Part 4:

Workshop full papers on Blast management in some West African Countries

Rice blast management in Burkina Faso from 1999 to 2002: Use of varietal resistance and training of agents and producers

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Abstract

Blast (*Magnaporthe grisea*) remains one of the main biotic constraints to rice production in Burkina Faso. Without any candidate for biological control that would be both effective and available to producers, the current approach in blast control tends to develop an integrated control strategy combining genetic resistance, cultural practices, and the use of alternative natural products instead of the conventional chemical control. Accordingly, from 1998 to 2002, the selection of genetic material resulted in seven varieties for upland rice conditions and 13 varieties for rain-fed lowland with an average to good resistance, and 15 fixed lines at the end the cycle for rain-fed lowland rice. About 10 NERICAs resistant under upland conditions were identified during the 2002 rainy season. A multidisciplinary approach including entomology, phytopathology and nematology allowed the development of a technological package for the integrated protection of rice. This package combines the use of neem kernel extracts against insects, organic matter and dried neem leaves against nematodes, and rice chaff ashes against blast. It was tested in the irrigated rice perimeters of Banzon and Karfiguéla in 2000 and it increased the yields by 9.3% to 18.5% depending on sites. Its economic profitability was established using the cost-benefit ratio which reached 1 to 3.38 on the first site (Banzon) and 1 to 1.50 on the second site (Karfiguéla). Such results offer a real solution for the integrated protection of irrigated rice culture against the three main groups of pests in Burkina Faso, while preserving the environment from dangerous pesticides.

In this approach for blast control, the training of producers and the field supervising agents from development structures was particularly emphasised. Therefore the training and re-training in the integrated management against rice pests reached about 100 technicians from the DRA (*Directions Régionales de l'Agriculture*) and numerous producers thanks to PVS (Participatory Varietal Selection) activities and the production of good-quality seeds.

Résumé

La pyriculariose (*Magnaporthe grisea*) demeure une des principales contraintes biotiques à la production du riz au Burkina Faso. En absence de candidat de lutte biologique efficace et accessible aux producteurs, l'approche de contrôle de la pyriculariose passe par le développement de la lutte intégrée combinant la résistance génétique, les pratiques culturales et l'usage de produits naturels alternatifs à la lutte chimique conventionnelle. C'est ainsi que de 1998 à 2002 le criblage du matériel génétique a permis de retenir 7 variétés de résistance bonne à moyenne en riziculture pluviale et 13 en riziculture de bas-fond ainsi que 15 lignées en fin de disjonction pour le riz de bas-fond. Une dizaine de NERICA résistantes en condition pluviale a été également identifiée au cours de la saison humide 2002. Une approche pluridisciplinaire impliquant l'entomologie, la phytopathologie et la nématologie a permis de mettre au point un paquet technologique de protection intégrée du riz. Ce paquet combine l'application des extraits d'amandes de neem contre les insectes, de la matière organique et de feuilles séchées de neem contre les nématodes, et de cendres de balles de riz contre la pyriculariose. Il a été testé sur les périmètres rizicoles irrigués de Banzon et de Karfiguéla en 2000 et a permis des gains de rendement de 9,3 % à 18,5 % selon les sites. Sa rentabilité économique a été établie sur la base du rapport coût-bénéfice qui est de 1 pour 3,38 sur le premier site (Banzon) et de 1 pour 1,50 sur le

second site (Karfiguéla). Ces résultats apportent une solution concrète à la protection intégrée du riz irrigué contre les trois principaux groupes de ravageurs au Burkina Faso, tout en préservant l'environnement des pesticides dangereux.

Dans l'approche de gestion de la pyriculariose un accent été mis également sur la formation des producteurs et des agents d'encadrement des structures de développement du Burkina. C'est ainsi que la formation et le recyclage en gestion intégrée des ravageurs du riz a touché une centaine de techniciens des DRA (Direction régionale de l'Agriculture) et de nombreux producteurs à travers les activités PVS (Participatory Varietal Selection) et la production de semences de qualité acceptable.

Introduction

In Burkina Faso, rice production ranks fourth after sorghum, millet and maize. Rice culture has numerous biotic constraints including diseases, among which blast is the most frequent (Pande 1997). In West Africa and more in Burkina Faso, blast is the disease that has the greatest economic impact on rice production (Notteghem 1985; Sy and Séré 1996). The farmers' losses usually reach 9 to 14% but they may exceed 20% with susceptible varieties. WARDA (1995) estimated the yearly yield loss due to this disease at more than 10 million US dollars. The development of technological packages of integrated management and variety resistance are the control methods chosen in Burkina Faso to fight this disease. The PVS approach to variety resistance is certainly the most profitable economically, the least damaging environmentally and the most easily adopted by the farmers.

The screening of segregated lines and NERICAs for their resistance to blast is one among other selection methods used to widen the genetic basis of rice. This is why the screening of 15 lines since 1999 and 16 NERICAs during the 2002 rainy season was implemented under a heavy pressure of natural inoculum (DITER procedure) in order to obtain new valuable material for the Burkina rice producers. Beyond extending the available genetic basis, the training of producers and supervising agents was used as a reliable means to transfer the technologies developed by researchers.

Screening the rice varieties selected in Burkina Faso for their resistance to blast

Justification

Most of the vegetal material selected before 1999 was not assessed under heavy pressure of *Magnaporthe grisea* for its resistance to blast. Extending the genetic basis of rice leads to the introduction and assessment of the varieties proposed by INGER in order to check their performances under Burkina conditions.

Objective

The main objective is to offer the producers a vegetal material tolerant or resistant to the most frequent diseases, including blast.

Material and methods

Thirty varieties selected before 1999 (15 for rain-fed lowland conditions and 15 for upland conditions), 15 segregated lines for rain-fed lowlands, and 15 NERICAs were screened for their resistance to blast from 1999 to 2002 (Table 29). Apart from the segregated lines, which were sown without repetition, the procedure was similar for all the other batches. The F3 and F4 generation lines have been sown every year since 1999 in Banfora under heavy pressure of natural inoculum of *Magnaporthe grisea* according to a DITER procedure (Notteghem 1985). The other batches (the varieties selected before 1999 and the NERICAs) were also sown in June or July in Farako-Bâ for the upland conditions and in Banfora for the rain-fed lowland conditions using a Fisher block design with four repetitions with a DITER-like infesting border. For each experiment the development of blast was followed at 35, 42, 49 and 56 days after sowing using a visual scale of 0 to 9 (IRRI 1996); the blast panicle neck lesions were also counted at 15 and 30 days after panicle emergence. The yield components were also measured (harvested grain weight, weight of 1000 grains, grain humidity). Statistical analysis of the data was performed with STATVIEW/SAS (Statistical Analysis System). Mean comparison was made with the SNK test. The regression analysis of the rice yield losses on blast incidence was used to complete the application of a scale allowing the final classification of varieties according to their resistance level to the two types of symptoms of the disease. Determination coefficient (R^2) was used to calculate percentage of yield loss due to blast. Four categories were outlined:

- 0 to 3% of losses due to blast = resistant varieties (GR)
- 3 to 5% of losses due to blast = moderately resistant varieties (MR)
- 5 to 7% of losses due to blast = susceptible varieties (S)
- more than 7% of losses due to blast = very susceptible varieties (VS).

Results

The field resistance screening allowed characterisation of the status of 15 genotypes under rain-fed upland conditions (Tables 30 and 31) and 15 for rain-fed lowlands (Table 32). The detailed results have already been presented at the 2nd biennial meeting of 4 R 2002.

Calculation of the loss took into account the determination coefficient of regression for the losses due to neck blast for which $R^2 = 0.25$.

Conclusion

Through the DITER procedure the status of 15 selected varieties has been determined. Among the 15 genotypes used, one presents good resistance to blast and 12 are moderately resistant with acceptable levels of yield losses.

Screening of NERICAs for their resistance to blast

Justification

The NERICAs have not yet been made available in the Burkina rice-growing zones. Their popularisation will have to be preceded by the preliminary screening study of their resistance to various diseases including blast. This is why a first batch of 13 NERICAs was tested under heavy pressure of natural inoculum.

Objective

This experiment was designed to determine the status of NERICAs under rain-fed upland conditions, particularly as concerns their resistance to blast which is the main rice disease in Burkina Faso.

Material and methods

Twenty rice varieties including 13 NERICAs (Table 5) were sown under rain-fed upland conditions at the Farako-Bâ station under a Fisher block design with four repetitions with a DITER-like infesting border. Four control varieties for blast susceptibility (CO 39, TOX 305510-1-1-1, FKR 2 and IR31851-96-2-3-2) and one resistant control (WAB 56-50) were used. The susceptible controls were sown at one-week

Table 29. Varieties and lines tested[†] under different rice-growing conditions between 1999 and 2002.

Rain-fed upland conditions	Lines in rain-fed lowlands	Rain-fed lowland conditions	NERICAs
	1- WAT 1176-B-INERA-B-B		
	2- WAT 1174-B-INERA-B-B		
	3- WAT 1181-B-INERA-B-B		
	4- WAT 1184-B-INERA-B-B		
	5- WAT 1189-B-INERA-B-B		
	6- WAT 1191-B-INERA-B-B		
	7- WAT 1193-B-INERA-B-B		
	8- WAT 1223-B-INERA-B-B		
	9- WAT 1242-B-INERA-B-B		
FKR 41	10- WAT 1244-B-INERA-B-B	FKR 19	WAB 365-B-4-H4-HB
	11- WAT 1249-B-INERA-B-B		
	12- WAT 1273-B-INERA-B-B		
	13- WAT 1275-B-INERA-B-B		
	14- WAT 1281-B-INERA-B-B		
	15- WAT 1282-B-INERA-B-B		
FKR 39	WAT 1176-B-INERA-B-B	FKR 14	WAB 450-11-1-P28-1-HB
FKR 35	WAT 1181-B-INERA-B-B	FKR 48	WAB 450-11-1-4-P41-HB
FKR 33	WAT 1184-B-INERA-B-B	FKR 32	WAB 450-24-2-2-P33-HB
FKR 29	WAT 1189-B-INERA-B-B	FKR2	WAB 450-1-B-P-103-HB
FKR 21	WAT 1191-B-INERA-B-B	IR32307-107-3-2-2	WAB 450-1-B-P-6-1-1
FKR 1	WAT 1193-B-INERA-B-B	CICA 8	WAB 450-1-B-P-6-2-1
FKR 37	WAT 1223-B-INERA-B-B	TOX 3093-35-2-3-3-1	WAB 500-13-1-1
WAB 96-31	WAT 1242-B-INERA-B-B	ITA 306	WAB 502-10-1-1
WAB 450-1-BP20-HB	WAT 1244-B-INERA-B-B	MRC 2663-2483	WAB 502-11-4-1
WAB 96-3	WAT 1249-B-INERA-B-B	BW 293-2	WAB 502-9-2-1
WAB 375-B-12-H5-1	WAT 1273-B-INERA-B-B	WABIR 12979	WAB 510-7-2-1
WAB 375-B-4-H2-HB	WAT 1275-B-INERA-B-B	BASMATI 217	WAB 513-12-2-1
WAB 96-24	WAT 1281-B-INERA-B-B	IR 2042-178-1	WAB 510-7-2-1
WAB 368-B-2-H1-HB	WAT 1282-B-INERA-B-B	IR 31851-96-2-3-2	WAB 513-12-2-1
IR 31851-96-2-3-2		TOX 3055-10-1-1-1-1	WAB 56-50 (FKR 37)[1]
			WAB 56-57
			WAB 510-7-2-1
			WAB 513-12-2-1
			WAB 56-50 (FKR 37)
			WAB 56-57

†Susceptible control variety for the NERICA = CO 39; FKR2; IR 31851-96-2-3-2; TOX 3055-10-1-1-1-1; Resistant control variety for NERICA (1) = WAB 56-50. * = other varieties included in the NERICA test.

Table 30. Classification of rain-fed upland rice varieties according to their field resistance level and yield losses.

Genotype	Resistance to leaf blast	Resistance to neck blast	Mean global yield loss (%)	Loss due to neck blast (25%) of global loss	Genotype status
FKR 41	GR[†]	MR	19.62	4.90	MR
FKR 39	GR	GR	11.31	2.85	GR
FKR 35	GR	GR	35.25	8.81	VS
FKR 33	GR	GR	18.85	4.71	MR
FKR 29	GR	MR	20.82	5.20	S
FKR 21	GR	GR	30.76	7.52	VS
FKR 1	GR	MR	33.11	8.27	VS
FKR 37	GR	GR	30.62	7.65	VS
WAB 96-31	GR	MR	27.08	6.77	S
WAB 450-1-	MR	MR	34.61	8.65	VS
WAB 96-3	GR	MR	13.29	3.32	MR
WAB375-B-12-	GR	GR	10.04	2.51	GR
WAB375-B-4	GR	GR	11.25	2.81	GR
WAB 96-24	GR	MR	26.63	6.65	S
WAB368	GR	GR	16.55	4.16	MR
IR31851-96-2-3-2	S	S	79.5	19.87	VS

† GR = good resistance; MR = moderate resistance; S = susceptible; VS = very susceptible; R = resistant.

Table 31. Incidence of neck blast in the Banfora rain-fed lowland during the 2000 and 2001 rainy seasons.

Genotype	Incidence of neck blast (%)				Resistance level[‡]
	2000		2001		
	15 DAH[†]	30 DAH	15 DAH	30 DAH	
FKR 19	0.70 c	16.8 c	0.9 c	3.1 c	MR
FKR 14	0.17 c	11.2 c	0.2 c	1.3 c	GR
FKR 48	0.10 c	5.7 c	0.1 c	4.0 c	GR
FKR 32	0.0 c	6.7 c	0.1 c	3.8 c	GR
WABIR 12979	0.3 c	6.4 c	0.2 c	0.7 c	GR
IR 32307-107-3-2-2	0.6 c	22.9 c	0.1 c	7.1 c	MR
CICA 8	0.5 c	10.3 c	0.1 c	4.0 c	GR
TOX 3093-35-2-3-3-1	0.0 c	8.0 c	0.1 c	6.0 c	GR
ITA 308	0.0 c	4.9 c	0.1 c	3.0 c	GR
MRC 2663-2483	2.1 c	45.9 ab	1.7 c	25.0 b	S
BW 293-2	0.3 c	3.8 c	1.3 c	41.9 a	S
FKR 2	7.9 b	38.3 b	1.2 c	27.3 b	S
BASMATI 217	0.8 c	16.5 c	6.0 c	28.3 b	MR
IR 2042-178-1	0.4 c	18.5 ab	1.9 c	13.4 c	MR
IR 31851-962-3-2	15.2 a	56.8 a	11.5 b	34.1 ab	S
TOX 3055-10-1-1-1-1	n.d.	n.d.	15.6 a	37.2 ab	S
Significance at 5%	n.d.	n.d.	VHS	VHS	
Coefficient of variation			100.8	45.7	

In a column, means followed by a common letter are not significantly different at 5% level of probability.
VHS = Very highly significant; n.d. = missing data.

[†] DAH = days after heading.

[‡] GR = good resistance; MR = moderate resistance; S = susceptible.

Table 32. Classification of the varieties screened under rain-fed lowland conditions.

Genotype	Resistance to leaf blast	Resistance to neck blast	Mean yield losses (%)	Losses (%) due to neck blast (r^2=0.25*)	Variety status
FKR 19	GR†	MR	17.76	4.44	MR
FKR 14	GR	GR	17.56	4.39	MR
FKR 48	GR	GR	14.55	3.63	MR
FKR 32	GR	GR	14.35	3.76	MR
WABIR 12979	GR	GR	17.35	4.37	MR
IR 32307-107-3-2-2	GR	MR	11.42	2.85	GR
CICA 8	GR	GR	13.94	4.74	MR
TOX 3093-35-2-3-3-1	GR	GR	14.01	3.50	MR
ITA 306	GR	GR	24.74	6.18	S
MRC 2663-2483	MR	S	17.11	4.28	MR
BW 293-2	MR	S	13.90	3.48	MR
FKR 2	S	S	14.02	3.50	MR
BASMATI 217	MR	MR	13.49	3.37	MR
IR 2042-178-1	MR	MR	19.83	4.96	MR
IR 31851-962-3-2	MR	S	22.05	5.51	S
TOX 3055-10-1-1-1-1	S	S	46.44	11.61	VS

† GR = good resistance; MR = moderate resistance; S = susceptible; VS = very susceptible; R = resistant.

intervals so that there would always be a reference control to compare with the long-cycle NERICAs. The development of blast was measured from tillering to panicle emergence using a visual scale from 0 to 9 (IRRI 1996) and the counting of neck blast lesions was done at 15 and 30 days after heading. The yield components were measured.

The statistical analysis of the data was performed with STATVIEW/SAS (Statistical Analysis System). The comparison of the means was made with the SNK test. The regression analysis of the rice yield losses on blast incidence was used to calculate the actual losses due to blast. Our scale for the final classification of varieties according to their resistance level to the two types of the disease and to the losses actually due to the disease allow us to distinguish four categories as indicated in the methodology section.

Table 33. The NERICAs and other varieties evaluated for their resistance to blast during the 2002 rainy season at Farako-Bâ.

WAB 365-B-4-H4-HB	WAB 510-7-2-1
WAB 450-11-1-P28-1-HB	WAB 513-12-2-1
WAB 450-11-1-4-P41-HB	WAB 30-24*
WAB 450-24-2-2-P33-HB	WAB 32-60*
WAB 450-1-B-P-103-HB	WAB 56-57*
WAB 450-1-B-P-6-1-1	CO 39 **
WAB 450-1-B-P-6-2-1	TOX 305510-1-1-1**
WAB 500-13-1-1	IR31851-96-2-3-2**
WAB 502-10-1-1	FKR 2**
WAB 502-11-4-1	WAB 56-50 (resistance control)
WAB 502-9-2-1	

* = other non-NERICA varieties included in the test; ** = susceptible control varieties.

Results and discussion

The analysis of variance showed significant differences among varieties for resistance to the two forms of blast (at 35, 42, 49 and 56 days after sowing for leaf blast and at 15 and 30 days after heading for neck blast). Table 34 presents the evolution of variances and the values of *P* at the probability level of 0.05%. Apart from the control varieties, all the NERICAs resisted leaf blast at each phenological stage. The mean severity of leaf blast varied from 0.21 to 1.90 from the tillering to the panicle emergence stages (Table 34). Maximum scores of 8.5 and 9 were recorded on three susceptible controls. For neck blast, average incidences of 14.34 and 17.57 were recorded respectively at 15 and 30 days after heading whereas the susceptible controls presented peak levels of 100%.

Leaf blast appeared at low intensity on eight varieties at 35 days after sowing with severity scores from 0.12 to 1.62 on the 0 to 9 scale. Maximum scores of 7 to 9 were recorded on the susceptible controls at the end of the tillering and panicle emergence stages whereas the varieties that were most severely attacked did not present severity above 3.6 (Table 35).

The regression analysis shows that leaf blast at the end-tillering and panicle emergence stages was strongly influenced by how severe the disease had been at the previous stages (Figures 7 and 8).

Table 34. Summarized results of the analysis of variance for the severity of leaf blast at 35, 42, 49 and 56 days after sowing (DAS) and for the incidence of neck blast. The analysis was made with 21 varieties with four repetitions.

Parameters	Leaf blast				Neck blast incidence	
	35 DAS	42 DAS	49 DAS	56 DAS	15 DAH[†]	30 DAH
Variance	0.255	1.743	3.562	6.388	1126.04	888.72
P	0.0001	0.0001	0.0001	0.0001	0.001	0.0001
Mean	0.21	0.73	1.36	1.90	14.34	17.57
Minimum	0	0	0	0	0	0
Maximum	2.50	7.5	8.5	9	100	100

[†] DAH = days after heading.

Table 35. Evolution of leaf blast on NERICAs during the 2002 rainy season (0–9 scale).

Variety	LB1[†] 35 DAS	LB2 42 DAS	LB3 49 DAS	LB4 56 DAS	Resistance level[‡]
CO 39**	1.62	4.87	7	8.87	VS
FKR 2**	0	1.12	2.62	3.75	MS
IR 31851-69-2-3-2**	0.37	1.50	3	4.25	MS
TOX 3055-10-1-1-1**	0.87	2.12	5.12	8.12	VS
WAB 30-24*	0	0	0.50	0.62	R
WAB 32-60*	0	0.25	0.50	0.50	R
WAB 365-B-4-H4-HB	0	0	0	0.12	R
WAB 450-11-1-P28-1-HB	0	0.25	0.37	0.37	R
WAB 450-11-1-4-P41-HB	0	0.12	0.50	0.75	R
WAB 450-24-2-2-P33-HB	0	0	0.12	0.12	R
WAB 450-1-B-P-103-HB	0	0.12	0.75	1	R
WAB 450-1-B-P-6-1-1	0.12	0.37	0.50	0.75	R
WAB 450-1-B-P-6-2-1	0.25	0.25	0.25	0.37	R
WAB 500-13-1-1	0.25	0.62	1	1.25	R
WAB 502-10-1-1	0	0	0.37	0.37	R
WAB 502-11-4-1	0	0.12	0.25	0.25	R
WAB 502-9-2-1	0	0.37	0.62	0.75	R
WAB 510-7-2-1	0.87	2	2.37	3.62	R
WAB 513-12-2-1	0	0.5	1.37	2	R
WAB 56-50 (resistant check)	0	0.25	0.25	0.25	R
WAB 56-57*	0.12	0.37	1.12	1.87	R
Coefficient of Variation	2.35	1.82	1.38	1.33	

[†] LB1 = leaf blast at tillering; LB2 = leaf blast at full tillering; LB3 = leaf blast at end tillering; LB4 = leaf blast at panicle emergence; DAS = days after sowing.
[‡] VS = very susceptible; MS = moderately susceptible; R = resistant.
* = other varieties not NERICAs included in the test; ** = susceptible control varieties.

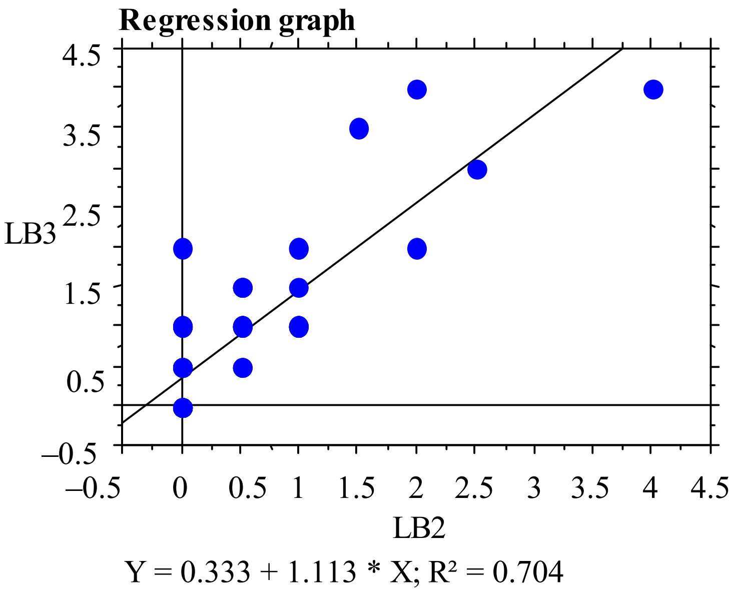

Figure 7. Regression of the effect of the leaf blast severity at the end-tillering stage on the severity at the full-tillering stage.

LB2 = Leaf blast at 42 days after sowing; LB3 = Leaf blast at 49 days after sowing.

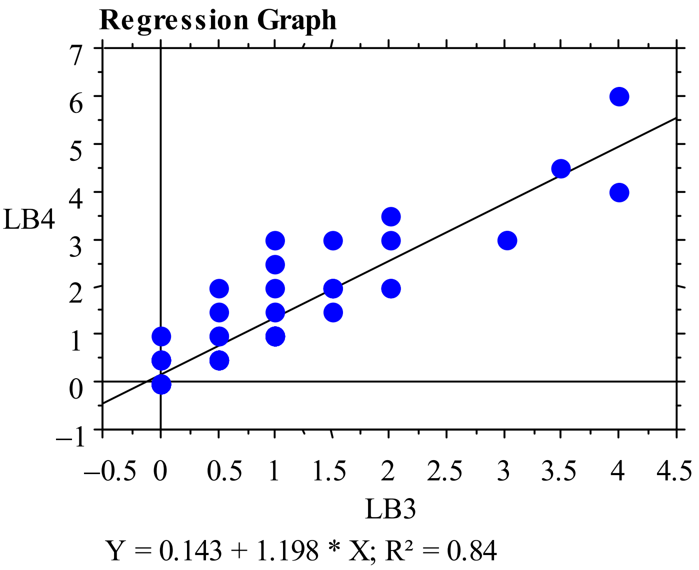

Figure 8. Regression of the effect of leaf blast severity at the panicle emergence stage on the severity at the end-tillering stage.

LB3 = Leaf blast at 49 days after sowing; LB4 = leaf blast at 56 days after sowing.

The evolution of the neck blast attack presented mean incidences of 0 to 7.5% at 15 days after heading according to the varieties tested and of 3 to 100% for the three controls (Table 36). At 30 days after heading the incidence of neck blast varied from 15 to 100% for the three controls and from 3 to 64% for the NERICAs. As can be noted, the susceptible control varieties CO 39 and TOX 3055-10-1-1-1 were completely burnt out, leading to a 100% yield loss. The IR 31851-96-2-3-2 control was not able to complete its cycle because of the lack of rain while the heavy pressure of neck blast as early as late heading contributed to complete destruction before cropping.

Unlike what happened in the former rainy seasons, the susceptible control variety FKR 2 resisted neck blast, presenting an average incidence of 3% at the first measurement (15 days after heading) and 15% at 30 days after heading.

Table 36. Incidence of neck blast at 15 and 30 days after heading (DAH).

Variety	Neck blast incidence		Resistance level[†]
	15 DAH	30 DAH	
CO 39**	100	100	VS
FKR 2**	3.1	15.01	S
IR 31851-69-2-3-2**	100	100	VS
TOX 3055-10-1-1-1**	7.5	100	VS
WAB 30-24*	0	3.3	R
WAB 32-60*	0.1	5.8	R
WAB 365-B-4-H4-HB	0	2.8	R
WAB 450-11-1-P28-1-HB	4.3	50.5	S
WAB 450-11-1-4-P41-HB	3.8	59.5	S
WAB 450-24-2-2-P33-HB	0	4.5	R
WAB 450-1-B-P-103-HB	5.8	8.0	R
WAB 450-1-B-P-6-1-1	0.1	1.0	R
WAB 450-1-B-P-6-2-1	0.1	1.9	R
WAB 500-13-1-1	0.2	4.6	R
WAB 502-10-1-1	0	0.8	R
WAB 502-11-4-1	0	1.4	R
WAB 502-9-2-1	0.3	5.3	R
WAB 510-7-2-1	0	0	R
WAB 513-12-2-1	6.8	13.3	S
WAB 56-50 (susceptible control)	0	7.1	R
WAB 56-57*	1.4	4.7	R
Coefficient of Variation	2.34	1.70	

[†] VS = very susceptible; S = susceptible; R = resistant.

The regression analyses of blast incidence on yield losses show that the combined effect of leaf and neck blast explains 89.4% of gross losses (Table 37). When compared with what happened in the preceding seasons (2000 and 2001) when the losses actually due to the disease did not exceed 30%, the 2002 rainy season presented some drought periods that contributed to the weakening of the rice seedlings while it also increased the incidence of blast on the susceptible controls. The grain losses show a significant variation according to varieties. Among the 15 NERICAs that completed their development cycles, an average gross loss of 25.65% was recorded. A 100% loss was recorded with the susceptible controls except with FKR 2 which showed a 19% loss only (Table 38). The separation with the SNK test of the means shows that the 16 NERICAs and the resistant control WAB 56-50 do not present any significant differences but differ from the three susceptible controls. The variety classification takes into account the loss recorded for the resistant control WAB 56-50 under blast heavy pressure and the criteria that were *a priori* retained to select the varieties (see classification scale). Table 38 presents the results obtained with 10 resistant and five moderately resistant NERICAs.

Table 37. Coefficients of regression determination (R^2) for the grain losses (L) on the severity and incidence of blast at the different phenological states of rice development.

	Independent variables LB and NB								
Dependent variable L	LB1[†]	LB2	LB3	LB4	LB1+ LB2+ LB3+LB4	NB1	NB2	NB1+NB2	LB + NB
R^2	0,31	0,37	0,56	0,61	0,62	0,81	0,33	0,893	0,894

[†] LB = leaf blast; NB = neck blast; 1 = tillering; 2 = full tillering; 3 = end tillering; 4 = panicle emergence; L = grain loss.

Conclusion

Among the 18 NERICAs tested, only 15 actually completed their development cycle. Blast pressure was nevertheless strong enough to differentiate the varieties according to their susceptibility level. Three among the four susceptible controls were burnt out as was the infesting border before maturity. When compared with the resistant control variety WAB 56-50, 10 NERICAs possessed a good resistance level and five were moderately resistant to blast.

Table 38. Classification of NERICAs according to their resistance level during the 2002 rainy season.

Variety	Resistance[†] to:		Mean losses (%)	Losses due to blast (%)	Variety status
	Leaf blast	Neck blast			
CO 39**	VS	VS	100	89.4	VS
FKR 2**	MS	S	19.06	17.04	S
IR 31851-96-2-3-2**	MS	VS	100	89.4	VS
TOX 3055-10-1-1-1**	VS	VS	100	89.4	VS
WAB 30-24*	R	R	9.07	8.11	R
WAB 32-60*	R	R	17.59	15.72	R
WAB 365-B-4-H4-HB	R	R	19.55	17.48	MR
WAB 450-11-1-P28-1-HB	R	MR	14.53	12.99	R
WAB 450-11-1-4-P41-HB	R	MR	11.22	10.03	R
WAB 450-24-2-2-P33-HB	R	R	8.28	7.40	R
WAB 450-1-B-P-103-HB	R	R	9.80	8.76	R
WAB 450-1-B-P-6-1-1	R	R	21.24	18.99	MR
WAB 450-1-B-P-6-2-1	R	R	18.37	16.42	MR
WAB 500-13-1-1	R	R	20.12	17.99	MR
WAB 502-10-1-1	R	R	8.65	7.73	R
WAB 502-11-4-1	R	R	10.95	9.79	R
WAB 502-9-2-1	R	R	9.0	8.05	R
WAB 513-12-2-1	R	S	18.5	16.54	MR
WAB 56-50 (susceptible control)	R	R	14.85	13.28	R
WAB 56-57*	R	R	7.81	6.98	R

[†] VS = very susceptible; S = susceptible; MR = moderately resistant; R = resistant.

Follow-up of rainfed lowland rice segregated populations for blast in Banfora

Justification

In order to extend the genetic basis of rice, the segregated lines were cultivated under a DITER design since 1999 and have become a productive source of resistant varieties at the end of the cycle.

Material and methods

During the 2001 crop, the five best panicles were picked out for each line, making a total of 75 panicles; they were sown in one per line without repetition under a DITER design. The infesting border was composed of the following susceptible varieties: TOX 3055-10-1-1-1 + IR 31851-96-2-3-2 + FKR 2. The development of blast was followed as previously from tillering to panicle emergence using a visual scale of 0 to 9 (IRRI 1996) and blast counting on the panicle neck was made at 15 and 30 days after heading.

Results

As shown in Table 39 the analysis of variance of blast severity and incidence shows highly significant differences at all the development stages of the plant. In spite of the screening performed each year since 1999 and the choice of the least affected line-heads, the supposedly fixed lines were still receptive to contamination by *M. grisea*. The severity of leaf blast varied from 0.9 to 3.5 and from 1.1 to 4.3 respectively at the end-tillering stage (LB3) and at the panicle emergence stage (LB4). The incidence of neck blast 30 days after heading varied from 0 to 12.44%. The infesting border had burnt out before the maturity stage. Apart from WAT 1281-B-FKR-B-B, which displayed a severity measure of 6, it may be stated that the 14 other lines are resistant at the leaf phase of the disease. At the heading stage, 10 among 15 lines present a rather good resistance to neck blast whereas four may be considered as moderately resistant and one as susceptible (Table 40).

Table 39. Results of the analysis of variance for blast severity and incidence on the segregated lines under rain-fed lowland conditions, rainy season 2002.

Parameter	LB1	LB2	LB3	LB4	NB1	NB2
	35 DAS	42 DAS	49 DAS	56 DAS	15 DAH	30 DAH
Mean	1.37	1.82	2.19	2.66	2.43	4.55
Variance	1.416	1.686	1.593	2.336	7.95	20.21
Minimum	0	0.5	0.5	1	0	0
Maximum	6.5	8.5	8.5	9	9.58	12.44
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
CV	0.87	0.71	0.58	0.57	1.16	0.99

LB = leaf blast; NB = neck blast; 1 = tillering; 2 = full tillering; 3 = end tillering; 4 = panicle emergence; DAS = days after sowing; DAH= days after heading.

Table 40. Means for blast severity and incidence on the rain-fed lowland segregated lines during the 2002 rainy season.

Lines	LB2[†]	LB2	LB3	LB4	Inc. NB1[‡]	Inc. NB2	Status[§]
WAT 1174-B-FKR-B-B	0.5	1.3	2	2	2.22	3.82	R
WAT 1176-B-FKR-B-B	0.1	0.7	1.2	1.5	1.83	3.04	R
WAT 1181-B-FKR-B-B	3.1	3.6	2.9	4.3	9.58	12.47	S
WAT 1184-B-FKR-B-B	1	1.1	1.8	2.2	2.59	8.96	MR
WAT 1189-B-FKR-B-B	1.4	1.7	1.8	1.9	2.29	3.36	R
WAT 1191-B-FKR-B-B	0.8	0.9	1.2	1.4	0.78	2.14	R
WAT 1193-B-FKR-B-B	2.2	2.2	2.2	2.2	1.41	4.32	R
WAT 1223-B-FKR-B-B	3	3.5	3.5	4	3.03	5.69	MR
WAT 1242-B-FKR-B-B	0.8	1.6	2.5	3.3	2.04	3.33	R
WAT 1244-B-FKR-B-B	1.6	2	2.4	2.9	1.95	3.79	R
WAT 1249-B-FKR-B-B	0.3	0.6	0.9	1.1	1.41	2.44	R
WAT 1273-B-FKR-B-B	0.7	1	1.4	1.7	0.47	2.75	R
WAT 1275-B-FKR-B-B	0.7	1.2	1.7	2.5	1.55	1.98	R
WAT 1281-B-FKR-B-B	2.6	3.3	4.6	6	1.15	5.71	MR
WAT 1282-B-FKR-B-B	0.7	1.2	1.5	1.7	3.82	3.86	MR
Infesting border	2	8.5	8.5	9	100	100	VS
CV	0.87	0.71	0.58	0.57	1.16	0.99	

[†] LB2 = leaf blast at full tillering; LB3 = leaf blast at end tillering; LB4 = leaf blast at panicle emergence.
[‡] Incidence of neck blast: NB1 = 15 days after heading; NB2 = 30 days after heading.
[§] VS = very susceptible; S = susceptible; MR = moderately resistant; R = resistant.

Multidisciplinary approach of the integrated protection of rice against insects, blast and nematodes

In parallel with these experiments, developing a technological package for the integrated protection of rice against insects, blast and nematodes became necessary. The technological package combines the application of neem kernel extract against insects, organic matter and dried neem leaves against nematodes, and rice chaff ashes against blast. Its efficacy (Tables 41and 42) and economic profitability were assessed.

Table 41. Efficacy of the technological package of integrated protection of rice against the most frequent pests during the 2002 rainy season..

Treatment[†]	Dead hearts, 60 DAT[‡] (%)		Onion tubes, 60 DAT (%)		Incidence of NB, 30 DAH (%)		Nematodes per g of root, 60 DAT	
	Banzon	Karfiguela	Banzon	Karfiguela	Banzon	Karfiguela	Banzon	Karfiguela
T1	5.3 a	4.1 a	0.4 a	11.9 a	8.0 ab	10.1 a	11 a	22 a
T2	3.8 a	1.4 b	0.7 a	6.0 b	5.7 b	8.1 a	8 c	5 b
T3	2.1 a	1.7 b	0.3 a	5.9 b	11.4 a	11.7 a	11 a	7 b

In a column, means followed by a common letter are not significantly different at 5% level of probability.

[†] T1 = the application of neem kernel extract against insects; T2 = organic matter and dried neem leaves against nematodes; T3 = rice chaff ashes against blast.

[‡] NB = neck blast; DAT = days after transplanting; DAH = days after heading.

Table 42. Evolution of yields (14% humidity) as a function of IPM package treatments applied at two sites, rainy season 2000.

Treatment[†]	Karfiguéla (t/ha)	Banzon (t/ha)
T1	5.78 a	6.31 b
T2	7.69 b	7.48 a
T3	6.32 b	7.48 a
Probability	0.0068	0.1462
Significance	HS	NS

In a column, means followed by a common letter are not significantly different at 5% level of probability. HS = highly significant; NS = not significant.

[†] T1 = the application of neem kernel extract against insects; T2 = organic matter and dried neem leaves against nematodes; T3 = rice chaff ashes against blast.

The gains in yield (Figure 9) obtained after chemical treatment and the application of the technological package in comparison with the control were respectively of 33% and 9.3% in Karfiguéla and of 18.5% in Banson (for treatments T2 and T3).

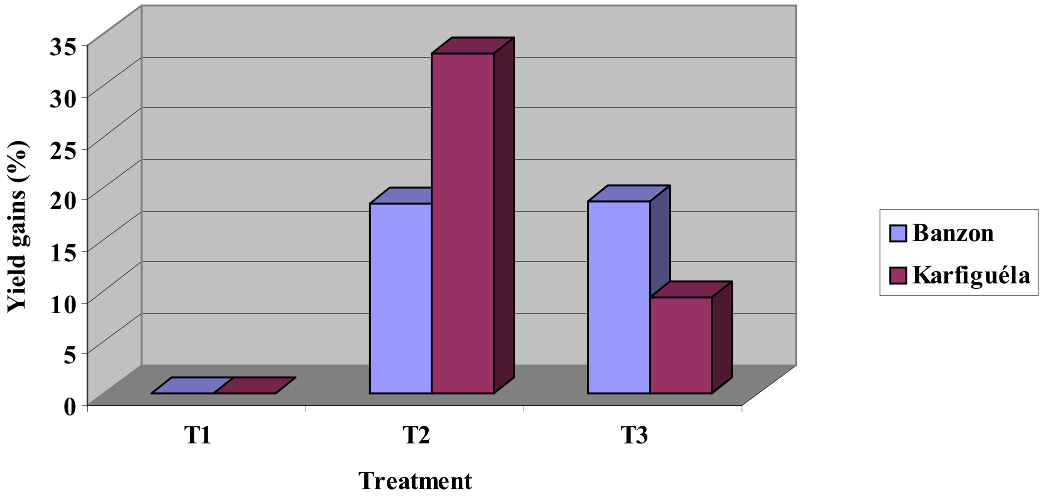

Figure 9. Gains in yield obtained with the technological package.

The release of a technological package requires a study of its economic profitability (Table 42). The data presented take into account the current price of paddy rice (105 Fcfa) and the cost of a phytosanitary treatment (Treatment 2) as compared to the use of natural pesticides such as neem, manure and rice chaff ashes which are locally available. The costs linked to the processing of these products were considered as family labour. The benefits generated by the different treatments were used in the calculation of the Cost:Benefit ratio (Table 43).

The chemical treatments (T2) yielded a benefit of 122,585 Fcfa, equivalent to that of the technological package in Banzon and 200,550 Fcfa as compared with 56,760 Fcfa for the technological package in Karfiguéla. However the very high cost of the imported chemical products inevitably leads to an economic loss after T2: the Cost:Benefit ratio reaches 1:4.10 in Banzon and 1:1.89 in Karfiguéla. This economic profitability of the technological package could be even further increased if its application was coupled with a procedure of phytosanitary supervising and of threshold intervention design (Dakouo *et al.* 1995). According to these authors, spraying the leaves with a mash of neem kernels would be necessary only after the attack had reached the level of 5% of dead hearts or 1% of white panicles. This new IPM package for rice protection presents the great advantage of protecting the environment and being environmentally sustainable as it is exclusively composed of non-polluting and biodegradable natural products.

Table 43. Economic profitability of the technological package of rice integrated protection, rainy season 2000.

Treatment†	Yield (t/ha)		Benefit (x 1000 Fcfa)		Treatment cost (x 1000 Fcfa)		Cost:benefit ratio	
	Banzon	Karfiguela	Banzon	Karfiguela	Banzon	Karfiguela	Banzon	Karfiguela
T1	6.31 a	5.78 b	–	–	–	–	–	–
T2	7.48 a	7.69 a	122.85	200.55	600.60	600.60	1:0.20	1:0.33
T3	7.48 a	6.32 a	122.85	56.76	30.00	30.00	1:4.10	1:1.89

In a column, means followed by a common letter are not significantly different at 5% level of probability.
†T1 = the application of neem kernel extract against insects; T2 = organic matter and dried neem leaves against nematodes; T3 = rice chaff ashes against blast.

Conclusion

The objectives targeted by the IPM technological package of integrated rice protection have been reached. Indeed the package combining organic matter, neem-derived products and the rice chaff ashes provides a good sanitary cover by considerably reducing the level of parasite/pest pressure while increasing rice yields.

These results offer a real solution to the integrated protection of rice against the three main types of pests (insects, diseases and nematodes) that can usually be found in the rice-growing perimeters of Burkina Faso.

The transfer of this rice protection package will be carried on for implementation in all the main irrigated perimeters of the country.

General conclusion and prospects

The field resistance of numerous selected varieties is well known. Susceptible and resistant genotypes have been identified and will be used as control references for future studies in rice breeding and phytopathology. The complete characterisation of the varieties screened in the field could be further developed through molecular biology tools.

The NERICAs tested during the 2002 rainy season present a good resistance to the Burkina *M. grisea* strains. After their behaviour has been confirmed in 2003, they will be tested under PVS procedures before being made available to the producers.

The training sessions offered were implemented in the frame of the PVS activities, of the “seed mini-doses in rice-growing” sessions and in a sub-regional project: IPPM (Integrated Plant and Pest management). From 1999 to 2002, 91 supervising agents and 649 producers were trained. The rice-growers training in IPM will be extended further.

The partnership with the Laboratoire de Pathologie et Biologie Moléculaire of WARDA will allow us to complete the characterisation of the varieties using the modern tools of molecular biology.

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Rice Blast Disease (*Pyricularia oryzae*) in The Gambia: Genotype by Environmental Reaction, Economic Significance and Management Strategies

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Résumé

La pyriculariose du riz causée par *Pyricularia oryzae* Cav. est une maladie majeure sous écosystème pluvial et de bas-fond en Gambie. Les pertes attribuées à la pyriculariose varient selon les localités et les variétés. En Gambie, même si l'importance économique de la pyriculariose du riz n'est pas publiée, des pertes de 100 % ont été observées dans certaines localités particulièrement soumis à des conditions de stress. Le programme de gestion des ravageurs (Pest Management Programme) de NARI (National Agricultural Research Institute), en collaboration avec l'Association pour le Développement de la Riziculture en Afrique de l'Ouest (ADRAO) a commencé à cribler des variétés de riz pluvial et de bas-fond pour leur résistance à la pyriculariose. Un programme de criblage de deux ans de six variétés, USEN (ACC.32560), BG90-2, ITA 212, IR 36, IR 64, et ARC5987 sous condition naturelle de bas-fond a démarré en 2000 tandis que le programme en condition pluviale a commencé en 2003. Aucune différence significative n'a été observée entre les traitements du rendement, mais des différences ont été notées dans l'effet des localités sur la réaction des variétés à la pyriculariose et sur leur rendement éventuel. Même si l'essai en condition pluviale n'a pas été concluante du fait du déficit pluviométrique, on a pu noter la même tendance de l'effet de l'environnement durant les premiers stades de croissance. La variété locale Prasana, tout en ayant la note de sévérité la plus élevée dans toutes les localités, se comporte beaucoup mieux à Dasilameh et à Sutusinjang qu'à Kanilai et Brikama ou la culture a été complètement détruite.

Summary

Rice blast *Pyricularia oryzae* Cav. is a major disease in the upland and lowland ecologies of the Gambia. Crop losses attributed to rice blast vary according to localities and varieties. In The Gambia although economic importance of rice blast has not been documented, yield losses of up to 100% have been observed in some locations especially under stress conditions. The Pest Management Programme (PMP) of NARI in collaboration with West African Rice Development Association (WARDA) started screening lowland and upland varieties for their resistance to blast. A two-year research programme screening six rice varieties, USEN (ACC.32560), BG90-2, ITA 212, IR 36, IR 64, and ARC5987 under natural lowland conditions was started in 2000 and while the upland work started only last season (2003). No significant difference was observed between treatment yields, but significant differences were seen in the effect of location on reaction of varieties to blast and their eventual yield. Although the upland trials did not do well due to shortage of rains, the same trend of environment by genotype reaction was observed during the early stages of the trials. The local check Prasana even though scoring the highest severity at all locations, performed much better in Dasilameh and Sutusinjang compared to Kanilai and Brikama sites where the crop was completely ravaged by the fungus

Background

The Republic of The Gambia is located on the Atlantic Coast of West Africa, between 14°0' and 16°38'W longitude, and 13°12' and 13°32'N latitude. The Republic of Senegal, with approximately 480 km of border, abuts it on the east, north and south. The Gambia has a total land area of 11 295 km^2 (about 1.04 million ha) of which 45% (about 558 000 ha) is arable; of that, 32% (about 178 560 ha) is currently used and 28% is forest and woodland (UNDP 1997).

The River Gambia, which originates from Guinea Conakry, is the most important water source for crop production. The country has a sub-tropical climate with a long dry season from November to May and a short rainy season from June to October. The average annual rainfall is about 850–1000 mm with mean temperatures of 21°–33°C. The River Gambia has a yearly occurring salinisation and subsequent flushing process over a long stretch of the river. During the dry season, the saline front moves upstream, reaching Kuntaur (about 260 km from Banjul) and in the wet season, the front is pushed downstream to about 80 km from Banjul. Tidal irrigation depends on the Atlantic tide movement, which pushes the water through the inlet gates or small creeks called 'bolons', distributing the water in the rice fields along both sides of the River Gambia.

Rice, the staple food of The Gambian people, accounts for 25–30% of total cereal production and occupies 56% of cultivated land (UNDP 1997). Most rice cultivation is along both sides of The Gambia River and has been steadily increasing from 18 950 tons in 1996 to 34 100 tons in 2000. At present the production figure stands at 10 100 tons for upland rice, 10 600 tons for swamp rice and 13 400 tons for irrigated rice (Table 44).

Rice in The Gambia is grown under diverse hydrological, climatic and edaphic conditions characteristic of the ecological zones of the country: upland, tidal swamps (lowland), mangrove swamps and the irrigated swamps. This diversity influences the spectrum and severity of pest and disease problems at any given site with rice blast being one of the most important disease problems (Bridge *et al.* 1978; Sanyang and Darboe 1999). The blast problem is generally more serious in the irrigated and upland ecologies—in the irrigated areas due to high planting densities, high fertiliser use and the high cropping intensity, and in the uplands where the disease is one of the primary constraints to increasing yield and yield stability of rice production in The Gambia. In the tidal swamps (including the mangrove and inland valley lowlands), which are the most important ecology in terms of area, blast is also a very important disease, especially in the lowlands.

Economic significance and management strategies

Rice blast (*Pyricularia oryzae* Cav.) is a major disease in the upland, irrigated and lowland ecologies of the Gambia. Crop losses attributed to rice blast vary according to localities and varieties. Agrios (1997) reported estimated yield losses of 50–90% from different parts of the world where blast is endemic while Jones (1987) reported an even wider yield loss range of 30–100%. In The Gambia, although the economic importance of rice blast has not been documented, yield losses of up to 100% have been observed in some locations, especially under stress conditions (Jobe *et al.* 2002). In fact some varieties in our blast trial of 2002/2003 were completely lost owing to the severity of the blast disease in some parts of the country as a result of water stress due to erratic rains.

Table 44. Rice production in ‘000 tons in The Gambia

Year	Upland	Swamp	Irrigated	Total
1982	4100	29 600	0	33 700
1983	2600	18 100	5400	26 100
1984	2200	8900	16 100	27 200
1985	3600	11 700	7800	23 100
1986	4250	12 750	7460	24 460
1987	1440	12 500	6500	20 440
1988	3880	20 000	5990	29 870
1989	2800	11 460	8340	22 600
1990	2390	12 010	6600	21 000
1991	2740	12 510	5740	20 990
1992	2000	12 500	4910	19 410
1993	1210	10 840	0	12 050
1994	3660	16 610	0	20 270
1995	3363	15 589	0	18 952
1996	4029	14 156	0	18 185
1997	6523	10 170	7446	24 139
1998	7990	10 846	7800	26 636
1999	8864	9424	13 365	31 653
2000	10 100	10 600	13 400	34 100

Source: Statistical Year Book of The Gambia (NASS 2001).

Screening work on rice varieties in The Gambia started in the late 1950s through various rice projects and the then-cereals research unit of the former department of agricultural research (DAR) and recently NARI; selection has been for blast-resistant varieties (Jobe *et al.* 2002). The Pest Management Programme (PMP) of NARI in collaboration with West African Rice Development Association (WARDA) also started a programme to screen some improved exotic upland and lowland rice varieties for their reaction to *P. oryzae* in The Gambia (ongoing).

The introduction of many exotic varieties by the above-mentioned institutions coupled with the recent massive introduction of hundreds of varieties through the Chinese Rice Technical Mission (CRTM) has nearly resulted in a total replacement of the landraces by improved exotic lines. Although varietal resistance appears to be the most economical way to control the disease, the fungus can produce new races which attack resistant varieties. This might explain the unpredictable behaviour (in multilocation trials) reported by Jobe *et al.* (2002) of varieties such as IR36 which was found to exhibit resistance elsewhere (Bonman *et al.* 1992). Occurrence of the disease is favoured by the use of high levels of nitrogen, the intensity of cropping in the irrigated areas, and non-flooding which results in stress conditions in the uplands. Farmers in The Gambia use very little seed dressing, if any. Some of the commercial and well-to-do farmers tried spraying their upland crops with fungicides in an attempt to control the rice blast disease.

As part of efforts to manage blast and other diseases of rice in The Gambia, NARI plans to study the variation in pathogenic strains of fungus in the country and to construct a screen house at NARI headquarters in Brikama (proposal submitted to WARDA during the 4Rs Meeting in 2002).

Genotype by environmental reaction

Varieties tested under the WARDA / NARI collaborative activities and through the many other programmes cited above have been reporting varied and mixed reactions of the same varieties in different locations of similar ecology and water regimes. The varieties USEN (ACC.32560), BG90-2, ITA 212, IR 36, IR 64, and ARC5987—tested under WARDA/NARI collaborative programme natural lowland conditions—showed no significant yield differences among treatments at any one location, but significant differences in the effect of location on reaction of varieties to blast and their eventual yield were reported by Jobe *et al.* (2002).

In the Kanilai and Brikama areas, varieties such as Prasana and Dingding Taringo (local Check) in the upland trials for 2002/2003 developed large spindle-shaped lesions with wide grayish centers. In the Ndemban and Sutusinjang areas, the same varieties developed very small lesions representing some tougher reaction by the plant to the fungus in these locations (Jobe and Darboe unpublished). Whether this reaction was due to difference in fungal strains or other environmental factors remains to be studied. In fact, in the Brikama area, the leaves of Prasana were killed by the coalescing and spread of the lesions.

Conclusions

In order to develop strategies for the deployment of durable blast resistance appropriate to The Gambia—and any West African country—there is a need to establish the existence and variability of pathogenic strains or races of the fungus at the regional and national levels. There is also a need for the exploitation of both conventional and biotechnological approaches to breeding for resistance. The employment of multiline varieties for the control of blast should also be considered. Even though it is argued that dirty crop multilines will lead to the evolution of new and complex pathogenic races, we must not forget that multilines can stabilise the racial composition of pathogen populations with simple races that carry one or a few genes for virulence being predominant and as a result offering effective long-term control.

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Pathogenic Variability of *Magnaporthe grisea* and Rice Screening for Resistance to Blast Disease in Nigeria

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Résumé

La pyriculariose du riz provoquée par *Magnaporthe grisea* (Hebert) Barr., est la maladie fongique la plus dévastatrice rencontrée par les paysans au Nigeria. Créer et introduire des variétés résistantes sont identifiées comme les seules options effectives de gestion durable de la pyriculariose au Nigeria. Bien que le criblage des cultivars de riz pour la résistance à la maladie ait été conduit pendant plusieurs années au Nigeria, le progrès dans le développement des variétés résistantes est généralement très lent. La compréhension appropriée de la variabilité au sein de la population de l'agent de la pyriculariose au Nigeria permettrait un programme plus dirigé pour les croisements pour la résistance. L'adoption de la technique de culture de cales peut contribuer à l'identification rapide de la diversité génétique du champignon responsable de la pyriculariose au Nigeria.

Abstract

Blast disease, caused by *Magnaporthe grisea* (Hebert) Barr., is the most devastating fungal disease of rice encountered by farmers in Nigeria. Breeding and introduction of blast-resistant rice varieties has been identified as the only effective option for the sustainable management of blast disease in Nigeria. Although screening of rice cultivars for blast resistance has been conducted for several years in Nigeria, progress in the development of blast-resistant varieties is generally very slow. Proper understanding of the pathogenic variability among the blast population in Nigeria would allow a more directed programme for breeding blast-resistant rice varieties. The adoption of callus culture technique may contribute toward rapid identification of genetic diversity of blast fungus in Nigeria.

Introduction

Rice is one of the most important staples in Nigeria. Recent statistical data show that an average Nigerian uses about 21 kg of rice per year (FAOSTAT 1999). For the past 5 years, Nigeria has produced about 3.2 million tons of paddy rice, making it the largest producer of rice in West Africa (FASonline 2002). However, Nigeria still needs massive imports to meet the growing local demands for rice products. In 2002 alone, Nigeria's import bill on rice peaked at $756 million. Several factors are responsible for the lack of self-sufficiency in rice production in Nigeria: low-input farming systems by resource-poor rice farmers, water-control problems, abiotic stresses, pests and diseases.

The high susceptibility of local rice varieties to diseases, especially blast (causative agent: *Magnaporthe grisea* (Hebert) Barr) is one of the major threats to rice production in Nigeria. In rice-growing areas of Nigeria, a blast disease outbreak could cause the loss of about 35–50% of rice yield, and in a serious outbreak of the disease, up to 100% of yield could be lost (WARDA 1999). Fungicides are available and widely used, especially in advanced countries, to protect rice from blast. However, the high cost of modern fungicides limits their application by poor farmers in countries like Nigeria. Breeding and introduction of blast-resistant rice varieties is currently accepted as a more sustainable approach to combating the disease.

The National Cereals Research Institute (NCRI) at Badeggi, Nigeria, has made remarkable contributions in the development and introduction of blast-resistant rice varieties for resource-poor rice farmers in Nigeria. Unfortunately, the blast-resistant rice cultivars often break down with time, and some varieties resistant to blast disease in one rice-growing location may be susceptible at another location. This lack of durable resistance to blast disease could be attributed to the high genetic diversity of the fungus (Ou 1979). There is a need, therefore, to characterise the pathogenic variability of the *Magnaporthe grisea* population in Nigeria.

Pathogenic variability in the blast population in Nigeria

Several decades ago, Awoderu (1970) made an attempt to identify the pathogenic variability of the blast population in Nigeria. He obtained 132 monoconidial cultures of blast fungus from diverse rice ecologies of Nigeria and used conidial suspensions (25 x 10^3 spores/ml) to inoculate selected sets of Nigerian and international differential cultivars. Through this approach, several races of the blast fungus were identified, with some having a high virulence spectrum (for example, NG-05 and NG-10), and others with very low virulence spectrum (for example, NG-01 and NG-02). Unfortunately, for several years now, there has not been any similar organised research on blast fungus at NCRI. Decisions on blast resistance or susceptibility of rice cultivars are now deduced from field observations of typical blast symptoms on leaves or panicles under natural infestation. Tables 45–47, for example, show the observations of blast incidence on rice cultivars during field trials conducted in 2001 at different locations. Cultivars with a blast score of 1–3 are considered resistant, while those with a score of 5–7 are susceptible. Several cultivars displayed contrasting reactions to blast disease at different locations (Tables 45–47). Thus, it could be concluded that the blast population in Nigeria is very diverse. However, the dearth of current information on the exact nature of blast disease incidence and severity in the different rice-growing areas of Nigeria makes it difficult to rely on such conclusions.

Methodology for the application of plant tissue culture for rapid identification of pathogen variability of blast fungus

The classical approach for characterisation of genetic diversity of blast fungus involves the use of differential varieties to conduct greenhouse and field screening of breeding lines for their reactions to blast isolates collected from diverse rice-growing areas. This approach could also be applied *in vitro*, using callus cultures induced from differential rice cultivars.

In our experiments (Kuta 2000), callus cultures obtained from seeds of rice varieties with contrasting reactions to blast disease, and cultures of blast fungal strains, differing in virulence to the rice varieties, were used to investigate host-pathogen interactions *in vitro*. The differential rice varieties and blast strains used are shown in Table 48.

Necrotic response

Necrotic reactions of rice cells were evaluated visually 48 hours after treatment with elicitor or after blast infection.

Table 45. Blast scores of rice cultivars at different locations (NCRI 2002).

Rice cultivar	Location A (Badeggi, North-Central Nigeria)	Location B (Amakama, Southeastern Nigeria)
B 5592F-5-ST-31-11	1	1
C 74 (FKR 26)	5	1
CNA 6675 (FKR 43)	0	1
CAN 6681	0	1
CO 39	1	1
CT 6775-5-17-4-2-SP	3	1
FKR 33	1	1
IDSA 27	1	1
IDSA 6	1	1
IR 39379-99-2-3-3-2	1	1
IR 52280-117-1-1-3	1	1
IRAT 136	1	1
IRAT 300	1	1
ITA 257	2	1
KAYBONNET	1	1
KENT	1	1
LEAH	1	1
TOX 3125-1-4-1-1-2-3-3	5	1
UPL R15	2	1
WAB 181-36	1	1
WAB 32-60	1	1
WAB 502-10-1-1	1	1
WAB 513-12-2-1	1	1
WAB 56-50	1	1
WAB 56-57	1	1

Table 46. Blast scores of rice lines in observational nursery at different locations (NCRI 2002).

Rice line	Location A (Tufa, North-Central Nigeria)	Location B (Amakama, Southeastern Nigeria)
63-83	5	1
CAN 4136	7	1
CAN 6719	5	1
CT 11231-35-2-M-M	3	0
DAIMABA DION	3	3
DAIMABA DROLE	3	3
DAINEKANNOUMANKA	3	3
DORSHSON	3	3
GBAHATO	3	3
IDSA 6	1	1
IDSA 91	3	3
IS 1001	1	3
M 22	3	3
TOX 3443-34-1-3-1	5	3
TOX 3449-72-2-1-3	5	1
WAB 326-B-B-12-H3	5	3
WAB 326-B-B-17-H1	5	1
WAB 337-B-B-13-H4	5	1
WAB 368-B-5-H1-HB	5	3
WAB 450-1-B-P-163-4-1	3	1
WAB 515-B-13A1-3	1	1
WAB 515-B-16A2-8	5	3
WAB 515-B-24A1-3	5	5
WAB 96-30	7	1

Table 47. Blast scores of upland rice lines in observational nursery at different locations in the same agro-ecological zone (NCRI 2002).

Line	Location A (Badeggi)	Location B (Tufa)
CT 1006-7-2-M5-1P-3-M	1	2
CT 6258-5-2-5-3-3P	1	1
BMPASC 105	0	2
FKR 14 (H418)	0	1
FKR 5	1	3
IDSA 77	1	2
IRAT 1-168	3	5
TGR 78	0	3
TGR 94	0	5
TOX 1010-14-4-7-4	1	2
TOX 1889-22-103-1	0	2
WAB 126-18-HB	1	2
WAB 128-B-B-2-HB	1	1
WAB 176-42-HB	1	5
WAB 181-50	0	7
WAB 224-12-H-HB	1	3
WAB 242-B-B-2-H2	0	4
WAB 272-B-B-1H2	1	7
WAB 272-B-B-7-H1	1	7
WAB 306-B-B-1-L3-L1-LB	0	7
WAB 326-B-B-11-H2	1	5
WAB 331-B-B-13-H3	1	7
WAB 337-B-B-13-H3	0	7
WAB 365-B-1-H1-HB	0	5
WAB 365-B-6-H2-H3	0	3
WAB 368-B-1-H3-H3	0	5
WAB 375-B-12-H2-1	0	5
WAB 376-B-13-H1-H1-HB	0	3
WAB 377-B-20-H5	0	2
WAB 488-114-2	0	2
WAB 492-119-1	0	2
WAB 506-137-1	0	1
WAB 383-10-1	0	1
WAB 586-5-1	1	2
WABC 165	1	1

Callus colonisation

Dual culture of rice callus and blast fungus was conducted by plating 2 pieces of calli (diameter 5 mm) 2 cm apart on a MS medium (Murashige and Skoog, 1962) in a Petri plate and plating in-between the calli, in the center, fungal mycelium (diameter 1 mm). The level of callus colonisation was estimated using the following scale:

0 – aerial hyphae absent
1 – aerial hyphae covers less than 25% of the surface of the calli
2 – aerial hyphae covers up to 50% of the surface of the calli
3 – aerial hyphae covers up to 75% of the surface of the calli
4 – aerial hyphae covers all the surface of the calli

In the assessment, the diameter and the structure of fungal colony were also considered.

Callus inoculation and collection of diffusate

Callus fragments (4-5 mg) were placed into a well of a 96-well tissue culture plate (“Linbro”, Flow Laboratories) containing 50 µl of distil water. Then another 50 µl of water or blast spore suspension (200 thousand spores/ml) was added to the callus fragments. The plate was then incubated in the dark for 18 hours at 23°C. Then, the liquid was collected with simultaneous removal of inoculum spores (Lapikova *et al.*, 1994) and is further referred to as “exometabolites” in this report.

Estimation of fungitoxicity of callus diffusate

Estimation of fungitoxicity of diffusate were conducted according to the method described by Lapikova *et al.* (1998). 80 µl of callus exometabolite was poured into wells of 96-well plate and 10 µl of freshly prepared spore suspension (3.5 × 104 /ml) was added. 10 µl of water was also added to the mixture and incubated for 5 h at 23C. Then under inverted microscope, the number of spores that germinated was counted in 5 replicates of 100 spores. The measure of fungitoxicity of diffusate was their capacity to inhibit fungal spore germination. The inhibition of germination was determined against spores incubated with 80 µl of water in place of a diffusate. All values are represented as means ± standard deviations (n = 5).

Results

Blast-induced necrotic response of calli was observed only during incompatible interactions (Table 48). In dual culture, colonisation of calli was observed only during compatible combinations. Mycelium growth was stimulated around the calli in compatible, but not in incompatible interactions As a result, the morphology of fungal colony in compatible interactions differs from that in incompatible ones. In addition, the formation of aerial hyphae of the fungus was stimulated by calli of susceptible but not resistant varieties.

Inoculation of calli of resistant rice cultivar Zenith with spores of avirulent *M.grisea* strain Ina168 resulted in the production of diffusate that significantly inhibited germination of blast spores (Table 49). On the other hand, diffusate from non-inoculated Zenith calli, just like water, did not inhibit spore germination.

Table 48. Differential rice varieties, differential strains of blast fungus and their types of interactions during blast infection of rice calli (S=compatibility, R=incompatibility)

Variety of rice	Differential blast strain				
	H5-3	Ken54-20	Kyu82-395A	Ina168	PH31
Zenith	R	R	R	R	S
Shin2	S	S	R	S	S
Aichi-Asahi	S	S	S	R	S
Maratelli	S	S	S	S	S

The callus-blast interaction that resulted in callus necrosis was considered as incompatible (R) interaction, while the interactions in which necrosis was not observed was tagged as compatible (S).

Table 49. Toxicity of diffusate from rice calli of cultivar Zenith to *Magnaporthe grisea* (18 h incubation of cells with the inoculum (fungal strain Ina 168) and 5 h incubation of test object (the same fungal strain) in cells diffusate)

Treatment	Inhibition of spore germination, %
Water	0 ± 3
Diffusate of non-inoculated cells	–2 ± 8
Diffusate of inoculated cells	70 ± 9

(Absolute spore germination in water 67 ± 2%).

Discussion

The gene-for-gene rice-blast interactions observed in our experiments with rice callus tissues may suggest that isolated rice cells retain the blast disease defense properties that are characteristic to intact rice plants. Such correlation of the disease resistance responses in cultured cells *in vitro* to that of intact plants has earlier been reported with other crops (Daub 1986; Spanos and Woodward 1997). The contrasting reactions of callus tissues of differential rice cultivars to blast infection *in vitro* could be exploited to develop a rapid method of identifying pathogen variability in blast population.

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