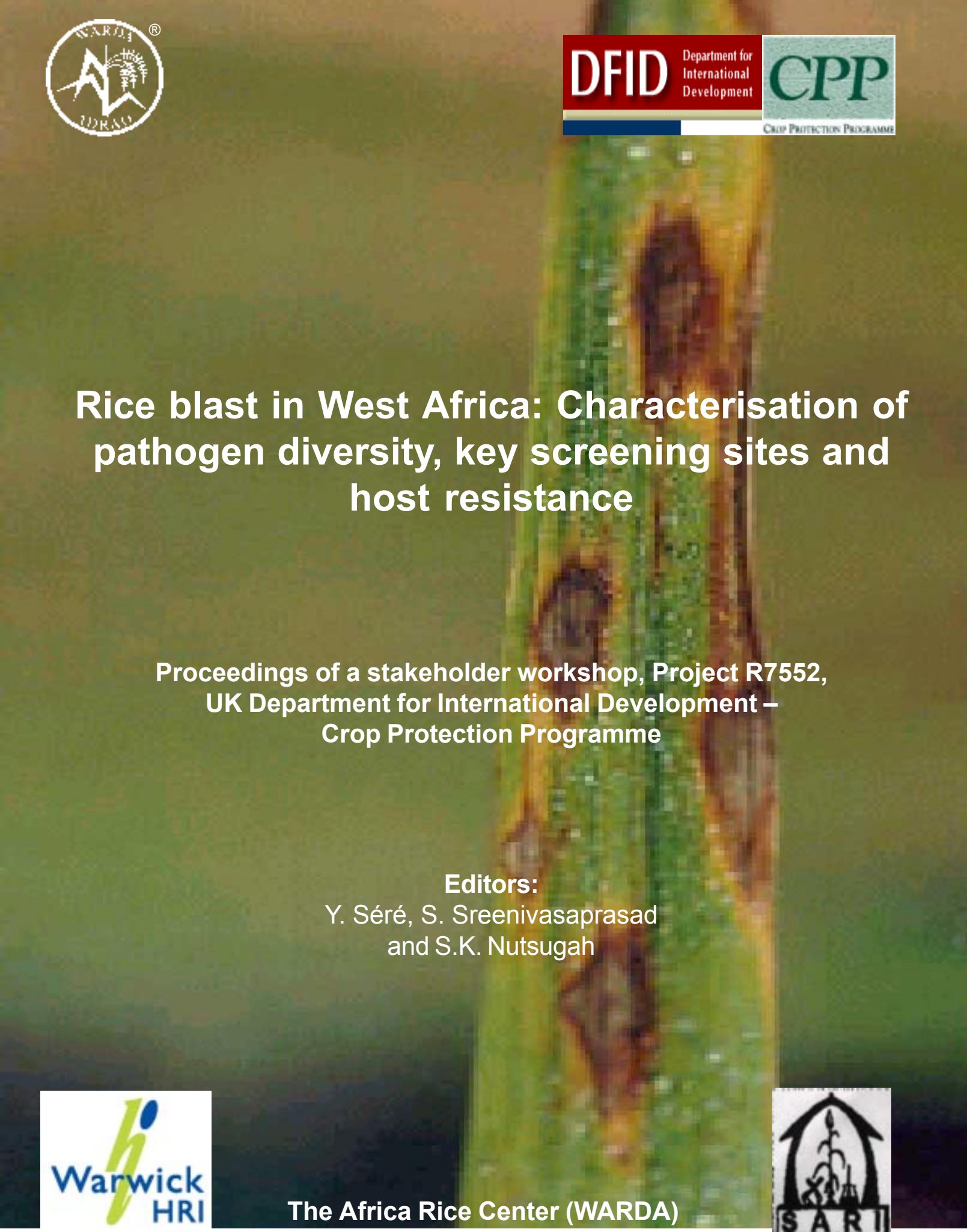

Rice blast in West Africa: Characterisation of pathogen diversity, key screening sites and host resistance

Proceedings of a stakeholder workshop, Project R7552, UK Department for International Development – Crop Protection Programme

Editors:
Y. Séré, S. Sreenivasaprasad
and S.K. Nutsugah

The Africa Rice Center (WARDA)

About The Africa Rice Center (WARDA)

The Africa Rice Center (WARDA) is an autonomous intergovernmental research association of African member states and also one of the 16 international agricultural research Centers supported by the Consultative Group on International Agricultural Research (CGIAR).

The mission of WARDA is to contribute to food security and poverty alleviation in sub-Saharan Africa (SSA), through research, partnerships, capacity strengthening, and policy support on rice-based systems, and in ways that promote sustainable agricultural development based on environmentally sound management of natural resources.

The *modus operandi* of WARDA is partnership at all levels. WARDA's research and development activities are conducted in collaboration with various stakeholders – primarily the National Agricultural Research Systems (NARS), academic institutions, advanced research institutions, farmers' organisations, non-governmental organisations, and donors – for the benefit of African farmers, mostly small-scale producers, as well as the millions of African families for whom rice means food.

The 'New Rice for Africa' (NERICA), which is bringing hope to millions of poor people in Africa, was developed by WARDA and its partners. The success of the NERICAs has helped shape the Center's future direction, extending its horizon beyond West and Central Africa into Eastern and Southern Africa. The creation of NERICA is in harmony with the spirit of the World Summit on Sustainable Development (WSSD), the Tokyo International Conference on Africa's Development (TICAD), the Millennium Development Goals (MDG), and the New Partnership for Africa's Development (NEPAD) for sustainable development. The African Rice Initiative (ARI) was launched in 2002 to promote the dissemination of NERICA and complementary technologies throughout SSA.

WARDA hosts ARI, the Regional Rice Research and Development Network for West and Central Africa (ROCARIZ), and the Inland Valley Consortium (IVC).

WARDA has its headquarters in Côte d'Ivoire and regional research stations near St Louis, Senegal, at the International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria, and at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) research station at Samanko, near Bamako, Mali.

For more information, visit *www.warda.org*

Main Research Center and Headquarters

WARDA/ADRAO
01 B.P. 2551
Bouaké 01
Côte d'Ivoire

Tel.: (225) 31 65 93 00
Fax: (225) 31 65 93 11
(225) 22 41 18 07
E-mail: *warda@cgiar.org*

WARDA Sahel Station

ADRAO
B.P. 96
St Louis
Senegal

Tel.: (221) 962 6493
(221) 962 6441
Fax: (221) 962 6491
E-mail: *warda-sahel@cgiar.org*

WARDA Nigeria Station

WARDA
c/o International
Institute of Tropical
Agriculture
(IITA)
Oyo Road, PMB 5320
Ibadan
Nigeria

Tel.: (234-2) 241 2626
Fax: (234-2) 241 2221
E-mail: *iita@cgiar.org*

WARDA Bamako

ADRAO
s/c International Crops
Research Institute for the
Semi-Arid Tropics
(ICRISAT)
B.P. 320
Bamako, Mali

Tel.: (223) 222 77 07
(223) 222 33 75
Fax: (223) 222 86 83
E-mail: *warda@cgiar.org*

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Cover: Leaf blast on a susceptible variety (Usen) at Farako-Bâ in Burkina Faso

WARDA
01 B.P. 2551
Bouaké 01
Côte d'Ivoire

Tel.: (225) 31 65 93 00
Fax: (225) 31 65 93 11
(225) 22 41 18 07
E-mail: *warda@cgiar.org*

Web-site: *http://www.warda.org/*

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Contents

Foreword

The demand for rice in sub-Saharan Africa is double the rate of population growth and rice consumption is growing faster than that of any other major staple. Rice is grown in all but the desert zone and is the main staple in eight West African countries. Rice accounts for 20–50% of the total calorific consumption and its availability and prices impact directly on the poor and are major food security issues in the region.

The estimated burden of malnutrition is slightly greater for females than for males, as anaemia affects mostly women in the reproductive stage. Malnutrition raises the risk of death and may reduce the physical and mental capacity of children. So, increasing rice production is essential since the poorest urban households in many African countries obtain a larger share of their cereal-based calories from rice than do higher-income households, and rice purchases represent a greater share of their total cash expenditures. Rice availability and rice prices have become a major determinant of the welfare of the poorest West African consumers, most of whom are the least food secure.

However, the average yield of 1.7 t ha^{-1} in the region is the lowest in the world and FAO estimates nearly 4 million tonnes of annual rice imports into West Africa worth more than US$ 1 billion per annum. Thus there is a clear gap between consumption and local production owing to a number of biophysical constraints. Blast caused by *Pyricularia grisea* (Cke) Sacc. (Teleomorphe: *Magnaporthe grisea* (Hebert) Barr) is one of the major constraints to rice production. Rice blast is found in all the rice-growing ecosystems of West Africa. It is more important in upland and rainfed lowland ecosystems than in other ecologies. It causes significant and unpredictable losses in rice fields mainly when farmers try to improve their traditional and poor-yielding system by using new varieties and fertilisers. Consequently this disease is one of the major constraints to intensification of rice cultivation.

Globally, using resistant cultivars mainly controls rice blast. However, where blast is prevalent, resistance breakdown due to high pathogen diversity is well documented. Recent global efforts have focused on determining the blast pathogen diversity, combining modern molecular-biotechnological approaches with the traditional pathological assays for efficient exploitation of host resistance.

In West Africa, a complete lack of understanding of the blast pathogen variability has hindered efforts towards the identification and development of blast-resistant cultivars adapted to local agro-ecological conditions. To address this, the West Africa Rice Development Association (WARDA–Africa Rice Center), Côte d'Ivoire; Savanna Agricultural Research Institute (SARI) and Crops Research Institute (CRI), Ghana; Horticulture Research International, UK and associated organisations have been involved in a collaborative strategic research program (Projects R7552/R6738) funded by the UK Department for International Development (DFID)–Crop Protection Programme (CPP).

In this program, considerable progress has been made on pathogen diversity, potential host resistances and characterisation of screening sites. Strong collaboration and partnership among WARDA, NARS and UK organisations, contributing to capability strengthening and a network of regional and international contacts, have been established. This provides an excellent opportunity for farmer participatory evaluation of blast-resistant material identified, utilisation of partial and major gene resistances and continued pathogen monitoring through technology and knowledge promotion to WARDA/NARS. These strategies coupled with appropriate cultural practices, including seed hygiene, would lead to sustainable blast management in the West African region.

Two targeted groups are the ultimate beneficiaries of the project: (i) the resource-poor smallholder farm families, (ii) the poor urban and rural consumers. The first group produces more than 80% of the region's rice. It includes women rice farmers who traditionally dominate rice cultivation in most of the Sub-Saharan African countries, but have been too often marginalised from technical change. They often form the lowest income subgroup and provide the major share of labor in most rice production systems, especially in low-input farming systems. The resource-poor smallholder farm families group is targeted as producers as well as consumers. For them, rice has become both a staple food crop and a cash crop. The second group of rural and urban consumers is not involved directly in rice production. They were targeted because rice has become more and more important in their diets, and rice availability and rice prices are essential to their livelihoods. Within each group, women and children are the most vulnerable subgroup and they suffer disproportionate poverty, with crucial consequences for malnutrition and for health.

The workshop on 'Strategic characterisation of blast pathogen diversity, key screening sites and host resistance', held on 5 March 2003 in Accra, Ghana, was the last part of the project activities aiming at achieving wider dissemination and uptake of outputs. In addition to the project collaborators and associated scientists from WARDA, SARI and CRI, participants included CSIR, MOFA and University of Ghana, Ghana, NARS from Burkina Faso (INERA), Nigeria (NCRI) and Gambia (NARI) and FAO–RAF, Accra. The importance of rice and production constraints in West Africa and the project background and objectives were covered in the opening session. This was followed by a number of presentations made in three technical sessions on breeding for blast resistance and varietal diffusion including impact assessment methodologies; blast project activities and outputs, and blast management in some West African countries. A final discussion session included presentations on CPP rice projects, cluster and blast follow-up activities as well as open discussion on further activities. Importance of blast as a major constraint was highlighted at this workshop and in subsequent discussions. The need to characterise the blast pathogen populations in key countries and use the knowledge and resources to identify, develop and deploy resistance as well as promotion of molecular–biotechnological tools to NARS/WARDA for long-term local monitoring was strongly emphasised by a range of stakeholders including NARS and WARDA scientists, CSIR, Ghana, FAO–RAF and FARA.

This volume includes the workshop report, full papers covering various presentations and useful appendices. It is intended to serve as a reference manual for scientists involved in rice blast research and management in Africa, particularly the West African region.

Y. Séré, S. Sreenivasaprasad and S.K. Nutsugah
Editors

Part 1:
Workshop report

Workshop report

Dr. Joseph Cobbina, CSIR-AFFS, Ghana chaired the opening session and stressed the growing importance of rice in Ghana and underlined the need to develop improved management methods for major pests and diseases including blast. Dr. Cobbina mentioned that in view of the considerable progress made in blast management work and the successful partnerships built among Ghanaian NARS, West African NARS, WARDA and UK institutes, there may be opportunities to seek support from the World Bank-funded Agriculture Sub-Services Sector Investment Programme (AgSSIP), CSIR and MOFA for further promotion of some of the outputs, particularly for capacity strengthening.

Dr. A.B. Salifu, Director, SARI, Ghana gave the Welcome Address:

Mr. Chairman, colleagues, fellow research scientists, distinguished invited participants, members of the press, ladies and gentlemen,
It is a great pleasure for me to welcome all of you and to express gratitude of the Council for Scientific & Industrial Research (CSIR) and its institutes, Savanna Agricultural Research Institute and Crops Research Institute, for your presence at this workshop on "Strategies for Development and Deployment of Durable Blast Resistance in West Africa".

When I sought to add a little more spice to my welcome address I discovered I could easily end up taking the whole day making statements only on the importance of rice in the daily lives of people across the entire globe. This is one crop whose farming is the largest single use of land for producing food. I have therefore cautioned and limited myself in the tendency for over-indulgence. However, one of the new things I found out during the process of putting things together is the meaning of the words TOYOTA and HONDA; not as we know them as vehicle types but as they relate to rice: Toyota means bountiful rice fields, Honda means the main rice field!

Mr. Chairman, ladies and gentlemen:

The demand for rice in the West African sub-region is growing faster than any other major staple for food and constitutes a major source of calories for the rural and urban poor. Rice is grown on approximately 4.3 million ha in 17 West African countries constituting the West Africa Rice Development Association (WARDA). The annual production is about 7.4 million tons of paddy. The FAO estimates there are 4 million tons of annual rice imports into West Africa worth more than US$ 1 billion per annum. Thus, there is a clear gap in local production and consumption that needs to be addressed by the African agricultural and development community and their international development partners. Indeed our own situation in Ghana is that we still spend in excess of US$ 100 million per annum for rice imports. Expanding domestic rice production through improved technologies is key to generating greater demand for employment and incomes throughout the rice commodity sector, particularly employment for urban and rural women folk.

Mr. Chairman, unfortunately the average yield of rice in the West African Sub-region is 1.7 tons per hectare. This is the lowest compared to the rest of the world. Mr. Chairman, the low yields in our sub region are not necessarily an act of God. A wide range of biophysical constraints reduces the yield potential in this region. One such constraint – and a major one for that matter – is the blast disease.

Blast is a widespread and serious disease across the West African rice farming system. It affects the crop at all stages of growth and development. There is seedling blast in irrigated rice fields, there is leaf blast in the upland and lowland, with panicle blast often present in all these rice-growing environments. Up to 77% of yield loss due to blast have been reported for West Africa. The situation is often exacerbated by frequent breakdown of resistance in instances where resistance to the disease has been incorporated in rice varieties. Mr. Chairman, in order to develop blast resistance that is stable over space and durable over time, it is critical to understand the diversity and dynamics of the pathogen populations and then identify resistance sources.

It is in this context that the UK Department for International Development (DFID)-Crop Protection Programme funded a collaborative research project on rice blast. The Horticulture Research International (UK), WARDA, the CSIR-Savanna Agricultural Research Institute (SARI) and the CSIR-Crops Research Institute (CRI) participated in the collaborative research in which the collaborating institutes undertook research on characterisation and identification of sites suitable for blast resistance screening in Ghana, Nigeria, Côte d'Ivoire and Burkina Faso.

Baseline data on the genetic and pathogenic diversity of the pathogen populations in and around key sites in these countries have been generated and the persistent dominant lineages as well as major pathotype groups identified. This has given a good understanding of the diversity and distribution pattern of the pathogen lineages and pathotypes across the rice growing regions in these countries and provides a framework for the identification and deployment of lineage – and location-specific resistance sources. The outputs generated have been disseminated to the National Agricultural Research System collaborators as well as to other NARS plant pathologists and breeders for possible deployment in their research programs. I am very confident that participants will find the detailed presentations very useful and interesting to the extent of their active contributions to the discussions that will follow.

Mr. Chairman, ladies and gentlemen, permit me to focus a little more on the importance of rice and rural livelihoods in Northern Ghana, the mandate of my institute. In the 1970s, rice was the single most important source of employment and income for rural and indeed urban people in the Northern Regions. In those days, the farmers of the north were occupied almost on a daily basis throughout the year. The decline of the rice enterprise in these areas in recent times is partly to blame for "*kayaye*" phenomenon and the grave poverty statistics quoted for the North. Indeed, I dare say that the occasional disturbance of the peace and security in these areas could also be a consequential by-product for as the sayings go "A hungry man is an angry man" and that "the devil finds work for idle hands".

Mr. Chairman, transformation of the livelihoods and reduction of poverty in the North can be achieved by genuine and concerted efforts by all concerned to bring back rice as a viable farming enterprise in the North in particular and the country as a whole. In terms of what it takes to make this positive upturn possible, research is already up with shoulder to the wheel. As contribution of its quota to the national agenda for development, CSIR-SARI with its mandate for research in Northern Ghana has worked assiduously and collaboratively in developing technologies to support the rice sector. Some of these efforts include:

- The release of a new rice variety called *Digan* during the last quarter of 2002. Apart from the higher stable yield and better cooking and nutritional characteristics offered by this variety, the Dagbani name given to it describes its versatility of doing well in diverse rice growing ecologies. This variety will surely support rice farmers in the North to improve production and productivity and contribute to government efforts to bring down rice imports by 30%.
- Ladies and gentlemen, I wish to recall that during his visit to Japan last year his Excellency the President of the Republic of Ghana was offered support to promote the New Rice for Africa (NERICA) rice in Ghana. In keeping with government efforts CSIR-SARI and CRI sent seed of 6 and 3 NERICA lines, respectively to the Irrigation Development Authority of MOFA at Ashiaman for multiplication and subsequent diffusion among farmers. The NERICA lines sent by CSIR-SARI were previously evaluated with more than 200 farmers.
- CSIR-SARI is currently championing the concept of Participatory Varietal Selection (PVS) for developing new rice varieties. The PVS approach gives collaborating farmers, processors and consumers the opportunity to evaluate and select preferred genotypes of rice for further testing. The approach reduces the time it takes for research to come out with new varieties and increases the acceptability and adoption of selected varieties by the wider community of farmers and consumers.

The involvement of DFID in Ghana's socio-economic development is immense and needs to be trumpeted by Ghanaians at all times. While thanking the DFID for its continued assistance I will emphasise that Ghana is an agricultural country. Therefore, any realignment in emphasis in DFID's development assistance particularly to Ghana should not depart completely from agriculture and allied activities.

Mr. Chairman, with these few remarks, I welcome you once again on behalf of CSIR and its institutes of SARI and CRI. I wish a successful workshop. It is a pleasure to see the impressive turnout for this workshop. Thank you very much.

Dr. S. Sreenivasaprasad, Blast Project Leader outlined the global importance of rice blast, problems faced in traditional approaches to blast management and the recent use of novel tools to better understand blast population structure to develop durable resistance, and explained the DFID-CPP funded rice blast project background and objectives.

First theme: Breeding for blast resistance and the process of varietal diffusion

1.1. Selection of intra-specific (*Oryza sativa* × *O. sativa*) and inter-specific (*O. sativa* × *O. glaberrima*) lines for their tolerance to blast in Burkina Faso

Presented by M. Sié, INERA Burkina Faso

Summary

*Blast is a major constraint to rice production in Burkina Faso. With increasing blast incidences recently, farmers had to abandon some varieties. In collaboration with WARDA and through the breeding-task force, we are using intraspecific (*sativa × sativa*) and interspecific (*glaberrima × sativa*) crosses to screen for rainfed lowland adaptability and pest and diseases resistance. We have screened 571 lines generated at WARDA over three seasons (2000–2002) at Banfora in southwest Burkina. From these we have selected 15 lines as potential candidates with good level of pest and disease resistance and useful agronomic characteristics. This breeding approach needs to be further developed to fully exploit the wide range of genes contributing to these traits.*

Discussion

The discussion focused on the choice of susceptible varieties for the infector rows.

If these susceptible varieties bear any resistant gene to some races, only the races that are able to overcome such resistant genes are spread by the infected rows. It is not possible to get a good picture of the entire pathogen population at the screening site.

Besides the importance of the check, integrating spatial and temporal aspects of the method was also emphasised so as

1. to develop the screening in different sites
2. to conduct the experiment over years in order to account for the ecological differences

1.2. Screening Strategy for Durable Resistance to Rice Blast Disease at WARDA

Presented by Y. Séré, WARDA

Summary

Rice blast is one of the most important biotic constraints in different rice ecologies in West African Sudan Guinean zone. The development of varieties with effective resistance is an important activity and the goal for rice pathologists and breeders. WARDA has developed a strategy to identify rice material with durable resistance to blast, which can be adopted directly by farmers and also used as donors in a breeding programme. This presentation will explain such an approach after focusing on the understanding of host-pathogen interaction that was used to develop the screening strategy.

Discussion

The resistance status of interspecific hybrids NERICA 6 and NERICA 7 was discussed since these varieties appeared to show a low level of horizontal resistance under the epidemiological conditions at M'bé.

It is important to notice that the performance of a variety has to be viewed in relation to the biotic and abiotic parameters of the environment (or more precisely of the site or ecological niches) that has been targeted. In particular the performance of NERICAs 6 and 7 will depend on the characterisation of the environment. Thus it is important to keep in mind the notion of slow diffusion of varieties in order to progressively follow its adaptation to the appropriate environment.

Another issue raised related to the number of races/pathotype found in West Africa. This presentation did not deal with individual pathotypes or races rather the virulence diversities at key sites. Diversity and distribution of pathotypes would be discussed in the next presentations, which deal with the results of the CPP project.

The issue of susceptible check was again discussed. It has been mentioned clearly that a good susceptible variety that could be used in the infector row is a variety, which does not possess a vertical resistant gene. Before choosing such a variety, it is necessary to conduct artificial inoculation on a set of susceptible varieties. Those that show susceptibility to diverse isolates are the best susceptible checks to be used in infector row.

1.3. Impact assessment of agricultural technology: Concept, methodology and application to rice pests and diseases

Presented by A. Diagne, WARDA

Summary

Once disparate approaches to impact assessment encompassing various sub-disciplines of economics and statistics are now converging to provide a single unified methodological framework within which the impact of various types of programmes, policy changes, and technologies on various behavioural, environmental and welfare outcomes can be assessed with a level of rigor satisfactory from both economic and statistical perspectives. A synthesis of some recent methodological developments is presented within one single coherent conceptual and methodological framework, including an application to the assessment of the adoption impact of disease resistance varietal technologies.

Discussion

Farmers attribute crop failure to various non-disease causes including witchcraft. Farmers in general have difficulty with the concept of 'plants being sick' and in the interviews farmers are asked about the symptoms and not diseases.

Second theme: DFID – CPP Blast project activities and outputs

2.1. Survey of Rice Blast and Varietal Screening in Ghana

Presented by S.K. Nutsugah on behalf SARI & CRI, Ghana

Summary

Surveys were conducted in farmers' fields, participatory varietal selection (PVS) rice nurseries and research trial plots which overlapped with WARDA/NARS screening sites during 2000-2002 cropping seasons to assess the incidence of rice blast and relative importance of various rice diseases in Ghana. In addition field trials were carried out on twenty or more PVS rice varieties during the same period at some of the key screening sites in Northern Region to assess their response to blast using local susceptible checks.

Two hundred and sixty-four fields were surveyed in all the major-rice growing areas across the entire country during the project life span. The incidence of blast varied considerably across these sites, which have been grouped into low, moderate and high blast areas with incidence levels of 1-10%, > 10-50% and > 50-100%, respectively. The high blast areas are Boama-Dumase, Offinso (Ashanti, [AR]), Abora, Brofiyedru/Bremang, Diaso, Nkwantanang, Treposo (Central, [CR]), Otumi (Eastern, [ER]), Galenkpegu, Kpachie, Nyankpala (Northern, [NR]), Bawku, Nyorigu (Upper East, [UER]), Fodome, Golokwati, Hohoe, Kpoeta, Santrokofi (Volta, [VR]), Datano, Sayerano and Tanoso (Western, [WR]) Regions. The low blast areas are Afere, Bibiani, Juabeso, Sefwi-Wiawso, Sui-ano #1, Sui-ano #2 (WR), Asikam, Daamang, Subi (ER) and Dromankoma (AR). Owuosabroso (WR) was identified as a moderate blast area. No blast was observed in Brong Ahafo, Greater-Accra and Upper West Regions. A key observation about the high incidence of blast in and around Hohoe was that the disease originated from the nursery beds and was transplanted to the fields by the farmers. The blast infection in most of the fields visited was observed at the seedling and vegetative growth stages. Few cases of blast infection were seen at panicle initiation and grain filling stages due to varietal and agro-ecological differences at the sampling sites.

High incidence of other foliar diseases notably brown spot, narrow brown leaf spot and false smut was observed. These diseases were observed predominantly at Afere, Datano, Nsuansua, Sayerano and Tanoso while brown spot was mostly dominant at Kade, Fumbisi, Manga, Navrongo, Sandema and Tono. High visual scores of 7-9 on the IRRI Standard Evaluation System for rice were recorded for brown spot at Santrokofi in most of the nursery beds. False smut prevalence was high in WR with visual scores of 3-5 during the 2002 cropping season.

Heavy yield losses (up to 100%) largely due to blast infection were reported from the farmers' records at some of the high blast areas notably Fodome, Hohoe, Santrokofi and Datano even though drought effect exacerbated the situation at the former three sites. The survey results suggest that Datano (WR), Hohoe (VR) and Nyankpala (NR) are blast hot spots and key sites for resistance screening which correlates with the diversity of the pathogen populations.

Blast incidence on the PVS rice varieties was generally low and the severity rating ranged between 1-3 suggesting their resistance/partial-resistance potential at the selected key screening sites in Northern Region. These improved varieties need to be tested sufficiently at other key sites across the country to benefit the resource-poor farmers and consumers.

Discussion

The issue of number of blast races was raised again. The 15 characterised isolates used in bioassays fell into three lineages. Dr Sreenivasaprasad would discuss the races/pathotypes in his presentation.

Relatedness of pathogen isolates causing different types of blast (e.g. leaf and panicle blast) was raised and whether varieties that are resistant to leaf blast are also resistant to panicle blast? Based on

observations during the surveys, panicle blast was observed in only one location in the Western Region and leaf blast was very common in Ghana. From the molecular characterisation work in general, leaf and panicle isolates fall into the same genetic lineage suggesting the utility of shared resistances.

Regarding the implications of the work in relation to the prevalence of blast pathotypes in different regions of Ghana, it had been stated that Warwick HRI has been able to identify or characterise the blast pathogen in different regions of Ghana. Invariably, the local varieties were found to be susceptible and the improved varieties were more or less resistant. Therefore promotion of improved varieties needs to be pursued vigorously.

2.2. Analysis of *Magnaporthe grisea* population structure in Côte d'Ivoire as a prerequisite for the deployment of varieties with durable blast resistance

Presented Dr. Y. Séré, WARDA

Summary

One of the critical issues in the relationship between rice and the blast pathogen is the instability of host plant resistance due to the pathogen variability. To focus on strategy based on varietal resistance, it is not only important to know the nature of the varieties but also to determine the nature of pathogen pressure at different screening sites. For achieving these objectives, a technique was developed in the field to analyse the virulence spectrum of the pathogen. This would help in screening site characterisation and completing the information relating to molecular analysis in order to develop an evaluation system for deployment of durable resistance.

Discussion

Importance of the approaches outlined in this communication for breeders was emphasised. Moroberekan is the main check used at WARDA for durable resistance to the rice blast fungus. It was observed that Moroberekan could possess two major genes for vertical resistance and a polygenic system (QTLs) responsible for horizontal resistance.

Whether the number of blast races/pathotypes in Côte d'Ivoire was identified and why not, particularly in view of the information mentioned for other parts of the world, except Africa, in the introductory talk given by the participant from Warwick HRI, was raised.

It was clarified that the objective of this work conducted in Côte d'Ivoire was not to identify races particularly as it was not possible at WARDA at the moment to perform pathotyping under controlled and confined conditions. The work deals with the identification of pathogen virulence diversity and the evaluation of their frequency through field trapping, without considering their association into individual races.

Meanwhile, using international differentials Dr Sreenivasaprasad's team had done this work for West African isolates as part of the collaborative effort and the results will be presented in next paper.

2.3. Diversity of blast pathogen populations in four West African countries and strategies for resistance management

Presented by S. Sreenivasaprasad, Warwick HRI, UK

Summary

During the last few years, global efforts have focused on determining the blast pathogen diversity combining modern molecular-biotechnological approaches with the traditional pathological assays for efficient exploitation of host resistance.

The key objectives of this collaborative strategic research programme funded by DFID-CPP (Project R7552/R6738) were to characterise and identify the key resistance screening sites used by WARDA and NARS. Assess the genetic and pathogenic diversity of the pathogen populations. A baseline collection of more than 350 blast pathogen isolates collected in and around key screening sites and surrounding farms from Côte d'Ivoire, Ghana, Burkina Faso and Nigeria has been established. DNA fingerprinting (MGR586 probe) for the identification of blast lineages (genetic groups and diversity) and pathotyping on international rice differentials for virulence diversity were used.

Blast lineages (genetic groups) varied from 2-5 per country with up to 16 lineages in 4 countries. In each country one or two lineages were dominant. For example, GH-1 (56%) was present across Ghana on up to 20 rice varieties/lines. BF-1 included more than 70% of isolates from rice, wild rice and weeds in Burkina Faso. CD-1 and CD-2 were 38 – 56% in Côte d' Ivoire comprising isolates collected over a five-year period. Distribution of some lineages was restricted, e.g. GH-2 (31%) mainly from Eastern Ghana, appearing at low frequency in Northern Ghana. Lineages common among the four countries were identified and nine distinct West African blast lineages designated WA1 - WA9. Among these, WA1, WA2 and WA3 are the major West African blast lineages and are present in two or more countries. In general, different types of blast were caused by isolates in the same lineage suggesting the utility of common resistances. Pathogen virulence diversity was high with 16–25 pathotypes in each country. IB (particularly IB-1) was the dominant pathotype group in Ghana and Nigeria. IC was the prominent (43%) pathotype group in Burkina, a range of pathotypes were present at certain sites (e.g. Farako-Ba). In Côte d' Ivoire IA, IB, IC and ID were 16–29%. Presence of blast pathogen on weedy rice O. longistaminata *and common weed hosts has been identified. Several of these isolates are closely related to rice pathogenic isolates and are pathogenic on rice under controlled conditions. Screening of a range of rice varieties under controlled conditions against West African blast lineage representatives and at some of the characterised sites (linked to and utilising PVS material) has led to the identification of potential resistances that need to be further tested/developed. Rice scientists world-wide have embarked on developing novel strategies such as 'lineage exclusion' being used/tested in Latin America and Asia as well as planting mixed stands of rice cultivars in China for blast resistance. Both these approaches are based on a sound understanding of the biodiversity of the pathogen populations. We have generated knowledge on pathogen diversity new to West Africa contributing to the global atlas on blast pathogen diversity and providing a framework for efficient utilisation of host resistance.*

Discussion

The work and the presentation were found very exciting. More clarification was requested concerning pathotype/race and lineage identifications. It was clarified that pathogen populations (isolates) from West African sites were first characterised by molecular methods and genetic groups (lineages) were identified. Representatives of these genetic lineages were virulence characterised on International Rice Differentials to identify pathotypes, which gives an understanding of the virulence diversity of the genetic lineages. The lineage and pathotype information together can then be used as a framework for identification, development and deployment of resistance.

Promotion of the molecular-biotechnological tools to West African scientists including NARS and WARDA was emphasised. It was clarified that the next phase of work revolves around technology promotion to WARDA, so that the genetic characterisation can be done in the region at WARDA linked to NARS (possibly including the Mali-Guinea belt) and that this is being discussed with CPP, WARDA, NARS and other stakeholders.

Third theme: Blast disease management in some West African Countries

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

Presented by K.B. Kaboré, INERA, Burkina Faso

Summary

Blast is a principal constraint to rice production in Burkina Faso. We are working on blast management through host resistance, cultural practices, IPM/ICM and training of farmers and extension staff. From the screening trials of National varieties, we have identified 7 and 13 varieties showing blast resistance in upland and lowland conditions, respectively. Similar activities with INGER material and NERICAs are in progress. During the 2002 humid season, up to 15 NERICAs showed resistance response to blast compared to the resistant check used. We have developed one IPM package targeting pests and diseases incorporating natural products. To ensure proper use of technologies developed, we have organised training sessions for farmers and extension staff in areas such as rice IPM for seed production, PVS work and the FAO ICM programme.

Discussion

Methodology used for yield losses and also the issue of choosing the resistant and susceptible checks were discussed. It is observed that it is good to have some common material in collaborative work, but additional resistant and susceptible checks may need to be used by each collaborator due to different epidemiological situations in each country (localities).

It was observed that beyond the pest and disease resistance, the general performance and deployment of NERICAs in Burkina Faso in the first year was interesting.

3.2. Rice Blast Disease in The Gambia: Genotype by Environmental Reaction, Economic Significance and Management Strategies

Presented by L.M.S. Jobe, NARI, Gambia

Summary

Rice blast, caused by Pyricularia grisea *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.

Discussion

The issue of multilines was discussed. It was mentioned that a survey by WARDA economists, social scientists and genetic resource personnel led to the finding that the farmers' perception about the varieties may be different to those of scientists. Farmers tend to have a population instead of varieties in their field, which may explain the low level of diseases in farmers' fields in certain instances. It was observed that blast is a problem mainly in high input systems with improved and homogenous varieties and fertilisers.

It was emphasised that utilisation of multilines as a strategy in blast management also needs a better understanding of blast population structure and the genetic basis of resistance so that to apply suitable resistant genes together in the same locality, as has been the case in China recently.

It was observed that the massive introduction of rice varieties from China has definitely resulted in some problems in The Gambia. There was concern about the potential negative impact and trans-continental spread of certain diseases e.g. Tungro virus, common in Asia are not yet reported in the West African sub region.

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

Presented by D.D. Kuta, NCRI, Nigeria

Summary

Blast disease, caused by Pyricularia grisea *(Cke) Sacc. (Teleomorphe:* 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 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.*

This paper reviews the importance of differential rice cultivars and differential strains of blast fungus in breeding for blast resistance, and also suggests low-cost effective innovative approaches for proper characterisation of blast fungus in Nigeria.

Discussion

It was mentioned that the work using rice callus cultures to study blast compatibility reactions was very interesting and it was clarified that the tissue culture approach was developed based on conventional greenhouse screening results. It was also observed that although the present study was restricted to selected differential cultivars, the method developed could be extended to other groups of compatible and incompatible interactions in rice blast.

It was suggested that a closer analysis of the lesion sizes, whether hypersensitive reaction could be recorded or not, would be useful. Discussion centered around the role of phenolic compounds, reactive oxygen species and whether phytoalexins that are known to be induced non-specifically will have a role in gene-to-gene interaction.

It was also clarified that necrotic lesions either limit or kill the blast fungus, as has been observed during the pathogen isolations from a large number of samples showing resistant necrotic lesions.

Discussion session: Outputs, linkages and further work

This session started with a presentation by Dr. Tim Chancellor, CPP – Technical Adviser on the CPP rice projects cluster. CPP funded and proposed rice projects in Africa and Asia on improved management of pests, diseases and weeds, natural enemy biodiversity and ICM and the opportunities for wider linkages and promotion and uptake of outputs within and across different projects and geographic locations were discussed. Dr. Sreenivasaprasad, on behalf of the blast project team, outlined the proposed follow-on work for sustainable management of blast, focusing on the promotion of molecular-biotechnological tools to WARDA and NARS, and regional capacity building for blast characterisation combining the utilisation of major and partial resistances.

This was followed by general discussion on further work, up take of outputs and linkages based around the two talks and the presentations made during the earlier sessions. The main discussion points and recommendations are summarised below:

- Strong emphasis on further utilisation of characterised sites, pathogen lineages and pathotypes identified and the potential resistances in further multi-location screening through WARDA and NARS activities to achieve durable blast resistance. Particularly promotion of resistance material identified through appropriate links with the proposed DFID-CPP funded rice ICM project on major biotic constraints.
- Strong demand for promotion of molecular-biotechnological tools to NARS and WARDA for blast characterisation and long-term local monitoring leading to regional capacity strengthening. Further support from DFID and other sources linked to national and regional programmes would immensely benefit a number of young scientists trained in molecular biology.
- On capacity strengthening, the consensus was that it would be cost and resource efficient to utilise the biotechnology facilities associated with WARDA, to start with, where NARS scientists will have access to conduct biotechnology studies as visiting scientists. Some NARS systems e.g. Burkina Faso (INERA), Nigeria (NCRI) and Ghana (AgSSIP-CSIR/MOFA) are in the process of establishing centralised biotechnology facilities that can be accessed by national scientists. Scientists from these organisations trained at WARDA could facilitate lateral transfer of technologies in-country through the local structure. If and where appropriate simultaneous transfer of technologies to WARDA and NARS could also be explored.
- Involvement of NARES staff in above activities will have to be at appropriate entry levels, within the 'global vision' of the project activities, depending on local priorities, resources and capacity.
- Networking of the different national laboratories or where possible, even at the sub-regional level to enhance local capacities to carry out certain activities within national systems. For example, Gambia and Senegal has an agreement that allow Gambian scientists to send samples to Senegal for pesticide residual analysis whilst Senegalese counterparts can also send samples of pesticides to confirm the analyses.
- A number of opportunities exist for linkages with NARS in Asia on durable blast resistance management.
- The blast characterisation molecular tool kit developed in partnership with WARDA and associated NARS could also be promoted to Bangladesh Rice Research Institute utilising the facilities built under DFID-PETRRA.
- Similarly, link up with NARS in India could be useful in developing work on R genes in NILs and R gene pyramids.
- The type of collaboration between Africa and Asia needs to be further discussed to provide a framework for efficient exchange and training.

- WARDA's position in view of the political situation in Côte d'Ivoire was clarified. WARDA now mandated as Africa Rice Center is continuing to function at two locations: Abidjan for administration and ICRISAT station in Mali for all research work previously conducted at M'bé research station. Field preparation is currently on-going for the upcoming season. Molecular-biotechnology work will be carried out utilising the facilities at the University of Mali.
- Among other diseases, brown spot was raised as an important issue in Ghana and that it has not been possible so far to attract international support despite expression of demand. It was mentioned that brown spot is not a problem of low-input systems only and that there were many instances of well-nourished crops succumbing to the disease. Bacterial blight, possibly related to the importation of some Chinese varieties and RYMV were mentioned as other problems in the region.
- Impact assessment of outputs from strategic projects such as the CPP funded blast work. At this level only the potential impact can be assessed for this period as the technology has not been at farmers' fields yet. Valuable information has been generated by this project and can be used to assess such potential impacts, by applying appropriate methodologies.
- Promotion of identified disease management technologies to farmers' fields can be started and impact monitored. This would require that the impact assessment research is put in place first to generate baseline markers before the diffusion of technologies so as to aid in future assessment.
- Deployment of modern biotechnology has huge potential in West African agriculture. Issues of biosafety regulations, environmental impacts of new pest management technologies, public perception of the use of genetically modified organisms (GMOs) and effect on biodiversity need to be addressed.
- Herbicide-tolerant transgenic rice was observed as an potentially interesting issue. However, DFID-CPP are currently seeking to enhance impact of existing outputs. Immediate major investment in transgenic crops may not be appropriate in the West African situation due to the absence of regulatory frameworks. Research on risk assessment of GMOs may be valuable. DFID-CPP recently commissioned a report on this issue and this report will shortly be available on their website.
- Health issues such as HIV/AIDS are becoming crucial for agriculture and highlight the need for less-labour intensive practices. This will be carefully considered in relation to the proposed integrated crop management (ICM) project.

Dr E. Otoo, CRI, National Rice Coordinator, Ghana gave the closing remarks:

Before a country or a group of people goes to war with another country or group, there is the need to use military intelligence information to size up the military strength or the strategy of the enemy. This will help to know how to prepare and be able to defeat the enemy. That is the reason why I am delighted to note that our scientists have done just that with the rice blast system.

Since this morning we have learnt that while importation of rice is costing our governments so much, local production of rice is below expectation. This is because rice production has several biotic constraints, including diseases. Of these diseases, blast is a big force to reckon with. From the deliberations this morning and this afternoon, we know that screening sites for the disease or pathogen have been characterised in various West African countries, including Ghana. We need to know the type of resistance, which must exist in a host plant in order to fight against the blast pathogen. It is gratifying to note that different lineages and pathotypes of the blast fungus have been identified and some amount of resistance observed in certain varieties. It is a very good idea that different West African countries have taken up the tasks of challenging the pathogen to increase yield. I think that this multi-lineage exclusion to control blast will, eventually, be not only inexpensive but also environmentally friendly.

That is why I think we must all put our hands together for UK's DFID-CPP for funding such a project. I feel that as we in the sub-region are struggling to feed ourselves by increasing crop yield through such projects, the DFID-CPP should continue to fund other such projects. On behalf of the organisers and on my own behalf I wish you all safe journey back.

Part 2:
Workshop full papers on Breeding for blast resistance and the process of varietal diffusion

Selection of intra-specific (*Oryza sativa* × *O. sativa*) and inter-specific (*O. sativa* × *O. glaberrima*) lines for their tolerance to blast in Burkina Faso

Moussa Sié, Blaise Kaboré, Drissa Sérémé, Yonnelle D. Moukoumbi
Institut de l'Environnement et de Recherches Agricoles (I.N.E.R.A.)
01 B.P. 910 Bobo-Dioulasso 01

Résumé

Trois types de riziculture sont pratiquées au Burkina Faso : la riziculture pluviale stricte, la riziculture irriguée avec maîtrise de l'eau et la riziculture de bas-fond. Cette dernière occupe 70 % des superficies cultivées et assure 48 % de la production rizicole du pays. Cependant, la mauvaise répartition spatio-temporelle des pluies et la forte pression parasitaire notamment la pyriculariose et la virose contraignent les populations rurales à abandonner progressivement certains bas-fonds.

La pyriculariose est une des plus importantes contraintes pour les riziculteurs ouest-africains en général et burkinabé en particulier en raison de l'importance sans cesse croissante des pertes occasionnées. C'est pour cette raison que la recherche de donneurs pour la résistance durable ou de cultivars dotés d'un bon niveau de tolérance en milieu réel se révèle comme une priorité.

L'étude a eu à identifier des idéotypes capables de s'adapter aux conditions de bas-fond à partir d'une caractérisation agromorphologique de 76 lignées intraspécifiques (*O. sativa* × *O. sativa*) et 493 lignées interspécifiques (*O. glaberrima* × *O. sativa*) issues de 18 croisements. Elles ont été testées en condition de bas-fond à Banfora durant les saisons humides 2000, 2001 et 2002. A l'issue de la première campagne, 96 lignées ont été retenues (14 intra et 77 inter) et ont été conduites dans les mêmes conditions en saison humide 2001 ; 15 lignées ont été retenues (6 intraspécifiques issues de 6 croisements, et 9 interspécifiques issues de 4 croisements). Ces lignées ont été testées en condition de bas-fond et en condition irriguée (plaine irriguée de Karfiguéla) en hivernage 2002. Le dispositif utilisé est le DITER modifié mis au point par Notteghem en 1977 avec IR 50 comme témoin sensible à la pyriculariose, BG 90-2 pour la RYMV et ITA 306 pour la cécidomyie.

Les lignées ont manifesté une faible sensibilité vis-à-vis des maladies et des attaques d'insectes. Les notes obtenues sur les lignées ont été largement inférieures à 5 pour la pyriculariose et la panachure jaune en saison humide 2000. Les individus *O. glaberrima* × *O. sativa indic*a (croisement WAS 127, WAS 131) et *O. sativa japonica* × *O. sativa indica* (WAS 115) paraissent les plus sensibles à la pyriculariose lors de la première campagne.

A l'issue de la campagne hivernale 2001, 15 lignées issues de 10 croisements dont 6 intraspécifiques et 9 interspécifiques ont été retenues. Cette sélection a porté sur les génotypes dotés d'un bon niveau de résistance aux maladies que sont la pyriculariose et la virose, aux insectes (foreurs de tiges et cécidomyie) et présentant les caractères agronomiques souhaités : précocité, résistance à la verse, bonne aptitude au rendement et bonne qualité de grain.

Cette étude a abordé à la fois l'adaptabilité des différents génotypes à la riziculture de bas-fond (sur la base de l'évaluation des caractères agronomiques), la tolérance aux maladies (pyriculariose et panachure jaune) et aux insectes (foreurs de tiges et cécidomyie), ce qui met en jeu un ensemble de gènes contrôlant ces caractères complexes. La sélection assistée par marqueurs moléculaires qui fera appel à la biologie moléculaire offre de nouvelles voies pour identifier, sélectionner et transférer les gènes impliqués dans la résistance à la pyriculariose. Ainsi l'on pourra gagner du temps et sauver de l'argent pour la réalisation de nos objectifs.

Abstract

Three types of rice cropping systems are practiced in Burkina Faso: rain-fed upland, irrigated lowland and rain-fed lowland. This last type is practiced over 70% of the cultivated land and provides 48% of the country's rice production. However farmers are progressively abandoning some inland valleys because of the poor spatio-temporal distribution of rains and the high disease pressure, particularly blast and Rice Yellow Mottle Virus (RYMV).

Blast is one of the most damaging constraints for rice-growers in West Africa in general and in Burkina Faso in particular because of the ever-increasing yield losses. This is why finding donors with long-lasting resistance or cultivars with a good tolerance level in field conditions is a priority.

This study was designed to identify ideotypes adapted to inland valleys. It was based on the agromorphological characterisation of 76 intraspecific (*O. sativa* × *O. sativa*) lines and 493 interspecific (*O. sativa* × *O. glaberrima*) lines obtained after 18 crosses in 2000. They were tested in the Banfora rain-fed lowland during the 2000, 2001 and 2002 rainy seasons. At the end of the first season, 96 lines were selected (14 intra- and 77 interspecific) and were grown under the same conditions in 2001. Fifteen lines were then selected (6 intraspecific from 6 crosses, and 9 interspecific from 4 crosses). These lines were tested under rain-fed lowland and irrigated conditions in the irrigated plain of Karfiguéla during the 2002 rainy season. The design used was a modified DITER as described by Notteghem (1977), using IR 50 as the susceptible control for blast, BG 90-2 for RYMV, and ITA 306 for gall midge.

The lines showed low susceptibility to disease and insect attacks. The scores obtained with these lines were well below 5 for blast and RYMV for the 2000 rainy season. The individual crosses that seemed to be most susceptible to blast during this first year were WAS 127 and WAS 131 (*O. glaberrima* × *O. sativa*) and WAS 115 (*O. sativa japonica* × *O. sativa indica*).

At the end of the 2001 rainy season, 15 lines (6 intraspecific and 9 interspecific) obtained from 10 crosses were selected. This selection was based on the genotypes with a good resistance level to diseases such as blast and RYMV, to insects (stem borers and gall midge), and which also had the following agronomic characteristics: short cycle duration, resistance to lodging, good yield potential, and good grain quality.

This study dealt with the adaptability of the different genotypes to inland valley rice cropping (as based on the evaluation of the agronomic characteristics), and with the tolerance to diseases (blast and RYMV) and insects (stem borers and gall midge), which involves a number of genes controlling these complex characters. Marker-assisted selection opens new ways to identify, select and transfer the genes that are linked to blast resistance. This will be a convenient way to save time and money in the implementation of our objectives.

Introduction

In Burkina Faso, rice cropping has been showing an unforeseen expansion in the last two decades, although its development has remained relatively low compared with other cereals. In fact, the area grown in rice is only 1.8% of the total area grown in cereals. Rice ranks fourth after sorghum, millet and maize in both cropping area and production (PSSA 1999). In Burkina Faso, the consumption per inhabitant, which was estimated at 4.5 kg/year in 1960, reached 8.5 kg/year in 1980 and 18.5 kg/year in 2000 (GBIKPI 1996, cited in Causse *et al.* 1997).

Rice production reached 97 000 tons in 1996–98; and will need to yield 305 000 tons of paddy in 2010 to meet a 10% yearly increase (Ministère de l'Agriculture 1999).

In the last 10 years, imports have reached 851 130 tons, corresponding to 130 385 million Fcfa. The mean yields increased from 0.8 t/ha in 1970 to 2.2 t/ha in 1998 (Traoré 2000).

Three types of rice cropping systems are practiced in Burkina Faso: rain-fed upland, irrigated lowland and rain-fed lowland. This last type is practiced over 70% of the cultivated land and provides 48% of the country's rice production (Sié 1999). The country has a high potential in rain-fed lowlands, especially in the south where annual rainfalls reach and often exceed 1000 mm.

The country's low rice yield is mainly due to socioeconomic, climatic and biotic constraints, but diseases also cause high yield losses. This is why the instability of resistance to blast is the main flaw in the introduced material as it leads to discarding of varieties a few years after their release. In addition, some new diseases such as Rice Yellow Mottle Virus (RYMV) have recently appeared and become a threat for the varieties currently cultivated under irrigated and rain-fed lowland conditions.

Within the scope of biodiversity extension, WARDA has implemented a number of interspecific crosses: *O. glaberrima* × *O. sativa japonica* for rain-fed upland conditions and *O. glaberrima* × *O. sativa*

indica for irrigated conditions. The new lines will have to be tested in the rain-fed lowland rice-cropping system in Burkina Faso.

The objective of this study was the testing of intra- and interspecific lines to identify the ideotypes adapted to rain-fed lowland rice-cropping; evaluation was based on their agromorphological characteristics and their resistance to diseases and insect attacks.

Material and methods

The experiment was held in the irrigated part of the Banfora rain-fed lowland in the Comoé area. It is a rudimentary system with buried compacted bunds.

The Comoé area has a climate of the south-sudanian type (Guinko 1984) tending to sudanian-guinean in the far south. Two seasons can be distinguished: a rainy season from April to October, and a dry season from November to March.

As it is situated between the 1000 and 1200 mm isohyets (CRPA 1994) the Comoé area is in the better-watered zone in Burkina Faso. From 1951 to 2000, the mean rainfalls in the Banfora area reached 1030 mm, and in the 2000 rainy season, 1200 mm. Most of the precipitation falls between June and September over 50 to 70 days of rain.

The rain-fed lowland soils are hydromorphic to pseudogley. Their texture is of a clay-loam type and the pH is between 3.5 and 5.4.

Vegetal material

The study aimed at testing these lines under rain-fed lowland conditions and at identifying the ideotypes adapted to that cropping system; it was based on the agromorphological characterisation of 76 intraspecific lines (*O. sativa* × *O. sativa*) and 493 interspecific lines (*O. glaberrima* × *O. sativa*) descended from 18 crosses. Their susceptibility to the main rice diseases, including blast and RYMV, was observed.

At the end of the 2000 rainy season, 91 lines (14 intraspecific and 77 interspecific) were selected. These lines were grown under the same conditions in 2001 after which 15 lines (6 intraspecific and 9 interspecific) descended from 10 crosses were selected. The 15 lines were tested in the Banfora valley in 2002. Tables 1 and 2 show the different crosses performed.

From the beginning, the lines originating in Saint-Louis (Senegal) were screened in semi-artificial conditions for RYMV susceptibility. The infesting border was made with a mixture of IR 50 and WITA 8, which are respectively susceptible to blast and RYMV.

Experiment management

Sowing was performed on two 2.4 m-long rows for each line. Sowing was direct using three seeds per planting hill separated by interspaces of 0.25 m × 0.25 m.

Sowing was implemented after a fertilisation of 200 kg/ha of NPK. Later 150 kg/ha of NPK were supplied in three 50 kg / ha applications: (1) just after the first weeding, (2) at the panicle initiation stage at 65–70 days after sowing (DAS), and (3) at booting stage. The infesting border was fertilised with 300 kg/ha of urea spread in three applications of 100 kg/ha each.

Experimental design

The experiment was run in a minor riverbed in the Banfora rain-fed lowland following a DITER design (Decreasing Inoculum Test for the Evaluation of Resistance) as fine-tuned by Notteghem in 1977. The lines to be tested and the controls were transplanted on two rows each, perpendicular to the infesting border in order to homogenise the spread of the disease and to assess the resistance level of the different lines. Controls were sown every 50 lines in order to assess the pressure level of the disease.

Table 1. Interspecific crosses.

Designation of cross	Type of cross†	Parents	No.of lines	Generation
WAB 878	*O. glaberrima* × *O. sj*	CG 14/IRAT 144	3 14	F_5 F_6
WAB 880	*O. glaberrima* × *O. sj*	CG 14/WAB 56-50	18 46	F_5 F_6
WAB 881	*O. glaberrima* × *O. sj*	CG 20/IRAT144 /	2 16	F_5 F_6
WAS 122	*O. glaberrima* × *O. si*	TOG 5681/3* IR 64	2 24 111	F_3 F_4 F_5
WAS 124	*O. glaberrima* × *O. si*	TOG 5681/3* IR 1529-680-3-2	25 8	F_4 F_5
WAS 126	*O. glaberrima* × *O. si*	TOG 5681/2* IR 64 // IR 31785-58-1-2-3-3	41	F_5
WAS 127	*O. glaberrima* × *O. si*	TOG 5681/2* IR 64 // IR 31851-96-2-3-2-1	2 21 43	F_3 F_4 F_5
WAS 131	*O. glaberrima* × *O. si*	TOG 5674/3* IR 31785-58-1-2-3-3	16 4	F_4 F_5
WAS 161	*O. glaberrima* × *O. si*	TOG 5681/ 4* IR 64	2 18	F_3 F_4
WAS 162	*O. glaberrima* × *O. si*	TOG 5681/ 4* IR 1529	1 8	F_3 F_4
WAS 163	*O. glaberrima* × *O. si*	TOG 5674/ 4* IR 31785	4	F_4
WAS 164	*O. glaberrima* × *O. si*	TOG 5675/ 3* IR 28	22	F_3
WAS 186	*O. glaberrima* × *O. si*	TOG 5681/ 5* IR 64	1 7	F_3 F_4
WAS 187	*O. glaberrima* × *O. si*	TOG 5681/ 5* IR 1529	4	F_4
WAS 189	*O. glaberrima* × *O. si*	TOG 5675/ 4* IR 28	5	F_4
WAS 190	*O. glaberrima* × *O. si*	TOG 7291/ 3* ITA123	6	F_4
WAS 191	*(O. si* × *glaberrima*) × O*. si*	IR 64 / TOG 5681 // 4* IR 64	16	F_4
WAS 192	(*O. si* × *glaberrima*) × *O. si*	IR 31785 / TOG 5674 // 4* IR 31785-58-1-2-3-3	3	F_4

† *O. si* = *Oryza sativa indica*; *O. sj* = *Oryza sativa japonica*.

Table 2. Intraspecific crosses.

Designation of cross†	Type of cross	Parents	No. of lines	Generation
WAT 1174 -B	*O. sj* × *O. si*	ITA 123 / TOX 3226-5-2-2-2	1	F_4
WAT 1176 -B	*O. sj* × *O. si*	ITA 123 / ITA 414	1	F_4
WAT 1181 -B	*O. si* × *O. si*	ITA 416 / TOX 3226-5-2-2-2	1	F_4
WAT 1184 -B	*O. si* × *O. si*	TOX 3093-35-2-3-3 / ITA 414	13	F_4
WAT 1187-B	*O. si* × *O. si*	TOX 3058-52-2-2-3-2 / ITA 414	1	F_4
WAT 1189 -B	*O. si* × *O. si*	TOX 3093-35-2-3-3 / TOX 3226-5-2-2-2	1	F_4
WAT 1191 -B	*O. si* × *O. si*	TOX 3093-35-2-3-3 / ITA 414	1	F_4
WAT 1193 -B	*O. si* × *O. si*	TOX 3093-35-2-3-3 / TOX 3876-56-1-4	1	F_4
WAT 1123	*O. si* × *O. si*	TOX 3090-135-1-3-2-2 / CISADANE// CK 73 / MATCANDU	1	F_4
WAT1223 -B	*O. si* × *O. si*	TOX 3399-108-3-3-2 / SPT 7106-2-3-3-1	1	F_4
WAT 1224 -B	*O. si* × *O. si*	TOX 3399-108-3-3-2 / IR 48028-B-B-126-3	1	F_4
WAT 1249 -B	*O. si* × *O. si*	TOX 3093-4-5-1-1 / TOX 3162-11-1-2-1-1	1	F_4
WAT 1273 -B	*O. si* × *O. si*	BW 348-1 / TOX 3233-46-3-3-4-2-2	1	F_4
WAT 1275 -B	*O. si* × *O. si*	SURASHA / TOX 3233-46-3-3-4-2-2	1	F_4
WAT 1281- B	*O. si* × *O. si*	PHALGUNA / TOX 3876-58-1-2	1	F_4
WAS 97	*O. sj* × *O. sj*	LAC 23 / ITA 123	4	F_4
WAS 99	*O. sj* × *O. si*	FOFIFA 62 / IR 64	1	F_6
WAS 105	*O. sj* × *O. si*	IR 47686-15-1-1 / BG 90-2	3	F_6
WAS 106	*O. sj* × *O. si*	ITA 305 / IR 32307-107-3-2-2	1	F_3
WAS 107	*O. sj* × *O. si*	ITA 305 / BG 90-2	4	F_6
WAS 108	*O. sj* × *O. si*	FOFIFA 62 / IR 1529-680-2-3	1	F_3
WAS 110	*O. sj* × *O. si*	FOFIFA 62 / JAYA	5	F_6

WAS 112	*O. sj* × *O. si*	LAC 23 / BG90-2	1	F_3
WAS 114	*O. sj* × *O. si*	ITA 305 / IR 13240-2-2-3	8	F_5
WAS 115	*O. si* × *O. sj*	BG 90-2 / ITA 305	1	F_3
WAS 116	*O. si* × *O. si*	GIGANTE / BG 380-2	1	F_3
WAS 117	*O. si* × *O. si*	JAYA / GIGANTE	1	F_3
WAS 121	*O. sj* × *O. si*	ITA 305 / BG 380-2	4	F_5
WAS 129	(*O. sj* × *O. si*) × *O. si*	FOFIFA 62/BG90-2 // IR 13240-108-2-2-3	6	F_5
WAS 137	*O. sj* × *O. si*	LAC 23/BG 90-2 // IR 28	1	F_3
WAS 138	*O. sj* × *O. si*	LAC 23/BG 90-2 // IR 64	1	F_3
WAS 140	*O. sj* × *O. si*	FOFIFA 62// 2*IR 1529-680-3-2	1	F_3
WAS 141	*O. sj* × *O. si*	ITA 305 / BG 380-2 // IR 32307-107-3-2-2	1	F_3
WAS 142	(*O. sj* × *O. si*) × *O. si*	ITA 305 / IR 13240-108-2-2-3 // IR 31785-58-1-2-3-3	1	F_5
WAS 146	(*O. si* × *O. sj*) × *O. si*)	BG 90-2 / ITA 305 // ITA 306	1	F_5
WAS 151	(*O. sj* × *O. sj*) × *O. si*	LAC 23/ITA 123 // IR 1529-680-3-2	1	F_5

† *O. si* = *Oryza sativa indica*; *O. sj* = *Oryza sativa japonica*.

The infesting border, sown along four continuous rows, was composed of a mixture of two varieties, one susceptible to blast (IR 50) and the other susceptible to RYMV (BG 90-2). The infesting border was sown 1 week before the varieties to be tested.

Observations

The agromorphological parameters observed were:

- mean number of tillers per seedling 60 days after sowing (T60)
- mean height of the mature plants (MH)
- sowing to heading duration (SHD)
- sowing to maturity duration (SMD)
- number of panicles per meter (P/M)
- panicle length (PL)
- panicle weight (PW)
- yield (Y)
- sterility level (St)
- number of dead hearts (DH)
- number of onion tubes (OT).

The assessment of the diseases was implemented as follows:

Rice blast

In order to assess blast in this study, we considered the 12 planting hills that made a row while the lines were distributed in two distinct batches of six seedlings each:

- Batch 1 of the row was set on the alleyway side and covered the first to the sixth planting hills.
- Batch 2 was set on the infesting border side and covered all the other rice plants, from the seventh to the twelfth hills.

The scoring was made from the tenth day after the outbreak of the first lesions (DAO) on the infesting border for leaf blast (corresponding to 30 DAS) and from the fifteenth day after heading (DAH) for neck blast (corresponding to 95 DAS). The INGER–IRRI (1996) Standard Evaluation System (SES) was used.

RYMV

To determine the RYMV level of infestation on the intra- and interspecific lines, the severity of the disease was scored at 30 DAS and 95 DAS with the evaluation system of varietal resistance to RYMV developed by IITA.

Measurement of the water table fluctuations

Two piezometers placed next to the two adjacent plots were used to measure the water table level every day during the entire vegetative cycle

Data analysis

Statistical analysis of the agromorphological and entomological data was performed with Genstat and Statistica. Excel was used for the phytopathological data and the fluctuations of the water table.

Results and discussion

Fluctuation of the water table

In the Banfora area, the rainy season is well under way in June, which is when its effects on the water table become perceptible. However, in our study, the follow-up of the water table was possible only from 19 July 2001. The results obtained show firstly, that the variations of the water table are dependent on the inland valley topological sequence (minor riverbed, slope bottom). The observations also show that the fluctuations of the water table during the rainy season can be schematised into three successive phases both at the level of the minor riverbed and at the bottom of the hill: a rising phase, a flooding phase, and a drying up phase.

Agromorphological evaluation of the lines

The projection of the individuals shows a distribution of the intra- and interspecific lines on axes 1 and 2 of the Principal Component Analysis (Figure 1). On axis 1, two line groups can be distinguished: there is a high concentration of lines on the positive side of axis 1 close to 0 and over the first centimeters on the negative side. Thus, two groups of lines can be distinguished on axis 1.

Group I is situated in the positive part and in the first centimeters of the negative side of the axis: it groups most of the vegetal material studied. This group contains lines with average to high levels of tillering, short compact and heavy panicles, with a seeding–heading cycle of average length.

Group II is situated at the negative end part of axis 1. The lines in this group are characterised by a low level of tillering, relatively long and meager panicles with a short seeding–heading cycle.

On the basis of their agromorphological characters and of their reaction to diseases and pests, 15 lines were selected and assessed in the third year (2002 rainy season).

The analysis of variance of the yields showed a non-significant difference with a coefficient of variance of 18.59 and a mean of 2937 kg/ha. The best variety was WAS 129-B-IDSA-B-WAS 1-1-FKR B-B, which yielded 3912 kg/ha.

Line screening for blast and RYMV

The parasite pressure was moderate over the three years. The IR 50 used in the infesting border showed very strong signs of neck blast, scored up to 7.

For the families from the crosses WAB 880 and WAB 881, the leaf blast scores (at 30 DAS) were identical for the two areas of observation: 0.11 on the alley side (B1) and 0.13 on the infesting border side (B2) ; the neck blast scores (at 95 DAS) were 0 for both B1 and B2. For the lines from the WAB 878 crossing, the leaf blast scores (at 30 DAS) were 0.35 (B1) and 0.59 (B2) and 0 at 95 DAS. This shows that the WAB 878 lines were slightly more susceptible to leaf blast than the controls and WAS 880 and 881. No individual presented any visual symptoms of neck blast or RYMV. These crosses took advantage of the high level of tolerance coming from their *japonica* (IRAT 144 or WAB 56-50) and *glaberrima* parents.

The *O. glaberrima* × *O. sativa indica* lines (crosses WAS 127, WAS 131) and the *O. sativa indica* × *O. sativa japonica* (from the 2000 selection) (WAS 115) were the most susceptible to blast during the first season (Tables 3 and 4). Eighteen intraspecific families among 36 (50%) presented no symptom of blast, all types being put together, whereas only one interspecific family (191) showed the same result (Tables 3 and 4).

Through these three seasons, RYMV pressure was low over the whole vegetative cycle of the rice plants. BG90-2, a particularly susceptible variety, did not present severe signs of RYMV. Twenty-five lines showed some susceptibility to the virus.

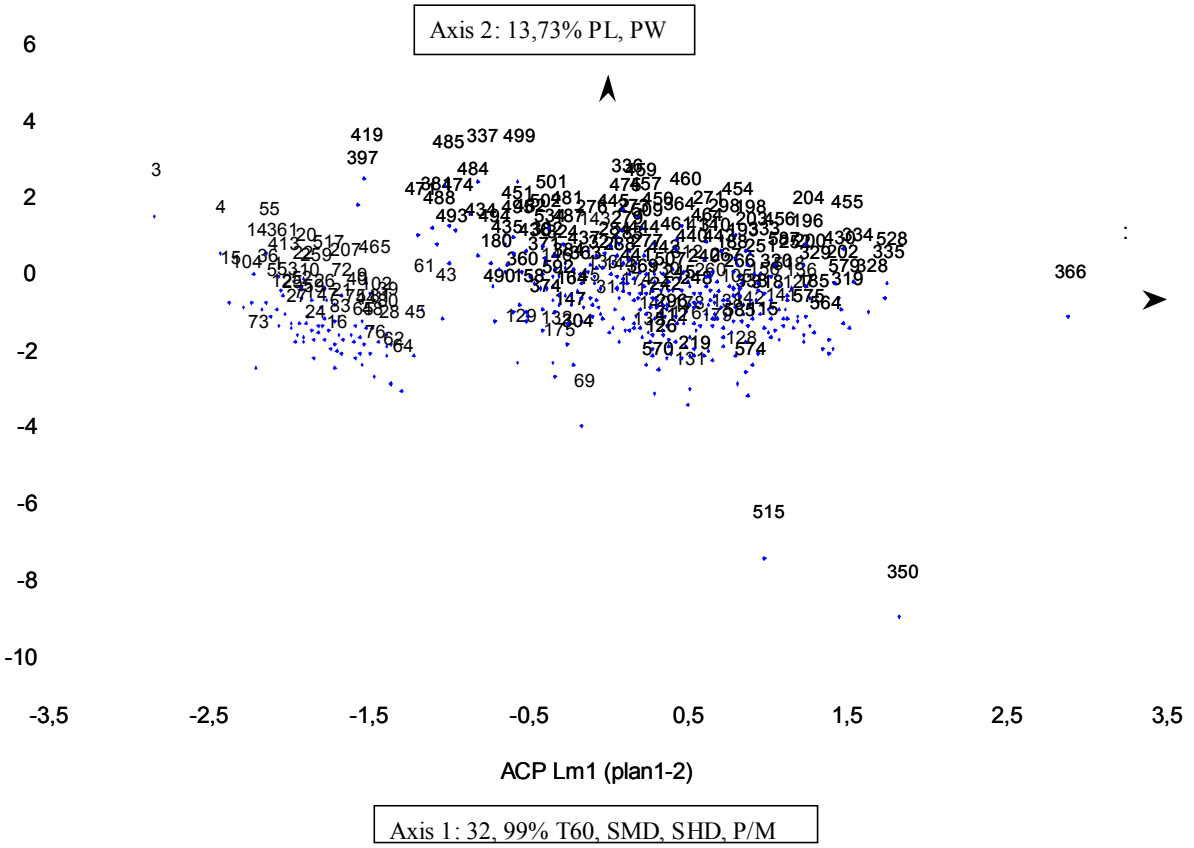

Figure 1. Distribution of the individuals on axes 1 and 2 of the Principal Component Analysis.

Table 3. Blast and RYMV incidence on interspecific progenies from *O. glaberrima* × *O. sativa japonica* (Banfora rain-fed lowland, wet season 2000).

Cross name	Blast disease				RYMV	
	On leaves (30DAS)		On necks (95DAS)			
	B1†	B2†	B1	B2	30 DAS	95 DAS
WAS 122	0.18	0.07	0.26	0.32	2	2.65
WAS 124	0.85	1.09	1.95	1.5	0.10	0.16
WAS 126	0.43	0.26	0.44	0.23	0.14	2.65
WAS 127	1.14	0.74	3.67	3.55	0.11	0.5
WAS 131	0.70	0.41	2.51	2.45	0.49	0.75
WAS 161	0	0	0	0	0.50	0.85
WAS 162	0.22	0	0	0	0	0.78
WAS 163	0.5	0.5	0	0	0	0
WAS 164	0.05	0.09	0.14	0.14	0.45	0.36
WAS 186	0.25	0.38	0.25	0	0.62	1.25
WAS 187	0	0	0.25	0	0	0.5
WAS 189	0.25	0.25	0.62	1.25	0	0
WAS 190	0.12	0.37	0.37	0.37	0.62	0.62
WAS 191	0	0	0	0	0	0
WAS 192	0	0	0	0	0	1.07

† B1 = Batch 1 of the row setting on the alleyway side. B2 = Batch 2 of the row setting on the alleyway side.

Table 4. Blast and RYMV incidence on intraspecific progenies from *O. sativa* × *O. sativa* (Banfora rain-fed lowland, wet season 2000).

Designation of cross	Blast disease				RYMV	
	On leaves (30DAS)		On necks (95DAS)			
	B1†	B2†	B1	B2	30 DAS	95 DAS
WAS 97	0	0	0	0	0	0
WAS 99	0	0	0	0	0	0
WAS 105	0.33	0.67	0	0	0	0
WAS 106	0	0	1	1	0	0
WAS 107	0.62	0	0	0	0	0
WAS 108	0	0	0	0	0	0
WAS 110	0.87	1	0	0	0	0
WAS 112	1	0	0	0	0	0
WAS 114	0	0.07	0.5	0.5	0	0
WAS 115	0	0	5	3	0	0
WAS 116	1	1	0	0	0	0
WAS 117	1	0	0	0	0	0
WAS 121	0	0	0.16	0.5	0	0
WAS 129	0.17	0.5	0.5	0	0	0
WAS 137	0	0	0	0	0	0
WAS 138	0	0	0	0	0	0
WAS 140	0	0	0	0	0	0
WAS 141	0.67	0	0.33	0	0	0
WAS 142	1	0	0.5	0.5	0	0
WAS 146	0	0	0	0	0	0
WAS 151	0	0	0	0	0	0
WAT 1174	0	0	0	0	0	0
WAT 1176	0	0	0	0	0	0
WAT 1181	0	0	0	0	0	0
WAT 1184	0	0	0	0	0	0
WAT 1189	1	0	0	0	0	0
WAT 1191	1	0	0	0	0	0
WAT 1193	0	0	0	0	0	0
WAT 1223	0	1	0	0	0	0
WAT 1242	0	0	0	0	0	0
WAT 1244	1	0	0	0	0	0
WAT 1249	0	0	0	0	0	0
WAT 1273	0	0	0	0	0	0
WAT 1275	0	0	0	0	0	0
WAT 1281	1	0	0	0	0	0
WAT 1282	0	0	0	0	0	0

† B1 = Batch 1 of the row setting on the alleyway side. B2 = Batch 2 of the row setting on the alleyway side.

Table 5. Evaluation of the 15 best interspecific and intraspecific lines in the Banfora rain-fed lowland (in 2000, 2001 and 2002).

Variety	2000									2001									2002								
	T60	P/m	SHD	MH	St	LB	NB	DH	OT	T60	P/m	SHD	MH	St	LB	NB	DH	OT	T60	P/m	SHD	MH	LB	NB*	DH	OT	Y
WAT 1176-B-FKR-B-B	79	74	90	103	3	0	0	1	5	59	57	86	133	0	1	0	0	54	90	55	88	135	2	3.48	1	24	3256
WAT 1184-B-FKR-B-B	58	54	84	147	3	0	0	1	5	72	55	93	124	5	3	0	5	41	115	59	86	114	2	3.8	1	15	3291
WAT 1191-B-FKR-B-B	73	72	86	113	5	0	0	1	7	71	48	88	90	0	1	0	0	22	135	47	87	102	2	4.37	2	20	3309
WAS 105-B-I-B-WAS-2-1-FKR-B-B	102	96	86	112	3	0	0	1	2	75	48	88	88	0	3	0	0	27	125	55	90	100	2	8.08	1	24	3251
WAS 114-B-I-B-WAS-1-5-FKR-B-B	77	65	86	115	3	0	7	1	5	90	50	88	103	0	1	0	7	27	122	54	86	111	2	5.16	1	15	3589
WAS 129-B-I-B-WAS-1-1-FKR-B-B	96	95	84	130	5	0	0	3	2	76	63	86	121	4	1	1	3	26	133	60	85	107	2	6.56	1	18	3912
WAS 122-1-1-WAS-6-1-FKR-B-B	44	41	86	114	1	0	0	0	14	73	54	91	116	0	3	0	0	29	132	49	85	113	2	4.95	1	30	3357
WAS 122-1-1-WAS-2-V-FKR-B-B	89	73	86	117	3	0	0	5	12	77	44	88	94	0	1	0	5	11	99	43	85	106	2	4.27	1	23	3035
WAS 122-1-1-WAS-B-FKR-B-B	65	53	87	122	3	0	0			61	47	92	112	0	1	0	0	29	97	43	85	106	2	3.54	1	24	2173
WAS 161-6-4-FKR-B-B	55	52	72	103	1	0	0	0	0	79	58	86	88	0	5	0	0	48	118	48	84	103	2	38.27	1	19	2277
WAS 161-8-9-3-FKR-B-B	94	86	87	106	3	0	0	0	13	112	55	88	85	0	2	0	3	55	126	50	83	91	2	6.05	1	19	2157
WAS 161-6-3-FKR-B-B	84	81	81	102	3	0	0	0	8	97	58	82	94	0	3	1	0	33	123	65	80	97	3	7,88	2	21	2128
WAS 163-B-5-3-FKR-B-B	66	52	93	89	3	1	0	0	10	65	50	88	91	0	3	0	0	58	115	38	89	94	2	8,36	1	22	3000
WAS 191-8 -3-FKR-B-B	71	69	93	88	3	0	0	3	11	75	62	85	96	5	5	0	0	45	130	55	82	100	3	3,03	1	16	2261
WAS 191-9-3-FKR-B-B	35	30	101	92	3	0	0	3	0	76	76	86	97	5	3	1	5	41	104	61	82	107	2	3,38	1	16	3053

T60 = mean number of tillers per seedling 60 days after sowing
SHD = sowing to heading duration
St = sterility level
LB = leaf blast score
DH = number of dead hearts
Y = Yield in kg per hectare
P/m = number of panicles per meter
MH = mean height of the mature plants
NB = Neck blast score (* = incidence in %)
OT = number of onion tubes

Concerning the onion tube variable, among the intraspecific lines (*O. sativa indica* × *O. sativa indica, O. sativa indica* × *O. sativa japonica,* and *O. sativa japonica* × *O. sativa japonica*), 16 crosses among 36 showed an average of 5 to 15% attack.

All the intra- and interspecific lines were susceptible to gall midge with scores from 4 to 58% in 2000. Such a source of damage therefore remains a serious problem in the inland valley ecology in Burkina Faso for which an adequate solution should be found.

Behaviour of the lines selected during the three rain seasons

Table 5 presents the behaviour of the 15 lines selected in 2001 over the three years considered: 2000, 2001 and 2002. The data show that during the first season the lines displayed some resistance to leaf blast as all the observations recorded were between 0 and 1. For neck blast, only the line WAS 114-B-IDSA-WAS 1-5-FKR-B-B reached the score 7.

During the second season, all lines showed signs of leaf blast with scores from 0 to 5. Two lines were scored 5. The neck blast attacks were low. During the third season, all the lines performed well for both leaf and neck blast except WAS 161-6-4-FKR-B-B which is susceptible to neck blast. Such data show that line susceptibility to blast changes according to seasons. Under such conditions, assessing the severity of the symptoms requires a wide approach in time (over years) and in space (in different environments) in order to ensure the selection of varieties with a stable resistance to blast.

Table 6 shows the weekly evolution of 15 lines as concerns leaf and neck blast. All lines presented a low score at the first evaluation date, 35 DAS. One week later, the score rose from 1 to 2 for two lines (WAS 122-1-1-WAS-2-V-FKR-B-B and WAS 161-8-9-3-FKR-B-B); the susceptibility to blast of the last line showed a steady increase.

At the fourth scoring, the lines WAS 161-6-3-FKR-B-B, WAS 163-B-5-3-FKR-B-B and WAS 191-8-3-FKR-B-B increased their susceptibility score from 1 or 2 to 3. All the other lines scored 2.

The susceptible control confirmed its susceptibility to the two forms of blast. For neck blast, WAS 161-6-4-FKR-B-B showed the highest susceptibility with an incidence score of 38.27.

Table 6. Blast disease score on 15 interspecific and intraspecific varieties in the Banfora rain-fed lowland during the wet season 2000.

Variety	Leaf blast score at different dates				Neck blast score (%)	
	22 Aug	29 Aug	3 Sept	10 Sept		
WAT 1176-B-FKR-B-B	1	1	2	2	0.20	3.48
WAT 1184-B-FKR-B-B	1	1	1	2	0.19	3.80
WAT 1191-B-FKR-B-B	1	1	2	2	0.18	4.37
WAS 105-B-I-B-WAS-2-1-FKR-B-B	1	1	2	2	0.23	8.08
WAS 114-B-I-B-WAS-1-5-FKR-B-B	1	1	2	2	0.19	5.16
WAS 129-B-I-B-WAS-1-1-FKR-B-B	1	1	1	2	0.16	6.56
WAS 122-1-1-WAS-6-1-FKR-B-B	1	1	1	2	0.22	4.95
WAS 122-1-1-WAS-2-V-FKR-B-B	1	2	2	2	0.22	4.27
WAS 122-1-1-WAS-B-FKR-B-B	1	1	1	2	0.26	3.54
WAS 161-6-4-FKR-B-B	1	1	2	2	0.20	38.27
WAS 161-8-9-3-FKR-B-B	1	2	3	2	0.16	6.05
WAS 161-6-3-FKR-B-B	1	1	1	3	0.18	7.88
WAS 163-B-5-3-FKR-B-B	1	1	2	3	0.19	8.36
WAS 191-8 -3-FKR-B-B	1	1	2	3	0.15	3.03
WAS 191-9-3-FKR-B-B	1	1	1	2	0.19	3.38
Control (TOX 30555-10-1-1)	4	6	8	8	1.22	70.25

Conclusion

During the 2000, 2001 and 2002 rainy seasons, intra- and interspecific lines were assessed. After an agromorphological assessment based on their susceptibility to insects, diseases and water table variations, a number of lines were selected for their adaptability to rain-fed lowland rice cropping systems.

In 2000, 15.06% of the intra- and interspecific lines were selected: 91 among 569 intra- and interspecific lines were selected, totaling 92. In 2001 rainy season, these 91 lines were grown, allowing a further selection of 15 lines that were re-evaluated in the Banfora inland valley in 2002. Among these, 14 were not too susceptible to leaf and neck blast (Table 6).

Intraspecific lines

1.	WAT 1176-B-FKR-B-B	*ITA 123/ITA 414*
2.	WAT 1184-B-FKR-B-B	FAROX 308-35-1-2/TOX 3226-5-2-2-2
3.	WAT 1191-B-FKR-B-B	TOX 3093-35-2-3-3/TOX 3226-5-2-2-2
4.	WAS 114-B-FKR-B-B	ITA 305/IR 13240-2-2-3
5.	WAS 129-B-FKR-B-B	FOFIFA 62/BG 90-2//IR 13240-108-2-2-3

Interspecific lines

6.	WAS 122-IDSA-1-B-FKR-B-B	*TOG 5681/3*IR 64*
7.	WAS 122-IDSA-1-2-FKR-B-B	*TOG 5681/3*IR 64*
8.	WAS 122-IDSA-1-WAS-6-1-FKR-B-B	*TOG 5681/3*IR 64*
9.	WAS 191-8-3-FKR-B-B	IR 64 // TOG 5681/4*IR 64
10.	WAS 191-9-3-FKR-B-B.	*IR 64 // TOG 5681/4*IR 64*

The weekly scores for blast confirmed the good tolerance level of the lines selected in 2002.

The joint work of many breeders from Mali, Burkina Faso, Togo and the ARI coordinator confirmed the good behaviour of the following jointly selected lines: WAS 122-IDSA-1-B-FKR-B-B, WAS 122-IDSA-1-2-FKR-B-B, WAS 122-IDSA-1-WAS-6-1-FKR-B-B and WAT 1176-B-FKR-B-B.

Prospect

These lines could be incorporated in a regional study within the breeders' network. They would benefit from the contribution of pathologists and entomologists before being selected as material with a stable selection level and thereafter become available to producers in PVS studies.

Potential use of molecular markers in rice breeding

Until now this study dealt with the adaptability of different genotypes to rain-fed lowland rice systems (by measurement of the agronomic characters), the tolerance to diseases (blast and RYMV) and to insects (stem borers and gall midge) that involve several genes controlling these complex characters. Marker-assisted selection, using molecular biology, offers new ways to identify, select and transfer the genes implied in blast resistance. The studies in quantitative genetics are now facilitated by the use of molecular markers (Sié 1997). The markers derived from Restriction Fragment Length Polymorphism (RFLP) present numerous qualities that make them the favourite markers for the creation of genetic linkage maps and for the follow-up of characters after crosses and backcrosses (Paszek 1996). Indeed the main genes for resistance to diseases and insects are mostly a group of genes controlling complex characters such as yield and quality (Young *et al.* 1992). The genes of disease resistance were the first gene categories to be mapped using RFLP. This could be explained because most of the resistance genes that were characterised

are controlled by alleles localised on a single locus and most are dominant genes easy to identify. The use of such markers is easy as their number is almost limitless, they are neutral, i.e. their allelic status has no influence on the phenotype, they are co-dominant (the allelic differences in a heterozygotic individual can be detected and are detectable whatever the origin of the tissue analysed or the development stage of the individual). Saturated maps have recently been built for a number of plants (Kurata *et al.* 1994; Causse *et al.* 1996).

The RFLP method can be used in plant breeding as the desired combination of genes can be obtained faster than with the classical methods, thus allowing a quicker selection of new varieties. Such an approach is particularly useful in the production system using a low level of input in which the problems due to insects, diseases and weeds are the main constraints.

Analysis of genetic diversity

The use of molecular markers has allowed characterisation of the diversity in the disease-causing populations as is the case for the strains of the fungus provoking blast. This is how Levy (1997, cited in Causse *et al.* 1997) obtained the genetic fingerprints of the profiles of a highly repeated sequence, *MGR 586*.

Romain *et al.* (1997) cited by Courtois (2001) were able, using molecular markers, to build a dendogram of the genetically linked groups. When inoculated, strains from the same line display very similar behaviour, and some resistance genes are effective against all strains belonging to the same line.

QTL use

Numerous characters show a continuous quantitative variation in segregating populations and are under the dependence of many loci that can interact among themselves and with the environment. The genetic mapping of segregating populations for these characters allows localisation of these loci or QTLs (Quantitative Trait Loci). Most maps use populations descending from backcrosses (F2) which are difficult to reproduce and do not provide good phenotypic values (de Vienne *et al.* 1999). Recent studies mostly used doubled haploids (DH) in order to establish genetic maps and to localise QTLs as, in particular for rice, the lines are genetically homozygotic after one single generation and can therefore be multiplied without segregation).

When QTLs have been identified for a group of qualitative characters, it is then possible to use their linked markers for breeding. According to de Vienne *et al.* (1999), using QTLs could allow researchers to attain the following objectives:

- improving performances by accumulating alleles favourable to yield, resistance to lodging and to pests.
- improving the stability of varieties by accumulating general QTLs in order to create high-yielding varieties for a large range of environments.
- improving the adaptation to a given environment, by accumulating QTLs specific to that environment.

Another interesting aspect in the use of QTLs is getting rid of the unfavourable breeding correlations. One of the correlations that could be improved is the unfavourable correlation between cycle duration and yield (de Vienne *et al.* 1999).

By limiting the use of QTL-based mapping to the evaluation of phenotypes in a single environment, the genotype × environment interaction could be neglected although it is an essential component influencing the expression of quantitative characters. The expression of alleles to QTLs throughout seasons and locations is an acute problem in plant breeding. Because of the cost of analysing different parameters for adaptability, the early use of the corresponding QTLs and of their genotype × environment interaction could be extremely helpful in plant breeding. It is therefore becoming evident that, with molecular biology, the use of information will favour a tighter collaboration between breeders, pathologists and molecular biologists (Robertson 1968).

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Screening Strategy for Durable Resistance to Rice Blast Disease at WARDA

Yacouba Séré[1], Abdoul Aziz Sy[2], Solomon Koffi Akator[1], Amos Onasanya[1], Kamelan Zai[1]
[1]WARDA, Africa Rice Center, B.P. 320, Bamako (Mali)
[2]Senior Research & Technology Officer/FAO-RAF, Accra (Ghana)

Résumé

La pyriculariose du riz est une des plus importantes maladies du riz dans la plupart des écosystèmes rizicoles d'Afrique de l'Ouest. La recherche de variétés dotées d'une résistance efficace fait l'objet d'une préoccupation constante des phytopathologistes comme des sélectionneurs. L'ADRAO s'est donc investie à mettre au point une stratégie visant à identifier du matériel résistant et utilisable directement par les producteurs, ou pouvant servir de donneurs dans les programmes de création variétale. La présente communication décrit cette stratégie après avoir fait le point sur les connaissances des relations hôte parasites qui ont servi de trame à la mise au point de cette technique d'identification de variétés dotées d'une résistance durable. Cette stratégie consiste à évaluer la résistance horizontale des variétés dont la résistance verticale a été surmontée.

Abstract

Rice blast is one of the most important diseases in most of the rice ecosystems of West Africa. The development of rice varieties with an effective resistance is on ongoing objective of plant pathologists and breeders. WARDA has thus developed a strategy aiming to identify resistant material to be used either directly by the producers, or as donors in the breeding programmes. The present communication describes this methodology, following a progress report on knowledge of the host-parasite relationships that were used to develop the screening strategy for durable resistance. This strategy consists of analysing the parameters of horizontal resistance for varieties on which vertical resistance is not operating.

Introduction

Rice blast is the most widespread disease of rice in the world. Caused by the pathogenic fungus *Pyricularia grisea* (Cke) Sacc. (Teleomorphe: *Magnaporthe grisea* (Hebert) Barr), it is found in most of the rice ecosystems of West Africa. Particularly dangerous in upland rice, it also causes serious damage in rain-fed lowland and irrigated systems, mainly when farmers seek to intensify the production by the use of improved varieties and fertilisers. Unexpected epidemic explosions still appear such has those observed within farmers' fields in the west of Côte d'Ivoire in 2002 (pers. comm., Bouet, Plant Pathologist, National Center of Agronomic Research).

One of the principal components of an integrated management system for this disease is varietal resistance. However, this can be unstable in space and in time according to the structure of the pathogen population. It is thus important to take this fact into account when wishing either to diffuse material to farmers or to provide donors to the breeders. In Japan, when the vertical resistance to blast fungus became ineffective for a variety, the variety proved even more sensitive than the sensitive local cultivars (Ezuka 1979).

The present communication aims to describe the strategy developed by WARDA to identify varieties with durable resistance. Before that, however, we summarise the knowledge of the host-pathogen relationship that was used in the conception of this screening technique.

Host-pathogen relationship within the couple *Oryza sativa/Magnaporthe grisea*

In genetic control of plant diseases, plant pathologists distinguish a specific or vertical resistance and a non-specific or horizontal resistance. The relations between rice and blast fungus conform so well to the principles governing the host-pathogen relationship that plant pathologists use to regard this couple as a model. The two kinds of resistance have been described for the couple *O. sativa/M. grisea.*

Vertical resistance is characterised by the existence of a differential interaction between the pathogen isolates confronted with the host varieties. It is qualitative, i.e. it results in an all-or-nothing reaction. Since it is controlled, in general, by a few genes of major effect, breeders frequently use it to create resistant materials. Unfortunately, it is not durable, since new pathogen races are able, in a relatively short time, to overcome it and destroy the efforts of many years of labour.

The existence of a monogenic (or oligogenic) resistance to the blast fungus has been largely confirmed by many studies (Wang *et al.* 1994; Naqvi *et al.* 1995; Pan *et al.* 1996; Yu *et al.* 1996; Liu *et al.* 2002). This oligogenic system is responsible for a qualitative, complete and non-durable resistance.

The position of each gene on the various chromosomes of rice was specified. For example, Yu *et al.* (1996) reported Pi-2(t) and Pi-4 on chromosomes 6 and 12 respectively, while Zu *et al*. (quoted by Pan *et al.* 1996) described Pi-zh(t) on chromosome 8. By the use of isogenic lines, Inukai *et al.* (1994) identified Pi-1 and Pi-2(t), which are allelic or strongly linked to Pi-z, then Pi-3 and Pi-4a(t) allelic or strongly linked to Pi-ta. Wang *et al.* (1994), through molecular analysis, discovered Pi-5(t) and Pi-7(t) respectively on chromosomes 4 and 11. Pi-8 is noted by Pan *et al.* (1996) on chromosome 8. More recently, Liu *et al.* (2002) showed that Pi-2(t) and Pi-9(t), two resistance genes efficient against many strains of *M. grisea*, are linked genes located on chromosome 6.

The host-pathogen relationships proceed according to the gene-for-gene principle illustrated in Table 7.

Table 7. Relationship between *Magnaporthe grisea* avirulence gene and *Oryza sativa* resistance genes, according to the gene-for-gene theory.

Pathogen avirulence gene	Host resistance genes	Reactions†
av-a+ & av-b	Without any resistant gene	+
	Pi-a only	+
	Pi-b only	-
	Pi-a and Pi-b	-
av-a & av-b+	Without any resistant gene	+
	Pi-a only	-
	Pi-b only	+
	Pi-a and Pi-b	-
av-a+ & av-b+	Without any resistant gene	+
	Pi-a only	+
	Pi-b only	+
	Pi-a and Pi-b	+

† + = compatible reaction (susceptibility); - = incompatible reaction (resistance).

By artificial inoculation of pathogen isolates to a range of suitable differential varieties, it is possible to identify avirulence genes which these isolates carry and thus to identify the races present within a given population.

Horizontal resistance is characterised by the absence of differential interaction. It is quantitative and stable. Its genetic determinism is polygenic. From the epidemiological point of view, it acts by slowing down the progression of the disease.

Such a system has been clearly shown in rice under different denominations: Field Resistance, Slow blasting, Quantitative Resistance, Non-specific Resistance and Partial Resistance. Scientists agree that this system is stable and durable, and that its genetic determinism is polygenic (Bonman 1992; Notteghem 1993; Wang *et al.* 1994).

An interesting variety in its behaviour with respect to the diseases (with durable resistance) is that it must not only be slightly attacked, but more especially, needs to express this aptitude on a broad geographical surface and/or during several cultural cycles. The stability of resistance is almost as important as the weakness of the attacks. This is why horizontal resistance is preferred to vertical resistance.

Various techniques have been used to characterise horizontal resistance. Evaluation of the infection rate of the epidemics is one method. While proceeding, for example, by evaluating the amount of disease over the time, it is possible to draw an exponential curve ($x = x_0 e^{rt}$), and define the epidemiological parameters related to the varieties and, in particular, to calculate the infection rate (r). However this rate is defined only during the logarithmic phase of the disease-progression curve. It can thus prove to be insufficient to characterise the aptitude of the varieties to slow down the progression of the epidemics for the adult plant resistance. Some prefer to use the value of the Area Under the Disease Progress Curve (AUDPC). Others take as a starting point the model by Eberhart and Russel (1966) developed for yield stability, in order to characterise the relationships between rice varieties and the blast pathogen.

Application to the screening for resistance

The strategy developed by WARDA is based on the principle that the two types of resistance (vertical and horizontal) can coexist within the same variety. For example, Moroberekan, a local variety of Côte d'Ivoire that is well known for the durability of its resistance to the blast fungus, has two dominant genes associated with a qualitative resistance and a polygenic system, quantitative trait loci (Qtl), affecting partial resistance (Wang *et al.* 1994).

It is then clear that differences in disease severity can come from different resistance mechanisms. Consequently, it is a mistake to base the choice of variety only on the disease score. Indeed, the small quantity of disease observed can be quite simply related to the fact that vertical resistance is effective against a large proportion of races of the pathogenic population (Chen and Line 1995).

The strategy (illustrated in Figure 2) consists of initially evaluating the vertical resistance of the material within blast nurseries. Each year, WARDA tests a few hundred varieties or lines provided by its Genetic Resources Unit and/or by the breeders.

The attacks are evaluated weekly according to the international scale (IRRI 1976). The varieties selected are those that receive a score of 3. As stated above, such a score can be for either horizontal resistance or effective vertical resistance against a part of the pathogen population.

The immune varieties are not selected because they could represent material having escaped the attack owing to the effectiveness of their vertical resistance against all the pathogenic population. They will be integrated in the nurseries of following years.

The tolerant material is then characterised for horizontal resistance by analysing the progression of the epidemic. They are tested in statistical trials led in Fisher blocks in the presence of the Moroberekan, the check for horizontal resistance.

Three epidemiological parameters are used: infection rate (r), the area under disease progress curve (AUDPC) and the maximum disease severity (x max).

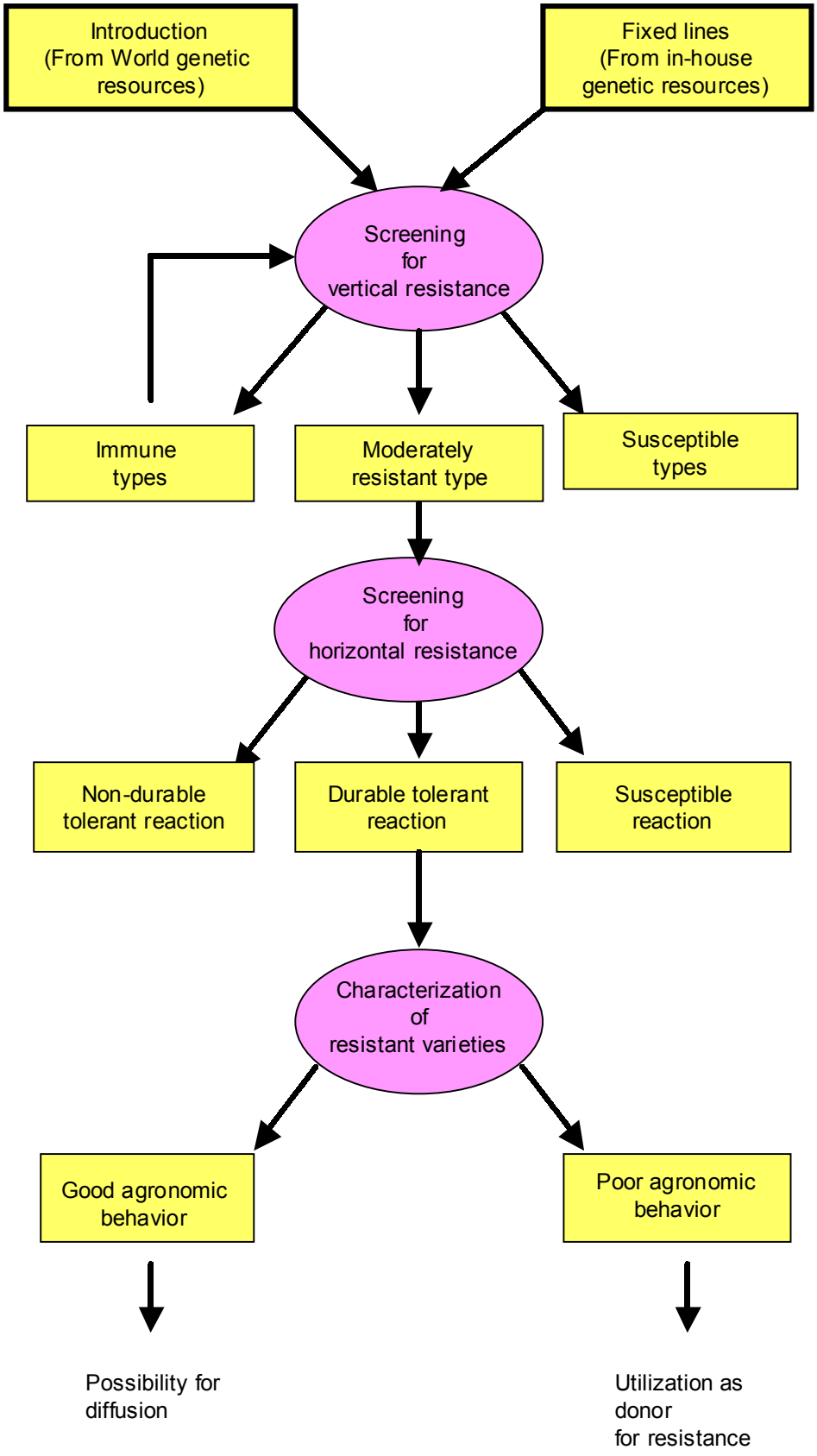

Figure 2. Steps of the screening strategy for durable blast resistance at WARDA

For example, in 2001, the comparative analysis of a hundred interspecific varieties and their parents (Table 8) led to the following results:

- 34 entries have a good level of horizontal resistance, including the interspecific varieties NERICA 1, NERICA 2, NERICA 3, NERICA 5 and their *sativa* parent (WAB 56-104), as well as the resistant check Moroberekan
- 72 entries, including NERICA 6, NERICA 7, the susceptible check and the *glaberrima* parent of interspecific, have a low level of horizontal resistance.

Table 8. Utilization of three epidemiological parameters to characterize the level of horizontal resistance of some rice varieties.

Parameters†			Conclusions		
x (max)	Infection rate	AUDPC	Type	Number	Example‡
+	+	+	Resistant	34	N1, N2, N3,
+	+	-			N5
+	-	+			Moroberekan
-	+	+			WAB 56-104
-	-	-	Susceptible	72	CG 20, CG14
-	-	+			Gigante
-	+	-			WAB 96-13-1
+	-	-			N6, N7

† % = as resistant as (or more resistant than) Moroberekan; & = less resistant than Moroberekan.
‡ N1 ... N6 = NERICA1 ... NERICA6.

Conclusion

The examination of the relationship between rice and the blast pathogen shows that unstable resistance and durable resistance can coexist within the same variety. If the plant does not have a specific resistance to the race with which it is confronted, the infection process proceeds normally until the production of the conidia enables the initiation of new lesions. The quantity of the disease obtained will then depend on the non-specific resistance. One can thus say that resistance during the first stages of the infectious process concerns resistance of the vertical type. When that it is overcome, the plant has a certain level of horizontal resistance with which to oppose the disease progression.

It is thus important to keep these facts in mind in the screening process for durable resistance. This is why the technique that we developed initially makes it possible to make sure that vertical resistance is overcome, before better determining the effects of horizontal resistance.

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Impact assessment of agricultural technology: Concept, methodology and application to rice pests and diseases

A. Diagne
WARDA, Africa Rice Center, B.P. 320, Bamako (Mali)

Abstract

Once disparate approaches to impact assessment encompassing various sub-disciplines of economics and statistics are now converging to provide a single unified methodological framework within which the impact of various types of programmes, policy changes, and technologies on various behavioural, environmental and welfare outcomes can be assessed with a level of rigour satisfactory from both economic and statistical perspectives. A synthesis of some recent methodological developments is presented within one single coherent conceptual and methodological framework, including an application to the assessment of the adoption impact of disease resistance varietal technologies.

Part 3:
Workshop full papers on DFID-CPP Blast project activities and outputs

Survey of Rice Blast and Varietal Screening in Ghana

S. K. Nutsugah[1], W. Dogbe[1], J. K. Twumasi[2], K. Dartey[2], J. Chipili[3], S. Sreenivasaprasad[3] and Y. Séré[4]
[1]Savanna Agricultural Research Institute, P. O. Box 52, Tamale, Ghana
[2]Crops Research Institute, P. O. Box 3785, Kumasi, Ghana
[3]Warwick HRI, Wellesbourne, Warwick CV35 9EF, UK
[4]West Africa Rice Development Association, 01 BP 2551, Bouaké, Côte d'Ivoire

Résumé

En vue d'évaluer l'incidence de la pyriculariose au Ghana, des surveillances ont été exercées en champs paysans, ainsi qu'au sein des pépinières de sélection variétale participative (VS) et de parcelles de recherche qui se situent au niveau des sites de criblage ADRAO/SNRA durant les saisons 2000-2002. En plus, des essais au champ ont été conduits sur 10 variétés dans certains des sites de criblage des régions du Nord et extrême Est du pays en vue d'évaluer leur réaction à la pyriculariose. Deux cent soixante quatre champs ont été surveillés dans toutes les principales régions productrices de riz de tout le pays pendant la durée du projet. L'incidence de la pyriculariose varie considérablement entre les sites, qui ont été regroupés en aires indemnes ou faiblement affectées, modérées et hautement touchées. Les zones les plus affectées sont Boama-Dumase, Offinso (Ashanti, [AR]), Abora, Brofoyedru, Bremang, Diaso, Nkwantanang, Treposo (Central, [CR]), Otumi (Eastern, [ER]), Galenkpegu, Kpachie, Nyankpala (Northern, [NR]), Bawku, Nyorigu (Upper East, [UER]), Fodome, Golokwati, Hohoe, Kpoeta, Santrokofi (Volta, [VR]), Datano, Sayerano et Tanoso (Western, [WR]). Les moins affectées sont Aferi, Bibiani, Juabeso, Sefwi-Wiawso, Sui-ano #1, Sui-ano #2 (WR), Asikam, Daamang, Subi (ER) et Dromankoma (AR). La maladie n'a pas été observée à Brong Ahafo, Greater-Accra et Upper West regions. Une observation importante au sujet de l'incidence élevée de la pyriculariose au niveau et autour de Hohoe est que la maladie provenait des lits de pépinières transplantées dans les champs par les paysans. Dans la plupart des champs visités, l'attaque de la pyriculariose était observée au stade plantules et au stade végétatif. Peu de cas d'infection furent observés à l'initiation paniculaire et lors du remplissage des grains du fait de différences variétales et agroécologiques au niveau des sites d'échantillonnage. Des lourdes pertes de rendement (plus de 100 %) dues en grande partie à la pyriculariose sont signalées par les paysans dans des zones de forte pression notamment Fodome, Hohoe, Santrokofi et Datano, même si l'effet de la sécheresse avait exacerbé la situation des 3 premières localités citées. Les résultats de la surveillance suggèrent que Datano (WR), Hohoe (VR) et Nyankpala (NR) sont des sites clés pour le criblage pour la résistance qui sont en corrélation avec la diversité des populations pathogènes. L'incidence de la pyriculariose sur les variétés des essais PVS est généralement faible avec des notes de sévérités comprises entre 1 et 3 suggérant leur résistance potentielle au niveau des sites clés de criblage de la région Nord. Ces variétés devraient être suffisamment testées en d'autres sites à travers le pays pour être utiles aux paysans à faibles ressources et aux consommateurs.

Abstract

Surveys were conducted in farmers' fields, participatory varietal selection (PVS) rice nurseries and research trial plots which overlapped with WARDA/NARS screening sites during 2000–2002 cropping seasons to assess the incidence of rice blast in Ghana. In addition, field trials were carried out on 10 or more PVS rice varieties during the same period at some of the key screening sites in Northern and Upper East regions to assess their response to blast.

Two hundred and sixty-four fields were surveyed in all the major-rice growing areas across the entire country during the project lifespan. The incidence of blast varied considerably across these sites, which have been grouped into no/low (0–3 predominant), moderate (4–6 predominant) and high (7–9 predominant) blast areas based on disease incidence and severity. The high-blast areas across the regions are Boama-Dumase, Offinso (Ashanti [AR]), Abora, Brofoyedru, Bremang, Diaso, Nkwantanang, Treposo (Central [CR]), Otumi (Eastern [ER]), Galenkpegu, Kpachie, Nyankpala (Northern [NR]), Bawku, Nyorigu (Upper East [UER]),

Fodome, Golokwati, Hohoe, Kpoeta, Santrokofi (Volta [VR]), Datano, Sayerano and Tanoso (Western [WR]). The low-blast areas are Aferi, Bibiani, Juabeso, Sefwi-Wiawso, Sui-ano #1, Sui-ano #2 (WR), Asikam, Daamang, Subi (ER) and Dromankoma (AR). No blast was observed in Brong Ahafo, Greater-Accra and Upper West regions. A key observation about the high incidence of blast in and around Hohoe was that the disease originated from the nursery beds and was transplanted onto the fields by the farmers. The blast infection in most of the fields visited was observed at the seedling and vegetative growth stages. Few cases of blast infection were seen at panicle initiation and grain-filling stages owing to varietal and agro-ecological differences at the sampling sites.

Heavy yield losses (up to 100%) largely due to blast infection were reported from the farmers' records at some of the high-blast areas—notably Fodome, Hohoe, Santrokofi and Datano—even though drought effect exacerbated the situation at the former three sites. The survey results suggest that Datano (WR), Hohoe (VR) and Nyankpala (NR) are blast hot spots and key sites for resistance screening, which correlates with the diversity of the pathogen populations.

Blast incidence on the PVS rice varieties was generally low and the severity rating ranged between 1 and 3, suggesting their resistance/partial-resistance potential at the selected key screening sites in Northern region. These improved varieties need to be tested sufficiently at other key sites across the country to benefit the resource-poor farmers and consumers.

Introduction

Rice is becoming increasingly an important staple food consumed throughout Ghana. The per capita consumption has risen from 13.9 kg/year in 1995 to 14.5 in 2000 (MOFA 2001). Ghana imports 564 000 mt paddy annually compared with domestic production of 180 000 mt. Import of milled rice alone was worth US$ 95 million in 1999 and it is likely to be slightly more currently. Rice production has expanded in recent times and it is being given the needed attention to achieve national food security, alleviate rural poverty and contribute to the overall economy through import substitution and foreign exchange conservation.

The area under rice production in Ghana is over 130 000 ha of which 61% a is rain-fed upland/ hydromorphic ecosystem, 21% rain-fed lowland and inland valleys ecosystem and 18% irrigated (MOFA 1999).

Rice production in Ghana is constrained by a number of biotic factors including diseases, pests and weeds. Blast disease caused by the fungus *Pyricularia grisea* (Rossman *et al*. 1990) (telemorph = *Magnaporthe grisea*) (Webster 1980) remains a threat to rice production in both temperate and tropical regions despite extensive research efforts directed toward controlling the disease (Teng 1994). The ability of the pathogen to infect rice at different stages of growth, and its adaptation to both upland and lowland rice ecosystems are indications of the plasticity of *P. grisea* to changing environments (Bonman *et al*. 1992; Teng 1994). In the West African sub-region, blast is recognised as a primary constraint to rice production causing 3.2-77.0% yield losses (Fomba and Taylor 1994).

Rice blast was first recorded in Ghana by Bunting and Dade (1925) and then Dade (1940). It was also observed by Leather (1959) and Piening (1962). It was listed as an important disease by Clerk (1974) and Oduro (2000).

Devastation of some rice cultivars by blast was observed in Northern Ghana in 1969. Since then various reports by Twumasi (1996, 1998), Twumasi and Adu-Tutu (1995), Nutsugah (1997 a, b) and Nutsugah and Twumasi (2001) have identified the disease as a serious threat to rice production in Ghana. Together with brown spot (*Bipolaris oryzae*), blast disease has again been recently listed as one of the serious constraints to rice production in the country (Gerken *et al*. 2001). These reports suggest the need for improved management of blast disease in Ghana if the target set for rice production is to be achieved.

Recognising this need, the UK Department for International Development (DFID)-Crop Protection Programme (CPP), managed by Natural Resources International, funded a collaborative research project on rice blast with West Africa Rice Development Association (WARDA), Côte d'Ivoire, Savanna Agricultural Research Institute (SARI) and Crops Research Institute (CRI), Ghana and Horticulture Research International (HRI), UK. As part of this project, nation-wide surveys were conducted during

2000-2002 cropping seasons in all the major rice-growing areas to assess the incidence of rice blast and relative importance of other rice diseases in Ghana. Furthermore, field trials and screenhouse testing were carried out on 20 or more rice varieties grown in the country to identify cultivars that would have high levels of resistance to the disease. This paper mainly reports the work done in Ghana by SARI and CRI in coordination with WARDA and HRI.

Materials and methods

Surveys

In October and November 2000 and May 2001, surveys were conducted in Asante-Akim and Ejura districts in Ashanti region and Sefwi and Wassa-Amenfi districts in Western region where the rice plants had reached the tillering or vegetative stage. In July 2001, more extensive surveys were conducted by a team of scientists from SARI, CRI and HRI on farmers' fields, participatory varietal selection (PVS) rice fields and research trials in seven regions of Southern Ghana, namely; Ashanti, Brong-Ahafo, Central, Eastern, Greater-Accra, Volta and Western regions. In September and October 2001, surveys were conducted in the northern part of the country in rice-growing areas of Northern, Upper East and Upper West regions. Final surveys were again conducted in the Southern and Northern sectors of the country in August and September 2002.

In all these surveys, each field was visually divided diagonally into four sectors, and 10-20 hills or plants from each sector were assessed for the presence or absence of the disease. If present, assessment was done according to the IRRI Standard Evaluation System (IRRI–SES). The visual rating for disease damage was made on a scale of 0–9. Survey sites were categorised into no/low (0–3 predominant), moderate (4–6 predominant) and high (7–9 predominant) blast areas based on disease incidence and severity. Diseased leaves were collected and some samples sent to HRI, UK for MGR586 fingerprint group or lineage determination and pathotype analysis. The remaining samples were kept in Ghana for isolation of the blast pathogen.

Isolation of **Magnaporthe grisea**

Blast lesions on diseased or infected leaves less than 1.0 cm in size were removed with a flamed scalpel. They were surface-sterilised in 1% sodium hypochlorite for 1.5 min, followed by three rinses in sterile distilled water before being placed on moist filter papers in Petri dishes at room temperature and incubated under alternating cycles of 12 h near ultra-violet (NUV) light and 12 h darkness for 48 h for sporulation. The spores were transferred onto Oat Meal Agar (OMA) plates containing chlortetracycline hydroxide and incubated as before for 2 wks. Some of the surface-disinfected lesions were incubated directly on Petri dishes containing OMA to which chlortetracycline hydroxide had been added.

Artificial inoculation studies

Test plants

Seeds of 20 or more rice varieties collected from rice-growing districts and from PVS nurseries were used in the inoculation studies in the screenhouse (Table 9).

The seeds were primed before being sown in plastic buckets. The seeds were soaked in tap water in beakers overnight. The next morning, the seeds that floated on the water surface were discarded, and only those that sank to the bottom of the beakers were sown. Fifteen seeds were sown per plastic bucket. Six days after germination, the seedlings were thinned to 10 seedlings per plastic bucket. Ammonia and NPK fertilisers were applied at standard rates. The varieties were also tested in the field for their reactions to the blast pathogen.

Table 9. Rice varieties screened against characterised *Magnaporthe grisea* isolates belonging to different lineages from Ghana.

Variety	Designation†
Viwornu	Local, Hohoe, VR
Mr. Harrow	Local, Aframso, AR
Mr. More	Local, Aframso, AR
Asante-mo	Local, Boama-Dumase, AR
Sika-mo	Improved, Boama-Dumase, AR
Agya-Amoa	Local, Sayerano, WR
Martin	Local, Gbi-Godenu, VR
IR 12979-24-1	Improved, NR
IDSA 46	Improved, NR
IRAT 216	Improved, NR
WAB 450-I-B-P91-HB	Improved, NR
Mendi	Local, NR
Gomba	Local, NR
Agona	Local, NR
WAB 450-I-B-P15-7-1-1	Improved, NR
Kleminson	Improved, NR
WAB 515-177-2	Improved, NR
IDSA 85	Improved, NR
WAB 56-50	Improved, NR
Agosanga	Local, NR

VR=Volta region, AR=Ashanti region, WR=Western region, NR=Northern region.

The pathogen

Fifteen isolates of the blast pathogen characterised at HRI were sub-cultured on OMA (Table 10). Conidia of *M. grisea* were examined under a binocular dissecting microscope and their identity confirmed by light microscopy. Conidia were placed on OMA plates containing aureomycin and incubated at room temperature for 14–21 d with 12 h NUV light and 12 h dark cycle.

The conidia were then gently washed with sterile distilled water and the suspensions were passed through cheesecloth. The spore concentration of the liquid containing gelatin was determined with a haemocytometer and adjusted to 10^5 conidia/ml. The suspensions were sprayed with HUMBROL spray Gun Kit on the seedlings grown in the plastic pots in the screenhouse. Inoculated plants were observed daily and after 14 d, disease assessment was done using the IRRI–SES scale of 0–9. The plant response was grouped into two categories, namely resistant (0–3) and susceptible (4–9).

Table 10. Details of site, host cultivar and lineage grouping of *Magnaporthe grisea* isolates from Ghana used in artificial inoculation studies.

Serial . no	HRI code	Source code	Host/cultivar	Site	Lineage
1	78	B175	Red rice	Bolgatanga	GH-1
2	79	B179	Red rice	Bolgatanga	GH-1
3	52	B159	TOX 3792-10-1-2-1-1-3-2	Hohoe	GH-1
4	50	B153	TOX 3880-38-1-1-2	Hohoe	GH-1
5	57	B167	TOX 4004-43-1-2-1	Hohoe	GH-1
6	55	B164	TOX 728-1	Hohoe	GH-1
7	70	B157	IR 12979	Hohoe	GH-1
8	27	B124	WAB 651-B-9-836	Kwadaso	GH-1
9	11	B18	Unknown	Tono	GH-4
10	51	B158	CK 73	Hohoe	GH-2
11	49	B152	TCA 80-4	Hohoe	GH-2
12	46	B149	TGR 75	Hohoe	GH-2
13	53	B161	TOX 3100-37-3-3-2-4	Hohoe	GH-2
14	73	B202	Red rice	Bolgatanga	GH-1
15	72	B201	Red rice	Bolgatanga	GH-1

Field trials

Assembly of germplasm for evaluation was done in April–May with subsequent seeding in June–July. The seeds were sown in rows 25 cm apart in a plot size of 5 × 2 m. Ten or more varieties were screened using randomised complete block design with 4 replications and 4 rows for each variety. Screening was done in selected PVS sites in Northern (Galenkpegu, Gbulung, Golinga, Nyankpala, Salaga, Tarkpaa and Tolon) and Upper East (Nyorigu and Tambalug) regions. These fields have been used as PVS nurseries for the past 4 years. Blast incidence observations were made during the crop progression with the levels recorded at least once every 4 weeks.

Results and discussion

Surveys

Results of blast surveys across Ghana are presented in Tables 11 and 12.

Table 11. Survey of rice blast in Southern Ghana, 2000-2001 cropping seasons.

Location	Variety	Incidence/Severity†
Ashanti region		
Adansi-Praso	Red rice	Moderate
Adugyama	Sika-mo	No/Low
	Local	No/Low
Aframso	Mr. More	Moderate
	Sika-mo	No/Low
	Mr. Harrow	No/Low
Amakom	Local	No/Low
Anyinasuso	Local	High
Biemso #2	Sika-mo	No/Low
Besease	Sika-mo	No/Low
Boama-Dumase	Sika-mo	No/Low
	Asante mo	High
Bronikrom	Local	No/Low
Dromankoma	Mr. More/Harrow	High
Kasei	Local	No/Low
Mmoframfadwen	Local	No/Low
Nkawie	Local	No/Low
Nobewam	Sika-mo	No/Low
Offinso-Kayera	Local	High
Tepa	Local	No/Low
Brong Ahafo region		
Atebubu	Mr. More	No/Low
Kwame-Danso	Mr. More	No/Low
Goaso	Asante-mo	No/Low
Central region		
Abora	Local	High
Agono-Port	Local	Moderate
Agyahamenso	Local	No/Low
Assin-Akonfudi	Sika-mo	No/Low
Assin-Dompem	Sika-mo	No/Low
Ayamfuri	Unknown	Moderate
Brofoyedru/Bremang	Unknown	High
Diaso	Red rice	High
Nkwantanang	Red rice	High
Treposo	Aberewa besi (Local variety)	High

Location	Variety	Incidence/Severity†
Eastern region		
Abaam	Red rice	No/Low
Abodom	Red rice	No/Low
Asikam	Red rice	High
Asutsuare	Sika-mo	No/Low
Daamang	Red rice	High
Ekoso	Red rice	No/Low
Kpong	GR-19	No/Low
Nkwantanan	Red rice	High
Otumi	Red rice	High
Subi	Red rice	High
Western region		
Adjakaa-Manso	Asante-mo	High
Aferi	Agya-Amoa	High
Apratu	Unknown	No/Low
Asaasetere	Unknown	No/Low
Asanta	Unknown	No/Low
Bibiani	PVS & Local	High
Datano	Sika-mo	No/Low
	Agya-Amoa	High
	Agya-Amoa	High
Juabeso	Agya-Amoa	Moderate
Kentenkrobu	Unknown	Moderate
Nkroful	Agya-Amoa	High
Nsuansua	Agya-Amoa	Moderate
Owuosabroso	Agya-Amoa	High
Sanyerano	Agya-Amoa	High
Sefwi-Wiawso	Agya-Amoa	High
Suiano #2 Suiano # 2	Agya-Amoa	High
Tanoso	Red rice	High
Tarkwa-Nsuaem	Unknown	No/Low
Greater-Accra region		
Dawhenya	GR 21	No/Low
Volta region		
Afife	Sika-mo	No/Low
Akpafu-Mempeasem	Viwornu	Moderate
Akpafu-Odomi	Viwornu	High
Akpafu-Todzi	Viwornu	Moderate
Fodome	Viwornu	High
Gbi-Godenu	Viwornu	High
Golokwati	Viwornu	Moderate
Hohoe	Viwornu	High
Jasikan	Sika-mo	No/Low
Kadjebi	Sika-mo	No/Low
Kpoeta	Viwornu	Moderate
	Perfumed rice	High
Santrokofi	Viwornu	High
Worawora	Sika-mo	No/Low

†Survey sites were categorized into no/low (0–3 predominant), moderate (4–6 predominant) and high (7–9 predominant) blast areas based on disease incidence and severity using IRRI–SES 0–9 scale.

Table 12. Survey of rice blast in Northern Ghana, 2000-2001 cropping seasons.

Location	Variety	Incidence/Severity†
Northern region		
Damongo	Unknown	No/Low
Galenkpegu	PVS/Research plot	High
Golinga	Research plot/farmer	No/Low
Kpachie	PVS plot/farmer	High
Nyankpala	PVS plot/Commercial	High
Salaga	Research plot/farmer	High
Upper East region		
Bawku	Unknown	High
Bolgatanga	Unknown	High
Fumbisi	Unknown	No/Low
Manga	Improved	No/Low
Navrongo	Unknown	No/Low
Nyorigu	PVS plot	High
Sandema	Unknown	No/Low
Tono	Unknown	No/Low
Wiaga	Unknown	No/Low
Upper West region		
Babame	Unknown	No/Low
Busa	Unknown	No/Low
Dorimon	Unknown	No/Low
Vieri	Unknown	No/Low
Wa	Unknown	No/Low

†Survey sites were categorised into no/low (0–3 predominant), moderate (4–6 predominant) and high (7–9 predominant) blast areas based on disease incidence and severity using IRRI–SES 0–9 scale.

2001

In Ashanti region, blast was prevalent and severe in the nurseries and on rice plants at the vegetative stage in and around Offinso district (Offinso-Kayera, Anyinasuso), Asante-Akim district (Boama-Dumase), Ejura/Sekyedumasi district (Aframso, Dromankoma) and Adansi-East district (Adansi-Praso) in decreasing order of importance. The disease occurred on the local variety Asante-mo that was popularly grown in the various localities surveyed. The disease was not found on any of the improved varieties.

No blast was observed in Brong-Ahafo region.

In Central region, blast was found to be devastating in the Upper Denkyira district (Treposo, Nkwantanum, Diaso, Brofoyedru, Bremang and Abora). In Assin district (Assin-Akonfudi and Assin-Dompem), blast was completely absent. This was probably because not only had the crop reached harvesting stage but also the variety widely cultivated in the district (*Odo*) appeared to look like *Sika-mo*, an improved variety.

In Eastern region, the disease caused a lot of damage in the Kwaebibirim district (Subi, Otumi, Nkwantanang, Daamang and Asikam). Rice blast was conspicuously absent in the Krobo area (Asutsuare and Kpong). Brown spot was the only disease prevalent.

In Western region, blast was very destructive in Bibiani, Sefwi Juabeso-Bia, (Sayerano, Datano, Juabeso, Aferi) and Sefwi-Wiawso (Sefwi-Wiawso, Tanoso, Nsuansua) districts where a very popular local rice variety—*Agya-Amoa*—is widely grown. At Datano, blast completely destroyed the integrated pest management demonstration trial set up by the Ministry of Food and Agriculture in 2001, resulting in no activity in 2002. The blast situation on local varieties at Bibiani PVS nurseries in the Western region was not different from that of Juabeso-Bia and Sefwi-Wiawso districts. At Adjakaa-Manso (Wassa Amenfi district) in the Western region, blast incidence and severity were very high. In the Wassa-Fiase district (Tarkwa Nsuaem mile 1–5, Wassa Dompim), Nzema district (Asaasetere, Nkroful) and Takoradi district, blast was absent. Brown spot and false smut were, however, prevalent.

In Volta region, high incidence and severity of blast were predominant in the farmers' fields in and around Hohoe district. Rice fields at Akpafu-Mempeasem, Akpafu-Odomi, Fodome, Gbi-Godenu, Hohoe, Golokwati and Kpoeta where local variety *Viwornu* is widely cultivated were highly infected by blast.

2002

In 2002, the status of rice blast in some of the districts visited in Southern Ghana was different from the observations made in 2001.

In Upper Denkyira district of Central region (Diaso), the disease incidence was comparatively higher than in 2001.

No blast was observed in Assin district. However, brown spot was widely observed. Blast was not observed in Sefwi, Wassa-Amenfi and Wassa-Fiase districts of Western region or in Krobo and Kwaebibirim districts of Eastern region. Brown spot, narrow brown leaf spot, false smut and grain discoloration syndrome were the predominant diseases encountered.

In Volta region, while there was no blast disease incidence at Afife, Kadjebi and Worawora, the situation was different in Hohoe district. Farms at Fodome, Kpoeta, Santrokofi, Gbi-Godenu, Akpafu-Odomi and the vicinity of Hohoe were severely blasted.

In Northern region, there was high incidence and severity at Galenkpegu and Kpachie with isolated incidence at Nyankpala on commercial and research fields. At Salaga, a key research-screening site was free from blast infection but the discard plot grown to improved variety Tox 3050 was heavily blasted. There was no blast incidence at Damongo.

In Upper East region, there was severe incidence and severity of blast in farmers' fields at Bawku and PVS plots and farmers' fields at Nyorigu. Generally, there was no blast at Manga, Navrongo, Tono, Sandema, Wiaga and Fumbisi. However, an isolated rice farm at Manga was completely destroyed by brown spot disease.

Blast disease was not observed in Upper West region.

Incidence

In all the surveys, it was observed that when blast was present, severity was highest in the nurseries and young plants and ratoons in the vegetative stage.

Thus seedling blast was recorded at a number of locations during these surveys. The results also suggest that Sefwi, Upper-Denkyira and Hohoe districts in Southern Ghana, Nyankpala and Kpachie in Northern region and Bawku and Nyorigu in Upper East region can be regarded as blast hot spots. Generally, the incidence/severity of rice blast varies across different locations in different years. These surveys have shown that the importance of blast in Ghana cannot be overemphasised. This is because during the surveys, the team predicted that the blast disease would devastate the IPM demonstration plots set up by MOFA in Sefwi and some farms in Hohoe district. The great loss caused by blast in Hohoe district appeared in the Daily Graphic in February 2002, entitled "Hohoe district rice farmers in distress" but it was totally attributed to drought. Usually, blasted rice plants, whether or not in erratic rainfall season, would appear to the untrained eye as a rice plant "burnt" by the scorching sun. This would give the

impression that the plants are suffering because of insufficient moisture. However, our survey results suggested that the major cause of rice crop failure in Hohoe district in 2001 was the high incidence and severity of blast. This was because the disease was very conspicuous during the rainy period of the surveys. Generally, it was also observed that where farmers planted local varieties, blast incidence and severity were much higher than where improved varieties were planted. This was probably why some villages and towns in the Upper East region did not experience blast incidence. In towns such as Navrongo, Sandema and Tono, which are close to Tono Irrigation Project, adoption of improved varieties appeared to be very high.

Field trials

The incidence and severity of leaf blast on 10 or more PVS rice varieties screened in the nurseries at key sites in Northern and Upper East regions in 2000–2002 rainy seasons are shown in Tables 13–15. The blast incidence in the nurseries was generally low. At Galenkpegu, the local variety *Agongima* was heavily infected with blast with a score of 7.0 while the remaining varieties fell within the score range of 1–3 (Table 14). Blast incidence was low at Gbulung (Table 13) irrespective of the varieties in the observation nursery. The blast incidence at Golinga was generally low with a score range of 2.0–3.5 (Table 15). The blast pressure at the observation nursery at Nyankpala was generally low and ranged between 1.0 and 3.0 (Table 15). The blast situation at Salaga was also low except that *Kleminson* had a moderate blast incidence of 4.0 (Table 14). At Tolon, the blast infection level was low as well (Table 13). At Tambalung, the blast infection was very high on the local varieties; *Kpukpla* had a score of 7.7, *Agongima* had 7.7, *Agona* had 7.5 and *Agosanga* had 7.0. The other varieties had low to moderate blast infections and ranged between 1.0 and 4.0 (Table 14). The blast incidence at Nyorigu was much higher on the local varieties; *Agosanga* had a score of 8.5, *Agona* had 9.0, *Agongima* had 5.0, *Kukuosumbog* had 9.0, *Kpukpla* had 7.0 and *Gambiaka* had 7.0 (Table 15). Two of the improved varieties—*WAB 96-5-1* and *WAB 586-1-1*—also recorded high blast incidence with each having a score of 7.0. The remaining varieties had mean scores of 1.0–3.0 (Table 15). The blast infection level at Tarkpaa was generally low (Table 14). A number of improved varieties showed resistance response to blast at some of the sites and under the conditions tested, although in other cases the general disease incidence level was low. These varieties need to be further tested under high disease-pressure situations combined with farmer participatory approaches to validate their performance.

Table 13. Blast incidence on PVS rice varieties screened in the disease observation nurseries at Gbulung and Tolon during 2000 rainy season.

Variety	Score (0-9)†	
	Gbulung	Tolon
WAB 450-24-3-2-P18-HB	1	1.0
WAB 450-I-B-P91-HB (NERICA 4)	1	–
IRAT 262	1	1.0
WAB 337-I-B-B-7-H4	1	1.0
WAB 570-35-53	1	–
IR 12979-24-1	1	1.0
WAB 450-I-B-P15-7-1-1	–‡	1.0
KLEMINSON	1	1.0
WAB 586-1-1	1	1.0
IRAT 216	1	1.0
Kpukpla (Local)	1	2.0
WAB 96-5-1	1	1.0
WAB 96-11	1	–
Mean (all varieties)	1.0	1.1
LSD (0.05)	0.0	0.53
CV (%)	0.0	33.20

†Visual scores of leaf blast incidence and severity were recorded using the IRRI Standard Evaluation System of 0-9; mean of four replicates.
‡Not tested.

Table 14. Blast incidence on PVS rice varieties screened in the disease observation nurseries at Galenkpegu, Salaga, Tambalug and Tarkpaa during 2001 rainy season.

Variety	Score (0-9)†			
	Galenkpegu	Salaga	Tambalug	Tarkpaa
WAB 450-24-3-2-P18-HB	1	1.0	3.5	2.0
WAB 450-I-B-P91-HB (NERICA 4)	1	1.0	3.0	1.0
WAB 450-I-B-P160-HB (NERICA 6)	1	1.0	2.0	1.0
WAB 450-I-B-P163-4-1	1	1.0	1.0	1.0
WAB 450-11-1-2-P41-HB	1	1.0	1.0	1.0
WAB 450-I-B-P38-HB-(NERICA 1)	1	2.0	1.0	1.0
WAB 450-I-B-P163-2-1	1	1.0	1.0	1.0
IRAT 262	1	1.0	2.0	1.0
WAB 515-13-13A_1-8	1	1.0	4.0	1.0
WAB 337-I-B-B-7-H4	1	1.5	1.0	1.0
WAB 570-35-53	1	2.0	1.0	2.0
IR 12979-24-1	1	1.5	1.5	1.0
WAB 450-I-B-P15-7-1-1	1	1.8	2.0	1.0
KLEMINSON	1	4.0	1.5	1.0
WAB 586-1-1	1	1.5	3.5	1.0
IRAT 216	1	1.0	–	–
Sika-mo	1	1.0	3.0	1.0
Gambiaka	2	1.5	1.0	2.0
Kpukpla (Local)	3	–‡	7.5	1.0
Kukuosumbog (Local)	2	–	3.0	1.0
WAB 96-5-1	1	–	3.5	2.0
IDESA 85	1	–	1.5	1.0
WAB 96-11	1	–	1.0	1.0
Agongima (Local)	7	–	7.5	1.0
Agona (Local)	2	–	7.5	1.0
Agosanga (Local)	3	–	7.0	–
Mean (all varieties)	1.5	1.4	2.9	1.2
LSD (0.05)	0.0	1.14	1.25	0.0
CV (%)	0.0	56.17	31.10	0.0

†Visual scores of leaf blast incidence and severity were recorded using the IRRI Standard Evaluation System of 0-9; mean of four replicates.
‡Not tested

Table 15. Blast incidence on PVS rice varieties screened in the disease observation nurseries at Nyorigu, Golinga and Nyankpala during 2002 rainy season.

Variety	Score (0-9)†		
	Nyorigu	Golinga	Nyankpala
WAB 450-24-3-2-P18-HB	3.0	2.0	3.0
WAB 450-I-B-P91-HB (NERICA 4)	1.0	2.0	1.0
WAB 450-I-B-P160-HB (NERICA 6)	1.0	2.0	2.3
WAB 450-I-B-P163-4-1	1.0	2.0	1.3
WAB 450-11-1-2-P41-HB	3.0	3.5	1.0
WAB 450-I-B-P38-HB-(NERICA 1)	3.0	2.5	1.0
WAB 450-I-B-P163-2-1	1.0	2.0	1.0
IRAT 262	3.0	2.0	1.0
WAB 515-13-13A$_1$-8	1.0	2.0	1.0
WAB 337-I-B-B-7-H4	1.0	–	1.0
WAB 570-35-53	3.0	2.5	1.5
IR 12979-24-1	3.0	2.0	1.0
WAB 450-I-B-P15-7-1-1	1.0	2.0	2.0
KLEMINSON	1.0	–	2.3
WAB 586-1-1	7.0	2.0	1.5
IRAT 216	1.0	2.0	1.3
Sikamo (Tox 3108-56-4-2-2-2)	1.0	–	1.8
Gambiaka	7.0	–	2.8
Kpukpla (Local)	7.0	2.5	2.5
NERICA 5	–‡	2.0	–
Kukuosumbog (Local)	9.0	2.0	2.8
WAB 96-5-1	7.0	–	3.0
IDESA 85	3.0	–	–
WAB 96-11	3.0	–	2.8
Agongima (Local)	5.0	–	–
Agona (Local)	9.0	–	–
Agosanga (Local)	8.5	–	1.8
Mean (all varieties)	3.6	2.1	1.8
LSD (0.05)	0.27	0.49	0.94
CV (%)	5.45	16.53	37.95

†Visual scores of leaf blast incidence and severity were recorded using the IRRI Standard Evaluation System of 0–9; mean of four replicates.
‡Not tested.

Artificial inoculation studies

Results of the artificial inoculation studies conducted in the screenhouse are presented in Table 16. It was very clear that out of the 10 local varieties tested, 70% showed no resistance to any of the 15 isolates of *M. grisea* used. These were *Viwornu*, *Asante-mo*, *Agya-Amoa*, *Mendi*, *Gomba*, *Agona* and *Agosanga*. They were highly susceptible to some of the isolates. Of the remaining three varieties, *Martin* and *Mr. More* showed resistance to 6 of the 15 isolates, while *Mr. Harrow* was resistant to three isolates. From these results, it appears as if *Mr. Harrow*, *Martin* and *Mr. More* were once improved varieties which had been grown in their respective localities for a long time and have had their resistance broken down.

The 10 improved varieties showed varying degrees of resistance to the 15 isolates. *Sika-mo* showed resistance to 14 isolates (93%) while *WAB 450-I-B-P91-HB* showed resistance to 10 isolates (67%). Of the remaining 8 varieties, *IDSA 85* and *IRAT 216* showed resistance to 9 isolates (60%) and *WAB 515-177-2*, *WAB 450-I-B-P15-7-1-1* and *IR 12979-24-1* were resistant to 8 isolates (53%). The remaining 3 varieties—*IDSA 46*, *Kleminson* and *WAB 56-50*—were resistant to 7 isolates (47%).

The inoculation studies need to be repeated and the varieties field-tested to firmly establish the above observations. However, the results clearly suggest the need for development and promotion of blast-resistant varieties and that, as much as possible, farmers must be encouraged to sow improved rice varieties to control the disease.

Table 16. Reactions of 20 rice varieties inoculated with 15 isolates of *Magnaporthe grisea* in the screenhouse.

Variety	*M. grisea* isolates														
	B 18	B124	B149	B152	B153	B157	B158	B159	B161	B164	B167	B175	B179	B201	B202
Viwornu	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Mr. Harrow	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R
Mr. More	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S
Asante-mo	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Sika-mo	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R
Agya-Amoa	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Martin	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R
IR 12979-24-1	R	S	R	R	S	S	S	R	R	R	S	S	S	R	R
IDSA 46	S	S	R	R	S	R	R	S	R	R	R	S	S	S	S
IRAT 216	R	S	R	R	R	S	S	R	R	R	S	S	S	R	R
WAB 450-I-B-P91-HB	R	S	R	R	R	S	R	S	R	R	S	S	S	R	R
Mendi	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Gomba	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Agona	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
WAB 450-I-B-P15-7-1	R	S	R	R	R	R	S	R	R	S	S	S	S	S	S
Kleminson	S	S	R	R	S	S	S	R	R	S	S	S	R	R	R
WAB 515-177-2	R	S	R	R	R	S	R	R	S	R	S	S	S	S	R
IDSA 85	R	S	R	R	S	S	S	S	S	R	R	R	R	R	R
WAB 56-50	S	S	R	R	R	R	S	R	S	R	R	S	S	S	S
Agosanga	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

†R, resistant (0–3) and S, susceptible (4–9) using the IRRI Standard Evaluation System of 0–9.

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Analysis of *Magnaporthe grisea* population structure in Côte d'Ivoire as a prerequisite for the deployment of varieties with durable blast resistance

Y. Séré[1], A. A. Sy[2], S.K. Akator[1], A. Onasanya[1], K. Zai[1], S. Sreenivasaprasad[3], S. K. Nutsugah[4] and J. K. Twumasi[5]
[1]Africa Rice Center (WARDA), B.P. 320, Bamako, Mali
[2]Senior Research & Technology Officer/FAO-RAF, Accra, Ghana
[3]Warwick HRI, Wellesbourne, Warwick, UK
[4]Savanna Agricultural Research Institute (SARI), PO. Box 52, Tamale, Ghana
[5]Crops Research Institute (CRI), PO. Box 3785, Kumasi, Ghana

Résumé

L'instabilité de la résistance de la plante-hôte est un phénomène fréquemment rencontré dans les relations entre le riz et l'agent responsable de la pyriculariose. Si l'on souhaite baser la stratégie de lutte contre cette maladie sur la résistance variétale, il est important non seulement de connaître la nature de la résistance portée par les variétés proposées à la vulgarisation, mais aussi la pression parasitaire dans les sites de criblage. La méthode classique d'analyse du spectre de virulence des populations pathogènes étant longue et délicate à conduire, une nouvelle technique a été mise au point. Elle a été utilisée pour caractériser les sites de criblage. Elle pourra servir pour compléter les informations relatives au phénotype moléculaire en vue de la mise au point d'un système d'évaluation permettant le déploiement des variétés résistantes dans des conditions durables.

Abstract

The instability of the resistance of the host plant is a phenomenon frequently observed in the relationship between rice and the blast pathogen. To focus on a control strategy based on varietal resistance, it is important not only to know the nature of the resistance carried by the varieties to be released, but also to determine the pathogen pressure in the screening sites. Since the traditional method of analysis of the virulence spectrum of the blast pathogen populations is laborious and sensitive, a new technique was developed. The method was used to characterise screening sites. It may be used to complement information relating to the pathogen's genetic lineage composition, in order to help in the development of an evaluation system for the deployment of resistant varieties in a durable way.

Introduction

One of the most remarkable phenomena in the relationship between rice and the blast fungus *Pyricularia grisea* (Cke) Sacc. (Teleomorphe: *Magnaporthe grisea* (Hebert) Barr) is the instability of the plant-host resistance, recognised as due to the pathogen variability. A clear understanding of the nature of this relationship is extremely important to develop a control strategy based on the use of varietal resistance. It is in fact important not only to know the nature of resistance carried by the varieties proposed for diffusion, but also to determine the nature of the disease pressure at the screening sites.

In the absence of such information, varieties that seem resistant initially become susceptible after a few years, illustrating the boom-and-bust cycle well known to plant pathologists and breeders.

The racial nature of *M. grisea* isolates can be determined by testing their pathogenicity on a suitable set of differential varieties. However, to have a complete picture of the composition of the pathogen population of a given zone, it is necessary to collect a large number of samples and carry out many

artificial inoculations. The traditional method of collection, culture and pathotyping is time consuming (Kiyosawa 1976). It is also difficult to conduct in the absence of standard pathotyping conditions.

Consequently we tested a technique consisting of exposing some varieties with known resistance to natural inoculum. This technique was then used to study the structure of *M. grisea* populations at screening sites in Côte d'Ivoire.

Materials and methods

Development of a simple technique to analyse blast fungus populations

The technique developed is based on the gene-for-gene theory previously illustrated for the relationship between rice and the blast fungus (Séré *et al*. 2003). When a set of varieties with known resistance genes is exposed to natural inoculum, the development of susceptible reaction on one of the varieties indicates the non-functional avirulence defined here as the presence of a corresponding virulence factor within the pathogen population.

Nineteen varieties with known resistance genes were chosen (Table 17). These were planted in small plots of 4 lines of 1 m with a spacing of 10 cm between the lines. Sowing was in continuous lines at a rate of 8 g of seed per linear meter. Thinning was done at 21 days after sowing to no more than 50 seedlings per line.

Lesions described as "bs" types by Kiyosawa (1976) are hypersensitive reactions indicating an incompatible relationship between the host plant and the pathogen (Séré 1999). It is only the presence of susceptible lesions (types bg, bG, pG) on a variety that indicates the presence of the corresponding virulence factor able to match this variety within the pathogen population.

Table 17. Resistance genes to the blast fungus and rice varieties containing the genes.

Locus	Rice resistance gene	Corresponding blast fungus virulence factor	Varieties type for each resistance gene
a	Pi-a	av-a+	Aichi Asahi
b	Pi-b	av-b+	BI 1
f	Pi-f	av-f+	St 1
i	Pi-i	av-i+	Ishikari Shiroke
k	Pi-k	av-k+	Kusabue; Kanto 51
	Pi-k^h	av-k^h+	K3
	Pi-k^m	av-k^m+	Tsuyuake
	Pi-k^p	av-k^p+	K2†; K60
	Pi-k^s	av-k^s+	Shin 2
m	Pi-m	av-m+	Chugoku 31‡
t	Pi-t	av-ta+	K59
	Pi-ta	av-ta^2+	K1; Yashiro Mochi
	Pi-ta^2	av-t+	Pi n°4
z	Pi-z	av-z+	Fukunishiki
	Pi-z^t	av-z^t+	Toride 1

Source: Sy and Séré (1996).
† K2 also has resistance gene Pi-a ‡ Chugoku also has resistance gene Pi-k

Experimentation of the trapping technique in pathogenic population structure study

Varieties and experimental sites

We focused on six varieties, either released or in the process of diffusion:

- DEMANBA, a local variety of the region of Man
- WAB 450-I B P-38-HB and WAB 450-11-1-P31-1-HB, two interspecific lines (from crossing *Oryza sativa* and *Oryza glaberrima*) known under the names of NERICA 1 and NERICA 2, respectively
- DOURADO PRECOCE, variety of Brazilian origin released since the 1970s
- WAB 56-125, a variety developed by WARDA and released in Côte d'Ivoire and known for its relatively stable resistance to the blast pathogen
- IRAT 13, a resistant variety used in many crosses as donor of resistance.

Moreover, 17 differential varieties were used along with these released varieties.

In 2000, the experiments were conducted in two locations: Man in the forest zone and M'Bé in the forest-savannah transition zone. Two sites were used in each locality. In Man, the situation on the research station was compared with that on a farmer's field. In M'Bé, the lowland-growing environment was compared with the upland one. In each site, one trial was conducted at the beginning of the growing season and another at the end of the season.

In 2001, a third site (Boundiali) in the savanna zone was included and three sowings were carried out at each experimental site—at the beginning, in the middle and at the end of the rice-growing period—in order to better understand the population dynamics of the blast pathogen.

Experimental design

Each individual trial was laid out in a Fisher block design with four replications. Each replication comprised plots of 3 lines of 2 m by variety sown in continuous lines at a rate of 2 g seed per linear meter. The lines were spaced at 20 cm.

The number of seedlings per line was examined 2 to 3 weeks after sowing and thinning was carried out to leave around 100 seedlings per line. Blast disease was examined and scored on a weekly basis.

In each diseased plot, 10 samples were taken randomly and stored in an icebox during the field trips and then in the refrigerator at the laboratory. Monospore cultures are carried out and sent to Horticulture Research International (HRI) in the UK for analysis of the molecular profiles. Owing to the poor rate of recovery of the isolates during the first samplings, the technique of isolate collection was modified as follows: as soon as the blast samples were collected in the field, pieces of lesions were placed directly in Petri dishes containing an agar medium amended with two antibiotics (aureomycin 50 mg/L and streptomycin 50 mg/L).

Results and discussion

Developing the trapping technique for population structure study

Foliar blast as well as neck blast was observed for the majority of varieties, although some of them did not mature because of a generalised scalding due to drought. There were compatible interactions between the blast population and certain varieties, and incompatible interactions with others. These results revealed the virulence factors (non-functional avirulences) av-a+, av-k+, av-ta+, av-z+, av-k^h+ and av-i+ associated with av-k^s+. Later, the factors av-ta^2+, av-b+, av-t+, av-f+ and the association av-k+ with av-m+ and ava+ with av-b+ were found (Table 18). Consequently, the trapping technique makes it possible to identify avirulence genes within a pathogen population, without prejudging the way in which these genes can be associated to constitute individual races. However, the following points need to be considered in interpreting the results.

Table 18. Response of rice varieties bearing known vertical resistance genes to the blast fungus, at Farako-Ba, Burkina Faso.

Varieties tested	Corresponding resistance genes	Virulence spectrum to leaf blast		Virulence spectrum to neck blast	
		60 DAS†	76 DAS†	79 DAS†	93 DAS†
Shin 2	Pi-ks	–‡	–	–	–
Aichi Asahi	Pi-a	av-a+	av-a+	av-a+	harvested
Kanto 51	Pi-k	av-k+	av-k+	*	av-k+
Kusabue	Pi-k	av-k+	av-k+	av-k+	av-k+
Fujisaka	Pi-i ; Pi-k^{s}	av-i+ ; av-k^{s}+	av-i+ ; av-k^{s}+	av-i+ ; av-k^{s}+	av-i+ ; av-k^{s}+
K1	Pi-ta	av-ta+	av-ta+	*	av-ta+
Pi n°4	Pi-ta^{2}	–	av-ta^{2}+	–	–
Ou 244	Pi-z	av-z+	av-z+	*	av-z+
Fukunishiki	Pi-z	–	–	–	–
Toride 1	Pi-z^{t}	–	–	*	–
K3	Pi-k^{h}	av-k^{h}+	av-k^{h}+	*	Wilted
Tetep	Pi-k^{h}	–	av-k^{h}+	*	Wilted
Bl 8	Pi-b	–	av-b+	–	–
Bl 1	Pi-b	–	av-b+	–	–
K59	Pi-t	–	av-t+	*	Wilted
St 1	Pi-f	–	av-f+	–	–
Chugoku 31	Pi-k ; Pi-m	–	av-k+ ; av-m+	–	–
Tsuyuake	Pi-k^{m}	–	–	av-k^{m}+	Harvested
Tongil	Pi-a ; Pi-b	–	av-a+ ; av-b+	*	–

† DAS= Days after sowing.
‡ Incompatibility (no symptom).
* Non headed.

The varieties Shin 2, Fukunishiki and Toride 1 were attacked neither by leaf blast nor by neck blast, i.e. genes av-k^s+, av-z+ and av-z^t+ are not included in the genetic structure of the pathogen population. However, Fujisaka, carrying both the resistance genes Pi-i and Pi-k^s, was attacked. In fact, according to the gene-for-gene theory, any race able to attack Fujisaka should also be able to attack Shin 2. The most likely explanation is the probable existence within Shin 2 of another resistance gene that can resist the races that attack Fujisaka and which are carrying av-k^s+.

In the same way, K3 and Tetep, which carry the same gene Pi-k^h, reacted differently with the same population, the disease being detected two weeks later on Tetep. Such a difference in behaviour between varieties with the same genetic background regarding their vertical resistance gene was found for Ou 244 and Fukunishiki both carrying Pi-z. The absence of lesions on Ou 244 attests to the probable existence in this variety of another resistance gene different from Pi-z.

The varieties Pi n°4, Bl 8, Bl 1, St 1 and Chugoku 31 were attacked by leaf blast but not by neck blast. However, Koga (1994a, 1994b) showed that the process of pathogenesis, from germination of the conidia to penetration and colonisation of tissues, proceeds in the same manner on both leaves and necks. The development of pathogen inside the tissues of the necks is even faster than that in leaves (Koga 1995). It is, thus, reasonable to assume that the genetic basis of the host-pathogen relationship is the same for the two kinds of organs. In fact, when artificial inoculations are done with known races, those that are pathogenic on leaves are also pathogenic on the neck (Ou and Nuq, quoted by Ou 1985). Moreover, Balal *et al*. (1977) showed that two dominant genes are responsible for resistance in leaves as in the neck.

Field studies (Bonman *et al.* 1989), however, have shown some cases where varieties are susceptible to one type of blast and resistant to the other. Such lack of correlation would come from differences in racial prevalence (Ou 1985) or in environmental conditions (Ahn and Rubiano 1984; Bonman *et al.* 1989). In the present study, the varieties attacked at the leaf level but resistant in the reproductive phase are those on which the disease appeared late on the leaves. As these organs are the main source of contamination of the necks, it is plausible that under these circumstances they might not provide sufficient inoculum to the panicles.

Using the trapping technique to study the blast pathogen population structure at different sites

In the application of the trapping technique to study the dynamics of the pathogen populations in screening sites in Côte d'Ivoire, the focus was first on the virulence spectrum as revealed by the number of varieties attacked.

The results obtained in 2000 (Table 19) indicated that the pathogen population at the Man research station site presents the greatest diversity with 99% of the varieties attacked for the early planting and 67% for later ones. The farmer's field at Man, where 80% of the varieties carried susceptible lesions, followed it. At M'Bé, the upland site (with 68% of diseased varieties for the first cycle and 10% for the second) was more damaged than the lowland one, which presented a narrow spectrum. This situation resembles previous observations by Séré (1999) in Burkina Faso, where the greatest diversity was found in research stations where a lot of varietal trials have been conducted for many years.

In 2001, Boundiali site in the savanna zone showed diversity almost as great as Man in the forest zone. These two sites are thus good sites for screening for resistance as it is likely that most races able to overcome the vertical resistance of the varieties will be found. In fact, the breakdown of the vertical resistance of the varieties enables one to appreciate their level of horizontal resistance (Van der Plank 1975).

The virulence spectrum was variable according to date of sowing, indicating that the structure of the population is not completely stable over time. For instance, in 2000, the maximum diversity was found at the beginning of the growing season; in 2001, when three planting periods were studied, the largest virulence spectrum was observed within the plots established during the middle of the season.

Table 19. Virulence spectrum of *Magnaporthe grisea* population at screening sites in Côte d'Ivoire during the 2000 and 2001 rice-growing seasons.

Site	Percentage of varieties attacked by sowing periods					
	Early		Intermediate		Late	
	2000	2001	2000	2001	2000	2001
Man Research Station	99	87	–	100	67	87
Man Farmer's field	80	70	–	100	80	91
M'Bé Upland site	68	0	–	48	10	91
M'Bé Lowland site	17	0	–	4	6	57
Boundiali	–[†]	96	–	100	–	61

[†] Not tested.

The next issue addressed was whether the technique can be used to quantify the pathogen population and thus to characterise each virulence factor by its frequency. As the number of lesions is proportional to the concentration of conidia (Ahn and Ou 1982; Pinnschmidt *et al*. 1993), the number of lesions formed on a variety could indicate the number of virulence factors able to overcome the resistance of this variety.

However, it should be recognised that the number of lesions per unit area is also an indicator of the level of horizontal resistance (Yeh *et al*. 1989), because it depends on the response of the variety to slow down the production of spores for the subsequent infections. Under these conditions, two varieties having the same vertical resistance but different levels of horizontal resistance may present different numbers of lesions, especially when the symptoms are observed after several series of auto infection.

Despite this consideration, Séré (1999) could compare the sites by the density of their pathogenic population through the comparison of the total lesions observed on the varieties. The results obtained made it possible to identify ideal screening sites like Farako-Ba in Burkina Faso which are characterised not only by the extent of the virulence spectrum, but also by the high density of their pathogen populations.

In order to allow a statistical comparison, in the tests of 2000, lesions indicating susceptibility as well as hypersensitivity that characterise the entire population were considered. The number of lesions for 100 leaves was subjected to analysis of variance using the IRRISTAT software developed by IRRI, after the application of transformations in conformity with the recommendations of Gomez and Gomez (1984), and after checking of the homogeneity of the variances. The analysis showed the existence of differential interaction between the varieties and the screening sites (Table 20) that characterise a vertical host-pathogen system.

Duncan's Multiple Range Test made it possible to compare the averages. The density of inoculum was larger at Man than at other sites, particularly in the plots planted early on the research station (Table 21).

The percentage of isolates retrieved from leaf symptoms increased from 12% to 94% with the improved isolation technique described above. Some of the monospore cultures were subjected to molecular analysis at HRI (Sreenivasaprasad *et al*. 2003). These isolates mainly belong to three of the five lineages previously described in Côte d'Ivoire by Chipili *et al*. (2001). The maximum diversity in lineages was found in the site where the maximum diversity of virulence genes was established. Thus, further isolate collection and analysis should be concentrated at these sites, in order to better appreciate the relationship between lineages and pathotypes and enable the development of the lineage exclusion method described by Zeigler *et al*. (1994).

Table 20. Analysis of variance for the number of total blast lesions for 100 leaves (x) based on values transformed to SQRT (x+0.5).

Origin of the variation	Degrees of Freedom	F calculated	Significance
Replication	2	2.02	Non-significant differences
Treatments	183	26.34	Highly significant differences
Varieties	22	70.94	Highly significant differences
Sites	7	307.95	Highly significant differences
Varieties × Sites	154	7.17	Highly significant differences
Error	366		
Total	551		

Table 21. Mean comparison of the number of total lesions for 100 leaves of some of the varieties tested in 2000 rice-growing season.

Site[†]	**Means by varieties**[‡]					
	NERICA 1	**NERICA 2**	**DEMANBA**	**DOURADO**	**IRAT 13**	**WAB 56-125**
Man Research station (E)	45.8a	4.2a	86.8a	86.8a	86.8a	20.8a
Man Research station (L)	1.5b	1.2a	7.5c	25.5b	12.2c	2.2b
Man Farmer's field (E)	2.2b	0.8a	61.8a	79.2a	30.8b	6.2b
Man Farmer's field (E)	1.5b	1.5a	63.5a	86.8a	30.8b	3.7b
M'Bé Upland (E)	2.8b	1.0a	37.5b	25.5b	17.5bc	2.8b
M'Bé Upland (L)	0.2b	0.1a	2.8cd	1.8c	1.5d	0.5b
M'Bé Lowland (E)	1.0b	0.1a	0.6d	0.5c	0.0d	1.5b
M'Bé Lowland (L)	1.0b	0.1a	0.2d	0.2c	0.2d	1.0b

[†] E = Early planting date; L = Later planting date.
[‡] Within a column, means followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test.

Conclusion

In view of the difficulties in determining the virulence structure of the pathogen population by the traditional assays, we developed a system of trapping, which consists of exposing varieties with known resistance genes to natural inoculum. This system is appropriate for determining the factors of virulence present within the pathogenic population, based on the reaction of the varieties, without prejudging their association into distinct races. It also makes it possible to specify the relative abundance of the virulence factors through the evaluation of the number of lesions by variety, even if this is also under the influence of partial resistance.

Applied to the study of various locations, the trapping technique makes it possible to characterise the sites either by the number of attacked varieties defining the virulence spectrum of their pathogen population, or by the density of this population revealed by the number of susceptible lesions observed. The populations are diverse at the Man site and Boundiali and these constitute ideal screening sites for durable resistance. These data on the virulence diversity are in conformity with the results obtained by molecular analysis on lineage composition of blast isolates (Chipili *et al*. 2001; Sreenivasaprasad *et al*. 2003). This trapping technique will allow a better orientation toward sampling methodology adopted for collecting isolates and analysing them by molecular tools.

This study highlights the complexity in the relationship between *M. grisea* and its host. The pathogen population shows variations in its virulence composition either during a rice-growing season or from one season to another. It is important to take this into account not only in the screening for resistant varieties, but also in the durability of resistances deployed.

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Diversity of blast pathogen populations in four West African countries and strategies for resistance management

S. Sreenivasaprasad[1], J. Chipili[1], S. Muthumeenakshi[1], Y. Séré[2], Z. Kamelan[2], K. Akator[2], S. K. Nutsugah[3], W. Dogbe[3], J. Twumasi[4], K. Dartey[4], N.J. Talbot[5] and A. E. Brown[6]
[1]Warwick HRI, Wellesbourne, Warwick, UK
[2]West Africa Rice Development Association, Bouaké, Côte d'Ivoire
[3] Savanna Agricultural Research Institute, Nayankpala, Ghana
[4]Crops Research Institute, Kumasi, Ghana
[5] University of Exeter, Exeter, UK
[6]The Queen's University of Belfast, Belfast, UK.

Résumé

Des phytopathologistes ouest africains du riz et un pathologiste spécialisé en biologie moléculaire des champignons au Royaume-Uni ont été impliqués dans un projet collaboratif sur la pyriculariose du riz financé par le Department for International Development-Crop Protection Programme (DFID/CPP) entre 1996 et 2000. Des activités de recherche stratégique soutenant une gestion améliorée de la maladie principalement à travers l'utilisation de la résistance de la plante hôte ont été conduites par ces projets (R6738 et R7552). Une collection de base de plus de 350 isolats du pathogène a été établie à travers les sites de criblage et les champs environnants en Côte d'Ivoire, au Ghana, au Burkina Faso et au Nigeria. L'empreinte ADN (sonde MGR 586) pour l'identification des lineages (groupes génétiques et diversité) ainsi que les tests pathologiques sur les différentielles internationaux de riz ont été utilisés pour caractériser la population pathogène ainsi que les sites de criblage.

Les lineages (groupes génétiques) varient de 2 à 5 par pays et vont jusqu'à 16 lineages pour 4 pays. Dans chaque pays, un à deux lineages sont prépondérants. Par exemple, GH-1 (56 %) est présent à travers le Ghana et sur près de 20 variétés (ou lignées) de riz. La distribution de quelques-uns des lineages est réduite. C'est le cas par exemple de GH-2 (31 %) prédominant à l'est du Ghana et apparaissant à faible fréquence au Nord Ghana. Des lineages communs aux 4 pays ont été identifiés et 9 lineages distincts en Afrique de l'Ouest ont été dénommés WA1 à WA9

La diversité de la virulence est élevée avec 16 à 25 pathotypes dans chaque pays. IB (et surtout IB-1) est le pathogroupe dominant au Ghana et au Nigeria. IC (43 %) était prépondérant au Burkina Faso où une série de pathotypes étaient présents dans quelques sites comme Farako-Bâ. En Côte d'Ivoire, IA, IB, IC et ID étaient présents entre 16 et 29 %.

La présence de l'agent responsable de la pyriculariose sur le riz sauvage *Oryza longistaminata,* et sur des graminées adventices a été confirmée. Plusieurs de ces isolats se rapprochent des isolats du riz et sont pathogènes sur le riz en condition d'inoculation contrôlée. Le criblage d'une série de variétés de riz en conditions contrôlée vis-à-vis de lineages représentatifs et dans des sites caractérisés a conduit à l'identification de résistances potentielles qui mériteraient d'être confirmées.

Les données générées sur la diversité de l'agent responsable de la pyriculariose sont nouvelles en Afrique de l'Ouest, et permettent de combler les lacunes dans l'établissement de la carte mondiale de la diversité du pathogène. La caractérisation de l'agent pathogène et des sites de criblage ainsi que l'identification de sources de résistance fournissent un cadre pour la mise au point et le déploiement durable de la résistance à la pyriculariose en Afrique de l'Ouest.

Abstract

Rice pathologists and breeders from West Africa and fungal molecular pathologists from UK have been involved in a Department for International Development-Crop Protection Programme funded collaborative programme (1996–2002) on rice blast. Strategic research activities underpinning improved disease management mainly through the utilisation of host resistance have been carried out in these projects (R6738 and R7552). A baseline collection of more than 350 blast pathogen isolates from key screening sites and surrounding farms in Côte d'Ivoire, Ghana, Burkina Faso and Nigeria has been established. DNA fingerprinting (MGR586 probe) for the identification of blast lineages (genetic groups and diversity) and pathotyping on international rice differentials for virulence diversity were used for the characterisation of pathogen populations and screening sites.

Blast lineages (genetic groups) varied from 2-5 per country with up to 16 lineages in 4 countries. In each country one or two lineages were dominant. For example, GH-1 (56%) was present across Ghana on up to 20 rice varieties/lines. BF-1 included more than 70% of isolates from rice, wild rice and weeds in Burkina Faso. CD-1 and CD-2 were 38 – 56% in Côte d' Ivoire comprising isolates collected over a five-year period. Distribution of some lineages was restricted, e.g. GH-2 (31%) mainly from Eastern Ghana, appearing at low frequency in Northern Ghana. Lineages common among the four countries were identified and nine distinct West African blast lineages designated WA1 - WA9. Among these, WA1, WA2 and WA3 are the major West African blast lineages and are present in two or more countries. In general, different types of blast were caused by isolates in the same lineage suggesting the utility of common resistances.

Pathogen virulence diversity was high with 16–25 pathotypes in each country. IB (particularly IB-1) was the dominant pathotype group in Ghana and Nigeria. IC was the prominent (43%) pathotype group in Burkina, a range of pathotypes were present at certain sites (e.g. Farako-Bâ). In Côte d'Ivoire IA, IB, IC and ID were 16–29%.

Presence of blast pathogen on weedy rice *Oryza longistaminata* and common weed hosts has been identified. Several of these isolates are closely related to rice pathogenic isolates and are pathogenic on rice under controlled conditions. Screening of a range of rice varieties under controlled conditions against West African blast lineage representatives and at some of the characterised sites (utilising PVS material) has led to the identification of potential resistances that need to be further tested/developed.

Data generated on blast pathogen diversity is new to West Africa, filling an important knowledge gap and contributes to the global atlas on rice blast. Characterised pathogen populations and key sites and the potential resistance sources identified provide a framework for development and sustainable deployment of blast resistance in the West African region.

Introduction

The demand for rice in sub-Saharan Africa is growing faster than for any other major staple, with consumption broadening across all socio-economic classes, including the poor. In West Africa, growth in demand for rice is double the rate of population growth. Rice availability and prices impact directly on the welfare of the poorest consumers in the region and is a major food security issue. Rice is the main staple in eight West African countries and accounts for 20–50% total calorific consumption. However, average yield of 1.7 t ha^{-1} in the region is lowest in the world due to a number of bio-physical constraints. FAO estimates nearly 4 million tonnes of annual rice imports into West Africa worth more than US$ 1 billion per annum. Thus there is a clear gap in local production and consumption that needs to be addressed by the African agricultural and development community and their International partners. WARDA's priority focus is the humid and sub-humid rice growing zone which represents approx. 83% of total rice area in West Africa. It is in this environment that vast majority of resource poor farmers grow rice. Within this zone, rainfed upland and lowland rice production systems cover 78%. Enhancing domestic rice production through improved technologies can generate greater demand for female employment and incomes throughout the rice commodity sector in the region. Blast and weeds are priority biotic constraints in the rainfed systems. Blast caused by *Pyricularia grisea* (Cke) Sacc. (Teleomorphe: *Magnaporthe grisea* (Hebert) Barr), occurs in all rice-growing ecologies and is a major constraint in upland environments

where predisposition factors could favour disease development to epidemic proportions. Varying levels of yield loss (e.g. 3.3–77%) have been reported in West Africa, depending on the country and agro-ecological conditions (Fomba and Taylor, 1994; Singh *et al*., 2000).

Globally rice blast is mainly controlled by using resistant cultivars and to a lesser extent by the application of fungicides. However, where blast is prevalent, resistance breakdown due to the high diversity of the virulent forms of the pathogen is well documented. With fungicides, socio-economic and environmental issues and potential pathogen resistance are of concern. During the last few years global efforts have focused on determining the blast pathogen diversity combining modern molecular-biotechnological approaches with the traditional pathological assays for efficient exploitation of host resistance (Levy *et al*., 1991; Chen *et al*., 1995; Roumen *et al*., 1997; Correa-Victoria *et al*., 2000; Mekwatanakarn *et al*., 2000; Gnanamanickam *et al*., 2000; Chipili *et al*., 2001; Sridhar and Singh, 2001; Yi-jun *et al*., 2001).

West Africa Rice Development Association (WARDA), Côte d'Ivoire; Savanna Agricultural Research Institute (SARI) and Crops Research Institute (CRI), Ghana, Horticulture Research International, UK and associated organisations have been involved in a collaborative strategic research programme (Projects R7552/R6738) funded by the UK Department for International Development (DFID)-Crop Protection Programme (CPP). The key objectives were to characterise and identify the resistance screening sites used by WARDA and National Agricultural Research System (NARS). Assess the genetic and pathogenic diversity of strategically collected pathogen populations. Identification of potential resistances and capability strengthening at partner organisations. This paper mainly describes the work done at HRI in co-ordination with WARDA, SARI and CRI on population and site characterisation and lineage-associated resistance.

Materials and methods

More than 350 *M. grisea* isolates were obtained from blast samples collected from various screening sites in Burkina Faso, Côte d'Ivoire, Ghana and Nigeria (Chipili *et al*., 2000, 2002). DNA was extracted from mycelial powder by the CTAB method (Valent *et al*., 1991; Hamer and Givan, 1990; Sreenivasaprasad, 2000). DNA digestion and Southern hybridisation with the MGR586 (kindly provided by Dr. J.E. Hamer, Purdue Univ./Paradigm Genetics, USA) probe were carried out following standard protocols (Levy *et al*., 1991). RAPD-PCR was carried out using seven different primers (A1, A3, A7, A11, A13, PAP2 and PAP3 following standard protocols (Chipili, 2000). Sequencing of the ribosomal DNA spacer regions was undertaken following standard procedures using conserved primers (White *et al*., 1990; Talhinhas *et al*., 2002). Virulence spectrum of the *M. grisea* isolates was determined on the international set of rice cultivars, with three replicates following the scale of Valent *et al*. (1991), and pathotype designations were assigned based on Ling and Ou (1969). Screening of popular varieties/breeding lines from West Africa along with four standard checks with seven characterised isolates (representatives of some of the West African lineages) was done using a randomised complete block design with five replicates per isolate. Scoring was done following Valent *et al.* (1991). For a detailed description of the methodologies see chapter by Muthumeenakshi *et al*. this volume.

Results and discussion

WARDA and NARS partners [linked to IPM-Task Force (TF) and Breeding-TF] carried out most of the survey, sampling and field activities with inputs and participation from HRI. Molecular analyses and pathogenicity work were carried out at HRI with inputs and participation from WARDA and NARS, which included training attachments. Characterisation of more than 350 (data on some isolates not shown) blast pathogen isolates collected in and around key WARDA and NARS screening sites lead to the identification of blast lineages and pathotypes and their distribution.

Genetic (lineage) diversity

MGR586 fingerprints of more than 300 *M. grisea* isolates from Ghana, Nigeria, Burkina Faso and Côte d'Ivoire (Tables 22–25), along with an international reference strain R (Isolate code 4375.R.26, provided by Dr. John Hamer) were generated. Presence and absence of each restriction fragment in the 0.7-20 kb size range was scored and the data matrix subjected to cluster analysis to identify genetic groups (lineages). Seventy-one *M. grisea* isolates from Ghana (collected from seven regions - Western, Eastern, Ashanti, Northern, Upper Eastern, Volta and Central, in the country where rice is grown were grouped into four distinct lineages (genetic groups) designated GH-1 to GH-4 (Table 1). GH-1 was the major lineage comprising 52% of all the isolates and was present in all but one of the regions (eastern region) where isolates were collected. Lineage GH-1 occurred on at least 24 rice cultivars (some of which could have related genetic background, for example Tox-related cultivars) and four unknown cultivars. Lineage GH-2 comprised 31% of the isolates sampled and except for three isolates from Asikam (eastern region); Kpachie and Galinkpeliga (both from northern region) all were from the Hohoe area in the Volta region recovered from seven different cultivars (two of which were Tox-related. Lineage GH-3 consisted of six isolates from Agya-amoah (Sayerano and Sehuri in western region), red rice in Otumi, eastern region and two unknown cultivars at Kyrikoraa, central region. Isolates B18 and B137 from unknown rice cultivars in Nyankpala (northern region) formed lineage GH-4. Further, five *M. grisea* isolates from Tanaso (western region), Otumi (eastern region), Kwadaso (Ashanti region) and Tono (northern region) from elephant grass, wild rice and known/ unknown rice cultivars produced 'atypical' fingerprint patterns with few (up to 9) MGR586 hybridising bands. At least three of these isolates were pathogenic to rice and belonged to pathotype groups IB and IC.

Twenty-three *M. grisea* isolates from Nigeria (Table 23) were fingerprinted of which 20 formed a major lineage, NI-1. One isolate, B58 from Badeggi, was quite distinct with less than 50% similarity to any isolate in lineage NI-1 and was designated NI-2. These isolates were collected from three main WARDA/ NARS screening sites (Badeggi, Oyo and Uyo) and a minor site Ikenene and were from 23 different rice cultivars. The majority of isolates (12 of 20) in lineage NI-1 were from Oyo mainly from Tox-related cultivars. Lineage NI-1 was present at the three main sites sampled. Two isolates, IKR10/2 and B12 from Ikenene and Badeggi, respectively gave fewer (up to 9) MGR586 hybridising bands compared to normal rice-pathogenic populations (30 –50 MGR586 hybridising bands).

In Burkina Faso 72 *M. grisea* isolates from more than 20 rice cultivars, some wild rice and weed hosts fell into two main lineages BF-1 and BF-2 and three minor lineages BF3 to BF-5 (Table 24). These isolates were collected mostly from two main screening sites Farako-Bâ and Banfora and three other sites Sideradougou, Vallée du Kou and Labola. BF-1 was the dominant lineage including approximately 75% of the isolates collected in both 1996 and 1997 seasons. BF-1 was present at all the sites that were sampled (except Labola) and on up to 18 rice cultivars, *Oryza longistaminata* as well as some weed hosts (*Bracharia sp, Paspalum scrobiculatum* and *Setaria pallidae-fusca*). 72% of the isolates in BF-1 were from Farako-Bâ site from 14 cultivars. Lineage BF-2 comprised 17% of isolates and was present on six rice cultivars, *O. longistaminata* and *Rottboellia* at Banfora, Farako-Bâ, and Sideradougou. Two isolates from Farako-Bâ (S297 from Aichi Asahi) and Labola (S501 from a local variety) sites formed lineage BF-3. Lineages BF-4 and BF-5 were represented by one isolate each from rice cultivars Usen and Delta, respectively at Farako-Bâ. Two blast isolates from *O. longistaminata* and *Bracharia* sp. gave 'atypical' MGR586 fingerprints.

In Côte d'Ivoire 139 *M. grisea* isolates were analysed from main screening sites at Man, M'bé and Korhogo, with a small number of samples from Boundiali, Danané, Gagnoa and Sakassou sites covering different rice-ecosystems (Table 4). These isolates were grouped into two major lineages CD-1 and CD-2 and three minor lineages CD-3, CD-4 and CD-5. Lineage CD-1 present at six sites and on at least 25 different rice cultivars was the dominant lineage (53% of isolates). And 70% of the isolates in CD-1 were from Man site present on up to 16 rice cultivars. Lineage CD-2 consisted of 51 isolates infecting 22 rice cultivars collected from Man, M'bé and Korhogo as well as Boundiali and Danané. Lineage CD-3

Table 22. Details of site, host cultivar, pathotype designation and lineage grouping of *Magnaporthe grisea* isolates from Ghana.

No.	Code	Site/Location	Date	Variety	Type of Blast	Molecular Type / Lineage		Patho-type
1	60021c	Otumi (near Kade), ER	26.07.01	Red rice	leaf	atypical	atypical	IC-13
2	5033	Tono, NR	xx.11.01	unknown	leaf	atypical	atypical	IC-29
3	5038	Tono, NR	xx.11.01	Wildrice	leaf	atypical	atypical	IB-25
4	60025	Tanaso (near Sefwi Wiawso), WR	21.07.01	Elephant grass	leaf	atypical	atypical	II-I
5	B336	Kwadaso, AR	19.03.97	WAB 638-9H36	leaf	atypical	atypical	*
6	60059	Aframso (near Ejura), AR	xx.09.01	Mr More	leaf	GH-1	WA-1	IB-1
7	60060	Dromankuma (near Ejura), AR	xx.09.01	Mr More	leaf	GH-1	WA-1	ID-1
8	60012a	Abora-Denkyira, CR	21.07.01	Unknown	leaf	GH-1	WA-1	IB-1
9	60012b	Abora-Denkyira, CR	21.07.01	Unknown	leaf	GH-1	WA-1	IB-1
10	60012c	Abora-Denkyira, CR	21.07.01	Unknown	leaf	GH-1	WA-1	IC-9
11	6007c	Kyirikoraa (near Diaso), CR	21.07.01	Unknown	leaf	GH-1	WA-1	IC-1
12	60051	Golinga (Nyankpala), NR	xx.09.01	Agongima	leaf	GH-1	WA-1	IB-9
13	60046	Nyankpala, NR	xx.09.01	Mendi	leaf	GH-1	WA-1	IB-1
14	60055	Salaga (Nyankpala), NR	xx.09.01	Tox 3050	leaf	GH-1	WA-1	IB-9
15	60056	Nuregu, UER	xx.09.01	Local rice	leaf	GH-1	WA-1	IC-9
16	60057	Nuregu, UER	xx.09.01	Mr More	leaf	GH-1	WA-1	IB-1
17	60019b	Kpoeta (Sanki school) Hohoe, VR	25.07.01	Perfume rice	leaf	GH-1	WA-1	IB-5
18	60019c	Kpoeta (Sanki school) Hohoe, VR	25.07.01	Perfume rice	leaf	GH-1	WA-1	IB-1
19	60061	Offinso Kayera near Offinso, WR	xx.09.01	Asante-Mo (local)	leaf	GH-1	WA-1	IB-13
20	60062	Anyinasuso, Offinso, WR	xx.09.01	Asante-Mo (local)	leaf	GH-1	WA-1	IB-1
21	60063	Agyakoa-Manso, Asankrangwa, WR	xx.09.01	Wassa-Mo	leaf	GH-1	WA-1	ID-13
22	B175	Bolgatanga, NR	12.09.97	Red rice	leaf	GH-1	WA-1	*
23	B179	Bolgatanga, NR	12.09.97	Red rice	leaf	GH-1	WA-1	IC-13
24	B201	Bolgatanga, NR	12.09.97	Red rice	leaf	GH-1	WA-1	IC-25

No.	Code	Site/Location	Date	Variety	Type of Blast	Molecular Type / Lineage		Patho-type
25	B236	Bolgatanga, NR	12.09.97	Red rice	leaf	GH-1	WA-1	*
26	B159	Hohoe, VR	16.09.97	TOX 3792-10-1-2-1-1-3-2	leaf	GH-1	WA-1	IB-1
27	B153	Hohoe, VR	16.09.97	TOX 3880-38-1-1-2	leaf	GH-1	WA-1	IA-2
28	B167	Hohoe, VR	16.09.97	TOX 4004-43-1-2-1	leaf	GH-1	WA-1	IA-1
29	B163	Hohoe, VR	16.09.97	WAB 340-B-B-9-L3-L1-LB	leaf	GH-1	WA-1	IB-5
30	B154	Hohoe, VR	16.09.97	WAB 450-24-3-2-P18-HB	leaf	GH-1	WA-1	IB-1
31	B157	Hohoe, VR	16.09.97	WABIR 12979	leaf	GH-1	WA-1	IB-1
32	B115	Kwadaso, AR	12.09.97	WAB 638-9-A36	leaf	GH-1	WA-1	IB-1
33	B124	Kwadaso, AR	12.09.97	WAB 651-B-9-B36	-	GH-1	WA-1	IB-45
34	B119	Kwadaso, AR	12.09.97	WAB 651-B-A-158	-	GH-1	WA-1	ID-13
35	B200	Bolgatanga, NR	12.09.97	Red rice	-	GH-1	WA-1	IA-88
36	B202	Bolgatanga, NR	12.09.97	Red rice	-	GH-1	WA-1	IB-1
37	B151	Hohoe, VR	12.09.97	Tox 3416-170-2-1-1	-	GH-1	WA-1	*
38	B165	Hohoe, VR	12.09.97	Tox 3440-171-1-1-1	leaf	GH-1	WA-1	IB-7
39	B164	Hohoe, VR	12.09.97	Tox 728-1	-	GH-1	WA-1	IB-1
40	5079	Santrokofi, VR	xx.11.01	Viwono	leaf	GH-1	WA-1	IB-1
41	5081	Gbi-Godenu (Hohoe) , VR	xx.11.01	Viwono	leaf	GH-1	WA-1	IB-1
42	5083	Sayerano, WR	xx.11.01	Agya-amoah	leaf	GH-1	WA-1	IB-45
43	60035	Asikam, ER	28.07.01	Unknown	leaf	GH-2	WA-3	IC-25
44	60054	Kpachie, NR	xx.09.01	Local rice	leaf	GH-2	WA-3	IC-29
45	60053	Galinkpeliga (Nyankpala), NR	xx.09.01	Mr More	leaf	GH-2	WA-3	IB-13
46	5008	Gbi-Godenu (Hohoe), VR	xx.11.01	Viwono	leaf	GH-2	WA-3	IB-61
47	5009	Gbi-Godenu (Hohoe) , VR	xx.11.01	Viwono	leaf	GH-2	WA-3	IB-61
48	60011a	Santrokofi Rd (Hohoe) , VR	25.07.01	Viwono	leaf	GH-2	WA-3	IB-61
49	60011b	Santrokofi Rd (Hohoe) , VR	25.07.01	Viwono	leaf	GH-2	WA-3	IG-1

No.	Code	Site/Location	Date	Variety	Type of Blast	Molecular Type / Lineage		Patho-type
50	6005a	Fodome rd (Electricity) Hohoe, VR	25.07.01	Viwono	leaf	GH-2	WA-3	ID-13
51	6005b	Fodome rd (Electricity) Hohoe, VR	25.07.01	Viwono	leaf	GH-2	WA-3	IB-61
52	6005c	Fodome rd (Electricity) Hohoe, VR	25.07.01	Viwono	leaf	GH-2	WA-3	*
53	6005d	Fodome rd (Electricity) Hohoe, VR	25.07.01	Viwono	leaf	GH-2	WA-3	IC-1
54	6009a	Santrokofi Rd (Hohoe) , VR	25.07.01	Viwono	leaf	GH-2	WA-3	IB-1
55	6009b	Santrokofi Rd (Hohoe) , VR	25.07.01	Viwono	leaf	GH-2	WA-3	*
56	6009c	Santrokofi Rd (Hohoe) , VR	25.07.01	Viwono	leaf	GH-2	WA-3	IB-1
57	60040	Santrokofi (Hohoe) , VR	25.07.01	Weed	leaf	GH-2	WA-3	IG-1
58	B152	Hohoe, VR	16.09.97	TCA 80-4	leaf	GH-2	WA-3	IB-13
59	B147	Hohoe, VR	16.09.97	TOX 3100-37-3-3-2-9	leaf	GH-2	WA-3	IB-61
60	B158	Hohoe, VR	16.09.97	CK 73	leaf	GH-2	WA-3	IB-9
61	B150	Hohoe, VR	16.09.97	ITA 321	-	GH-2	WA-3	IB-61
62	B149	Hohoe, VR	16.09.97	TGR 75	-	GH-2	WA-3	IB-21
63	B161	Hohoe, VR	12.09.97	Tox 3100-37-3-3-2-4	-	GH-2	WA-3	IB-13
64	6007b	Kyirikoraa (near Diaso), CR	21.07.01	Unknown	leaf	GH-3	WA-2	IH-1
65	6007d	Kyirikoraa (near Diaso), CR	21.07.01	Unknown	leaf	GH-3	WA-2	IB-9
66	60021b	Otumi (near Kade), ER	26.07.01	Red rice	leaf	GH-3	WA-2	ID-9
67	5010	Sayerano, WR	xx.11.01	Agya-amoah	leaf	GH-3	WA-2	IC-9
68	5082	Sehuri, WR	xx.11.01	Agya-amoah	leaf	GH-3	WA-2	IA-9
69	60013a	Nsuoansua near Sefwi Wiawso, WR	20.07.01	Agya-Amoah	leaf	GH-3	WA-2	IF-1
70	B137	Nyankpala, NR	13.09.97	unknown	leaf	GH-4	WA-5	*
71	B18	Tono, NR	12.09.97	unknown	leaf	GH-4	WA-5	IC-17

ER, NR, WR, AR, CR, UER and VR – Eastern, Northern, Western, Ashanti, Central, Upper Eastern and Volta regions, respectively
- Unknown; * Not tested

Table 23. Details of site, host cultivar, pathotype designation and lineage grouping of *Magnaporthe grisea* isolates from Nigeria.

No.	Code	Site/Location	Date	Variety	Type of Blast	Molecular Type/ Lineage		Patho-type
1	IKR10/2	Ikenene	1996	IKR10/2	leaf	atypical	atypical	II-1
2	B12	Badeggi	13.11.96	IRAT 168	leaf	atypical	atypical	II-1
3	B2	Uyo	13.11.96	# 85795	leaf	NI-1	WA-1	IB-8
4	B1	Uyo	13.11.96	# 86791	leaf	NI-1	WA-1	IA-80
5	B3	Uyo	13.11.96	# 86901	leaf	NI-1	WA-1	IG-2
6	B7	Uyo	13.11.96	# 87091	leaf	NI-1	WA-1	IB-1
7	B5	Uyo	13.11.96	# 87153	leaf	NI-1	WA-1	IB-1
8	B61	Badeggi	xx.10.97	CO 39	leaf	NI-1	WA-1	IC-30
9	B14	Badeggi	13.11.96	FARO 43	leaf	NI-1	WA-1	IA-9
10	B13	Badeggi	13.11.96	FARO 48	leaf	NI-1	WA-1	IC-13
11	B89	Oyo	xx.10.97	ITA 321	leaf	NI-1	WA-1	IA-65
12	B86	Oyo	xx.10.97	TOX 3055-10-1-1-1-1	leaf	NI-1	WA-1	IA-8
13	B99	Oyo	xx.10.97	TOX 3107-39-1-2-1-1	leaf	NI-1	WA-1	IB-42
14	B96	Oyo	xx.10.97	TOX 3370-54-3-1-2	leaf	NI-1	WA-1	IB-1
15	B90	Oyo	xx.10.97	TOX 3388-112-1-1-1-2	leaf	NI-1	WA-1	ID-16
16	B100	Oyo	xx.10.97	TOX 3422-3-2-5-3-2-2-1	leaf	NI-1	WA-1	IB-1
17	B84	Oyo	xx.10.97	TOX 3441-123-2-1-2-1-1-1	leaf	NI-1	WA-1	IB-25
18	B94	Oyo	xx.10.97	TOX 3732-34-1-3-2	leaf	NI-1	WA-1	*
19	B82	Oyo	xx.10.97	TOX 4331-WAT-91-3-1-2-1	leaf	NI-1	WA-1	ID-1
20	B83	Oyo	xx.10.97	TOX 4332-WAT-15-2-3-1-1	leaf	NI-1	WA-1	IB-3
21	B88	Oyo	xx.10.97	WAB 907-B-B-9-H4-3	leaf	NI-1	WA-1	ID-6
22	B98	Oyo	xx.10.97	WITA 8	leaf	NI-1	WA-1	IB-1
23	B58	Badeggi	xx.10.97	FARO 49	leaf	NI-2	WA-6	IA-29

* Not tested

Table 24. Details of site, host cultivar, pathotype designation and lineage grouping of *Magnaporthe grisea* isolates from Burkina Faso.

No	Code	Site/Location	Date	Host/Variety	Part	Molecular type/Lineage		Patho-type
1	S320	Farako-Bâ	1996	*Brachiaria sp*	leaf	atypical	atypical	*
2	S529-2	Sideradougou	1997	*Oryza longistaminata*	leaf	atypical	atypical	IC-32
3	S315	Farako-Bâ	1996	4418	leaf	BF-1	WA-1	*
4	S370	Farako-Bâ	1996	Aichi asahi	leaf	BF-1	WA-1	*
5	S311	Farako-Bâ	1996	Caloro	leaf	BF-1	WA-1	IC-1
6	S343	Farako-Bâ	1996	Caloro	leaf	BF-1	WA-1	*
7	S371	Farako-Bâ	1996	Caloro	leaf	BF-1	WA-1	*
8	S373	Farako-Bâ	1996	Caloro	leaf	BF-1	WA-1	*
9	S341	Farako-Bâ	1996	Caloro	leaf	BF-1	WA-1	*
10	S349	Farako-Bâ	1996	Dourado	leaf	BF-1	WA-1	IC-10
11	S1308	Banfora	03.10.97	Dular	neck	BF-1	WA-1	*
12	S295	Farako-Bâ	1996	Dular	leaf	BF-1	WA-1	IC-1
13	S308-XL	Farako-Bâ	1996	Dular	leaf	BF-1	WA-1	*
14	S309	Farako-Bâ	1996	Dular	leaf	BF-1	WA-1	*
15	S1317	Banfora	04.10.97	FKR 19	neck	BF-1	WA-1	IC-13
16	S336	Farako-Bâ	1996	FKR 28	leaf	BF-1	WA-1	IC-30
17	S356	Farako-Bâ	1996	FKR 28	leaf	BF-1	WA-1	*
18	S340	Farako-Bâ	1996	FKR 28	leaf	BF-1	WA-1	*
19	S303	Farako-Bâ	1996	K3	leaf	BF-1	WA-1	IC-9
20	S364	Farako-Bâ	1996	K3	leaf	BF-1	WA-1	*
21	S338	Farako-Bâ	1996	K3	leaf	BF-1	WA-1	*
22	S296	Farako-Bâ	1996	Kanto 51	leaf	BF-1	WA-1	*
23	S359	Farako-Bâ	1996	Kanto 51	leaf	BF-1	WA-1	*
24	S360	Farako-Bâ	1996	Kanto 51	leaf	BF-1	WA-1	IC-1
25	S517-1	Sideradougou	1997	Local variety	leaf	BF-1	WA-1	IC-1
26	S586-1	Koumadougou	1997	Local variety	leaf	BF-1	WA-1	*
27	S387	Vallée du Kou	1996	*O. longistaminata*	leaf	BF-1	WA-1	IB-32
28	S389	Vallée du Kou	1997	*O. longistaminata*	leaf	BF-1	WA-1	IB-64

No	Code	Site/Location	Date	Host/Variety	Part	Molecular type/Lineage		Patho-type
29	S519	Sideradougou	1997	*O. longistaminata*	leaf	BF-1	WA-1	IA-1
30	S528	Sideradougou	1997	*O. longistaminata*	leaf	BF-1	WA-1	IC-25
31	S567-1	Banfora	1997	*O. longistaminata* close to Caloro	leaf	BF-1	WA-1	IG-1
32	S572	Banfora	1997	*O. longistaminata* close to Usen	leaf	BF-1	WA-1	*
33	S576	Banfora	1997	*O. longistaminata* close to Usen	leaf	BF-1	WA-1	IA-121
34	S579-1	Banfora	1997	*O. longistaminata* close to Usen	leaf	BF-1	WA-1	*
35	S328	Farako-Bâ	1996	*Paspalum scrobiculatum* close to Pekin	leaf	BF-1	WA-1	*
36	S326	Farako-Bâ	1996	*P. scrobiculatum* close to Raminad	leaf	BF-1	WA-1	IA-105
37	S323	Farako-Bâ	1996	*P. scrobiculatum*	leaf	BF-1	WA-1	*
38	S321	Farako-Bâ	1996	Pekin	leaf	BF-1	WA-1	*
39	S403	Farako-Bâ	1996	Pekin	leaf	BF-1	WA-1	IB-9
40	S414	Farako-Bâ	1996	Pekin	leaf	BF-1	WA-1	*
41	S415	Farako-Bâ	1996	Pekin	leaf	BF-1	WA-1	*
42	S334	Farako-Bâ		Pekin	leaf	BF-1	WA-1	*
43	S365	Farako-Bâ	1996	Pi No.4	leaf	BF-1	WA-1	*
44	S366	Farako-Bâ	1996	Pi No.4	leaf	BF-1	WA-1	IH-1
45	S367	Farako-Bâ	1996	Pi No.4	leaf	BF-1	WA-1	IC-22
46	S508	Sideradougou	1997	*Setaria pallidae-fusca*	leaf	BF-1	WA-1	IF-2
47	S552	Banfora	1997	Sha-tiao-tsao	leaf	BF-1	WA-1	IF-1
48	S333	Farako-Bâ	1996	Toride 1	leaf	BF-1	WA-1	IB-1
49	S374	Farako-Bâ	1996	Toride 1	leaf	BF-1	WA-1	*
50	S301-L	Banfora	1997	Tsuyuake	leaf	BF-1	WA-1	IC-17
51	S302	Farako-Bâ	1996	Tsuyuake	leaf	BF-1	WA-1	IC-27
52	S351	Farako-Bâ	1996	Usen	leaf	BF-1	WA-1	*
53	S353	Farako-Bâ	1996	Usen	leaf	BF-1	WA-1	IC-9
54	S354	Farako-Bâ	1996	Usen	leaf	BF-1	WA-1	*

No	Code	Site/Location	Date	Host/Variety	Part	Molecular type/Lineage		Patho-type
55	S299	Farako-Bâ	1996	Yashiro -mochi	leaf	BF-1	WA-1	*
56	S300-XL	Farako-Bâ	1996	Yashiro -mochi	leaf	BF-1	WA-1	IG-2
57	S1310	Banfora	03.10.97	Caloro	neck	BF-2	WA-2	*
58	S293	Farako-Bâ	1996	Caloro	leaf	BF-2	WA-2	IG-1
59	S346	Farako-Bâ	1996	Dourado	leaf	BF-2	WA-2	IG-1
60	S350	Farako-Bâ	1996	Dourado	leaf	BF-2	WA-2	IA-105
61	S348	Farako-Bâ	1996	Dourado	leaf	BF-2	WA-2	*
62	S538	Banfora	1997	Fukunishiki	leaf	BF-2	WA-2	*
63	S378	Farako-Bâ	1996	IRAT 13	leaf	BF-2	WA-2	IB-1
64	S547-2	Banfora	1997	Ishikari shiroke	leaf	BF-2	WA-2	*
65	S357	Farako-Bâ	1996	K60	leaf	BF-2	WA-2	*
66	S520-3	Sideradougou	1997	*O. longistaminata*	leaf	BF-2	WA-2	*
67	S313	Farako-Bâ	1996	*Rottboellia sp*	leaf	BF-2	WA-2	*
68	S314	Farako-Bâ	1996	*Rottboellia sp*	leaf	BF-2	WA-2	IG-1
69	S297	Farako-Bâ	1996	Aichi asahi	leaf	BF-3	WA-4	IH-1
70	S501	Labola	1997	Local variety	leaf	BF-3	WA-4	IA-80
71	S310	Farako-Bâ	1996	Usen	leaf	BF-4	WA-7	*
72	S316	Farako-Bâ	1996	Delta	leaf	BF-5	WA-8	*

* Not tested

Table 25. Details of site, host cultivar, pathotype designation and lineage grouping of *Magnaporthe grisea* isolates from Côte d'Ivoire.

No	Code	Site/Location	Date	Host/Variety	Type of Blast	Molecular type/ Lineage		Patho-type
1	WW6	CNRA (Man)	20.06.01	*Andropogon gayanus*	leaf	atypical	atypical	*
2	358	CNRA (Gagnoa)	08.01.01	*Brachiaria mutica*	leaf	atypical	atypical	*
3	369	Rte Abidjan (Gagnoa)	08.01.01	*Eleusine indica*	leaf	atypical	atypical	*
4	WW11	Korhogo	22.06.01	*Oryza barthii*	leaf	atypical	atypical	*
5	384	Key Site Gagnoa)	08.01.01	*Rottboellia exalta*	leaf	atypical	atypical	II-I
6	972	Key Site (Gagnoa)	08.01.01	*Rottboellia exalta*	leaf	atypical	atypical	*
7	WW2	Gagnoa	21.06.01	*Rottboellia exaltata*	leaf	atypical	atypical	*
8	S1351	M'bé	06.01.98	B40	leaf	CD-1	WA-1	*
9	S1337	M'bé	10.12.97	Bouake 189	leaf	CD-1	WA-1	*
10	S20	Man	13.09.96	Caloro	neck	CD-1	WA-1	*
11	S76	Man	13.09.96	Caloro	neck	CD-1	WA-1	*
12	S69	Man	13.09.96	Chugoku 31	neck	CD-1	WA-1	*
13	S8	Man	13.09.96	CO 39	neck	CD-1	WA-1	*
14	2081	CNRA (Man)	05.10.00	Dêmanba	leaf	CD-1	WA-1	*
15	2375	CNRA (Man)	16.12.00	Dêmanba	leaf	CD-1	WA-1	*
16	2251 (1)	Farmer's field (Man)	30.11.00	Dêmanba	leaf	CD-1	WA-1	IA-29
17	2251 (2)	Farmer's field (Man)	30.11.00	Dêmanba	leaf	CD-1	WA-1	*
18	2269 (2)	Farmer's field (Man)	30.11.00	Dêmanba	leaf	CD-1	WA-1	*
19	2278 (2)	CNRA (Man)	30.11.00	Dêmanba	leaf	CD-1	WA-1	*
20	2319 (1)	Farmer's field (Man)	16.12.00	Dêmanba	leaf	CD-1	WA-1	*
21	2319 (2)	Farmer's field (Man)	16.12.00	Dêmanba	leaf	CD-1	WA-1	*
22	2319 (3)	Farmer's field (Man)	16.12.00	Dêmanba	leaf	CD-1	WA-1	*
23	S28	Man	13.09.96	IDSA	neck	CD-1	WA-1	IA-13
24	S22	Man	13.09.96	Ishikari shiroke	neck	CD-1	WA-1	*
25	S31	Man	13.09.96	K 3	neck	CD-1	WA-1	*
26	S2	Man	13.09.96	K 3	neck	CD-1	WA-1	*
27	S1285	Korhogo	12.10.97	K59	neck	CD-1	WA-1	*
28	S64	Man	13.09.96	Kanto 51	neck	CD-1	WA-1	IC-13
29	S16	Man	13.09.96	NP 125	neck	CD-1	WA-1	*
30	MS 1170	Boundiali	-	Pekin	leaf	CD-1	WA-1	IG-1
31	MS1038	Key Site (Danané)	08.01.01	Pekin	leaf	CD-1	WA-1	IB-61
32	S1287	Korhogo	12.10.97	Pekin	neck	CD-1	WA-1	ID-13
33	2090	Farmer's field (Man)	05.10.00	Raminad	leaf	CD-1	WA-1	*
34	2206	CNRA (Man)	02.11.00	Raminad	leaf	CD-1	WA-1	IA-45
35	2252 (1)	Farmer's field (Man)	30.11.00	Raminad	leaf	CD-1	WA-1	*
36	2252 (2)	Farmer's field (Man)	30.11.00	Raminad	leaf	CD-1	WA-1	*

No	Code	Site/Location	Date	Host/Variety	Type of Blast	Molecular type/ Lineage		Patho-type
37	2252 (3)	Farmer's field (Man)	30.11.00	Raminad	leaf	CD-1	WA-1	*
38	2252 (4)	Farmer's field (Man)	30.11.00	Raminad	leaf	CD-1	WA-1	*
39	2295 (1)	Farmer's field (Man)	16.12.00	Raminad	leaf	CD-1	WA-1	*
40	2295 (2)	Farmer's field (Man)	16.12.00	Raminad	leaf	CD-1	WA-1	*
41	2295 (3)	Farmer's field (Man)	16.12.00	Raminad	leaf	CD-1	WA-1	*
42	2321 (2)	Farmer's field (Man)	16.12.00	Raminad	leaf	CD-1	WA-1	*
43	S42	Man	13.09.96	Raminad Str. 3	neck	CD-1	WA-1	*
44	S1277	Korhogo	12.10.97	Sha-tiao-tsao	neck	CD-1	WA-1	*
45	S14	Man	13.09.96	ST 1	neck	CD-1	WA-1	*
46	2353	CNRA (Man)	16.12.00	Tetep	leaf	CD-1	WA-1	IC-1
47	2304 (1)	Farmer's field (Man)	16.12.00	Tetep	leaf	CD-1	WA-1	*
48	2304 (2)	Farmer's field (Man)	16.12.00	Tetep	leaf	CD-1	WA-1	*
49	2304 (3)	Farmer's field (Man)	16.12.00	Tetep	leaf	CD-1	WA-1	*
50	2336 (1)	Farmer's field (Man)	16.12.00	Tetep	leaf	CD-1	WA-1	II-1
51	2336 (2)	Farmer's field (Man)	16.12.00	Tetep	leaf	CD-1	WA-1	*
52	1685	Key Site (Danané)	xx.12.00	Usen	neck	CD-1	WA-1	*
53	S25	Man	13.09.96	Usen	neck	CD-1	WA-1	*
54	S10	Man	13.09.96	Usen	neck	CD-1	WA-1	*
55	S1336	M'bé	10.12.97	Usen	leaf	CD-1	WA-1	*
56	2064	CNRA (Man)	05.10.00	NERICA 1	leaf	CD-1	WA-1	*
57	2390	Farmer's field (Man)	16.12.00	NERICA 1	leaf	CD-1	WA-1	IA-5
58	2263 (1)	Farmer's field (Man)	30.11.00	NERICA 1	leaf	CD-1	WA-1	*
59	2263 (2)	Farmer's field (Man)	30.11.00	NERICA 1	leaf	CD-1	WA-1	*
60	2409 (1)	Plateau (M'bé)	20.12.00	NERICA 1	neck	CD-1	WA-1	*
61	2409 (2)	Plateau (M'bé)	20.12.00	NERICA 1	neck	CD-1	WA-1	*
62	2409 (3)	Plateau (M'bé)	20.12.00	NERICA 1	neck	CD-1	WA-1	IB-1
63	1987	CNRA (Man)	11.09.00	NERICA 1	leaf	CD-1	WA-1	IA-109
64	386	Key Site (Gagnoa)	08.01.01	WAB 56-125	leaf	CD-1	WA-1	*
65	2086	Farmer's field (Man)	05.10.00	WAB 56-125	leaf	CD-1	WA-1	ID-13
66	2123	Bas-fond (M'bé)	10.10.00	WAB 56-125	leaf	CD-1	WA-1	*
67	2254 (1)	Farmer's field (Man)	30.11.00	WAB 56-125	leaf	CD-1	WA-1	*
68	2254 (2)	Farmer's field (Man)	30.11.00	WAB 56-125	leaf	CD-1	WA-1	*
69	2266 (2)	Farmer's field (Man)	30.11.00	WAB 56-125	leaf	CD-1	WA-1	*
70	2348 (1)	CNRA (Man)	14.12.00	WAB 56-125	leaf	CD-1	WA-1	*
71	2348 (3)	CNRA (Man)	14.12.00	WAB 56-125	leaf	CD-1	WA-1	*
72	2348 (5)	CNRA (Man)	14.12.00	WAB 56-125	leaf	CD-1	WA-1	*
73	MS 1172	Boundiali	-	WAB 56-125	leaf	CD-1	WA-1	IF-1
74	S1278	Korhogo	12.10.97	WAB 651-B-A 83	neck	CD-1	WA-1	IB-29

No	Code	Site/Location	Date	Host/Variety	Type of Blast	Molecular type/ Lineage		Patho-type
75	S32	Man	13.09.96	WAB 99-14	neck	CD-1	WA-1	*
76	S1343	Sakassou	16.12.98	Weed	neck	CD-1	WA-1	IA-112
77	S1330	M'bé	xx.10.97	WITA 11	leaf	CD-1	WA-1	*
78	S1338	Sakassou	16.12.97	WITA 11	neck	CD-1	WA-1	IB-29
79	S1341	Sakassou	16.12.97	WITA 9	neck	CD-1	WA-1	IB-13
80	MS1037	Danané	09.01.01	Yashiro Moshi	leaf	CD-1	WA-1	*
81	S1291	Korhogo	12.10.97	Yashiro-mochi	neck	CD-1	WA-1	ID-13
82	S1333	M'bé	10.12.97	B40	leaf	CD-2	WA-2	*
83	S51	Man	13.09.96	Caloro	neck	CD-2	WA-2	*
84	S1293	Korhogo	12.10.97	Caloro	neck	CD-2	WA-2	IC-13
85	S1273	Korhogo	09.10.97	Cisadane	neck	CD-2	WA-2	IA-9
86	S50	Man	13.09.96	CO 39	neck	CD-2	WA-2	*
87	2196	Plateau (M'bé)	10.10.00	Dêmanba	leaf	CD-2	WA-2	II-1
88	2229 (1)	Farmer's field (Man)	30.11.00	Dêmanba	leaf	CD-2	WA-2	*
89	2229 (2)	Farmer's field (Man)	30.11.00	Dêmanba	leaf	CD-2	WA-2	IB-9
90	2251 (3)	Farmer's field (Man)	30.11.00	Dêmanba	leaf	CD-2	WA-2	*
91	2251 (4)	Farmer's field (Man)	30.11.00	Dêmanba	leaf	CD-2	WA-2	*
92	2352 (1)	CNRA (Man)	16.10.00	Dêmanba	leaf	CD-2	WA-2	*
93	2352 (2)	CNRA (Man)	16.10.00	Dêmanba	leaf	CD-2	WA-2	IA-1
94	2406 (3)	Plateau (M'bé)	20.12.00	Dêmanba	leaf	CD-2	WA-2	*
95	2406 (4)	Plateau (M'bé)	20.12.00	Dêmanba	leaf	CD-2	WA-2	*
96	2413 (3)	Plateau (M'bé)	20.12.00	Dêmanba	leaf	CD-2	WA-2	ID-9
97	S6	Man	-	Dourado	neck	CD-2	WA-2	*
98	S75	Man	13.09.96	Fukunishiki	neck	CD-2	WA-2	*
99	S35	Man	13.09.96	IDSA 13	neck	CD-2	WA-2	*
100	S27	Man	13.09.96	IDSA 6	neck	CD-2	WA-2	*
101	S33	Man	13.09.96	IRAT 13	neck	CD-2	WA-2	*
102	S60	Man	13.09.96	K 59	neck	CD-2	WA-2	*
103	1025	Man	08.01.01	Moroberekan	leaf	CD-2	WA-2	II-I
104	S17	Man	13.09.96	Moroberekan	neck	CD-2	WA-2	*
105	S1331	M'bé	10.12.97	OB 677	leaf	CD-2	WA-2	*
106	S1294	Korhogo	12.10.97	Pekin	neck	CD-2	WA-2	*
107	S9	Man	13.09.96	Raminad Str. 3	neck	CD-2	WA-2	IC-1
108	S523-1	-	xx.09.96	Rice close to *O. longistaminata*	-	CD-2	WA-2	*
109	S1300	Boundiali	12.10.97	Sha-tiao-tsao	leaf	CD-2	WA-2	*
110	S24	Man	13.09.96	Sha-tiao-tsao	neck	CD-2	WA-2	*
111	S47	Man	13.09.96	Sha-tiao-tsao	neck	CD-2	WA-2	*

No	Code	Site/Location	Date	Host/Variety	Type of Blast	Molecular type/ Lineage		Patho-type
112	1868	Key Site Boundiali)	27.07.00	Tetep	leaf	CD-2	WA-2	*
113	2112	Farmer's field (Man)	05.10.00	Tetep	leaf	CD-2	WA-2	*
114	2231	Farmer's field (Man)	30.11.00	Tetep	leaf	CD-2	WA-2	II-1
115	S1282	Korhogo	12.10.97	Toride 1	neck	CD-2	WA-2	*
116	S30	Man	13.09.96	TOX 1011-4 A2	neck	CD-2	WA-2	IA-80
117	MS1032	Key Site (Danané)	-	Tox 1011-4A2	leaf	CD-2	WA-2	*
118	MS1001	Man	13.04.00	Usen	leaf	CD-2	WA-2	ID-9
119	S77	Man	13.09.96	Usen	neck	CD-2	WA-2	*
120	2344	CNRA (Man)	16.12.00	NERICA 1	leaf	CD-2	WA-2	IB-45
121	1798	Key Site (Boundiali)	xx.12.00	WAB 56-125	-	CD-2	WA-2	ID-9
122	2113	Farmer's field (Man)	05.10.00	WAB 56-125	leaf	CD-2	WA-2	*
123	2148	Plateau (M'bé)	10.10.00	WAB 56-125	leaf	CD-2	WA-2	*
124	2323	Farmer's field (Man)	14.12.00	WAB 56-125	leaf	CD-2	WA-2	*
125	2290 (1)	Farmer's field (Man)	16.12.00	WAB 56-125	leaf	CD-2	WA-2	*
126	2290 (2)	Farmer's field (Man)	16.12.00	WAB 56-125	leaf	CD-2	WA-2	*
127	2290 (3)	Farmer's field (Man)	16.12.00	WAB 56-125	leaf	CD-2	WA-2	*
128	2290 (5)	Farmer's field (Man)	16.12.00	WAB 56-125	leaf	CD-2	WA-2	*
129	2348 (2)	CNRA (Man)	14.12.00	WAB 56-125	leaf	CD-2	WA-2	ID-9
130	S1	Man	13.09.96	WAB 56-125	neck	CD-2	WA-2	*
131	S74	Man	13.09.96	WAB 56-125	neck	CD-2	WA-2	*
132	2411 (2)	Plateau (M'bé)	20.12.00	WAB 56-125	leaf	CD-2	WA-2	*
133	2229 (5)	Farmer's field (Man)	30.11.00	Dêmanba	leaf	CD-3	WA-4	*
134	S56	Man	13.09.96	Pi No. 4	neck	CD-3	WA-4	*
135	S1297	Korhogo	12.10.97	Aichi asahi	neck	CD-4	WA-3	*
136	S554	-	-	Caloro	leaf	CD-4	WA-3	*
137	S1284	Korhogo	12.10.97	K3	neck	CD-4	WA-3	IC-9
138	S1288	Korhogo	12.10.97	WAB 56-125	neck	CD-4	WA-3	IB-17
139	WW13	Korhogo	22.06.01	*Oryza barthii*	leaf	CD-5	WA-9	II-1

- Unknown; * Not tested

comprised isolates 2229(5) and S56 from Man site on rice cultivars Demanba and Pi No.4, respectively. Four isolates including three on rice cultivars K3, WAB 56-125 and Aichi Asahi at Korhogo formed lineage CD-4. Isolate WW13 from *Oryza barthii* at Korhogo formed lineage CD-5.

Overall, blast lineages (genetic groups) varied from 2-5 per country with up to 16 lineages in 4 countries. In each country one or two lineages were dominant. For example, GH-1 (56%) was present across Ghana on up to 20 rice varieties/lines comprising isolates collected over a five-year period. BF-1 included more than 70% of isolates from rice, wild rice and weeds in Burkina Faso. CD-1 and CD-2 were 38–56% in Côte d' Ivoire comprising isolates collected over a five-year period. Distribution of some lineages was restricted, e.g. GH-2 (31%) mainly from Eastern Ghana, appearing at low frequency in Northern Ghana. In general, different types of blast (e.g. foliar and neck) were caused by isolates in the same lineage suggesting the utility of common resistances. Lineages common among the four countries were identified and nine distinct West African blast lineages designated WA1 - WA9. Lineage WA-1 (Figures 3 and 4) was present in Ghana (GH-1), Burkina Faso (BF-1), Côte d'Ivoire (CD-1) and Nigeria (NI-1). WA-2 in both Ghana (GH-3) and Côte d'Ivoire (CD-2). WA-3 also in Ghana (GH-2) and Côte d'Ivoire (CD-4). WA-4 in Côte d'Ivoire (CD-3) and Burkina Faso (BF-3). The rest of the lineages WA-5 to WA-9 were distinct to each of the countries. Among these, WA1, WA2 and WA3 are the major West African blast lineages and are present in two or more countries (Table 26). This has clear implications not only for the identification, development and deployment of resistances but also to develop methodologies and the framework for restricting the potential spread of the pathogen through seed material.

Pathogenic (pathotype) diversity

The virulence spectrum and pathotype designations of representatives of some prevailing lineages of the rice blast pathogen in Ghana, Nigeria, Burkina Faso and Côte d'Ivoire was determined using the eight international rice differentials (Tables 22–25). In Ghana, 25 pathotypes were recorded from more than 27 rice cultivars and also from a weed and wild rice at sites across seven regions (Table 22). Volta regions with 12 pathotypes and northern region with nine were most diverse. A number of isolates showed distinct virulence spectrum defined by a particular pathotype, but there were also some examples where different isolates belonged to the same pathotype. For example, IB-1 (27% of isolates tested) was recorded in all regions except the eastern region. Isolates 5038 and 60040 from wild rice and weed, respectively were pathogenic on the differentials. The most frequently observed pathotype groups were IB and IC (59% and 19% of the isolates tested, respectively).

In Nigeria, 16 pathotypes were observed from three sites Badeggi, Oyo and Uyo and were from 20 different cultivars (Table 23). IA and IB were the most prominent pathotype groups (23% and 41% respectively). Except for IB-1 represented by five isolates, each of the pathotypes was represented by only one isolate. Two isolates, B12 and IKR10/2 recovered from rice were non-pathogenic on the differentials as well as susceptible checks B40 and CO39 and were designated as pathotype II-I (Ling and Ou, 1969).

In Burkina Faso, 23 pathotypes from up to 16 different cultivars were identified from two main sites Farako-Bâ and Banfora as well as two other sites Sideradougou and Vallée du Kou. Some pathotypes (for example IA-105, IB-1, IC-1, IC-9, IG-1 and IH-1) were represented by two or more isolates whereas the rest were represented by one isolate only. IC was the most frequently (42%) observed pathotype group. Blast isolates S576, S387, S389, S528, S529-2 and S567-1 from wild rice at Banfora, Vallée du Kou and Sideradougou as well as isolates collected from weeds *Paspalum* (S326) and *Setaria* (S508) were found to be pathogenic on the differentials (Table 24).

In Côte d'Ivoire 23 pathotypes from at least 18 rice cultivars and an unknown weed were recorded from seven sites characterised. IA (24%) and IB (22%) were not only the most frequently occurring pathotype groups, but also quite diverse with nine and seven pathotypes, respectively. At Man site 13 pathotypes belonging to diverse pathotype groups IA, IB, IC and ID and at Korhogo six pathotypes present on seven different cultivars were recorded (Table 25).

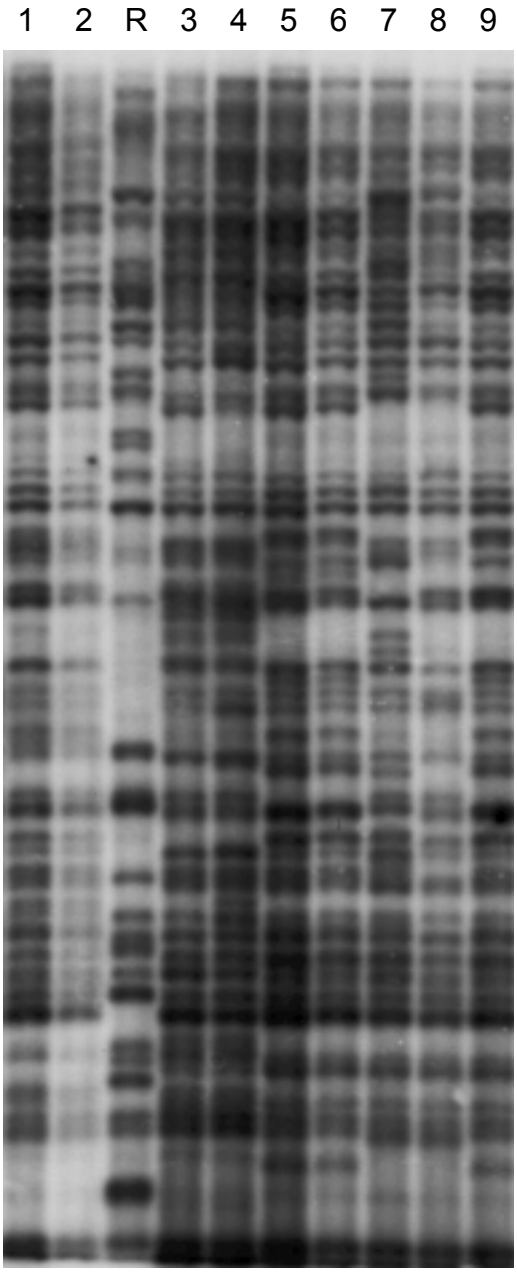

Figure 3. MGR586 fingerprints of a set of blast isolates from Nigeria (1 and 2), Ghana (3-8) and Côte d'Ivoire (9) showing high similarity and these isolates belong to a dominant West African lineage WA-1 (= NI-1, GH-1 and CD-1).

Note: R is an international reference isolate distinct from the rest of the isolates.

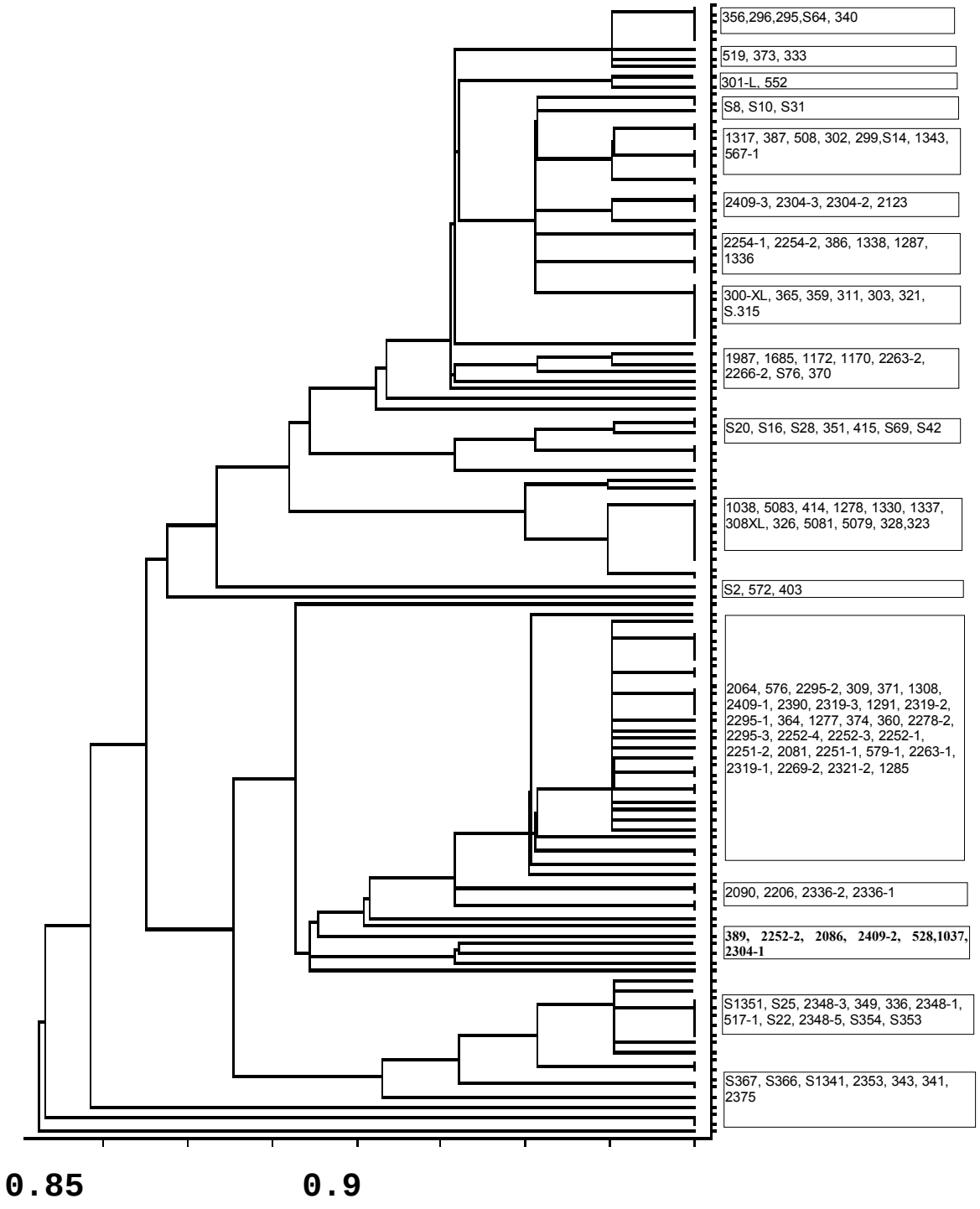

Figure 4. Dendrogram showing the close relatedness of a large collection of blast isolates from various sites in Côte d'Ivoire and Burkina Faso.

Note: These isolates fit into a dominant West African lineage WA-1 (= CD-1 and BF-1), based on 85–100% similarity in MGR586 fingerprint patterns. Isolate details are in Tables 24 and 25.

In total 52 pathotypes from 156 isolates were observed from the four West African countries (Table 27). The number of isolates pathotyped varied from 22 to 64 in each of the countries. Around 65% of pathotypes were represented by single isolates and others by more than one isolate. In some cases the same pathotype, for example IB-1 was recovered from twenty different lines in the four countries. For instance, IB-1 was identified from two cultivars (IRAT13 and Toride1) from Burkina Faso; from cultivars CK73 and WAB 450-IBP from Côte d'Ivoire; from twelve different cultivars from Ghana and from five different cultivars from Nigeria. Likewise IA-80 was recovered from three different cultivars each from Burkina Faso, Côte d'Ivoire and Nigeria. In other cases the same pathotype was recovered from different lines in the same country at the same site. For example IB-13 was recorded from two different cultivars (Tox 3100-37-3-3-2-4 and TCA 80-4) at Hohoe in Ghana and IG-1 from three different cultivars (Caloro, Dourado and *Rottboellia* sp) at Farako-Bâ in Burkina Faso.

In general, pathogen virulence diversity was high with 16–25 pathotypes in each country (Table 27). IB (particularly IB-1) was the dominant pathotype group in Ghana and Nigeria. IC was the prominent (43%) pathotype group in Burkina and a range of pathotypes was present at certain sites (e.g. 12 at Farako-Bâ). In Côte d'Ivoire IA, IB, IC and ID were 16–29%. The prominent pathotype groups in the West African region were IB, IC and IA (38, 21 and 15%, respectively). This understanding of the virulence diversities of major lineages and sites is critical to the efficient utilisation of host resistance.

Blast lineage-pathotype relationships

Combined analysis of the lineage-pathotype data has provided some understanding of the lineage-pathotype relationships. In Ghana and Nigeria, the lineage-pathotype relationships showed a similar trend. Pathotypes represented in lineage GH-1 were mostly IB group (up to 68%) and some of these were closely related (e.g. IB-1, IB-5, IB-7 and IB-9) differing by a few compatibility reactions on the international differentials. Some of the other isolates in this lineage originating from Tox-related cultivars at Hohoe also expressed related pathotypes (e.g. IA-1 and IA-2). Similarly, pathotypes represented in lineage GH-2 were mostly IB group (68%) and were related. Lineage GH-3 represented diverse pathotypes from groups IA, IB, IC, ID, IF and IH (Table 1).

In Nigeria, lineage NI-1 represented 15 pathotypes. Although these pathotypes originated from 19 different cultivars, a number of these isolates showed related international virulence grouping patterns, for example, pathotypes IA-8, IA-9, IA-65, IA-80; IB-1, IB-3, IB-8, IB-25 and IB-42; ID-1, ID-6 and ID-16. *M. grisea* isolates originating from the same site showed lesser degree of genetic diversity, although their virulence patterns varied. For instance, eleven isolates collected from Oyo from 11 cultivars expressed nine pathotypes, but all belonged to lineage NI-1 with very high MGR586 fingerprint similarity (Table 23).

Twenty-three pathotypes identified in Burkina Faso were represented by lineages BF-1, BF-2 and BF-3. The lineage-pathotype relationships observed in Burkina Faso were more complex compared to Nigeria and Ghana. Lineage BF-1 represented 21 pathotypes of groups IA, IB, IC, IF, IG IH which originated from up to 15 rice cultivars and some non-rice hosts. There were few cases where isolates from different cultivars but same site expressed the same pathotype. For example, isolates from cultivars Caloro, Dular, Kanto 51 were pathotype IC-1 and isolates from cultivars Usen and K3 were IC-9 at Farako-Bâ. Of the five isolates tested in lineage BF-2, S293, S346 and S314 from two rice cultivars and a weed *Rottboellia* at Farako-Bâ gave the same pathotype IG-1. Isolates S350 and S378 were pathotypes IA-105 and IB-1, respectively (Table 24).

In Côte d'Ivoire, pathotypes IA-5, IA-13, IA-29, IA-45, IA-109, IC-1, IC-13 and ID-13 identified at Man from different rice cultivars belonged to CD-1. A range of other pathotypes belonging to groups IA, IB, IF and IG from Boundiali, Danané, M'bé and Sakassou were also recorded indicating high virulence diversity in lineage CD-1. Out of eleven isolates in lineage CD-2, isolates 2413(3), ms1001, 1798 and 2348(2) from different cultivars and sites gave the same pathotype ID-9, whilst the rest of the isolates gave different pathotypes of IA, IB and IC groups (Table 25).

Table 26. Diversity and distribution of blast lineages in four West African countries.

		West African Lineages								
Country*	**Country-wise lineages**	**WA-1**	**WA-2**	**WA-3**	**WA-4**	**WA-5**	**WA-6**	**WA-7**	**WA-8**	**WA-9**
		Number of Isolates								
Nigeria	NI-1	20								
(23)	NI-2						1			21
	GH-1	37								
Ghana	GH-2			21						
(71)	GH-3		6							66
	GH-4					2				
	CD-1	74								
	CD-2		51							
Côte d'Ivoire	CD-3				2					132
(139)	CD-4			4						
	CD-5								1	
	BF-1	54								
	BF-2		12							
Burkina Faso	BF-3				2					70
(72)	BF-4							1		
	BF-5								1	
(305)		185	69	25	4	2	1	1	1	1 289

*Number of isolates analysed per country/West Africa.
A total of 305 isolates were analysed and 289 showed typical rice-pathogen like fingerprints (with 30–50 MGR586 hybridising bands).
These isolates were categorised into 16 country-wise lineages. Lineages common in more than one country were identified and 9 West African lineages WA-1 toWA-9 designated, where WA-1 = CD-1, BF-1, GH-1 and NI-1; WA-2 = CD-2, BF-2 and GH-3; WA-3 = CD-4 and GH-2; WA-4 = CD-3 and BF-3; WA-5 = GH-4; WA-6 = NI-2; WA-7 = BF-4; WA-8 = BF-5; WA-9 = CD-5.
16 isolates showed atypical fingerprints (with only 2–9 MGR586 hybridising bands) – 2 isolates in Nigeria, 5 Ghana, 7 Côte d'Ivoire and 2 Burkina Faso.
See Tables 22-25 for more details of the isolates and distribution of the lineages at different sites within each country.

Table 27. Diversity and distribution of blast pathotypes in four West African countries.

WA/ Country WA	International Pathotypes Number of isolates per Pathotype																Total No. of Pathotypes*		Total No. of Isolates
IA-1	IA-2	IA-5	IA-8	IA-9	IA-13	IA-29	IA-45	IA-65	IA-80	IA-88	IA-105	IA-109	IA-112	IA-121			15		23
N	-	-	-	1	1	-	1	-	1	1	-	-	-	-	-			5	5
G	1	1	-	-	1	-	-	-	-	-	1	-	-	-	-			4	4
BF	1	-	-	-	-	-	-	-	-	1	-	2	-	-	1			4	5
CD	1	-	1	-	1	1	1	1	-	1	-	-	1	1	-			9	9
WA	IB-1	IB-3	IB-5	IB-7	IB-8	IB-9	IB-13	IB-17	IB-21	IB-25	IB-29	IB-32	IB-42	IB-45	IB-61	IB-64	16		60
N	5	1	-	-	1	-	-	-	-	1	-	-	1	-	-	-		5	9
G	17	-	2	1	-	4	4	-	1	1	-	-	-	2	6	-		9	38
BF	2	-	-	-	-	1	-	-	-	-	-	1	-	-	-	1		4	5
CD	1	-	-	-	-	1	1	1	-	-	2	-	-	1	1	-		7	8
WA	IC-1	IC-9	IC-10	IC-13	IC-17	IC-22	IC-25	IC-27	IC-29	IC-30	IC-32						11		33
N	-	-	-	1	-	-	-	-	-	1	-							2	2
G	2	3	-	2	1	-	2	-	2	-	-							6	12
BF	4	2	1	1	1	1	1	1	-	1	1							10	14
CD	2	1	-	2	-	-	-	-	-	-	-							3	5
WA	ID-1	ID-6	ID-9	ID-13	ID-16												5		15
N	1	1	-	-	1													3	3
G	1	-	1	3	-													3	5
BF	-	-	-	-	-													-	-
CD	-	-	4	3	-													2	7
WA	IF-1	IF-2															2		4
N	-	-																-	-
G	1	-																1	1
BF	1	1																2	2
CD	1	-																1	1

WA/ Country WA	International Pathotypes Number of isolates per Pathotype		Total No. of Pathotypes*	Total No. of Isolates
WA	IG-1	IG-2	2	9
N	-	1	1	1
G	2	-	1	2
BF	4	1	2	5
CD	1	-	1	1
WA	IH-1		1	3
N	-		-	-
G	1		1	1
BF	2		1	2
CD	-		-	-
WA			52	87	147
N			16	20
G			25	63
BF			23	33
CD			23	31

WA, West Africa; N, Nigeria; G, Ghana; BF, Burkina Faso; CD, Côte d'Ivoire
156 blast isolates were pathotyped on International differentials A, Raminad Str. 3; B, Zenith; C, NP-125; D, Usen; E, Dular; F, Kanto 51; G, Sha-tiao-tsao and H, Caloro.
Disease reactions were scored based on Valent *et al.* (1991). Pathotype designations were based on the nomenclature of Ling and Ou (1969).
147 isolates were pathogenic on the differentials and 9 (Nigeria 2 isolates, Ghana 1 and Côte d'Ivoire 6) were non-pathogenic and were assigned II-1.
*A total of 87 international pathotypes were recorded in four countries. Following the identification of pathotypes present in more than one country, 52 (15 pathotypes belonging to group IA, 16 IB, 11 IC, 5 ID, 2 IF, 2 IG and 1 IH) distinct pathotypes were recorded in West Africa among the isolates pathotyped.

- Pathotype not recorded

Screening site characterisation

Based on the diversity and distribution of the blast genetic groups (lineages) and pathotypes (Tables 22–25) the following key sites across the four countries surveyed are suitable for blast resistance screening. In Ghana, Volta region (Hohoe) with major lineages GH-1 and GH-2 and 12 pathotypes belonging to five pathotype groups (IA, IB, IC, ID and IG) is a high diversity area/site. In the northern region (Nyankpala and Bolgatanga) lineages GH-1, 2 and 4 and nine pathotypes (IA, IB and IC groups) were recorded. Although seven pathotypes belonging to groups IA, IB, IC and ID were recorded in the Western region (Sayerano and Sehuri) one of the major lineages GH-2 was not observed, which needs to be further monitored.

In Nigeria, the dominant lineage NI-1 was present at all three main sites sampled. At Oyo nine pathotypes of groups IA, IB and ID at Uyo four pathotypes of groups IA, IB and IG were recorded. At Badeggi four pathotypes of groups IA and IC and also lineages NI-1 and 2 were recorded. Further characterisation work in Nigeria would provide an improved assessment of blast pathogen diversity at key sites.

In Burkina Faso, Farako-Bâ exhibited all five lineages and 12 pathotypes representing a range of genetic and virulence diversities. At Banfora and Sideradougou both major lineages BF-1 and BF-2 and five pathotypes each were recorded.

In Côte d'Ivoire, Man and Korhogo displayed high blast pathogen diversity with various lineages as well as 13 and six pathotypes, respectively belonging to four international pathotype groups (IA, IB, IC and ID).

Magnaporthe grisea *isolates on weeds and weedy rices*

During the course of these investigations, approximately 10% of the *M. grisea* isolates obtained from rice, wild rice (*Oryza longistaminata* and *O. barthii)* and non-rice hosts (e.g. *Paspalum*, *Setaria*, *Rottboellia* and *Bracharia*) gave 'atypical' MGR586 fingerprint patterns with reference to the host from which they originated (Tables 22–25). Typically, rice-pathogenic isolates show 30-50 MGR586 bands whereas non-rice pathogenic isolates (e.g from weeds) show one to only a few (up to 9) MGR586 bands, and these two groups of isolates are thought to be genetically distinct (Borromeo *et al*., 1993). However, we identified a number of isolates that differ from this pattern. For example, some isolates from rice gave only one to nine MGR586 bands. Isolates from wild rice gave both rice pathogen-like (30-50 bands) as well as non-rice pathogen-like (up to 9) MGR586 fingerprints. Similarly, isolates from weeds also gave rice pathogen-like (30–50 bands) as well as non-rice pathogen-like (up to 9) MGR586 fingerprints. These *M. grisea* isolates, along with a limited number of reference isolates from rice and non-rice hosts were further characterised using additional molecular markers namely ribosomal DNA-internal transcribed spacer 1 (rDNA-ITS1) sequence data and RAPD PCR profiles. In greenhouse tests using the international rice differential set along with CO39 and B40 as checks, some of these isolates were found to be pathogenic to rice (Tables 22, 24 and 25) under controlled conditions.

Combined application of the molecular analyses and pathogenicity tests has revealed the presence of blast pathogen (up to 10% of pathogen populations) on weedy rices and common weed hosts. Several of these isolates are closely related to rice pathogenic isolates in their genetic profile and are pathogenic to rice. The epidemiological significance of these isolates and their impact on blast/weed management as well as their origin and potential role in pathogen evolution, merit further investigations.

Blast incidence and host resistance

Incidence and severity of blast across key rice growing areas in Ghana have been surveyed and areas of low - high blast incidence have been identified (see Nutsugah *et al*, this volume), overlapping the pathogen population characterisation at key screening sites. Blast pathogen virulence structure has also been studied

at key sites Man and M'bé in Côte d'Ivoire developing methodologies for capturing the pathogen diversity and to utilise horizontal resistance (see articles by Sere *et al*., this volume). Blast lineage/pathotype data generated in corresponding investigations can be used for identification and utilisation of vertical resistance leading to the combined deployment of horizontal and vertical resistances.

Screening of a range of rice varieties under controlled conditions against West African blast lineage representatives and at some of the characterised sites linked to and utilising PVS material has led to the identification of potential blast resistances (Table 28; also see Nutsugah *et al*., this volume). Some of this material can be further tested/developed through WARDA/NARS partnership and proposed IPM/ICM activities.

Capacity strengthening and dissemination

WARDA pathology staff have been provided research training attachments at HRI to gain experience in laboratory analyses of *Magnaporthe grisea* and the use of molecular diagnostic tools and an African Pathologist (Zambian Ministry of Agriculture) has been trained to PhD level. Senior pathologists from WARDA and SARI also visited HRI to gain first-hand experience of the molecular–biotechnological tools for fungal pathogen characterisation. As part of this exercise inputs have been made into assessing the potential of putative West African differentials being developed by WARDA to capture blast pathogen genetic diversity. Pathological and preliminary molecular biological methodologies and material were provided to WARDA and NARS as appropriate for in-country use.

Outputs have been presented and disseminated at a wide range of national (e.g. Ghana Science Association Biennial Meeting 2001), regional (e.g. Regional Rice Research Review, 2000 and 2002; EU Rice Conference 2002) and international (e.g. Global Food Security Conference 2002; International Plant Pathology Congress, 1998 and 2002) scientific foray. Close interaction with the ROCARIZ network (IPM-TF and Breeding-TF) and linkages with FAO-RAF, CSIR-AgSSIP, FARA, AATF and NARS in Asia have been established which would help further develop the blast management work in the West African region applying both upstream and downstream technologies.

Perspectives

Rice scientists world-wide have embarked on developing novel strategies for blast resistance such as 'lineage exclusion' being used/tested in Latin America and Asia as well as planting mixed stands of rice cultivars in China (Zeigler *et al*., 1994; Zhu *et al*., 2001). Both these approaches are based on a sound understanding of the biodiversity of the pathogen populations. We have generated knowledge on pathogen diversity new to West Africa contributing to the global atlas on blast pathogen diversity (Figure 5) and providing a framework for efficient utilisation of host resistance.

In this programme, considerable progress has been made on pathogen diversity, potential host resistances and characterisation of screening sites within the West African region. Strong collaboration and partnership among WARDA, NARS and UK organisations, contributing to capability strengthening and a network of regional and international contacts have been established. This provides an excellent opportunity for farmer participatory evaluation of blast resistant material identified, further utilisation of partial and major gene resistances and continued in-country/in-region pathogen monitoring through technology and knowledge promotion to WARDA/NARS by HRI (Figure 6). We also have the potential to link up with NARS in Asia for utilising mutual knowledge and experiences through related DFID funded programmes. This is critical to the development of durable blast resistance in the West African region as integrated management of major biotic constraints of rice needs to be further developed, for example the potential impact of blast populations present on wild rice and weeds. These strategies coupled with appropriate cultural practices including seed hygiene would lead to sustainable management of rice blast.

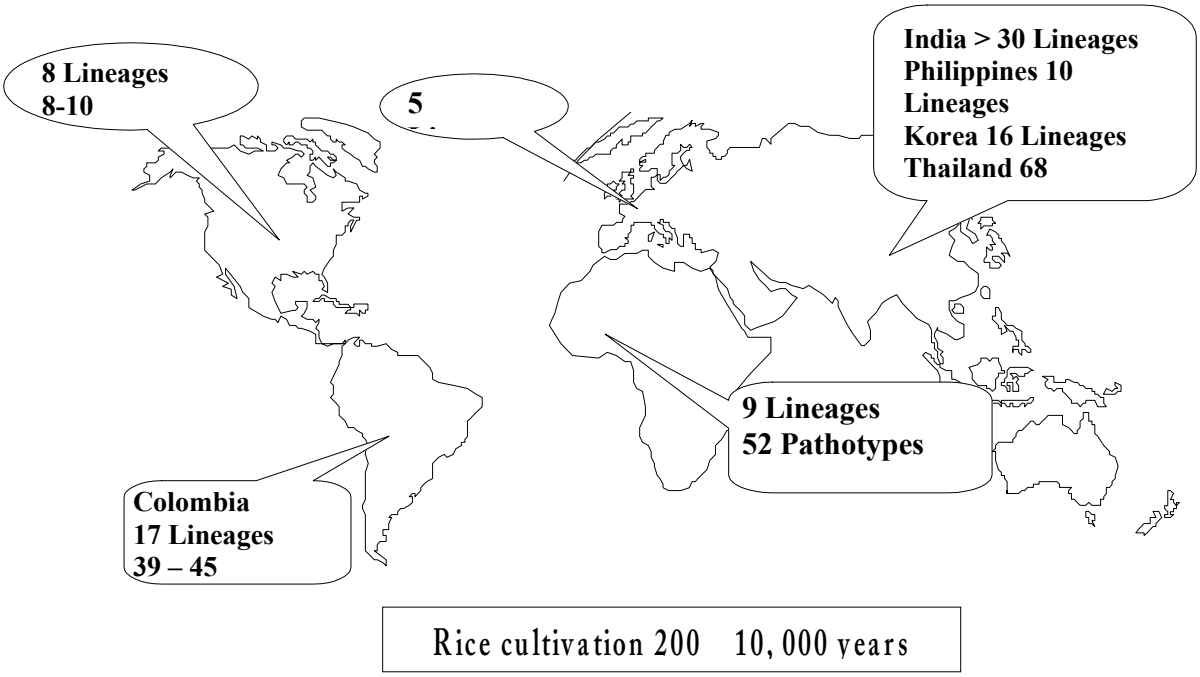

Figure 5. Blast pathogen lineages and pathotypes recorded across the globe from various rice growing ecologies.

Note: Data for West Africa has been contributed through this programme funded by DFID-CPP.

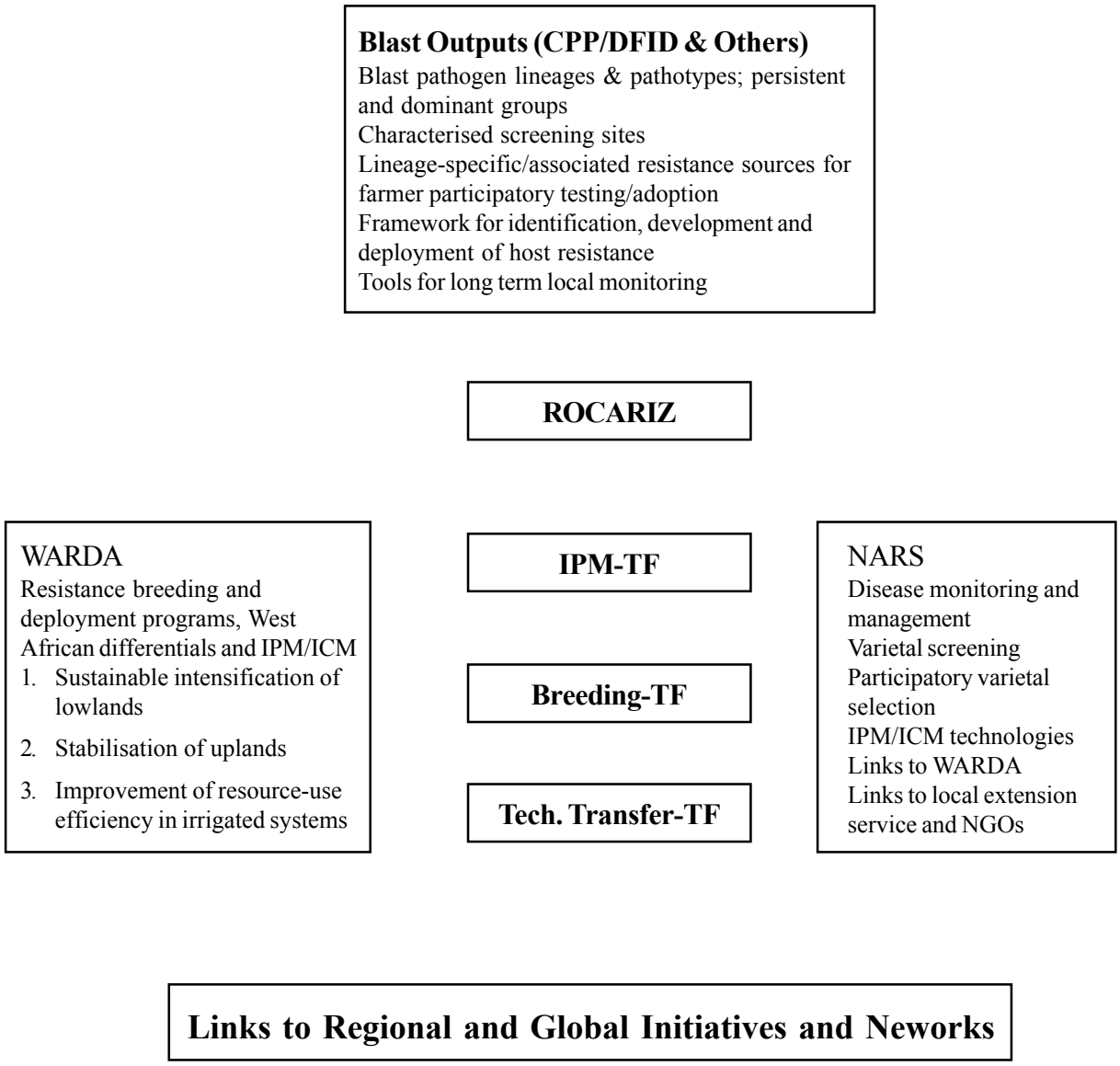

Figure 6. Pathway for Dissemination, Uptake and Adoption of Present and Future Outputs

Table 28. Response of a range of rice varieties provided by WARDA and NARS to characterised blast isolates.

		Score (mean)+ Blast Isolates						
No	Variety	2390	S2	2348(1)	B167	2229(2)	S75	6009a
1	CG14	0.5	0.1	0.2	0.3	0.5	0.5	3.1
2	ROHYB 185-B-20-2	0	3.6	0.6	0	-	0.7	0.3
3	ROHYB 185-B-20-1	4.3	0	1.6	0	-	2.1	0.2
4	ROHYB 185-B-24-1	0.3	2.3	0.2	0	-	0.4	0.2
5	ROHYB 181-B-5-B-1	0.2	2.4	2.8	0.1	0.1	1.5	0
6	*HARROW	3.5	0	0	0.4	1.4	0.4	2.4
7	*MOORE	3.5	0.1	2.5	2.4	1.7	0.1	2.7
8	WAB450-IB-P-38-HB NERICA1	0.3	3.7	0	0.8	1.8	0.4	0.3
9	BOUAKE 189	0.2	0	1.6	0.8	0	0.3	0.2
10	*AGONA	2.3	0	0	3.0	2.8	1.7	-
11	WAB450-I-B-15-7-1	-	2.5	1.4	2.3	-	0.2	2.5
12	OB 677	1.0	0.3	0.7	2.5	1.9	1.3	1.2
13	ROHYB 181-B-8-2	0	0.1	0.3	0.4	0.1	0.2	0
14	ROHYB 185-B-24-3	0.5	3.1	0.1	0	-	1.5	0.4
15	*SIKAMO (NP)	2.6	1.9	0.1	1.8	3.1	0.4	3.5
16	ROHYB 181-B-8-B-1	0.3	0.5	0.9	0	0.7	0	0.3
17	*IR 12979-24-1	3.3	0.2	1.9	2.8	-	1.5	-
18	*IDSA 46	-	-	-	-	2.7	-	-
19	ROHYB 185-B-20-3	-	0.8	1.7	0.4	1.1	0.1	0.1
20	*GR 18	0.1	0	1.7	0.4	0.1	0.1	-
21	*KPUKPULA	1.7	0.1	0.2	2.7	2.6	1.3	-
22	ROHYB 181-B-9-3	-	-	3.0	0	2.8	0.5	0.8
23	WAB 56-50	0.7	0.6	0	0.1	1.3	0.3	2.1
24	*GR 19	1.4	0	1.7	1.1	0.6	0.3	-
25	ROHYB 181-B-3-B-1	-	0.1	2.9	0.1	0.6	1.6	0.7
26	*ASANTE-MO (NP)	2.3	0	0.1	0.4	3.6	1.2	3.9
27	ROHYB 181-B-2-B-1	0.2	0	0.4	0.1	-	0.8	0
28	ROHYB 194-B-4-B-1	0.4	0.7	0	0.6	-	0.1	0.4
29	ROHYB 185-B-23-B-1	0.5	0.6	0.5	0.4	-	0	0.2
30	ROHYB 185-B-24-2	0.1	0.3	0	0	-	1.0	0.4
31	*GOMBA	2.7	1.0	2.0	1.0	2.3	0.4	-
32	*KLEMENSON	1.4	3.3	0.7	1.4	3.6	0.8	2.8

33	*ITA 330	0	2.9	0	0.4	0.1	1.4	-
34	*IRAT 216	0.4	0.1	0.4	0.3	0.7	0	1.6
35	*AGYA-AMOAH	2.0	0.6	0	1.5	3.5	0	1.4
36	WAB 56-104	0.9	0.2	0	0.7	-	0.1	4.1
37	MOROBEREKAN	2.3	0.6	0	0.6	-	0.9	3.1
C1	WITA 9	4.5	0.2	4.2	2.6	3.6	1.9	4.6
C2	WAB450-IB-P-20-HB NERICA 7	0.2	0.1	3.4	2.0	1.1	1.0	1.3
C3	CO39	2.4	2.7	2.5	1.4	2.5	1.2	2.9
C4	B40	3.7	2.1	3.9	1.2	3.6	1.7	4.0

*Varieties provided by SARI/CRI, Ghana
Other varieties were provided by WARDA, Côte d'Ivoire
C1 – C4 are varieties used as standard checks
Disease reactions were scored using the 0-5 scale, where 0 - 1 and 2 - 5 are resistant and susceptible reactions, respectively (Valent *et al*., 1991)
- Not tested
+REML Variance Components Analysis indicated a number of resistant reactions were significant with reference to the standard checks used
Isolate details - 2390: WA-1/GH-1, IA-5. S2: WA-1/GH-1. 2348(1): WA-1/GH-1. B167: WA-1/GH-1, IA-1. 2229(2): WA-2/CD-2, IB-9. S75: WA-2/CD-2. 6009a: WA-3/GH-2, IB-1. For further information refer to Tables 22 and 25.

Importance of blast as a major constraint was highlighted at the Regional Rice Research Review, WARDA in April 2002 and more recently at the rice blast project stakeholder workshop in Accra during March 2003. NARS pathologists and breeders specifically emphasised the need to characterise the blast pathogen populations in the key countries not covered so far and use the knowledge and resources to identify, develop and deploy resistance. Promotion of molecular – biotechnological tools to NARS/WARDA for long-term local monitoring has also been strongly emphasised by a range of stakeholders. WARDA senior management have expressed support for the proposed technology promotion and characterisation at WARDA. FAO/RAF-TCP, CSIR-AgSSIP, Ghana, FARA and AATF are other avenues to further develop and strengthen this approach and discussions with some of these programmes have indicated strong support and potential for wider linkages. BRRI, Bangladesh have expressed interest in developing work on blast pathogen characterisation and disease management. This provides an excellent opportunity to promote the knowledge and tools developed in partnership with WARDA and associated NARS to Asian NARS.

Discussions are ongoing among CPP management, project collaborators and other stakeholders on 'Technology promotion to WARDA and NARS for rice blast pathogen monitoring and sustainable utilisation of host resistance'.

Adaptation of current protocols and promotion of molecular tools and methodologies for long-term blast genetic characterisation in-country.

Strategic blast population characterisation at sites in key countries linked to wider regional/ national/WARDA's initiatives.

Training of NARS scientists in molecular biotechnological tools through links with WARDA leading to further in-country promotion.

Identification of lineage-specific resistance sources, utilisation of IRRI-NILs carrying major resistance (R) genes and wider linkages (e.g. Asian NARS) on utilising R genes and gene pyramids.

Focusing on building regional capacity in blast characterisation to underpin improved blast management.

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Dr. J. Chipili has recently re-joined the Zambian Ministry of Agriculture.

***For correspondence**

Dr. S. (Prasad) Sreenivasaprasad, Research Leader & Project Co-ordinator, Environmental Microbiology, Warwick HRI, Wellesbourne, Warwickshire CV35 9EF, United Kingdom, Tel.: 01789-470382, Fax.: 01789-470552, E. Mail: ss.prasad@hri.ac.uk

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

Kaboré K. Blaise and Sié Moussa
INERA, Programme Riz et Riziculture, Station de Farako-Bâ
B.P. 910 Bobo-Dioulasso, Burkina Faso
Email: progriz@fasonet.bf

Abstract

Blast caused by *Pyricularia grisea* (Cke) Sacc. (Teleomorphe: *Magnaporthe grisea* (Hebert) Barr) 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 provoquée par *Pyricularia grisea* (Cke) Sacc. (Teleomorphe: *Magnaporthe grisea* (Hebert) Barr) 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 *M. 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 *M. 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-I-B-P103-HB	WAB 56-57*
WAB 450-I-B-P6-1-1	CO 39 **
WAB 450-I-B-P6-2-1	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.

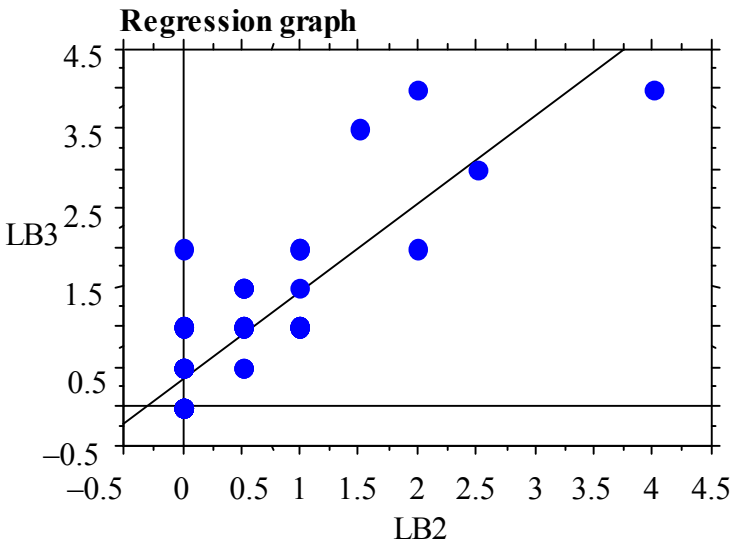
Table 35. Evolution of leaf blast on NERICAs during the 2002 rainy season (0–9 scale).

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-I-B-P103-HB	0	0.12	0.75	1	R
WAB 450-I-B-P6-1-1	0.12	0.37	0.50	0.75	R
WAB 450-I-B-P6-2-1	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.

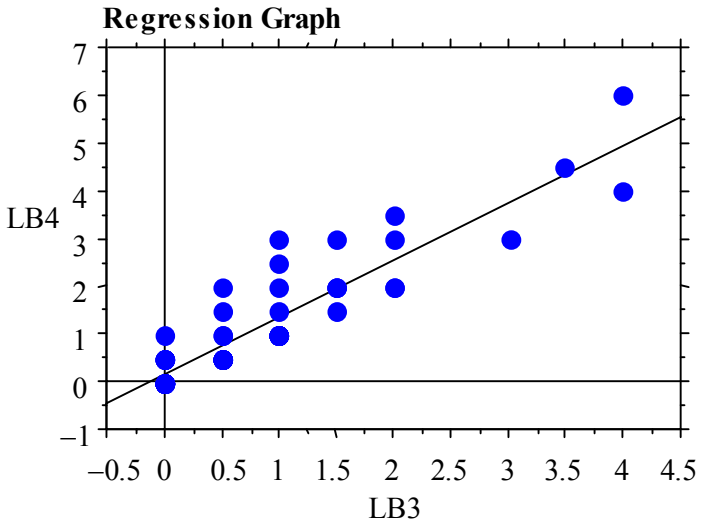
LB2 = Leaf blast at 42 days after sowing; LB3 = Leaf blast at 49 days after sowing.



Y = 0.333 + 1.113 * X; R^2 = 0.704

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.



Y = 0.143 + 1.198 * X; R^2 = 0.84

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-I-B-P103-HB	5.8	8.0	R
WAB 450-I-B-P6-1-1	0.1	1.0	R
WAB 450-I-B-P6-2-1	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-I-B-P103-HB	R	R	9.80	8.76	R
WAB 450-I-B-P6-1-1	R	R	21.24	18.99	MR
WAB 450-I-B-P6-2-1	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).

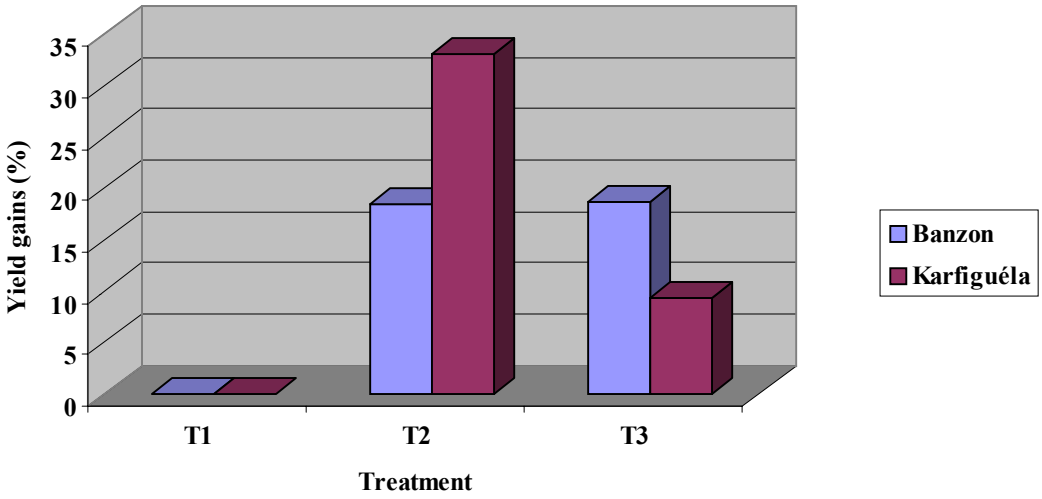


Figure 9. Gains in yield obtained with the technological package.

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 in The Gambia: Genotype by Environmental Reaction, Economic Significance and Management Strategies

Lamin M. S. Jobe
Senior Scientific Officer and Pest Management Programme Officer, National Agricultural Research Institute (NARI), PMB 526, Serrekunda, The Gambia. Email: lmsjobe@Yahoo.com

Résumé

La pyriculariose du riz causée par *Pyricularia grisea* (Cke) Sacc. (Teleomorphe: *Magnaporthe grisea* (Hebert) Barr) 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 caused by *Pyricularia grisea* (Cke) Sacc. (Teleomorphe: *Magnaporthe grisea* (Hebert) Barr) 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 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 Africa Rice Development Association (WARDA) also started a programme to screen some improved exotic upland and lowland rice varieties for their reaction to *P. grisae* 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

D. D. Kuta, E. D. Imolehin, L. Agboire and A. S. Gana
Crop Biotechnology and Stress Physiology Unit, National Cereals Research Institute, Badeggi, PMB 8 Bida, Niger State, Nigeria. E-mail: ncribiotechunit@yahoo.com

Résumé

La pyriculariose du riz provoquée par *Pyricularia grisea* (Cke) Sacc. (Teleomorphe: *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 *Pyricularia grisea* (Cke) Sacc. (Teleomorphe: *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 *M. 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-I-B-P163-4-1	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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Part 5:
Appendices

Workshop Programme

Time	Topic	Resource person
Registration and Opening session		
08:30-09:00	Registration	
09:00-09:10	Welcome address	Dr. A.B. Salifu, SARI
09.10-09:20	Chairman's address	Dr. Joseph Cobbina, CSIR
09:20-09:30	Project background and objectives	Dr. S. Sreenivasaprasad, HRI
Breeding for blast resistance and varietal diffusion		
09:30-10:00	Selection of intra-specific (*Oryza sativa* × *O. sativa*) and inter-specific (*O. sativa* × *O. glaberrima*) lines for their tolerance to blast in Burkina Faso	Dr. M. Sié, INERA
10:00-10:30	Screening Strategy for Durable Resistance to Rice Blast Disease at WARDA	Dr. Y. Séré, WARDA
10:30-11:00	Impact assessment of agricultural technology: Concept, methodology and application to rice pests and diseases	Dr. A. Diagne, WARDA
11:00-11:15	COFFEE BREAK	
DFID-CPP Blast project activities and outputs		
11:15-11:45	Survey of Rice Blast and Varietal Screening in Ghana (joint presentation by SARI and CRI)	Dr. S.K. Nutsugah, SARI SARI/CRI
11:45-12:15	Analysis of *Magnaporthe grisea* population structure in Côte d'Ivoire as a prerequisite for the deployment of varieties with durable blast resistance	Dr. Y. Séré, WARDA
12:15-12:45	Diversity of blast pathogen populations in four West African countries and strategies for resistance management	Dr. S. Sreenivasaprasad
12:45-13:45	LUNCH BREAK	
Blast management in some West African countries		
13:45-14:15	Rice blast management in Burkina Faso from 1999 to 2002: Use of varietal resistance and training of agents and producers	Dr. K.B. Kaboré, INERA
14:15-14:45	Rice Blast Disease in The Gambia: Genotype by Environmental Reaction, Economic Significance and Management Strategies	Dr. L.M.S. Jobe, NARI
14:45-15:15	Pathogenic Variability of *Magnaporthe grisea* and Rice Screening for Resistance to Blast Disease in Nigeria	Dr. D.D Kuta, NCRI
15:15-15:30	COFFEE BREAK	
Discussion session - Outputs, Linkages and Further work		
15:30-15:45	DFID-CPP Rice projects cluster	Dr. T.C.B. Chancellor, DFID-CPP
15:45-16:00	Further work for sustainable blast resistance/ management	Dr. S. Sreenivasaprasad
16:00-17:00	Outputs, Linkages, Further work and Recommendations Open Discusion	led by Prof. K.A. Oduro, Univ. of Ghana
17:00-17:15	Closing remarks and Farewell	Dr. E. Otoo, CRI

Rice blast pathogen *Magnaporthe grisea*: Methodologies for assessing genetic and pathogenic diversity

*S. Muthumeenakshi, J. Chipili and S. Sreenivasaprasad**
Warwick HRI
Warwickshire, CV35 9EF,UK.

Summary

Methodologies used for characterisation of genetic and pathogenic diversity of *Magnaporthe grisea* populations are described. These include isolation and storage of *M. grisea* isolates, various steps in MGR586 DNA fingerprinting and pathotyping under controlled conditions. Some of these methods can be further adapted to suit varying laboratory conditions in developing countries.

1. Isolation of *Magnaporthe grisea* from blast samples and storage

The infected plant part for e. g. leaf, stem or sheath is cut into small pieces around the area showing the blast lesion including the edge of the lesion. All pieces from a sample are placed in a small Petri dish. Surface sterilisation and washings are done in this container. Surface sterilisation with 1% sodium hypochlorite (bleach) for 1:30 min is followed by 3 washes with sterile distilled water. Then these plant pieces are placed in Petri dishes lined with moist filter papers and incubated at 25^{o}C for 24h to encourage sporulation. After incubation, these plant pieces are examined under stereo-dissecting microscope. Abundant *M. grisea* growth and sporulation can be seen from and around the lesions with grey, dense and bushy appearance (Fig. 10).

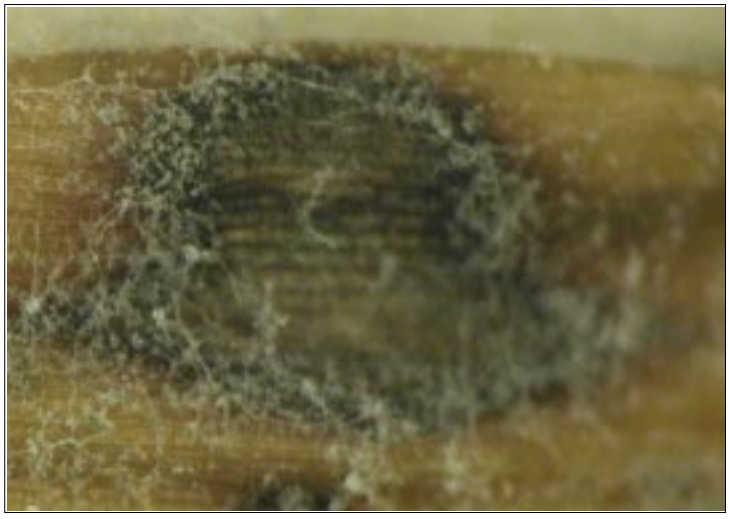

Figure 10. Growth and sporulation of *Magnaporthe grisea* from a blast lesion, following incubation in a moist chamber during the pathogen isolation process.

A sterile moistened needle is used to pick some conidia by brushing the needle across the sporulating lesion. The conidia are placed on oatmeal agar plate containing aureomycin. Plates were incubated at 25°C for about 7-10 days with 12 h darkness and 12 h light. The identity of *M. grisea* can be verified by checking the conidia under light microscope.

Mono-conidial cultures from the field isolate are derived by streaking a loopful of conidial suspension across a water agar (4% w/v) plate in a 'W' pattern, thus spreading the conidia. A guideline can be drawn on the undersurface the plate. Following 24h incubation at 25^{O}C, a germinating conidium can be easily picked and subcultured on to a fresh OMA plate amended with aureomycin using a fine scalpel. *M. grisea* cultures are preserved for long term storage as dried cultures on filter papers. Several sterile filter paper squares (approx. 0.8 cm^2) are placed around the actively growing edge of colonies on OMA plates. The culture is allowed to grow over the filter papers (7-10 days). The filter papers are then removed under aseptic condition and placed in a small Petri dish, in a desiccator and allowed to dry thoroughly. The dried filter papers are then stored at -20^{O} C until required (Valent *et al*., 1991).

2. DNA extraction, electrophoresis and quantification

2.1. Liquid culture preparation

Each *M. grisea* isolate was grown in 2 X Yeast Extract Glucose medium (YEG) (1 l contained glucose, 10.0 g; yeast extract, 2.0 g). Approximately ten plugs (5 mm in size) from an actively growing culture on antibiotic OMA medium were transferred to 100 ml of 2 X YEG medium contained in 250 ml Erlenmeyer flasks and grown at 25°C for 7 days in an orbital shaker (120 rpm). Mycelium was harvested by filtration through No. 3 Whatman filter paper and immediately frozen in liquid nitrogen. The frozen mycelium was pulverised, freeze-dried and ground to a fine powder using a sterile pestle and mortar. The mycelial powder was stored at -20°C until needed.

2.2. DNA extraction

CTAB method (Valent *et al*., 1991; Hamer and Givan, 1990; Sreenivasaprasad, 2000) described below is widely used with *M. grisea* isolates.

1. Mix approx. 300 mg mycelial powder with 4 ml CTAB buffer prewarmed to 65° C in a suitable centrifuge tube. Sterile disposable needles can be used to aid in mixing. Add 40 µl ß-mercaptoethanol in hood.
2. Incubate the tubes at 65°C for 30 min; mix once every 10 min.
3. Add 4 ml CHISAM, stand tubes uncapped for 2 min and mix by incubating on a platform shaker for 15 min (see notes).
4. Centrifuge for 30 min at 13,000 - 18,000 *g*.
5. Transfer the upper aqueous phase into a fresh centrifuge tube, add equal volume of isopropanol and mix.
6. Centrifuge for 10 min at 10,000 *g* to pellet DNA.
7. Discard the supernatant, vacuum dry the pellet briefly to remove traces of isopropanol.
8. Dissolve the DNA pellet in 600 µl sterile distilled water and add 100 µl RNase (20 mg ml^{-1} stock), incubate at 37° C for 30 min.
9. Transfer DNA to a 2.0 ml microfuge tube and add an equal volume of phenol : chloroform : isoamyl alcohol (25:24:1) and mix well by inversion.

10. Centrifuge for 15 min at 12,000 - 14,000 *g*.
11. Collect aqueous phase, add equal volume of CHISAM and incubate on a shaker for 15 min.
12. Centrifuge as above and collect aqueous phase.
13. To precipitate DNA, add 1/10 volume of 3 M sodium acetate, pH 5.2 and 2.5 volumes of cold 100% ethanol (stored at -20° C), mix well and incubate at -20° C for 15 min.
14. Pellet DNA by centrifugation for 5 min as in step 10.
15. Discard supernatant, wash pellet with 1 ml cold 70% ethanol (stored at -20° C) and centrifuge for 2 min as in step 10.
16. Vacuum dry the pellet briefly to remove traces of ethanol.
17. Dissolve the pellet in 300 µl of TE 8 by incubating on a rotawheel/platform shaker; electrophorese a 5 µl aliquot.

2.3. Materials and notes for DNA extraction

1. CTAB buffer: 2% CTAB (hexadecyltrimethylammonium bromide), 100 mM Tris, 10 mM EDTA and 700 mM NaCl. Add ß-mercaptoethanol (1% by volume) just before use (mercaptoethanol is toxic and smells horribly; work in fume-hood, wear gloves).
2. CHISAM: 24:1 (v/v) chloroform/ isoamyl alcohol mixture (volatile; work in fume-hood, wear gloves).
3. Phenol equilibrated to neutral pH can be purchased from Sigma. Store phenol in amber-coloured bottles under a layer of extraction buffer. Phenol is highly toxic and appropriate health and safety regulations such as wearing gloves, goggles, lab coat and working in fume-hood should be followed while handling.
4. RNase solution - Dissolve Ribonuclease I-A (Sigma) 20 mg ml^{-1} in TN buffer (10 mM Tris and 10 mM NaCl, pH 7.5), boil for 15 min to make it DNase free and allow to cool at room temperature for 1 h to permit renaturation of RNase. Store RNase solution at -20° C.
5. TE 8: 10 mM Tris and 1 mM EDTA, pH 8.0.
6. 20 x SSC: 3 M NaCl and 0.3 M sodium citrate, pH 7.0
7. Ethidium bromide: Use a 10 mg ml^{-1} stock (ethidium bromide is a carcinogen; purchase of stock solutions is recommended; adequate care should be taken in handling and disposal).
8. Avoid excess agar with the inoculum while setting up liquid culture for DNA extraction.
9. Break the frozen mycelium into as small pieces as possible to increase the efficiency of freeze drying. Do not allow the frozen mycelium to thaw.
10. While taking powered mycelium for DNA extraction, leave out agar pieces, if any.
11. Some freeze dried mycelial samples do not grind well; using liquid nitrogen (take adequate precautions while handling) or sterile sand while grinding helps the process.
12. Complete mixing of the mycelial powder and extraction buffer into a homogenate increases the efficiency of DNA extraction.
13. Using pipetteman for aliquoting/adding chloroform damages the rubber O-rings; preferable to use a pasteur pipette.
14. After adding chloroform or chloroform and isoamyl alcohol (CHISAM) to the homogenate, wait a couple of minutes before closing the cap and mixing; else the caps pop-off. With hot chloroform the tubes can even explode; loosen the cap to release the pressure, in between mixing.
15. While taking the aqueous layer, leave a clear layer of the inter/solvent phase; purity of the extracted DNA depends on avoiding these contaminants.

16. Use cut pipette tips or sterile transfer pipettes, while removing the aqueous phase, to avoid shearing of DNA.
17. Do not over dry DNA pellets; leads to problems in dissolving the DNA. Leaving the tubes on a rotawheel for several hours at room temperature or warming at 37-55° C for 15 - 30 min is useful; but do not pipette the solution up and down as it shears the DNA. Undissolved residues, if any, can be removed by brief centrifugation and collect the supernatant into a clean tube.
18. If the extracted DNA is not sufficiently pure (i.e. not amenable to restriction enzyme digestion and/or PCR amplification), repeat phenol : chloroform extraction and ethanol precipitation. Alternatively, Qiagen columns (QIAquick) can be used to purify 5-10 µg DNA, following the manufacturer's instructions.

2.4. Agarose gel electrophoresis and DNA quantification

DNA samples were electrophoresed on 0.8% agarose gels prepared with 1 X Tris Borate-EDTA (TBE) pH 8.3 (Tris, 108 g; Boric acid, 55 g; EDTA, 7.44 g) and using 1 X TBE as the running buffer. All agarose gels were processed in a constant electric field, in a horizontal configuration. The gel contained ethidium bromide, the DNA intercalating fluorescent dye at a concentration of 0.5 ìg ml^{-1}. The stock solution for ethidium bromide was purchased ready prepared (e.g. Life Tech, UK). For loading agarose gels, 4 X loading buffer (100 ml contained 40 ml 10 X TBE, 60 ml of 50% glycerol and 250 mg bromophenol blue) was added at an appropriate volume to the samples.

The DNA samples were viewed using an UV transilluminator (254 nm) and photographic records made when necessary. The concentration of DNA in a sample was estimated by ethidium bromide fluorescence comparing the intensity of known quantities of marker DNA and/or by spectrophotometry. Absorbance was recorded at 260 nm and 1 OD corresponds to 50 µg ml^{-1} of double-stranded DNA (Sambrook *et al*., 1989). DNA extracted as above is usually high quality and is suitable for a range of molecular analysis such as fingerprinting and RFLPs by hybridisation, RAPDs, AFLPs and SSRs by PCR as well as nucleotide sequencing. We have also adapted two other DNA extraction methods (incorporating DNA clean-up columns from Qiagen) which are simple and quick and DNA preparations from these can also be used for various applications mentioned above.

3. Molecular typing

3.1. DNA restriction and Southern blotting

Genomic DNA (2-3 µg) was digested with 20-25 U of *Eco* RI (Boehringer Mannheim) at 37°C overnight. Digested DNA (usually 20 µl reactions) was fractionated by electrophoresis in 0.8% agarose gels for 24 h at 60 V with *Hin*d III-digested ë DNA (Boehringer Mannheim) as a molecular size marker. On completion of electrophoresis, mobility (cm) of the molecular size marker was recorded using an UV transilluminator. The bottom left-hand corner of the gel was cut to record its orientation.

DNA was denatured by soaking the gel for 45 min in denaturing buffer containing 1.5 M NaCl and 0.5 N NaOH with constant gentle agitation (up to 1 l denaturing solution/gel) at room temperature. The gel was rinsed twice with de-ionised water and then soaked for 30 min in a neutralization solution containing 1 M Tris and 1.5 M NaCl (pH 7.4) at room temperature with constant gentle agitation. The neutralization step was repeated for 15 min with fresh neutralization solution. When removed from this solution, the gel was inverted and placed on a platform covered with 3 layers of Whatman No. 1 filter paper which acted as a wick by dipping into transfer buffer (10 X SSC) held in a tray. Nylon membrane

(Hybond N, Amersham) was cut to the size of the gel, rinsed with sterile distilled water, soaked in 10 X SSC for 5 min and placed over the gel. Air bubbles between the gel and the membrane were expelled and the edges of the gel were covered with strips of parafilm to ensure buffer flows through the gel only. Four layers of Whatman No. 3 filter paper, cut to the size of the nylon membrane, were soaked in 2 X SSC and placed on top of the membrane. To draw the transfer buffer through the gel 2-ply tissue paper folded to the size of the gel was stacked to approximately 6 cm on top of the filter paper and parafilm at the edge of the gel. A weight of approximately 500 g was placed on the tissue pad (Sambrook *et al*., 1989). Capillary transfer proceeded for 16-20 h, at the end of which the membrane was removed and the orientation of the gel and positions of the slots were marked. The membrane was then air-dried on benchkote (Sambrook *et al*., 1989) for about 20 min. DNA fragments, which transferred from the gel, were immobilized on the membrane by baking for 90 min in an oven at 80°C (Gallenkamp) and also by UV cross-linking at 35 mw cm^{-2} on an Appligene transilluminator. The membrane was then wrapped loosely in aluminium foil and stored at room temperature until use. Use of N^+ membranes, alkali blotting (Sambrook *et al*., 1989) and maintaining the membrane wet throughout the process further improves the resolution level of the fingerprints.

3.2. Preparation of MGR586 probe and hybridization

MGR586 probe (Levy *et al*., 1991) was labelled by the random priming method with 50 ìCi Redivue (^{32}P) dCTP (Amersham) using the rediprime DNA labelling system (Amersham) according to the manufacturer's instructions. Removal of the unincorporated ^{32}P improved the fingerprint resolution and background.

Pre-hybridization solution (6X SSC, 5 X Denhardt's solution, 0.5% SDS and 100 µgml^{-1} denatured herring sperm DNA) was prepared by dissolving one hybridization buffer tablet (Amersham) in 10 ml sterile distilled water. Pre-hybrization of membranes bearing restriction fragments was carried out in 10 ml pre-hybridization solution per membrane in a cylinder in an hybridization oven (Hybaid) at 65°C for at least 3 h. The labelled probe was denatured (boiled for 5 min and flash cooled on ice) and added to the pre-hybridization solution. Hybridization was performed overnight at 65°C. Post-hybridization washes consisted of three washes of 30 min each in 2 X SSC and 0.1% SDS and three washes of 30 min each in 0.1 X SSC and 0.1% SDS.

3.3. Autoradiography and analysis of fingerprints

After the final post-hybridization wash, the membrane was placed on backing paper (non-absorbent side of Whatman benchkote) and wrapped with cling film. It was then exposed to X-ray film (Genetic Research Instrumentation) using intensifying screens at -70°C. Autoradiographs were developed after 12 – 48 h. Similarity between MGR fingerprints among the *M. grisea* isolates was calculated using the formula: $S_{xy} = 2n_{xy}/n_x+n_y$, where n_{xy} is the number of shared fragments, and n_x and n_y are the number of fragments in isolates x and y. Further, presence and absence of all hybridization bands in the 0.8-20 kb size range was scored and a binary matrix computed. Cluster analysis by the group average method, UPGMA was performed to generate dendrograms. Based on the fingerprint patterns, similarity values (approx. above 75%) and grouping on the dendrograms, genetic groups (lineages) were identified (Levy *et al*., 1991).

3.4. RAPD- PCR and data analysis

A range of primers e.g. A1 (5' CAGGCCCTTC 3'), A13 (5' CAGCACCCAC 3') and B10 (5' CTGCTGGGAC 3') were used for RAPD PCR, as it is essential to build a composite picture of RAPD profiles for each isolate. These primers can be ordered from commercial companies such as Sigma-Genosys or Operon. Reaction mixtures contained 5 ìl of genomic DNA (10 - 20 ng), 5 ìl of 10 X Dynazyme buffer (Flowgen), 0.5 ìl of 10 mM dNTP mix, 2.5 ìl of primer (15 ng $ìl^{-1}$ stock), 0.5 ìl (1 U) of Dynazyme DNA polymerase and 36.5 ìl SDW to give a total volume of 50 ìl. Alternatively red-Taq ready mix (e.g. Sigma) that incorporates all of the PCR components can be used to achieve better consistency and to avoid cross-contamination etc. Amplification cycles were as follows: initial denaturation of 2 min at 95°C; 35 cycles of 1 min at 94°C, 30 s at 40°C and 2 min at 72°C followed by a final extension of 5 min at 72°C. After amplification the products (20 ìl) were separated in 20 x 20 cm 1.4% agarose gels for about 2 h at 100 V. Molecular size marker Type VI (Boeringher Manheim) was loaded appropriately with every set of samples.

Each amplified fragment was treated as a separate character. DNA fragments of the same size were assumed to represent the same genetic locus and scored as a common fragment. For cluster analysis, a binary matrix with presence (1) and absence (0) of each fragment was constructed for all the isolates for each primer was generated and data from all ten primers was combined. Pairwise similarity coefficients were computed and the dendrogram was generated by cluster analysis following the group average method (UPGMA) using the Genstat programme (Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK).

3.5. Other markers and adaptations

PCR based markers such as AFLPs, SSR-PCR and REP-PCR as well as sequence data from ribosomal RNA gene block spacer regions are also useful for characterisation of *M. grisea* isolates and would offer varying levels of resolution. We have screened a wide range of AFLP and SSR-PCR primers against *M. grisea* isolates and some primer combinations are more informative to assess genetic diversity. Further, methodologies used for preparation of mycelial material, DNA extraction, electrophoresis and MGR586 hybridisation/fingerprinting could be adapted (Muthumeenakshi *et al*., unpublished) to suit the requirements of laboratories in developing countries.

4. Pathotyping and resistance screening

4.1. Inoculum preparation

For spray inoculation of rice seedlings in the pathotyping experiments, aqueous conidial suspensions (10^5 conidia ml^{-1}) were prepared from 2-3 week old cultures of *M. grisea*. The conidial suspensions contained 0.1% (w/v) gelatin to facilitate adhesion of the conidia to the leaves. Conidia from each mono-conidial *M. grisea* isolate grown on Oat Meal Agar plates were collected by washing with sterile distilled water containing gelatin and the suspensions were filtered through Miracloth to remove mycelial fragments. Conidia were counted using a haemocytometer and, where necessary, the suspensions were adjusted to 10^5 conidia ml^{-1}.

4.2. Inoculation, disease scoring and pathotype designation

Pathotyping of *M. grisea* isolates was undertaken using the international rice differential set : A, Raminad Str. 3; B, Zenith; C, NP-125; D, Usen; E, Dular; F, Kanto 51; G, Sha-tiao-tsao and H, Caloro including B40 and CO39 which were used as standard checks (seeds obtained from WARDA). The eight international rice differentials selected from different rice growing regions by a co-ordinated effort provides a common set of cultivars for comparing the virulence spectrum (uniform pathotype designations) of *M. grisea* populations in different parts of the world.

Ten seedlings of each international rice differential variety and checks were grown to three-to-four leaf stage (18-21 days after planting) under greenhouse conditions in a plastic tray using John Innes No. 2 compost and were replicated three times. The seedlings were spray inoculated (using a badger airbrush) with 30 ml aqueous conidial suspension (i.e. 10 ml per tray) held inside polythene bags. Controls included seedlings sprayed only with the gelatin solution. Following spray inoculation, the polythene bags were sealed-up for two days to maintain high humidity. The polythene bags were removed after 48 h and the trays were maintained in growth chambers with a 12h photoperiod and set at 25ºC.

Host responses were scored visually for lesion type, 7 days after inoculation. Disease reaction was based on a 0-5 scale (Valent *et al*., 1991). A score of 0 and 1 being recorded as an incompatible (R) reaction. Lesion type 2 or greater or if the majority of seedlings exhibited fully sporulating lesions was recorded as a compatible (S) reaction. Pathotype designation was based on the nomenclature of Ling and Ou (1969), who developed the system for differentiating *M. grisea* by their reactions (virulence spectrum) on the international set of rice differentials.

The international pathotypes/races are designated as 'I' (for international), followed by A, B, C, D, E, F, G, or H - each representing one of the differentials, according to the first susceptible variety when the eight varieties are examined in the same sequence and a specific number to indicate a particular virulence pattern. For example an *M. grisea* isolate that shows S, R, R, R, R, R, R, R reactions on A, B, C, D, E, F, G, H, respectively is designated as IA-128. Based on the eight differential varieties, with each variety showing either susceptible (S) or resistant (R) reaction, a maximum of 256 races (2^8) of pathotypes/races can be differentiated. The 256 pathotypes/races consist of 128, 64, 32, 16, 8, 4, 2 and 1 races in the IA, IB, IC, ID, IE, IF, IG and IH groups, respectively. Pathotype II-1 was added to accommodate *M. grisea* isolates incompatible to all eight differentials (Ling and Ou, 1969).

4.3. Resistance screening

Various rice cultivars provided by WARDA and NARS in Ghana were screened against some of the characterised blast isolates from different lineages following the seedling model used for pathotyping, with five replicates and a randomised block design. Mean disease reactions, subjected to REML variance components analysis, are expressed on the basis of the 0-5 scale of Valent *et al*. (1991).

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Dr. J. Chipili has recently re-joined the Zambian Ministry of Agriculture.

***For correspondence**

Dr. S. (Prasad) Sreenivasaprasad, Research Leader & Project Co-ordinator, Environmental Microbiology, Warwick HRI, Wellesbourne, Warwickshire CV35 9EF, United Kingdom, Tel.: 01789-470382, Fax.: 01789-470552, E. Mail: ss.prasad@hri.ac.uk.

Poster

Population structure of the rice blast pathogen in West Africa and utilisation of host resistance

J. Chipili, S. Sreenivasaprasad*, S. Muthumeenakshi
Horticulture Research International, Warwick CV35 9EF, UK
Y. Séré, WARDA, Côte d'Ivoire; S.K. Nutsugah, SARI & J. Twumasi, CRI, Ghana
A.E. Brown, Queen's Univ, Belfast, UK & N.J. Talbot, Univ of Exeter, UK

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Blast affected rice crop

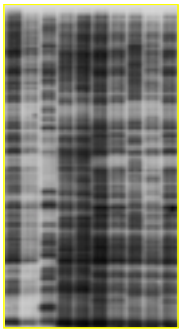
- In West Africa rice consumption is increasing and production remains low.
- Blast caused by *Magnaporthe grisea* is a serious problem in various ecologies
- Molecular tools and pathological assays used to characterise pathogen populations
- Collaborative research linking West African institutes and UK organisations.

Some of the key sites

Materials and Methods

- Strategic blast sampling in and around rice screening sites
- MGR586 fingerprinting (300 isolates) for genetic diversity
- Pathotyping (150 isolates) on international differentials
- Response of rice varieties/lines to lineage representatives.

Results



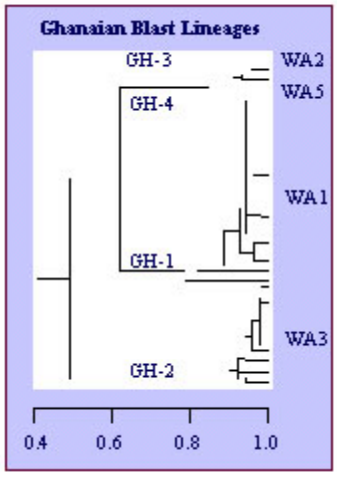
MGR586 profiles of blast isolates

- Blast lineages (genetic groups) varied from 2-5 per country; up to 16 lineages in 4 countries
- In each country one or two lineages were dominant

  GH-1 (56%) was present across Ghana on up to 20 rice varieties/lines

  BF-1 included more than 70% of isolates from rice, wild rice and weeds in Burkina Faso

  CD-1 and CD-2 were 38 – 56% in Côte d' Ivoire comprising isolates collected over a five year period
- Distribution of some lineages was restricted, GH-2 (31%) mainly from Eastern Ghana; appearing at low frequency in Northern Ghana
- Lineages common among the four countries were identified and nine distinct West African blast lineages designated WA1 - WA9



- High pathotype diversity, 16 - 25 in each country

  IB (particularly IB-1) was the dominant pathotype group in Ghana and Nigeria

  IC was the prominent (43%) pathotype group in Burkina, a range of pathotypes were present at certain sites (e.g. Farako-Ba)

  In Côte d'Ivoire IA, IB, IC and ID were 16 - 29%.

Conclusions and Future

- WA1, WA2 and WA3 are the major West African blast lineages
- Different types of blast caused by isolates in the same lineage
- Rice pathogenic blast populations present on wild rice/weeds
- A range of varieties/lines show resistance response to blast lineage representatives tested, and at characterised sites
- Framework for efficient utilisation of host resistance
- Regional extension of blast pathogen characterisation
- Participatory testing of resistant material and exploitation of major gene resistance.

Project R7552

DFID Department for International Development
CPP

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Abbreviations

CRI	Crops Research Institute, Ghana
CSRI	Council for Scientific and Industrial Research, Ghana
DFID-CPP	Department for International Development–Crop Protection Programme, United Kingdom
HRI	Horticulture Research International, United Kingdom
INERA	Institut de l'Environnement et de Recherches Agricoles, Burkina Faso
NARI	National Agricultural Research Institute, The Gambia
NARS	national agricultural research system(s)
NCRI	National Cereals Research Institute, Nigeria
SARI	Savanna Agricultural Research Institute, Ghana
WARDA	West Africa Rice Development Association, now the Africa Rice Center

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CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo (Mexico, DF, Mexico)
CIP	Centro Internacional de la Papa (Lima, Peru)
ICARDA	International Center for Agricultural Research in the Dry Areas (Aleppo, Syria)
ICLARM	WorldFish Center (Penang, Malaysia)
ICRAF	World Agroforestry Centre (Nairobi, Kenya)
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics (Patencheru, India)
IFPRI	International Food Policy Research Institute (Washington, DC, USA)
IITA	International Institute of Tropical Agriculture (Ibadan, Nigeria)
ILRI	International Livestock Research Institute (Nairobi, Kenya)
IPGRI	International Plant Genetic Resources Institute (Rome, Italy)
IRRI	International Rice Research Institute (Los Baños, Philippines)
ISNAR	International Service for National Agricultural Research (The Hague, Netherlands)
IWMI	International Water Management Institute (Colombo, Sri Lanka)
WARDA	The Africa Rice Center (Bouaké, Côte d'Ivoire)

WARDA – The Africa Rice Center

01 B.P. 2551, Bouaké 01, Côte d'Ivoire
01 B.P. 4029, Abidjan 01, Côte d'Ivoire

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