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Full Length Research Paper

Analysis of *Magnaporthe oryzae* population structure in Benin

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The study aimed at analyzing *Magnaporthe oryzae* population structure in Benin Republic, using Near Isogenic Lines and differential rice varieties in order to characterize the virulence spectrum of blast populations, as well as identifying the best blast disease hotspot screening sites. Blast trapping nurseries using 30 Near Isogenic Lines and 2 other rice varieties were setup in 4 blast disease hotspots (Kokey, Kandi, Ouedeme and Bagou) in Benin. The experiment was setup in a Fisher block design with three replicates. Blast disease was evaluated weekly from 21 days after sowing. The blast pathogen pressure was generally higher in hotspots/sites in the Northern part of Benin. The races of *M. oryzae* in 3 sites (Kokey, Kandi and Bagou) were able to overcome 9 resistance genes (*Pi1*, *Pi7*, *Pi5*, *Pikp*, *Pia*, *Pita2*, *Piks*, *Pi3* and *Pik*). However, in Ouedeme, the *M. oryzae* races were able to overcome 13 resistance genes (*Pi1*, *Pi7*, *Pi5*, *Pikp*, *Pia*, *Pita2*, *Piks*, *Pi3*, *Pik*, *Pita*, *Piz*, *Pikh* and *Pikm*). Ouedeme site was therefore identified as the best site for use to screen for durable resistance to blast disease in Benin Republic. This information is useful for development of durable resistant variety to blast disease in Benin.

Key words: *Magnaporthe oryzae*, pathogenicity, disease hotspots, resistance genes, durable resistance.

INTRODUCTION

Blast disease caused by *Magnaporthe oryzae* remains a threat to rice production worldwide despite extensive research efforts at its control (Koide et al., 2010). *M. oryzae* is highly specific to rice, although certain strains that do not attack rice can harm weeds in the rice field. Most infections occur on the leaves, causing diamond-shaped lesions with a gray or white center to appear, or on the panicles which turn white and die before being filled with grain (Nutsugah et al., 2008). Once on a rice plant, the fungus rapidly produces thousands of spores, which are carried readily through the air, by wind or rain, onto neighboring plants (Nutsugah et al., 2008). Blast was first reported in Asia more than three centuries ago and is now present in over 85 countries. It is highly adaptable to environmental conditions and can be found in irrigated lowland, rain-fed upland, or deepwater rice fields (Sere et al., 2004b). Blast can survive on seeds and can easily move over borders if proper safety checks are not in place. While it is present nearly everywhere rice is grown, blast is more of a problem in the temperate flooded and tropical upland cropping systems, marked by cooler climates (Sere et al., 2007). Rainy periods or periods of high humidity also favor the disease. Certain cultural practices that encourage blast development include excessive use of nitrogen through chemical fertilizers, and inadequate spacing often practiced under rice intensification (Piotti et al., 2005).

In the West African sub-region, blast is recognized as a primary constraint to rice production causing 3.2 to 77% yield losses (Sere et al., 2007). Deployment of resistant cultivars integrating good cultural practices is the most effective and economical way to combat the blast disease. However, breakdown of resistance is common due to the dynamic nature of the pathogen in responding to the host genotype and environment (Zeigler and

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Table 1. Identity of rice varieties including Near Isogenic Lines and their known blast resistance genes.

S/N	Variety	Resistance gene
1	C105 TTP-1	*Pita*
2	IRBL1-CL/CO	*Pi1*
3	IRBLta-Ya/CO	*Pita*
4	IRBL7-M/CO	*Pi7*
5	75-1-127	*Pi9*
6	IRBLzt-IR56/CO	*Pizt*
7	IRBL5-M/CO	*Pi5*
8	IRBLsh-Fu/CO	*Piz*
9	IRBLkp-K60/CO	*Pikp*
10	Co39	*Pia*
11	IR1529	*Pi33*
12	IRBLta2-Pi/CO	*Pita2*
13	IRBLks-CO/CO	*Piks*
14	K 59	*Pit*
15	C101 A51	*Piz5*
16	IRBLsh-S/CO	*Pish*
17	Tetep	*Pikh,Pi1,Pita+*
18	Moroberekan	*Pi5,Pi7*
19	IRBLkh-K3/CO	*Pikh*
20	IRBLta2-Re/CO	*Pita2*
21	C101 LAC	*Pi1*
22	IRBLb-IT13/CO	*Pib*
23	C102 TTP	*Pita*
24	C104 PKT	*Pi3*
25	IRBLz5-CA/CO	*Piz5*
26	IRBLkm-Ts/CO	*Pikm*
27	Nato	*Pii*
28	IRBLta2-IR64/CO	*Pita2*
29	St 1	*Pif*
30	IRBLk-Ku/CO	*Pik*
31	TOG5681	Unknown
32	NERICA1	Unknown

Correa, 2000). Understanding the diversity and dynamics of the pathogen populations and identification of resistance sources based on this knowledge is critical to the development of blast resistance that is stable over space and durable over time.

The present study is aimed at analyzing *M. oryzae* population structure in Benin Republic using Near Isogenic Lines and differential rice varieties. Besides, the study also aimed to characterize the virulence spectrum of blast populations as well as identifying the best blast disease hotspot screening sites.

MATERIALS AND METHODS

Research location

The study was carried out by Plant Pathology Unit, Africa Rice Center, Cotonou, Benin. Four field experiments were conducted from July to November 2009 at Bagou, Kandi, Kokoy and Ouedeme in the Republic of Benin.

Plant materials

The 32 rice varieties used for the study include Near Isogenic Lines (NILs) of CO39 genetic background obtained from IRRI and other host differential varieties (Table 1). The NILs are lines with indica cultivar CO39 genetic background that carried one or more known resistance gene.

Experimental design

The experiment was setup in a Fisher block design with three replicates as described by Sere et al. (2007).

Disease assessment

Blast disease was examined and scored subsequently at weekly intervals using the 0 to 9 (0-1 resistant, R; 2 intermediate, h; 3-9 highly susceptible, HS) scale rating system (Sere et al., 2007).

Statistical analysis

The blast incidence data was subjected to analysis of variance and Student Newman and Keuls means comparison test (Abdi and Williams, 2010) to compare the mean averages using statistical analysis system (SAS) software (Zhu and Kuljaca, 2005).

RESULTS

Blast pathogen population structure at different sites

Analysis of variance revealed a significant interaction ($P \leq 0.01$) between the sites and rice varieties, meaning that *M. oryzae* population structure and pressure were not the same in all the four sites (Table 2). *M. oryzae* virulence was at highest at Bagou site with 2.25 blast incidence score, followed by Kandi with 2.17, Kokoy with 2.05 and Ouedeme with 1.95 respectively (Table 3).

Characterization of the blast pathogen population of Bagou

The Student-Newman-Keuls range test showed the existence of 5 distinct groups (a-group; b-group; c-group; d-group; e-group) of NILs/varieties (Tables 4 and 5). On the basis of epidemiological perspective, the a-group with blast incidence of 3.02 to 3.08 corresponded to the virulence factor (highly susceptible), the b-group of 2.67 blast incidence also corresponded to the virulence factor (susceptible), the c-group with blast incidence of 1.87 to 1.95 indicated the moderate (intermediate) virulence, while d-group and e-group with 1.46 and 1.34 blast incidence, respectively indicated the incompatible reaction (resistance) (Tables 4 and 5). It means that there

Table 2. Analysis of variance of blast disease incidence/severity.

Source	DF	F value
Site (S)	3	71.65**
Variety (V)	31	134.77**
S x V	93	13.01**
Error	256	-

**: Significant at P≤0.01.

Table 3. Mean comparison of varietal blast incidence scores and the experimentation sites.

Sites	Mean blast incidence
Bagou	2.25±0.06[a]
Kandi	2.17±0.05[b]
Kokoy	2.05±0.06[c]
Ouedeme	1.95±0.06[d]

In a column, mean followed by a common letter are not significantly different at the 5% level by Student-Newman-Keuls range test.

were compatible interactions between the blast population and certain NILs/varieties, and incompatible interactions with others. Besides, 9 resistance genes (*Pi1*, *Pi7*, *Pi5*, *Pikp*, *Pia*, *Pita2*, *Piks*, *Pi3* and *Pik*) were overcome by blast pathogen races at Bagou and certainly throughout the region (Tables 4 and 5).

Characterization of the blast pathogen population of Kandi

The Student-Newman-Keuls range test showed the existence of 4 distinct groups (a-group; b-group; c-group; d-group) of NILs/varieties (Tables 4 and 5). The first a-group with 3.08 blast incidence corresponded to highly susceptible NILs/varieties, b-group with blast incidence of 2.61 to 2.67 represents susceptible NILs/varieties while the c-group with 1.87 to 1.95 blast incidence characterrized intermediate NILs/varieties and d-group with 1.58 blast incidence formed the resistance NILs/varieties (Tables 4 and 5). Blast pathogen races at Bagou and Kandi sites overcame 9 resistance genes (*Pi1*, *Pi7*, *Pi5*, *Pikp*, *Pia*, *Pita2*, *Piks*, *Pi3* and *Pik*). This indicates similar blast pathogen population structure in Bagou and Kandi sites.

Characterization of the blast pathogen population of Kokoy

The Student-Newman-Keuls range test showed the existence of 3 distinct groups (a-group; b-group; c-group) of NILs/varieties to reflect the virulence spectrum of blast pathogen population in this area (Tables 4 and 5). The first a-group with 2.61 to 3.03 blast incidence correspondded to highly susceptible NILs/varieties while b- and c-groups were with blast incidence of 2.61 to 2.04 and 1.34 to 1.67 respectively formed the resistance NILs/varieties (Tables 4 and 5). Blast pathogen races at Kokoy overcame 9 resistance genes (*Pi1*, *Pi7*, *Pi5*, *Pikp*, *Pia*, *Pita2*, *Piks*, *Pi3* and *Pik*) same as those overcame at Bagou and Kandi sites (Tables 4 and 5).

Characterization of the blast pathogen population of Ouedeme

The Student-Newman-Keuls range test showed the existence of 3 distinct groups (a-group; b-group; and c-group) of NILs/varieties that reflect blast pathogen population virulence spectrum in the area (Tables 4 and 5). The first a-group with 2.24 to 2.67 blast incidence corresponded to highly susceptible NILs/varieties, b-group with 1.86 to 2.19 blast incidence represents the intermediate NILs/varieties, and c-group with 2.19 to 1.68 blast incidence formed the resistance NILs/varieties (Tables 4 and 5). Blast pathogen races at Ouedeme overcame 15 resistance genes (*Pi1*, *Pita*, *Pi7*, *Pi5*, *Piz*, *Pikp*, *Pia*, *Pita2*, *Piks*, *Pikh*, *Pita2*, *Pi1*, *Pi3*, *Pikm*, and *Pik*) as compared to Bagou, Kandi and Kokoy sites that overcame 9 resistance genes (Tables 4 and 5). This consequently revealed the existence of greater blast pathogen diversity in Ouedeme site as compared to Bagou, Kandi and Kokoy sites.

DISCUSSION

Pathogenicity and virulence diversity are essential factors in determining blast pathogen population structure and virulence spectrum as revealed by this study. This means that the density of blast pathogen population varied from one site to another, indicating that the number of disease lesions which is proportional to the concentration of conidia varied across sites (Kennedy et al., 2000). Thus the number of lesions formed on a variety could indicate the number of virulence factors able to overcome the resistance of this variety (Sere et al., 2004a). These findings possibly suggest more virulence factors in blast pathogen population throughout the study sites. Since the number of NILs/varieties susceptible at Ouedeme was higher than that of Bagou, this suggests the possibility that Ouedeme has more virulence factors in blast pathogen population than Bagou.

Therefore, Ouedeme is the best site followed by Bagou site for screening for durable resistance as most blast pathogen races in these two sites were able to overcome the vertical resistance in the NILs/varieties. IRBLta2-Pi/CO, IRBLta2-Re/CO and IRBLta2-IR64/CO with *Pita2* resistant gene have different reactions across sites.

Table 4. Mean comparison of blast incidence scores of the NILs/varieties at the different experimentation sites.

NILs/Varieties	Resistance genes	Means by NILs/varieties‡			
		Bagou	Kandi	Kokoy	Ouedeme
105 TTP-1	*Pita*	1.95±0.08^{c}	1.95±0.08^{c}	1.58±0.001^{c}	2.12±0.001^{b}
IRBL1-CL/CO	*Pi1*	3.08±0.0001^{a}	2.67±0.06^{b}	2.61±0.06^{a}	2.41±0.07^{a}
IRBLta-Ya/CO	*Pita*	1.46±0.11^{d}	1.87±0.001^{c}	1.77±0.09^{b}	2.41±0.07^{a}
IRBL7-M/CO	*Pi7*	3.02±0.05^{a}	2.61±0.06^{b}	3.03±0.05^{a}	2.41±0.07^{a}
75-1-127	*Pi9*	1.87±0^{c}	1.87±0^{c}	1.46±0.11^{c}	0.71±0^{c}
IRBLzt-IR56/CO	*Pizt*	1.87±0^{c}	1.58±0.001^{d}	1.67±0.09^{c}	2.11±0.13^{b}
IRBL5-M/CO	*Pi5*	3.08±0^{a}	3.08±0^{a}	3.03±0.05^{a}	2.41±0.07^{a}
IRBLz-Fu/CO	*Piz*	1.87±0^{c}	1.87±0.001^{c}	1.67±0.09^{c}	2.24±0.25^{a}
IRBLkp-K60/CO	*Pikp*	3.08±0^{a}	2.61±0.06^{b}	3.03±0.05^{a}	2.54±0.11^{a}
Co39	*Pia*	3.08±0.001^{a}	3.08±0^{a}	3.03±0.05^{a}	2.41±0.07^{a}
IR1529	*Pi33*	1.87±0^{c}	1.95±0.08^{c}	1.46±0.11^{c}	1.30±0.29^{c}
IRBLta2-Pi/CO	*Pita2*	3.08±0.0001^{a}	2.61±0.06^{b}	2.67±0.06^{a}	2.54±0.11^{a}
IRBLks-CO/CO	*Piks*	3.08±0.0001^{a}	3.08±0.001^{a}	2.79±0.06^{a}	2.61±0.13^{a}
K 59	*Pit*	1.87±0^{c}	1.58±0^{d}	1.77±0.09^{b}	1.95±0.08^{b}
C101 A51	*Piz5*	1.87±0^{c}	1.95±0.08^{c}	1.77±0.09^{b}	1.68±0.10^{c}
IRBLsh-S/CO	*Pish*	1.87±0.001^{c}	2.04±0.08^{c}	1.67±0.09^{c}	0.88±0.17^{c}
Tetep	*Pikh,Pi1,Pita*	1.87±0.0001^{c}	1.87±0^{c}	1.46±0.11^{c}	1.46±0.12^{c}
Moroberekan	*Pi5,Pi7*	1.34±0.11^{e}	2.04±0.08^{c}	1.67±0.09^{c}	0.71±0^{c}
IRBLkh-K3/CO	*Pikh*	2.67±0.06^{b}	1.87±0.001^{c}	1.67±0.09^{c}	2.54±0.11^{a}
IRBLta2-Re/CO	*Pita2*	1.87±0^{c}	1.58±0^{d}	1.34±0.11^{c}	2.47±0.13^{a}
C101 LAC	*Pi1*	3.08±0^{a}	3.08±0^{a}	2.67±0.06^{a}	2.67±0.06^{a}
IRBLb-IT13/CO	*Pib*	1.87±0.001^{c}	1.95±0.08^{c}	2.04±0.08^{b}	1.47±0.41^{c}
C102 TTP	*Pita*	1.87±0^{c}	1.95±0.08^{c}	2.12±0^{b}	2.19±0.07^{b}
C104 PKT	*Pi3*	3.08±0.001^{a}	3.08±0^{a}	3.03±0.05^{a}	2.41±0.07^{a}
IRBLz5-CA/CO	*Piz5*	1.87±0.001^{c}	1.87±0^{c}	1.77±0.09^{b}	2.11±0.14^{b}
IRBLkm-Ts/CO	*Pikm*	1.87±0^{c}	1.95±0.08^{c}	1.58±0^{c}	2.33±0.20^{a}
Nato	*Pii*	1.87±0^{c}	1.95±0.08^{c}	1.77±0.09^{b}	0.71±0^{c}
IRBLta2-IR64/CO	*Pita2*	1.87±0.001^{c}	1.95±0.08^{c}	1.77±0.09^{b}	2.18±0.16^{b}
St 1	*Pif*	1.87±0^{c}	1.58±0^{d}	1.58±0^{c}	1.86±0.16^{b}
IRBLk-Ku/CO	*Pik*	3.08±0^{a}	2.61±0.06^{b}	2.61±0.06^{a}	2.41±0.07^{a}
TOG5681	Unknown	1.87±0^{c}	1.87±0^{c}	1.67±0.09^{c}	1.34±0.12^{c}
NERICA1	Unknown	1.87±0^{c}	1.95±0.08^{c}	1.77±0.09^{b}	0.71±0^{c}

‡ Within a column, means followed by the same letter are not significantly different at 5% level by Student-Newman-Keuls range test.

These reactions which have been predicted in previous studies suggest possible existence of another resistance gene(s) in IRBLta2-Re/CO and IRBLta2-IR64/CO in addition to *Pita2* (Sere et al., 2004a; Ishizaki et al., 2005; Hayashi and Fukuta, 2009).

Besides, NIL IRBL5-M/CO with resistance gene *Pi5* and NIL IRBL7-M/CO with resistance gene *Pi7* were all susceptible in all the four sites while their donor Moroberekan was resistant across all sites. This result suggests that durable resistance to blast disease could be achieved through gene pyramiding, since individual gene is easily overcome by the pathogen (Bormans et al., 2004; Sere et al., 2007). Moreover, TOG5681 and NERICA1 showed that both have potential for durable resistance to blast disease. Consequently, the deployment of NERICA1 to farmers' fields would be appropriate to replace the susceptible varieties currently used in Benin, while TOG5681 could serve as potential blast resistance donor in rice breeding program.

Conclusion

There is a great diversity in *M. oryzae* on the sites studied, and this diversity varied from one site to another. Pathogen pressure is greater on the three sites in the Northern than the Southern site. Bagou and Ouedeme sites are good sites for screening for blast resistance in Benin, as it is likely that most races found were able to overcome the vertical resistance of the varieties. The

Table 5. Blast pathogen virulence spectrum at the experimentation sites.

NILs/varieties	Resistance genes	Virulence spectrum			
		Ouedeme	Bagou	Kandi	Kokoy
C105 TTP-1	*Pita*	In	In	In	R
IRBL1-CL/CO	*Pi1*	S	HS	S	S
IRBLta-Ya/CO	*Pita*	S	R	In	In
IRBL7-M/CO	*Pi7*	S	HS	S	HS
75-1-127	*Pi9*	R	In	In	R
IRBLzt-IR56/CO	*Pizt*	In	In	R	R
IRBL5-M/CO	*Pi5*	S	HS	HS	HS
IRBLz-Fu/CO	*Piz*	S	In	In	R
IRBLkp-K60/CO	*Pikp*	S	HS	S	HS
Co39	*Pia*	S	HS	HS	HS
IR1529	*Pi33*	R	In	In	R
IRBLta2-Pi/CO	*Pita2*	S	HS	S	S
IRBLks-CO/CO	*Piks*	S	HS	HS	S
K 59	*Pit*	In	In	R	In
C101 A51	*Piz5*	R	In	In	In
IRBLsh-S/CO	*Pish*	R	In	In	R
Tetep	*Pikh,Pi1,Pita+*	R	In	In	R
Moroberekan	*Pi5,Pi7*	R	R	In	R
IRBLkh-K3/CO	*Pikh*	S	S	In	R
IRBLta2-Re/CO	*Pita2*	S	In	R	R
C101 LAC	*Pi1*	S	HS	HS	S
IRBLb-IT13/CO	*Pib*	R	In	In	In
C102 TTP	*Pita*	In	In	In	In
C104 PKT	*Pi3*	S	HS	HS	HS
IRBLz5-CA/CO	*Piz5*	In	In	In	In
IRBLkm-Ts/CO	*Pikm*	S	In	In	R
Nato	*Pii*	R	In	In	In
IRBLta2-IR64/CO	*Pita2*	In	In	In	In
St 1	*Pif*	In	In	R	R
IRBLk-Ku/CO	*Pik*	S	HS	S	S
TOG5681	Unknown	R	In	In	R
NERICA1	Unknown	R	In	In	In

R= Resistant; HS= high susceptible; S= susceptible; In= intermediate.

varieties with resistance genes *Pi1*, *Pita*, *Pi7*, *Pi5*, *Piz*, *Pikp*, *Pia*, *Pita2*, *Piks*, *Pikh*, *Pita2*, *Pi1*, *Pi3*, *Pikm*, and *Pik* are not stable at all sites in Benin and should therefore be avoided. This information is useful for development of durable resistant variety to blast disease in Benin.

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