

Screening of rice germplasm for blast resistance in Nigeria

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Abstract. *Gabriel MG, Alhasan U, Mary Y, Munsur Y, Olufunmilayo A. 2022. Screening of rice germplasm for blast resistance in Nigeria. Asian J Agric 6: 1-6.* Rice blast (*Magnaporthe oryzae* T.T. Hebert) M.E. Barr) is an important destructive disease of rice that can lead to 80% yield loss. Germplasm responds differently to blast fungus. This study aimed to screen rice germplasm for blast resistance in Nigeria. The four genotypes, namely Institute for Research in Tropical Agriculture, France (IRAT) 109, JAMILA, Federal Agriculture Research Oryza (FARO) 52, and FARO 66, were evaluated in a completely randomized design with three replications in the screen house of the Department of Crop Protection, Institute for Agricultural Research Samaru, Nigeria. Data were collected on plant height, the number of plants infected with a blast, seedling vigor, tillering ability, blast disease score, and leaf blast estimated. Analysis of variance showed a highly significant difference ($P \leq 0.01$) for seedling vigor (0.03**) and disease index (17.24**), while significant ($P \leq 0.05$) variation was observed for a number of the leaf (3.79*). In contrast, there was no significant ($P > 0.05$) variation for plant height and tillering ability. The highest PCV (Phenotypic Coefficient of Variance) and GCV (Genotypic Coefficient of Variance), also broad-sense heritability, were observed in leaf blasts. IRAT 109 (0.6) depicted a high resistance, JAMILA moderately susceptible (Blast score 4.0), while FARO 52 (7.3) and FARO 66 (6.1) were susceptible. A significant difference among genotypes implies sufficient variation among the genotype screened, suggesting that progress can be made following selection.

Keywords: *Magnaporthe oryzae*, *Oryza sativa*, resistance, rice, screening

INTRODUCTION

Rice (*Oryza sativa* L.), with a genome size of 430 Mb ($2n = 24$), is the most widely consumed staple food for a large part of the world's human population (Amanullah et al. 2016; Perera and Dahanayake 2016). It may have originated in China and is now cultivated worldwide (Smith 2006). Global rice production is estimated to be 755.5.0 million tons per year, harvested from 162.1 million ha in more than 100 countries, with average productivity of 4.7 tons/ha (FAO 2019). In Africa, 14.2 million ha and 17.1 million ha of the land area were cultivated, with 33.2 million tons and 38.5 million tons harvested in 2018 and 2019, respectively, with similar productivity of 2.3 tons/ha. For example, Nigeria had rice production on a land area of 3.3 million ha and 5.3 million ha, with 6.8 million tons and 8.4 million tons harvested and a productivity of 2.03 tons/ha and 1.6 tons/ha in 2018 and 2019, respectively (FAO 2018; 2019).

Rice is an important staple food crop for more than half of the world's population, and it provides 27% of the calories in low and middle-income countries (Patil and Sharanagouda 2017; Susanto et al. 2017; Estiati 2019; Weerakoon and Somaratne 2020). Therefore, yield loss of rice production represents a significant threat to food security. Furthermore, it is stated that rice production must increase by 40% in 2030 to meet the ever-increasing demand (Khush et al. 2001). Hence population is increasing at an alarming rate, making food security a major challenge in the future.

Disease and pests are among the most important limiting factors that affect rice production. More than 70 diseases carried by fungi, bacteria, viruses, or nematodes have been reported on rice, and in severe cases, these losses could be up to 70-80% in some rice ecosystems (Deepak and Prasanta 2017). Among rice diseases, a blast is one of the most devastating worldwide (Fahad et al. 2019). Its wide destructiveness under conducive conditions results in yield loss ranging from 1-50% in various environmental conditions (Skamnioti and Gurr 2009). It is indicated that the genetic control of blast resistance is complex due to major and minor genes with complementary or additive effects and their environmental interactions (Zewdu et al. 2018).

Though chemical control has been successful, it adds to the cost of cultivation and contaminates the environment (Nalley et al. 2016). Deployment of host plant resistance genes is considered the best option for managing the disease, most of which are distributed in clusters (Raboin et al. 2016; Bano et al. 2017). The rapid changes in the virulence characteristics of the blast population raise a continuous threat to the effectiveness of existing blast-resistant varieties. Moreover, for the control of blast, breeding resistant varieties is an effective approach to reduce the use of pesticides and minimize rice losses due to this disease.

Continuous studies on blasts are important to overcome this disease and sustain rice production in the future.

Therefore, this study aimed to screen four genotypes of rice germplasm for blast resistance in Nigeria.

MATERIALS AND METHODS

Study site

The research was conducted in 2019 at the Institute for Agricultural Research (IAR) screen house, Ahmadu Bello University Zaria, Kaduna State, located in Samaru on 11°11'N, 7°38'E and 686 m above sea level in the Northern Guinea Savannah Ecological Zone of Nigeria. The area's average annual rainfall is about 1,058 mm, distributed within 160 days (Olanuga 1979).

Plant materials

The plant materials comprised of four rice genotypes obtained were Institute for Research in Tropical Agriculture, France (IRAT) 109, Federal Agriculture Research Oryza (FARO) 52, and FARO 66. In addition, those genotypes were obtained from African Rice (International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria), and JAMILA was obtained from the Zaria. The Rice blast resistance of each genotype is presented in Table 1.

Procedures

Fungal isolation

Plant samples were collected from a rice blast-infested field in Dogarawa, Bomo Village, and Samaru Kaduna State, Nigeria. Diseased leaves and nodes of rice panicles were placed on wet filter papers in a Petri dish for sporulation (Ou 1985).

Media preparation

Potato Dextrose Agar with streptomycin (PDAs) growth media was used. First, 200 g of sliced peeled potatoes were weighed in 1 l of water and boiled for 30 minutes. It was then filtered out, and 20 g of agar and 20 g of Dextrose were weighed into the solution and mixed well. The media was then autoclaved at 121°C for 15 minutes and left to cool to about 40 °C. It was then dispensed into sterile Petri dishes with a diameter of 9 cm (Ou 1985).

Leaf preparation

Infected leaf samples were cut into small portions, and sodium hypochlorite was added to them for three minutes and rinsed three times with distilled water (Ou 1985).

Culturing

Leaf samples were placed on the media in a petri-dish in the microflow chamber. Then the samples were taken to an incubator for observation of blast and viewed under a microscope (7-14 days) (Ou 1985).

Inoculation

The inoculum was harvested from the cultured plate and blended (mixed) in 200 mL of water, and the solution was sieved using a muslin cloth. River sand was sieved and sterilized using the oven. It was then used to create injury (rubbing) on the leaf's surface to aid in proper penetration

of the inoculum. Next, the inoculum was sprayed on the surface of the plant leaf. And the residue was also used to inoculate the soil in the screen house. The inoculum's strength was determined using a hemocytometer (Gowrisri et al. 2019).

Spore storage and count

The cultured pathogen was subcultured into a McCartney bottle using a sterile picking pin. It was then kept for further use not to lose the pathogen (Gowrisri et al. 2019). The inoculum's strength was determined using a hemocytometer, and the spore count (50,000 spores/mL) was calculated (Smith et al. 1988).

Screening of genotype of rice

The four rice genotypes were screened for blast disease during the dry season of 2019 (February-March). The genotypes were raised in a completely randomized design with three replications in a 14 cm diameter wide and 12 cm deep pot. Five seeds of each of the materials were planted in 2 rows of 5 pots each. FARO 52 Plants were used as spreaders and inoculated with conidia harvested from the mycelia of a *Magnaporthe oryzae* (T.T. Hebert) M.E. Barr isolate. The test material (IRAT 109) surrounded six stands of susceptible rice cultivars as spreader rows. At the fourth-leaf stage (3-4 weeks after sowing), the seedlings were sprayed with spores of *M. oryzae* with about 30-40 mL of the spore suspension of the blast pathogen, and soil inoculation was done alongside the leaf inoculation. Water was sprayed 3-4 times a day to maintain high humidity. Inoculated seedlings were monitored to develop blast lesions (Gowda et al. 2015). The disease reaction of each genotype was recorded after 30 days of inoculation, following a standard 0-9 scale (SES IRRI 2013) (Table 2). In addition, data were collected on plant height (cm), seedling vigor (scale of 1-9), tillering ability (1-9), number of leaves affected, and leaf blast (1-9) as described by SES IRRI (2013).

Data analysis

The agronomical and physiological data collected were subjected to analysis of variance (ANOVA) using the General Linear Model procedure of the Statistical Analysis System (SAS 2002). Fisher's protected least significant difference (LSD) test was used for comparison.

Randomized complete block design (RCBD) linear model: $y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$

Where,

y_{ijk} = Response of the experimental i^{th} treatment unit with the j^{th} replicate and k^{th} block.

μ = The overall mean

α_i = Effect of treatment

β_j = The Effect of block j

e_{ijk} = Random error

i = Number treatment unit;

j = Number of replication;

k = Number of block (Kittiwat et al. 2018).

Table 1. Description of four rice genotypes studied (Toungos 2016)

Characters	Genotype			
	IRAT 109	FARO 52	FARO 66	JAMILA
Rice blast resistance	Resistance	Highly susceptible	Susceptible to blast	Moderately Susceptible to blast
Medium maturity	Medium maturity between 90-100 days	Medium Maturity (100-110)	Medium Maturity (100-110)	Medium maturity between 90-100 days
Iron toxicity	Not resistant to toxicity	Resistant to Iron toxicity	Moderately tolerant to iron toxicity	Not resistant to iron toxicity
Yield	Medium Yielding	High Yielding	High Yielding	High-yielding landrace
Grain	Fat white grain	Moderately long white-grain	Medium, slender grains	Long White-grain
Submergence	-	Susceptible	Tolerant	-

Table 2. Description of the standard evaluation system scale for rice blast disease scoring (SES IRRI 2013)

Grade	Disease severity	Host response
0	Please say something	Highly Resistant
1	Small brown specks of pinpoint 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 is the same as in 2, but a 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

Phenotypic and genotypic variability

The phenotypic and genotypic coefficient of variation were estimated according to Burton and Devane (1953) as follows:

$$\sigma_g^2 = \frac{MS_g - MS_e}{r}$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

Where:

$$\sigma_g^2 = \text{Genotypic variance}$$

$$\sigma_p^2 = \text{Phenotypic variance}$$

$$\sigma_e^2 = \text{error variance}$$

$$MG_g = \text{Mean squares of genotypes}$$

$$MS_e = \text{Mean square due to error}$$

$$r = \text{number of replications}$$

$$PCV = \frac{\sqrt{\sigma_p^2}}{X} \times 100$$

$$GCV = \frac{\sqrt{\sigma_g^2}}{X} \times 100$$

Where:

PCV = Phenotypic coefficient of variation

GCV = Genotypic coefficient of variation

X = Grand mean value of the trait

Heritability

Heritability in a broad sense (h_b^2) for five characters was computed using the formula adopted by Allard (1960) as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where:

h_b^2 = heritability in a broad sense

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

RESULTS AND DISCUSSION

Analysis of variance

The result of the analysis of variance (ANOVA) revealed highly significant ($P < 0.01$) variation for seedling vigor, number of infected leaves, and leaf blast. On the other hand, no significant ($P < 0.05$) difference was depicted by plant height and tillering ability (Table 3).

Estimated variance component

The result of the estimated variance component for genotypic variance range from 0.01 (seedling vigor) to 8.52 (leaf blast), while phenotypic variance range from 0.01 to 11.80 (plant height) (Table 4). The PCV value computed for the five traits ranged from 12.83 for seedling vigor to 65.24 for leaf blast, while GCV ranged from 5.93 tillering ability to 64.88 for leaf blast. The value of the phenotypic coefficient of variation was generally slightly higher than the corresponding genotypic coefficient of variation for all traits studied. High GCV was observed for leaf blast (64.88) and a number of the leaf (59.41), while moderate GCV was observed for seedling vigor (10.14). High PCV was observed for leaf blast (65.24) and a number of the leaf (61.21) while tillering ability (27.90), seedling vigor (12.83), and plant height (13.54) showed moderate PCV (Table 4).

Heritability in a broad sense

Broad sense heritability (Hb) estimates the total contribution of genetic variance to total phenotypic variance ranging from 4.52 tillering ability to 98.88 leaf blast. The heritability was high for leaf blast (98.88), the number of the leaf (94.20), and seedling vigor (62.50), which might be due to environmental influence on the expression of the traits. On the other hand, low heritability in the broad sense was observed for plant height (21.54) and tillering ability (4.52) (Table 4).

Mean performance of genotypes

Plant height has a mean of 25.38 and ranges from 23.88-29.25; seedling vigor has a mean of 0.90 and ranges from 0.8-1.0; tillering ability has a mean of 10.30 and ranges from 6.10-12.30, several leaves have a mean of 2.26 and ranged from 2.10-3.60 while leaf blast has a mean of 4.50 and ranged from 0.60-7.30 (Table 5). The significant difference for the traits studied at the 5% probability level was further confirmed by the mean comparison test using the respective LSD values. The mean performance indicated the different responses to the blast as there was variation.

Discussion

The average Nigerian rice productivity is still very low compared to other rice-producing countries worldwide. That is mainly due to insufficient improved rice varieties, disease, and other environmental factors affecting rice productivity. As a result, the present study screened four rice genotypes for resistance to rice blast and pattern of genetic variance present in the rice genotype. The presence of highly significant among the genotype for all characters except for tillering ability and plant height, which was non-significant, implies considerable variation among the genotype. Furthermore, the GCV values were relatively lesser than PCV for all traits. However, the difference between the PCV and GCV was relatively low for tillering ability, plant height, and vegetative vigor. That implies that the marked influence of environmental factors for the phenotypic expression of genotype was low; therefore, there is a higher chance of improving this trait through selection based on the phenotypic value of the traits. On the other hand, the difference in magnitude between the PCV and the GCV values was relatively high for the number of leaf and leaf blasts (Table 5) (Ikramullah et al. 2011).

Table 3. Mean squares of rice genotype screened for blast fungus

Source	Degree of freedom	Plant height	Seedling vigor	Tillering ability	Number of leaves affected	Leaf blast
Replications	1	1.98	0.01**	9.68	0.02	0.02 ^{ns}
Genotype	3	18.52	0.03**	16.52	3.79*	17.24**
Error	3	13.44	0.01	15.77	0.22	0.19 ^{ns}

Note: **: highly significance difference at ($P > 0.01$) probability level, *: significance difference at ($P > 0.05$) probability level, ns: no significant

Table 4. Variance component of rice genotype for blast fungus

Traits	Variance component					
	σ_e^2	σ_g^2	σ_p^2	GCV %	PCV %	Hb
Plant height	13.44	2.54	11.80	6.28	13.54	21.54
Seedling vigor	0.01	0.01	0.01	10.14	12.83	62.50
Tillering ability	15.77	0.37	8.26	5.93	27.90	4.52
Number of leaves affected	0.22	1.79	1.90	59.41	61.21	94.20
Leaf blast	0.19	8.52	8.62	64.88	65.24	98.88

Table 5. Mean performance of rice genotype for blast fungus

Traits	Genotype	Mean	Range	CV%
Plant height	FARO 52	23.93	23.88-29.25	16.96
	FARO 66	23.88		
	IRAT 109	24.47		
	JAMILA	29.25		
	Mean	25.38		
	LSD	13.7		
Seedling vigor	FARO 52	0.80	0.8-1	0.01
	FARO 66	0.80		
	IRAT 109	1.00		
	JAMILA	1.00		
	Mean	0.90		
	LSD	0.00		
Tillering ability	FARO 52	6.10	6.10-12.30	38.56
	FARO 66	12.30		
	IRAT 109	12.00		
	JAMILA	10.80		
	Mean	10.30		
	LSD	12.64		
Number of leaves affected	FARO 52	3.60	2.10-3.60	20.85
	FARO 66	2.90		
	IRAT 109	2.40		
	JAMILA	2.10		
	Mean	2.26		
	LSD	1.49		
Leaf blast	FARO 52	7.30	0.60-7.30	9.77
	FARO 66	6.10		
	IRAT 109	0.60		
	JAMILA	4.00		
	Mean	4.50		
	LSD	1.40		

A high heritability estimate was observed for seedling vigor, leaf number, and leaf blast. The high estimated heritability value for these traits indicated that the variation observed was mainly under genetic control and was less influenced by the environment. Hence, the possibility of progress from a selection. That may be attributed to the uniform environment in the screen house (Muhder et al. 2020). Furthermore, high heritability was observed for leaf blast, leaves, and seedling vigor. It suggests that selection based on these characters would be effective for future crossing programs. This result agrees with Tuhina-Khatun et al. (2015) and Kamara et al. (2017), who also reported high broad-sense heritability in rice.

Observations recorded 30-40 days after sowing based on leaf blast severity following SES IRRI (2013) scale showed that IRAT 109 is highly resistant, and this was visible both in growth and vigor. The resistant ability of these genotypes may be genetics as it suppresses the organism's development causing these diseases. Although FARO 66 is susceptible, it was less susceptible when compared to FARO 52, which is the most susceptible among the four genotypes. The fungus was visible 40 days after sowing on the leaves, while JAMILA was moderately susceptible. This result agrees with Spyridon et al. (2009), who reported that varietal differences significantly contributed to the resistance or susceptibility of the rice to leaf blast and also in line with the work of Gbadeyan et al.

(2018), who worked on screening of blast and genotype by environment interaction of rice. The different Response of the rice genotypes used in this study is important in selecting resistant varieties. These findings inspire further genetic studies to improve the genotypes through hybridization and selection programs.

In conclusion, the study highlighted rice germplasm with blast resistance in Nigeria and significant genetic variations for agronomically important traits, such as seedling vigor, leaf blast, and the number of leaves among the four rice genotypes that act as a pointer. Furthermore, the promising genotypes of IRAT 109 exhibited a significant level of resistance than JAMILA, FARO 52, and FARO 66. Hence, IRAT 109 can be considered a candidate for a blast-resistant variety for possible progress.

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