0.71

Disease assessment

Leaf blast severity recorded for 25 upland rice landrace accessions against rice blast disease is shown in Table 4 and 5. The result showed that none of the accessions screened was resistant or highly resistant to rice blast disease according to the Standard Evaluation System of International Rice Research Institute, 2013. Accession 1, 2, 7, 10, 22 were found to be moderately resistant against rice blast disease while eleven accessions were found to be moderately resistant. Nine accessions were susceptible to the disease and none of the accession is highly susceptible. The moderate resistant accessions can be exploited in a breeding programme for the development of blast disease-resistant for commercial cultivars after a proper genetics analysis has been done and their agronomic traits have been found desirable. Haq et al. (2002) gave a similar report when they screened twenty-five rice varieties and discovered that is only two lines were highly resistant. Mohanta et al. (2003) screened 32 rice accessions; three accessions were highly resistant, 12 were resistant, 16 moderately susceptible. The severity of rice blast disease is well pronounced in a low temperature and high humidity environment (Sarinya et al., 2021). The research stations agro-ecological zones are characterized by low temperature and high relative humidity. This could be the reason for low resistance of the crop to the disease. Rice blast disease was ranked as the most destructive rice disease limiting rice grain yield due to its high occurrence in a favourable environment. Identification of resistant upland rice accessions is one of the strategies to adopt in reducing the cost of rice production by inquiring agrochemicals in controlling the diseases and increase the rice grain yield per unit area. Screening and identification of rice blast disease donors from upland rice landraces accessions is highly essential in the development of cultivars that are resistant to the diseases. The development of rice varieties that are resistant to *Magnarporte oryzae* involves the integration of several resistance qualitative and quantitative genes into individual cultivar (Joshi et al., 2009).

Table 4 Leaf blast severity recorded across the four locations for two cropping seasons using Standard Evaluation System of International Rice Research Institute, 2013

Accession Code	Reaction to leaf blast (0-9 scale)	Disease Reaction	Accession Code	Reaction to leaf blast (0-9 scale)	Disease Reaction
Acc. 1	3	Moderately Resistant	Acc. 14	5	Moderately Susceptible
Acc. 2	3	Moderately Resistant	Acc. 15	7	Susceptible
Acc. 3	6	Susceptible	Acc. 16	6	Susceptible
Acc. 4	5	Moderately Susceptible	Acc. 17	5	Moderately Susceptible
Acc. 5	6	Susceptible	Acc. 18	4	Moderately Susceptible
Acc. 6	5	Moderately Susceptible	Acc. 19	6	Susceptible
Acc. 7	3	Moderately Resistant	Acc. 20	6	Susceptible
Acc. 8	7	Susceptible	Acc. 21	4	Moderately Susceptible
Acc. 9	6	Susceptible	Acc. 22	3	Moderately Resistant
Acc. 10	3	Moderately Resistant	Acc. 23	4	Moderately Susceptible
Acc. 11	7	Susceptible	Acc. 24	5	Moderately Susceptible
Acc. 12	4	Moderately Susceptible	Acc. 25	4	Moderately Susceptible
Acc. 13	4	Moderately Susceptible			

Table 5 Summary of upland rice landrace accessions screened against *Magnaporthe oryzae* across the four locations for two cropping seasons according to Standard Evaluation System of International Rice Research Institute (2013)

(SES-IRRI Score rating)	Disease reaction	Accessions
0	Highly Resistant	-
1	Resistant	-
2-3	Moderately Res istant	Acc. 1, Acc. 2, Acc. 7, Acc. 10, Acc. 22.
4-5	Moderately Susceptible	Acc. 4, Acc. 6, Acc.12, Acc. 13, Acc. 14, Acc. 17,Acc. 18, Acc. 21, Acc. 23, Acc. 24, Acc. 25
6-7	Susceptible	Acc. 3, Acc. 5, Acc. 8, Acc. 9, Acc. 11, Acc. 15, Acc. 16, Acc. 19, Acc. 20.
8-9	Highly Susceptible	-

Table 6 presents mean grain yield and some other yield components of upland rice landrace accessions screened against rice blast disease across four locations for two cropping seasons. Accession 1 (14.63) had the highest mean grain yield followed by accession 15, 7, 2, 13,23,10,16 with the mean grain yield of 14.43,14.02, 13.13, 12.13, 12.16, 12.20, and 12.00 grams per plant respectively while accession 22 had the least mean grain yield followed by accession 19, 3, and 14 respectively. All the accessions that were moderately resistant to rice blast disease recorded high mean grain yield and other yield component traits except accession 22. The high mean grain yield recorded in the accessions that are moderately resistant to rice blast disease could be as a result of lesser lesions on the leaves thereby increasing the leaf area available for photosynthesis, hence the grain yield tends to increase (Agbowuro et al., 2019).

These landrace accessions could be utilized as parent materials for the development of heterotic upland rice hybrids with rice blast resistance and higher grain yield. Accession 15 was susceptible to rice blast but it gave a high mean grain yield (15.43). The high grain yield potential in accession 15 could be incorporated into the accession that is moderately resistant to rice blast disease with lesser grain yield through hybridization and later backcrossed for the development of new high yield and blast resistance cultivars for commercialization.

Table 6 Mean grain yield and some other yield components of upland rice landrace accession screen against rice blast disease across the four locations for two cropping seasons

Accession Code	GY/P (g)	W1000S (cm)	NT/P	PW/P (g)	G/P	PH (cm)
Acc. 1	14.63	42.62	7.92	4.58	60.83	134.5
Acc. 2	13.13	32.38	3.79	4.01	54.46	98.5
Acc. 3	9.28	40.69	6.96	4.17	61.41	100.5
Acc. 4	11.18	34.33	3.71	3.89	49.60	105.4
Acc. 5	10.10	35.52	6.25	3.99	48.14	112.5
Acc. 6	10.09	38.55	5.78	3.50	47.00	96.78
Acc. 7	14.02	37.96	3.93	3.00	46.12	94.5
Acc. 8	11.43	38.00	4.46	2.62	45.12	120.56
Acc. 9	10.10	39.29	5.62	3.34	48.18	118.9
Acc. 10	12.20	40.97	8 .21	2.45	44.31	96.5
Acc. 11	11.40	36.49	6.23	4.01	56.80	108.5
Acc. 12	10.83	38.27	4.89	2.89	50.01	87.9
Acc. 13	12.13	33.58	4.62	7.23	53.45	98.6
Acc. 14	9.50	32.02	2.95	2.34	53.85	124.6
Acc. 15	14.43	39.06	5.88	5.03	57.67	117.8
Acc. 16	12.16	37.39	4.93	2.63	51.84	120.5
Acc. 17	11.09	35.88	3.20	5.45	53.21	114.8
Acc. 18	10.80	29.22	3.92	4.88	46.80	89.7
Acc. 19	9.06	35.91	3.05	3.24	48.90	91.4
Acc. 20	11.25	34.31	7.19	6.93	61.27	106.9
Acc. 21	9.80	37.16	3.25	3.99	49.78	109.6
Acc. 22	8.80	39.23	3.37	2.50	41.45	111.18
Acc. 23	12.00	33.50	4.97	3.18	50.23	138.9
Acc. 24	11.60	34.23	5.89	1.89	48.79	120.5
Acc. 25	10.8	33.48	2.04	2.67	44.40	85.9

GY/P = Grain yield per plant; W1000S = Weight of 1000 seeds; NT/P = Number of tiller per plant, PW = Panicle weight; G/P = Grain per panicle; PH = Plant height.

Conclusion

This present research work revealed that none of the upland rice landrace accessions grown in the prominent rice-growing area in different part of Nigeria were highly resistant or resistant to rice blast disease under field artificial inoculation in rainforest and derived guinea savanna agro-ecological zones for the period of two cropping seasons. Only four accessions of the 25 accessions screened were found to be moderately resistant. One of the four moderately resistant accessions gave low grain yield. The moderately resistant accessions with high mean grain yield could be exploited in disease resistance breeding programs using plant breeding techniques for the development of varieties and hybrids that are resistant to the blast disease with high yield for sufficient rice production. Farmers should be growing the accessions that are moderately resistant accessions in Southwestern Nigeria to reduce the cost of production and increase yield pending the time rice blast disease rice varieties will readily available and affordable.

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