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African Journal of Plant Science

*Full Length Research Paper*

# Relationship between rice blast severity and the rice growth stage for accurate selection of rice breeding material for improved rice production in Africa

**Murielle FANTODJI[1]*, Bonaventure Cohovi AHOHUENDO[1], Drissa SILUE[2] and Andreas von TIEDEMANN[3]**

[1]Department of Plant Pathology and Plant Protection, Crop Sciences Institute, University of Abomey-Calavi, 01 BP 526 Cotonou, Benin.
[2]Plant Pathology Unit, Africa Rice Center, B.P.2551, Bouaké 01, Côte d'Ivoire.
[3]Department of Crop Sciences, Division of Plant Pathology and Crop Protection, University of Göttingen, Grisebachstraße 6, 37077 Göttingen, Germany.

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**To improve the disease resistance of adapted rice genotypes, fourteen rice genotypes selected from previous field screening and four controls (two resistant and two susceptible) were screened in greenhouse trials against ten Beninese and four East African pathogenic isolates at rice seedling and tillering stages. Results show that across isolates, no significant difference was observed between disease severity on tested genotypes at both growth stages while a significant difference at *P <0.05* was observed between disease severity on tested genotypes and susceptible controls. In fact, among tested genotypes, twelve previously resistant under field conditions were also resistant under greenhouse conditions, and more resistant ones were OU244, ARICA 5, IRAT 104, and PiN°4. In addition, among all tested genotypes that were resistant, two (OU244 and RIL249 MORO) with R genes, *Piz* and *Pi5t*, were particularly promising. Besides, at the seedling stage and across isolates, genotype OU244 and IRBLZ-Fu harboring the same R-gene *Piz* displayed resistance and susceptibility reactions and the same results were observed between genotypes RIL249 MORO and IRBL5-M harboring the same R gene *Pi5t* but only with Beninese isolates. Also, an incompatibility reaction was observed between susceptible controls and some Beninese isolates. In conclusion, screening for resistance at the rice tillering stage appears a suitable protocol for the reliable selection of rice breeding material for improved rice production in Africa. Also, results lead to first selecting rice genotype OU244 as the most stable and promising that could be used for rice improvement against rice blast in Africa; and to further initiate identification of R gene (s) involved in the resistance of the tested genotypes and avirulence (Avr) genes in the isolates used in the current study.**

**Key words:** *Magnaporthe Oryza,* disease severity (DS), area under disease progress curve (AUDPC); seedling; tillering.

## INTRODUCTION

In Sub-Saharan Africa, rice is becoming an important staple food with demand being higher than production (Seck et al., 2012) and an expected future significant growth. Despite the need to increase, rice production in Africa also faces many biotic and abiotic constraints. Thus, among the biotic stresses affecting rice productivity, rice blast, a devastating fungal disease caused by *Magnaporthe oryzae (M. oryzae)*, is the most important constraint concerning geographical distribution and yield losses caused (Séré et al., 2013). The amount of

production annually lost because of blast is sufficient to give food to 60 million people (Nalley et al., 2016). The disease can damage rice at any growth stage including at seedling, early tillering, and heading stages (Chuwa et al., 2015). Thus, a recent study has shown that screening germplasm at the seedling stage under greenhouse conditions is not sufficient to identify leaf blast-resistant genotypes and suggests conducting additional evaluations at other stages including tillering (Luangmanee et al., 2016).

This type of disease's severity usually peaks around maximum tillering where the heavy infection is often destructive to rice yield (Hwang et al., 1987). The impact of leaf blast before the flowering stage is particularly severe as the formation of yield components takes place (Evans, 1975). Therefore, it is necessary to evaluate the levels of resistance to leaf blast at different growth stages of rice, mainly at the seedling and tillering stages for reliable selection of high-yielding material. Besides, the duration of the disease cycle is about 7 to 14 days after exposure to the pathogen (Cécile et al., 2008). Thus, both single and multiple assessments of disease resistance have been reported in previous investigations. For a single evaluation, the evaluation times would be at 7 to 8 DAI (Shirasawa et al., 2012; Aram et al., 2013a, b) and 8 to 10 DAI (Puri et al., 2009). For multiple evaluations, the assessment of the disease was carried out at 7 DAI and repeated at 14 DAI (Zhan et al., 2012). There were differences in the number of assessments and it was not conclusive. Therefore, setting the number of evaluations of blast symptoms after inoculation is also an issue to find out. Since eradicating the disease is technically not feasible, lowering its impact is one of the major goals of control strategies. This may be reached first by pyramiding available resistance (R) genes, which were rarely reported to fail, and second by cultivating resistant or tolerant genotypes (Ghazanfar et al, 2009). This strategy requires a continuous identification of new sources of resistance genes against the diverse pathogen races. Thus, to screen germplasm for efficient resistance sources/genes, a suitable, reliable, and handy phenotyping system is required, which takes into account the more complex stress impact in the field and the growth stage of the crop.

To this end, the ultimate goal of the current research was to provide a reliable protocol for identifying germplasm with stable resistance across all plant developmental stages. Specifically, this study aimed at (1) assessing the level of resistance of rice genotypes to blast isolates from East and West Africa, and (2) determining the correlation between phenotypic data obtained in the greenhouse at seedling and tillering stages.

## MATERIALS AND METHODS

The level of resistance of eighteen rice genotypes to four East African and ten West African *M. oryzae* isolates was investigated under greenhouse conditions at two rice vegetative stages, namely seedling and tillering. Thus, the material and methodology used were as follows:

### Germplasm tested

In total, eighteen rice genotypes (Table 1) were tested under controlled conditions. Rice genotypes ARICA 1-5 were released in several Sub-Saharan countries and are being grown by farmers. Moroberekan is an old genotype grown in West Africa and is resistant to blast since decades. IRAT 104 is a genotype developed by IRAT in France (now CIRAD) that was grown in several West African countries. Among this set of eighteen genotypes to be tested, twelve were selected based on their strong resistance phenotype observed in eight African countries while two were either resistant or susceptible (Awandé et al., 2020) and the remaining four were resistant and susceptible controls. Seeds were provided by Africa Rice Center, Cotonou, Benin. Resistance of this set of genotypes to *M. oryzae* was assessed under controlled greenhouse conditions at two rice vegetative stages, namely seedling (five weeks maximum after sowing) and tillering (ten weeks maximum after sowing).

### Experimental design for disease resistance screening

For this experiment, rice seeds were disinfected in 3% of sodium hypochlorite and rinsed with sterile distilled water and kept in Petri dishes filled with moistened filter papers. The Petri dishes were kept in the dark at 25-32°C for two weeks and watered as per need. Then, eight seedlings of each line were transplanted into seedling trays filled with soil (compost, peat, and sand at a ratio of 2:1:1). The plants were fertilized using liquid fertilizer composed of 3% of NPK Hakaphos (15-10-15) and 3% of 6.5% iron chelate (Fe-EDDHMA) two and three weeks after transplanting. The trays were then kept in a greenhouse at 25±5°C with a photoperiod of 14/10 hours (day/night) for five (seedling stage, 3 to 4 leaves) and ten (tillering stage, maximum number of tillers) weeks. Sowing for the tillering stage was performed five weeks before sowing for the seedling stage to logically let the inoculation be done for both growth stages at the same time.

The experiments were conducted using a randomized complete block design with eight replications of each genotype and three experimental repetitions were conducted.

### *Magnaporthe oryzae* isolates

Two sets of isolates (Table 2) were used for screening the germplasm under greenhouse conditions: one set from East Africa (Uganda, Rwanda, and Tanzania) with four isolates and another from West Africa (Benin) comprising 10 isolates.

The West African set provided by Africa Rice, was

*Corresponding author. E-mail: tatianafantodji@gmail.com. Tel: +22963488220.

**Table 1.** Rice genotypes tested under controlled conditions.

| Rice genotype | Known resistance gene | Type | Source | Reference |
|---|---|---|---|---|
| ARICA1 | Unknown | Newly improved lines | AfricaRice | Kumashiro et al. (2013) |
| ARICA2 | Unknown | Newly improved lines | AfricaRice | Kumashiro et al. (2013) |
| ARICA3 | Unknown | Newly improved lines | AfricaRice | Kumashiro et al. (2013) |
| ARICA4 | Unknown | Newly improved lines | AfricaRice | Kumashiro et al. (2013) |
| ARICA5 | Unknown | Newly improved lines | AfricaRice | Kumashiro et al. (2013) |
| OU244 | *Piz* | Old line | AfricaRice | Ou et al. (1971) |
| IRBLZ-Fu | *Piz* | Monogenic line | IRRI | Fukuta (2009) |
| RIL249MORO | *Pi5t* | Near isogenic line | IRRI | Jeon et al. (2003) |
| IRBL5-M | *Pi5t* | Monogenic line | IRRI | Fukuta (2009) |
| WAB56-104 | Unknown | Old line | AfricaRice | - |
| IRAT104 | Unknown | Resistant and traditional varieties | CIRAD | - |
| IRAT13 | *Pi, Pi8t* | Resistant and traditional varieties | CIRAD | Fukuta et al. (2009) |
| FUKUHIKARI | *Pita, Pik, Pi9(t)& Piz* | Japanese differential | AfricaRice | Cho et al. (2007) |
| PiN°4 | *Pita-2, Pish* | Japanese differential | AfricaRice | Kiyosawa (1969) |
| CO39 | *Pia, PiCO39* | Old line and susceptible control | IRRI | Chauhan et al. (2002) |
| MARATELLI | - | Old line and susceptible control | CIRAD | - |
| TETEP | *Pita, Pi5t, Pita-2, + others* | Old line and resistant control | AfricaRice | Yamada et al. (1976) |
| Moroberekan | *Pi5t+Pi7t+ Pi44+Pi12+ others* | Old line and resistant control | AfricaRice | Séré et al. (2013) |

International Rice Research Institute (IRRI), Philippines ; Centre International de Recherche pour le Développement (CIRAD), France, AfricaRice Plant Pathology laboratory, Benin.
Source: Authors

selected among 105 isolates representing the Africa-wide diversity of thefungus. These ten isolates match all resistance genes found in 54 differential varieties and the resistance of 10 traditional varieties (Odjo et al., unpublished). It is thus expected that the chances are high that any genotype that resists to all of them will resist anywhere in Africa. The East African set was provided by the Crop Protection Division at the Georg-August University of Göttingen, in Germany and was selected based on their pathogenicity reaction pattern on various R-genes.

**Inoculum preparation**

The methodology previously described by Onaga et al. (2015) was used. The isolates were grown on Petri dishes containing V8 agar medium (for 1 L, 100 ml vegetable juice, 2 g calcium carbonate, 15 g agar, and 900 ml distilled water) and 0.2 g streptomycin sulfate added after autoclaving at 120°C for 20 minutes and cooling). The photoperiod for growing isolates was 14/10 hours (day/night) at 25-30°C for a maximum of 10 days and conidia were harvested with sterile distilled water and filtrated through two layers of Miracloth. The concentration of conidia was assessed by counting under the microscope using a hemocytometer. The inoculum concentration of 1x105 conidia per ml was used for inoculating plants at both growth stages and the inocula contained 1% of Tween 20% added before inoculation.

**Inoculation method and incubation conditions**

Inoculations were performed as previously described by Onaga et al. (2015). Rice genotypes were spray-inoculated with selected isolates at five and ten weeks after sowing (at seedling and tillering stages, respectively) as described by Luangmanee et al. (2016) with some slight modifications. After incubation in the dark in a moist cabinet with high relative humidity (RH) averaging at 94% for 24 h, plants were transferred to a greenhouse (25±5°C with a Relative Humidity of 88% and artificial light and darkness cycle of 14/10 hours) for 12 days.

**Disease assessment**

Thus, lesion types reflecting disease severity were assessed at both seedling and tillering stages from the 4th to 12th day after inoculation (DAI) at two days intervals at the seedling stage on 3 to 4 leaves and at the tillering

**Table 2.** Origin of *M. oryzae* blast isolates used for screening of resistance to blast in Africa.

| Code | Isolate designation | Country of origin | Collection site | Year of collection | Cultivar of origin | Database |
|---|---|---|---|---|---|---|
| IPP1030 | 505UGA09 | Uganda | Unknown | 2009 | Unknown | APP |
| IPP1133 | 505RWA11 | Rwanda | Cyabayaga | 2011 | Yunikeng | APP |
| IPP1143 | 564UGA11 | Uganda | Doho-Butaleja | 2011 | K98 | APP |
| IPP1177 | TAN211.16 | Tanzania | Keyla | 2011 | V40 | APP |
| BN0252 | Lok20.3 | Benin | Lokossa | 2010 | Wita11 | AfricaRice |
| BN0050 | OUED10.17.4 | Benin | Ouèdèmè/Lokossa | 2010 | TOG8543 | AfricaRice |
| BN0066 | OUED10.28.3 | Benin | Ouèdèmè/Lokossa | 2010 | TOG7656 | AfricaRice |
| BN0119 | OUED10.26.6 | Benin | Ouèdèmè/Lokossa | 2010 | IRGC103973 | AfricaRice |
| BN0040 | OUED10.15.7 | Benin | Ouèdèmè/Lokossa | 2010 | IRGC104252 | AfricaRice |
| BN0013 | 1B | Benin | Monkassa/Malanville | 2011 | Unknown | AfricaRice |
| BN0082 | 183A | Benin | Djougou | 2012 | BL19 | AfricaRice |
| BN0094 | 32A | Benin | Bétérou | 2012 | BL19 | AfricaRice |
| BN0204 | 37A | Benin | Bétérou | 2012 | BL19 | AfricaRice |
| BN0202 | 7A | Benin | Bétérou | 2012 | BL19 | AfricaRice |

(APP): Plant Pathology Department/Georg-August University Göttingen; Benin isolates are representative of rice blast genetic diversity in African collection.
Source: Authors

stage on the three youngest leaves. For all greenhouse trials performed, lesions type was measured according to the rating scale 0-5 (Chaudhary et al., 2005) where 0-2 = Resistant (R), 2.1-3 = Moderately Resistant (MR), and 3.1-5 = Susceptible (S). To compare the relative levels of resistance of the tested rice genotypes to blast, blast severity data were converted to the area under disease progress curve (AUDPC) according to the formula described by Shaner and Finney (1977):

$$AUDPC=\sum [(0.5) (Yi+1+ Yi) (Ti+1-Ti)$$

Where, Y = disease severity at time i and T = time (days) of the assessment.

### Statistical analysis

Statistical analysis of data were done using Statistica Package version 13.2. The resistance of rice genotypes was analyzed using the non-parametric Kruskal-Wallis test. The dependent t-test was run to analyze the effect of growth stages on rice blast severity.

## RESULTS

Results obtained at the seedling stage (Figure 1) with the East African isolates indicated that leaf blast development progressed gradually and significantly from the 4th to 12th DAI on the susceptible controls MARATELLI and CO39, whereas no significant differences in resistance with the same isolates were observed between all tested genotypes from 4th to12th DAI (Figure 1). Thus, disease scores on the tested genotypes ranged from 0.0 to 1.4, while on the susceptible controls, they ranged from 0.9 to 5.0.

Results similar to those above were obtained at the tillering stage with the East African isolates (Figure 1) where blast development progressed gradually and significantly from the 4th to 12th DAI on the susceptible controls MARATELLI and CO39, whereas no significant differences in resistance with the same isolates were observed between all tested genotypes from 4th to12th DAI (Figure 1). Diseases scores of 0.0 to 1.9 were obtained on the tested genotypes, while the same scores at the seedling stage were obtained on the susceptible controls. Additionally, results obtained with East African isolates indicated that leaf blast disease severity peaks at 12 DAI, especially on susceptible controls at both rice growth stages (Figure 2). Moreover, at 12 DAI, significant differences in disease resistance were observed between susceptible controls and tested genotypes at $P<0.05$ according to the Kruskal-Wallis test at both growth stages. An example is between IRAT104 and susceptible controls after being challenged with East African isolate IPP1133 (Figure 2). Thus, all 12 tested genotypes (ARICA1 to ARICA5, OU244, RIL249 MORO, WAB56-104, IRAT 104, IRAT13, FUKUHIKARI,

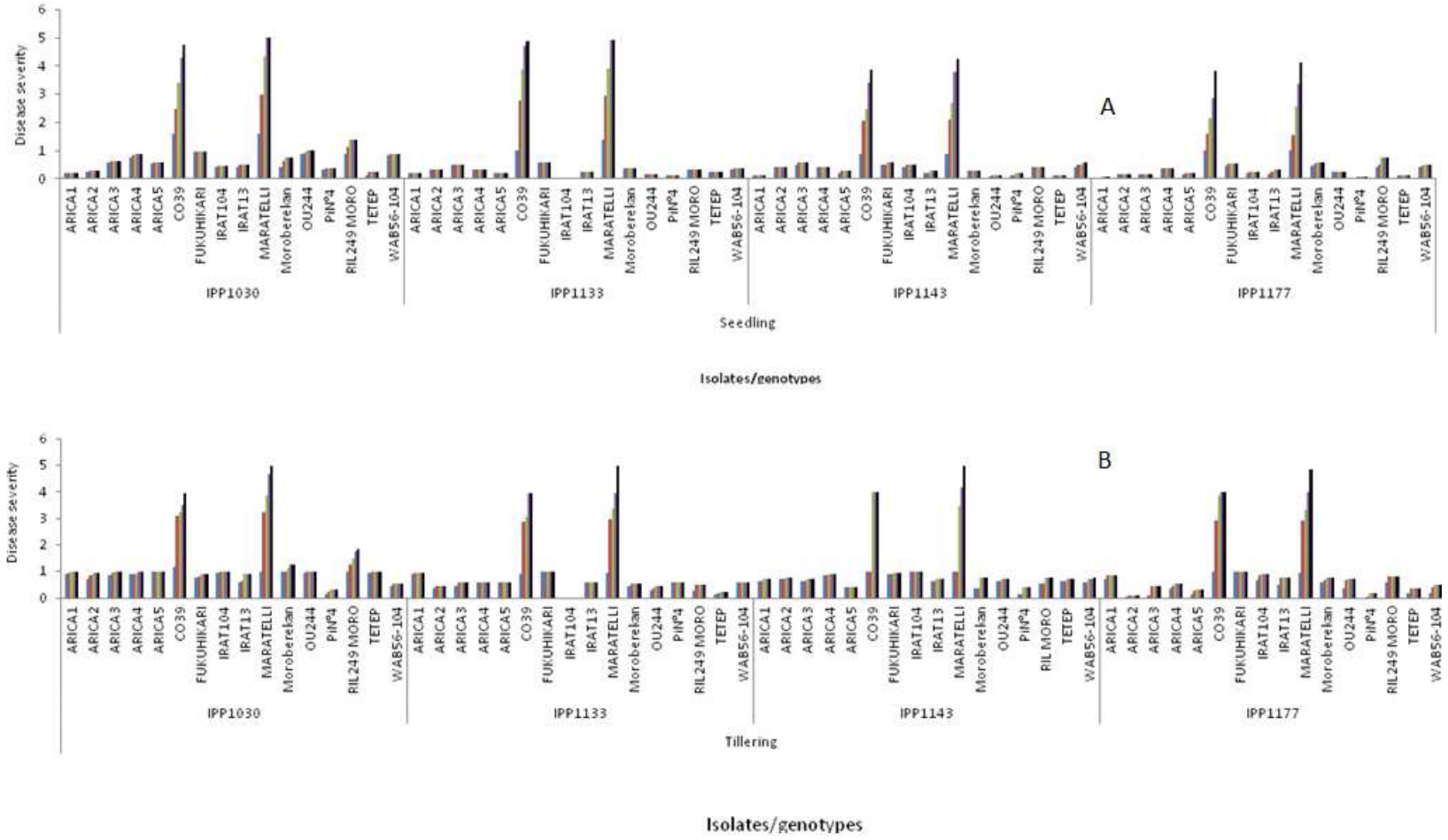


**Figure 1.** Leaf blast severity at (A) seedling and (B) tillering stages of rice genotypes challenged under greenhouse conditions with four East African rice blast isolates IPP1030; IPP1143; IPP1133; IPP1177; DS 4, 6, 8, 10 and 12 = Disease severity at 4, 6, 8, 10 and 12 DAI. CO39 and MARATELLI were used as susceptible controls and and Moroberekan as resistant controls. Data shown are the means of three replications.
Source: Authors

and PiN°4) were similarly resistant at seedling and tillering stages and more resistant genotypes were PiN°4, OU244, and IRAT104.

Results obtained with indigenous (Beninese) isolates are similar to those obtained above with East African isolates. Thus, the results indicated also that leaf blast development progressed gradually and significantly from the 4th to 12th DAI on the susceptible controls MARATELLI and CO39 at both growth stages, whereas no significant differences in resistance with the same isolates were observed between all tested

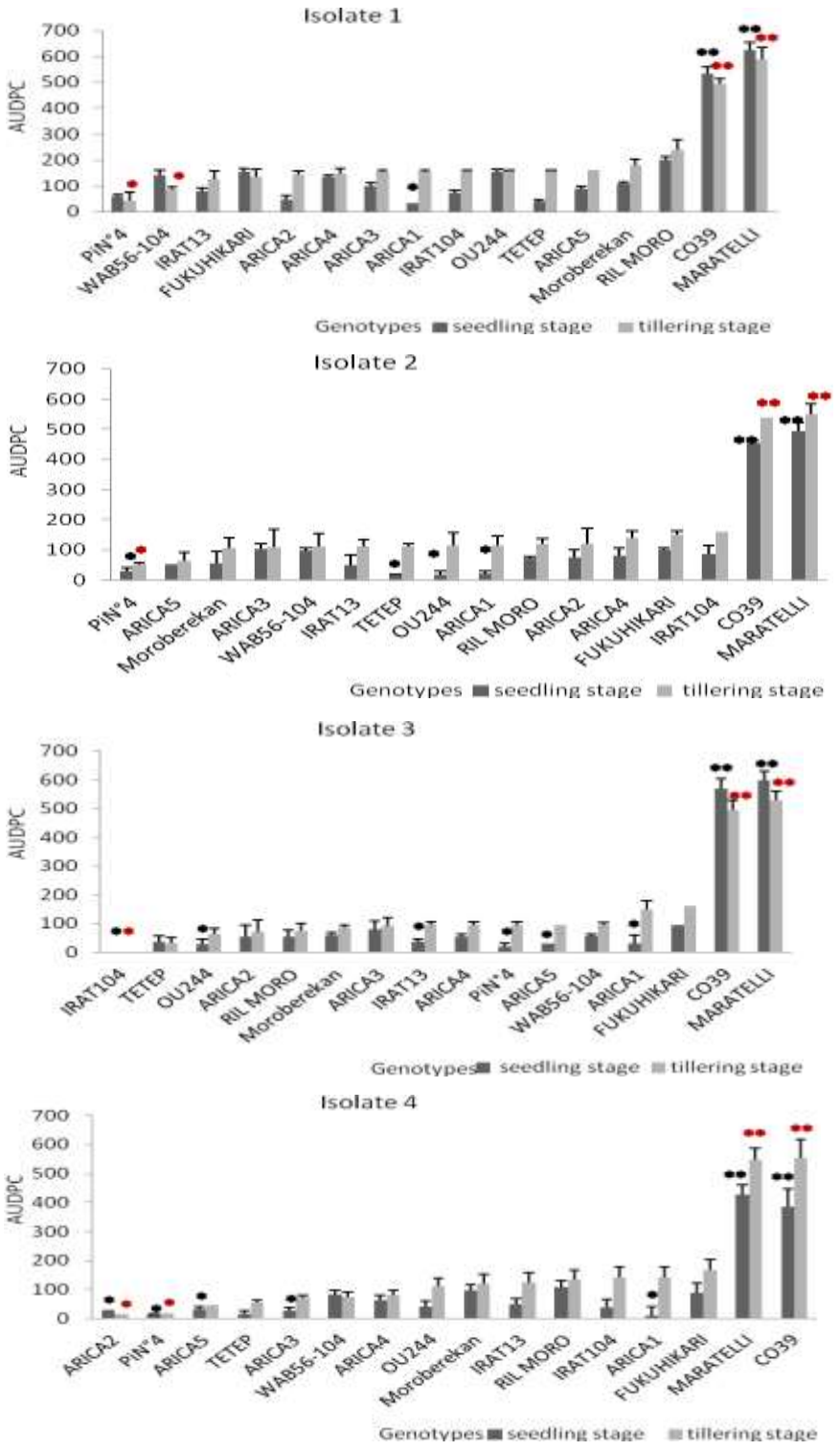


**Figure 2.** Leaf blast disease progress curve at rice seedling and tillering stages of rice genotypes challenged under greenhouse conditions with East African rice blast isolates and isolate 1 = IPP1030, isolate 2 = IPP1143, isolate 3 = IPP1133, isolate 4 = IPP1177. CO39 and MARATELLI were used as susceptible checks TETEP and Moroberekan as resistant checks. Data shown are the means of three replications. The data followed by black or red stars indicate respectively significant differences at seedling and tillering stages at P< 0. 05 according to non-parametric Kruskal-Wallis test and error bars correspond to the standard deviation.
Source: Authors

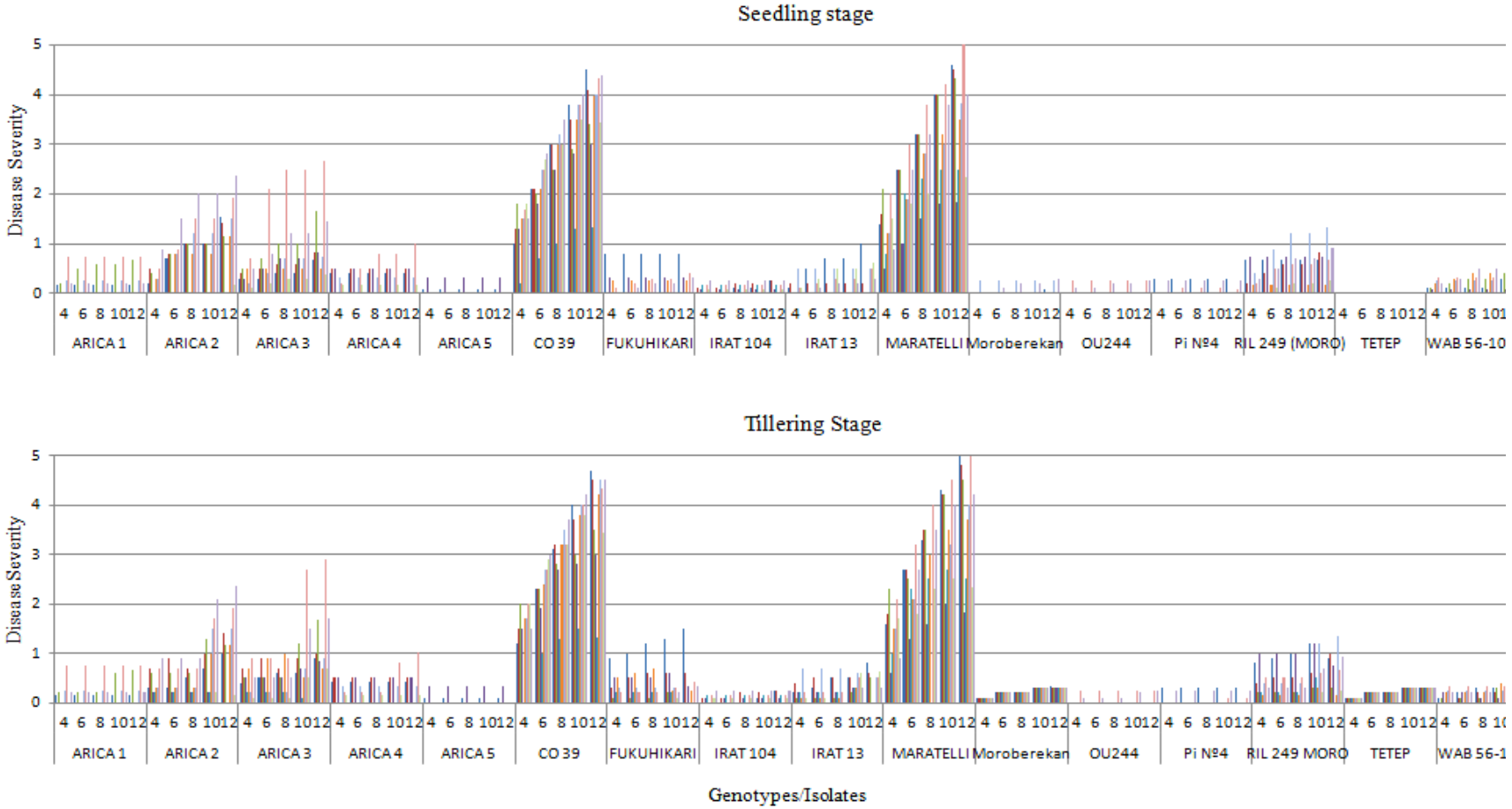


**Figure *3*.** Leaf blast severity at seedling stage of 16 rice genotypes challenged under greenhouse conditions with 10 Beninese rice blast isolates namely BN0040 (1), BN0050 (2), BN0082 (3), BN0252 (4), BN0094 (5), BN0013 (6), BN0119 (7), BN0204 (8), BN0066(9), BN0202 (10) at rice seedling and tillering stage. CO39 and MARATELLI were used as susceptible checks and Moroberekan as resistant checks. Data shown are the means of two replications and errors bars indicate standard deviations. Significant difference at P<0.05 according to Kruskal-Wallis test. DSI + number 1 to 10= Disease Severity Isolates 1 to 10 at seedling stage and DSI + number 1' to 10'= Disease Severity Isolates 1 to 10 at tillering stage.
Source: Authors

genotypes from 4th to12th DAI (Figure 3). Disease scores on the tested genotypes ranged from 0.0 to 2.7 at the seedling stage and from 0 to 2.9 at the tillering stage indicating that all tested genotypes are resistant; while the susceptible controls MARATELLI and CO39 showed susceptibility reactions (DS from 0.5 to5.0) with all isolates except BN0252, BN0094, and BN0066Thus, at both growth stages and at 12 DAI where the disease reached its peak, disease severity of 1.8 and 3.0, 1.3 and 2.5 were obtained on CO39 and MARATELLI (Figure 3) after

**Table 3.** Comparison of leaf blast resistance at seedling stage and at 12 DAI of two set of rice genotypes (OU244, IRBLZ-Fu) and (RIL249 MORO, IRBL5-M) challenged with four East African blast isolates [ (1)=IPP1030; (2)=IPP1143; (3)=IPP1133; (4)=IPP1177] and ten Beninese isolates (5)=BN0040, (6)=BN0050, (7)=BN0082, (8)=BN0252, (9)=BN0094, (10)=BN0013, (11)=BN0119, (12)=BN0204, (13)= BN0066, (14)=BN0202] at rice seedling stage. Data shown are the means of three replications.

| Genotypes | Known R gene(s) | DS1 | DS2 | DS3 | DS4 | DS5 | DS6 | DS7 | DS8 | DS9 | DS10 | DS11 | DS12 | DS13 | DS14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Moroberekan | *Pi5t+Pi7t+ Pi44+Pi12+ others* | 0.7 | 0.3 | 0.4 | 0.6 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.0 | 0.0 | 0.3 |
| RIL MORO | *Pi5t* | 1.4 | 0.4 | 0.3 | 0.7 | 0.7 | 0.8 | 0.0 | 0.8 | 0.0 | 0.2 | 1.3 | 0.7 | 0.3 | 0.9 |
| ARICA1 | None | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.0 | 0.7 | 0.0 | 0.0 | 0.0 | 0.3 | 0.8 | 0.0 | 0.2 |
| ARICA2 | None | 0.3 | 0.4 | 0.3 | 0.2 | 1.5 | 1.4 | 1.2 | 0.0 | 0.0 | 1.2 | 1.5 | 1.9 | 0.2 | 2.4 |
| ARICA3 | None | 0.6 | 0.6 | 0.5 | 0.2 | 0.7 | 0.8 | 1.7 | 0.8 | 0.0 | 0.5 | 0.8 | 2.7 | 0.4 | 1.5 |
| IRAT13 | *Pib, Pi8t* | 0.5 | 0.3 | 0.2 | 0.3 | 1.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 | 0.5 | 0.6 | 0.3 |
| FUKUHIKARI | *Pita, Pik, Pi9(t)& Piz* | 1.0 | 0.6 | 0.6 | 0.5 | 0.8 | 0.0 | 0.0 | 0.3 | 0.0 | 0.3 | 0.0 | 0.4 | 0.0 | 0.3 |
| CO39 | *Pia, PiCO39* | 4.8 | 3.9 | 4.9 | 3.8 | 4.5 | 4.1 | 3.4 | 3.0 | 1.3 | 4.0 | 4.0 | 4.3 | 3.5 | 4.4 |
| TETEP | *Pita, Pi5t, Pita-2, + others* | 0.3 | 0.1 | 0.2 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| OU244 | *Piz* | 1.0 | 0.1 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.0 | 0.3 |
| ARICA4 | None | 0.9 | 0.4 | 0.3 | 0.4 | 0.4 | 0.5 | 0.0 | 0.5 | 0.0 | 0.0 | 0.3 | 1.0 | 0.2 | 0.0 |
| ARICA5 | None | 0.6 | 0.3 | 0.2 | 0.2 | 0.1 | 0.0 | 0.0 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| IRAT104 | None | 0.5 | 0.5 | 0.0 | 0.2 | 0.3 | 0.3 | 0.0 | 0.1 | 0.2 | 0.0 | 0.0 | 0.2 | 0.1 | 0.3 |
| PiN°4 | *Pita-2 and Pish* | 0.4 | 0.2 | 0.1 | 0.1 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.3 |
| WAB56-104 | None | 0.9 | 0.6 | 0.4 | 0.5 | 0.3 | 0.0 | 0.4 | 0.1 | 0.0 | 0.5 | 0.3 | 0.3 | 0.0 | 0.6 |
| MARATELLI | None | 5.0 | 4.3 | 4.9 | 4.1 | 4.6 | 4.5 | 4.3 | 1.8 | 2.5 | 3.5 | 3.8 | 5.0 | 2.3 | 4.0 |
| IRBLZ-FU | *Piz* | 4.0 | 4.1 | 3.4 | 3.6 | 3.8 | 4.0 | 3.5 | 3.2 | 4.3 | 4.7 | 4.3 | 3.9 | 4.1 | 3.7 |
| IRBL5-M | *Pi5t* | 0.7 | 0.9 | 0.4 | 0.6 | 3.2 | 3.0 | 3.5 | 4.2 | 3.8 | 4.5 | 3.9 | 3.1 | 4.0 | 3.4 |

Rating scale 0 to 5 was used and phenotypes were ranged as follows: resistant (R) = 0.0 to 2.0; (S) = 4 to 5; (DS): Disease severity.
Source: Authors

challenged with isolates BN0252 and BN0094, respectively. With isolate BN0066, incompatibility reaction was observed only on the susceptible control MARATELLI with DS of 2.3 at both growth stages. In fact, among all tested genotypes with Beninese isolates, genotypes OU244, ARICA5, IRAT 104, and PiN°4 were highly resistant across all isolates and growth stages as did the resistant controls Moroberekan and TETEP.

Interestingly, all 12 tested genotypes selected based on their resistant phenotype under field conditions in eight African countries (Awandé et al., 2020) are also resistant under greenhouse conditions. Besides, contradictory results (resistance and susceptibility) were observed at the seedling stage and across isolates between rice genotypes OU244 and IRBLZ-Fu harboring the same R-gene *Piz*. The same results were observed between genotypes RIL249 MORO and IRBL5-M harboring the same R gene *Pi5t* after being challenged with Beninese isolates (Table 3).

## DISCUSSION

Screening various local rice genotypes for resistance to rice blast disease under greenhouse conditions is common (Ghazanfar et al., 2009; Haggag and Tawfik, 2014; Wu et al., 2017). However, disease resistance screening results at various stages of plant growth are rare. One report showed that significant differences in resistance levels to the disease exist between seedling and tillering stages (Luangmanee et al., 2016) and that some genotypes that were moderately resistant at the seedling stage became moderately susceptible at tillering, while some others that exhibited moderate resistance at the seedling stage maintained the same

phenotype at tillering stage. Also, as resistance levels increase with leaf age (Kato et al., 1969; Roumen et al., 1992) and blast infection by different races of the pathogen became increasingly reduced on either leaf of adult plants or older leaves when rice plants at different growth stage, rice genotypes were screened in the current study for resistance at two vegetative stages of rice growth, that is, seedling and tillering. Thus, in the current study, all genotypes resistant at the seedling stage also resisted at the tillering stage and the more resistant ones were OU244, ARICA 5, IRAT 104, and PiN°4. Thus, the results of the current study confirm Luangmanee et al., (2016) and Chuwa et al., (2015) findings and show the importance of testing rice genotypes at both growth stages for identifying stable resistance gene sources. Additionally, genotypes ARICA1-5, OU244, RIL249 MORO, WAB56-104, IRAT104, IRAT13, FUKUHIKARI, and PiN°4 that were resistant in fields of eight countries (Awandé et al., 2020) also resisted after artificial inoculation with all tested isolates at both growth stages; and these results show that these genotypes have broad-spectrum resistance and might be useful to improve rice production in Africa. Beninese isolates used in the current study represent the Africa-wide diversity for pathogenicity (Odjo et al., unpublished) and match all together all resistance genes present in 54 differential genotypes. We thus think that any genotype that resists these Beninese isolates is likely to resist anywhere in Africa. Thus, all the twelve resistant genotypes in the current study might resist to blast fungus anywhere they might be grown in Africa as they resist to the Beninese isolates. More again, among all the tested genotypes that were resistant through all the conducted experiments, two (OU 244 and RIL249 MORO) harboring R genes, *Piz* and *Pi5t*, were particularly promising as monogenic and could be useful in the breeding program for efficient rice blast disease management. Besides, the contradictory results (resistance/susceptibility) observed between genotypes OU244 and IRBLZ-Fu at the seedling stage and across isolates, then between RIL249 MORO and IRBL5-M after challenged with only Beninese isolates, are similar to those obtained previously under field conditions (Awande et al., 2020). These previous results might be because these genotypes harbor different R genes or maybe genotypes OU244 and RIL 249 MORO harbor additional R gene(s) that make their reaction different from the others. Thus, the precedent results are relevant as they might allow us to first select rice genotype OU244 with R gene *Piz* as the most stable breeding material useful for rice improvement against rice blast anywhere in Africa.

Thus, as it is unclear which gene(s) is involved in the resistance of OU244 and RIL249 MORO, identifying the R gene(s) involved in this genotype and also in the other genotypes that have shown resistant phenotype in the current study would provide some valuable clues for breeders to develop sustainable blast resistant germplasm. Rice genotype IRAT13 harbors *Pib, Pi8* (t) (Fukuta et al., 2009), and the two genes might be responsible for the strong observed resistance. The strong resistance in PiN°4 could be due to the efficiency of the resistance gene pyramid *Pita-2* and *Pish* it harbors.

More interestingly, an incompatibility reaction was observed between susceptible control MARATELLI and three Beninese isolates, namely BN0252, BN0094, and BN0066, while the same results were observed between susceptible control CO39 and two Beninese isolates, namely BN0252 and BN0094. One interpretation of these results is that the R genes, *Pi-CO39* and *Pia*, known to be in CO39 (Chauhan et al., 2002) might be responsible for its observed incompatibility with those isolates. For MARATELLI, an explanation could be that this cultivar harbors an unknown R gene that prevents its attack by these two isolates. So, based on the gene-for-gene theory (Silué et al., 1992), where a dominant resistance gene in the host corresponds to an avirulence gene in the pathogen, further identification of resistance genes in the tested genotypes and avirulence genes in the isolates used might be necessary to effectively identify breeding material.

To summarize, the protocol used in this study enabled us to efficiently assess disease development and accurately screen genotypes for resistance. Thus, in the current study, no significant differences at $p<0.05$ were observed between leaf blast resistance expressed by the tested genotypes at both seedling and tillering. All the tested genotypes were resistant across growth stages and isolates and the more resistant ones were OU244, ARICA 5, IRAT 104, and PiN°4. Therefore, among all the tested genotypes that were resistant through all the conducted experiments, two (OU244 and RIL 249 MORO with R genes, Piz and Pi5t) were particularly promising as monogenic. Besides, the contradictory results observed first between OU244 and IRBLZ-Fu across isolates and then between RIL249 MORO and IRBL5-M with only Beninese isolates lead to finally the selection of OU244 as a promising and more stable rice genotype that might be useful for rice improvement against rice blast. Also, an incompatibility reaction was observed, especially between susceptible control MARATELLI and three Beninese isolates leading to further identification of resistance genes in the tested genotypes and avirulence genes in the isolates used.

## CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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