R6738: Identification and characterisation of key screening sites for blast and scald resistance in West Africa

Final Technical Report

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Executive Summary
In response to the demand evidenced by a request from the West Africa Rice Development Association (WARDA), this project was established to characterise the molecular and phenotypic variability of rice leaf scald (*Monographella albescens*) and rice blast (*Magnaporthe grisea*) populations at selected WARDA and NARS rice trial sites. This characterisation was intended to identify those sites best suited to use for the screening of rice varieties for disease resistance traits, in support of improvement and promotion of environmentally-benign methods of rice disease control in the region.

Three trial sites in Nigeria and three in Ghana (the latter being trial sites for the DFID-PSP study on farmer-participatory variety selection for upland rice) were surveyed throughout the duration of the project, and samples of the two pathogens subjected to molecular and pathotypical analyses. Additional samples were also obtained from nearby farmers fields for comparative purposes. Blast samples collected from screening sites in Côte d' Ivoire and Burkina Faso were also characterised for their genetic and pathogenic diversity. Dr. Y. Séré, the Principal Pathologist at WARDA, completed a three month training and research attachment on blast analysis in the UK, Mr. J. Chipili was qualified to Ph.D. level and Dr. S. Nutsugah of the Savanna Agricultural Research Institute (SARI), Ghana completed a 3-week training attachment to the UK for familiarisation with the blast and scald analytical techniques being employed.

The project enables, for the first time, generation of profiles of both molecular and pathotypical variability of *M. albescens* populations across a broad geographical region. The data indicate a high degree of genetic and phenotypic uniformity both within and between populations in West Africa, rather than the existence of distinct 'races'. Reference strains obtained from Colombia and the Philippines also fell within the range of variability observed for the W. African populations. As a consequence of these findings, is was concluded that all of the W. African sites examined are suitable for use in future scald-resistance screening trials.

Assessment of rice cultivars has demonstrated that, while all cultivars are at least partially susceptible to *M. albescens*, a number of the cultivars held within local germplasm collections show above-average resistance to lesion development. Identification of less susceptible cultivars will facilitate exploitation of this material, which may be used as donor germplasm for field resistance traits. Future uptake pathways to develop these findings include WARDA / NARS breeding programmes.

Molecular and pathological tools were applied to characterise more than 200 *M. grisea* isolates from a range of rice cultivars/breeding lines, weedy rices and weeds collected from key WARDA and NARS screening sites in Ghana and Nigeria. At WARDA's request, additional samples from sites in Burkina Faso and Côte d'Ivoire were also characterised, under add-on funding (see Additional Outputs, 1998).

Based on MGR586 fingerprint analysis, seven blast pathogen lineages were identified from Burkina Faso, five from Côte d'Ivoire, three from Ghana and two from Nigeria. Analysis of the virulence spectrum of representative *M. grisea* isolates led to the identification of 28 pathotypes in Burkina Faso, 11 in Côte d' Ivoire, 21 in Ghana and 16 in Nigeria indicating a high degree of pathotype diversity. Data generated so far has provided a much improved understanding of the lineage-pathotype relationships. Pathotype heterogeneity appears to be common in most lineages. *M. grisea* isolates from weedy rices and weeds were found to be pathogenic on rice, indicating the potential of some of these hosts to harbour *M. grisea* populations pathogenic to rice.

This study has provided the baseline data on the *M. grisea* lineage and pathotype diversity and distribution new to this region. This has enabled characterisation of a number of key sites used in the regional and national breeding programmes. In addition, this will also permit further monitoring essential to understand any shifts in the blast pathogen populations. The putative blast resistant germplasm identified by the IPM/Breeding-Task Forces of WARDA, or utilised under the INGER-AFRICA programme, can be tested against lineage representatives to identify potential donors for lineage-exclusion tests. This would pave the way to develop a structured blast resistance breeding and deployment programme appropriate to the diversity and distribution of the pathogen populations.

Background

Rice is an increasingly important crop in West Africa, with production undergoing a considerable degree of intensification. The rice blast pathogen, *Magnaporthe grisea*, was identified by the West Africa Rice Development Association (WARDA) as the primary fungal pathogen of rainfed rice crops across the region. Rice leaf scald, caused by *Monographella albescens*, was observed to be less damaging on an annual basis, but comprised a ubiquitous presence capable of serious outbreaks under favourable local conditions, and is commonly present even on more blast-resistant rice cultivars. Typical scald symptoms are illustrated in figure 1.

No information was available regarding the genetic and pathological variability of the *M. albescens* populations in W. Africa, and only very limited assessment of pathological variability within Asian strains has been published. Minimal information was available on the *M. grisea* populations. R6738 was therefore established to provide data on these populations, through assessment of the two fungal species at existing WARDA and NARS trial sites in Nigeria and Ghana. This present work was intended to provide baseline information on their variability across the region, and to identify trial sites best suited for screening rice germplasm for resistance to the two disease pathogens. Identification of other pathogens that may present a future threat to the resultant blast / scald resistant material was also sought.

Figure 1: Typical symptoms of rice leaf scald on material collected in W. Africa. Lesions generally progress from leaf tips and margins, developing characteristic light-and-dark striations.

Despite its world-wide occurrence, relatively little is known about the molecular and pathological variability of *M. albescens*. Most previous research has been directed towards empirical testing of rice lines for resistance or tolerance (e.g. Das, 1976; Verma and Singh, 1982; Singh and Gupta, 1983), often with conflicting reports for given cultivars, suggesting a degree of geographic specialisation in the pathogen populations. Some more rigorous testing has been conducted under controlled conditions. Bonman *et al.* (1990), used an *in vitro* method to assess the susceptibility of a range of cultivars to specific isolates of *M. albescens*. Although Bonman *et al.* were able to demonstrate a range of susceptibility in the cultivars tested, with none showing complete resistance, only four isolates were used in the tests – all collected from one country. Previous work (DFID-funded project R5336) on the biology and infection processes of *M. albescens* in Côte d' Ivoire, conducting pathogenicity tests of selected isolates against W. African rice varieties indicated that it might be possible to differentiate fungal strains on the basis of their virulence. In that project, the molecular variability of *M. albescens* was assessed for the first time. The results indicated that this approach might provide a useful tool for comparison of *M. albescens* populations (Turner and Black, 1996; Turner and Black, 2000). The current project utilised these findings in assessing the *M. albescens* populations at the trial sites in Nigeria and Ghana, as outlined above.

As noted above, *M. grisea* has been the subject of intensive research world-wide. Breeding for stable blast resistance has been extremely difficult and there have been conflicting opinions on the diversity and stability of blast pathotypes (Zeigler *et al*., 1995). Hamer *et al*. (1989) discovered repetitive DNA elements in the *M. grisea* genome and one such element, MGR586 has been widely used to partition the blast pathogen diversity into distinct lineages in a number of geographic locations (Levy *et al*., 1991, 1993; Zeigler *et al*., 1995; Roumen *et al*., 1997).

The pathotype diversity results, together with the recent definition of lineages have allowed a higher resolution of the pathogen population structure and an understanding of the virulence diversities of the various lineages. Novel breeding strategies based on 'lineage-exclusion' are being tested in various rice growing regions with a view to prolonging the durability of resistant cultivars (Zeigler *et al*., 1995; Gnanamanickam *et al*., 1998). The 'lineage-exclusion' method proposes bringing together into cultivars those genes that are each effective against an entire 'lineage', so that durable resistance can be achieved against all the lineages prevalent in a region (Levy *et al*., 1993; Zeigler et al., 1994). Under project R5698: Molecular Differentiation of Rice Blast Pathotypes (Brown, 1995), molecular and pathotyping protocols were established and a range of *M. grisea* isolates originating from India, Sri Lanka and West Africa were characterised. The current project utilised the outputs of X0236 to characterise the blast pathogen diversity at some of the WARDA and NARES rice screening sites in West Africa.

Project Purpose

Improved methods for the control of rice pathogens developed and promoted, by means of improving the ability of breeders to generate rice material appropriate for the ecosystems and farmers' preferences, and which is resistant to local pathogen populations.

The project outputs are directed towards uptake by WARDA and its member NARS. WARDA is responsible for the co-ordination and promotion of rice research and development throughout West Africa and as such is ideally suited to promote use of the project outputs. The specific objectives of this project are to provide information on the variability and distribution of the rice blast and scald pathogen populations at rice screening sites, in order to identify those sites best suited to use in future resistance screening programmes, for the development of suitable disease resistant cultivars. The information generated within the time-scale of the current project will be utilised by breeding programmes extending for a number of years thereafter.

Research Activities

1: Trial site surveys:

In each year of the project, three trial sites in Nigeria were surveyed and sampled by members of the WARDA IPM taskforce. Project staff also surveyed and sampled trial sites in Ghana each year, working in partnership with Dr. Twumasi and Dr. Dartey of the Crops Research Institute (CRI) in the forest zone, and with Dr. Nutsugah of the Savanna Agricultural Research Institute (SARI) in the Sahelian zone. Additional sampling of nearby farmers' fields was conducted, to permit comparison of pathogen populations in farmers' fields with those on the trial sites. Blast samples from rice varieties/breeding lines, wild rice and weeds were also collected from WARDA screening sites in Côte d' Ivoire and Burkina Faso through surveys co-ordinated by WARDA.

2. Isolation of M. grisea and M. albescens:

Isolations of *M. grisea* and *M. albescens* were made from the collected material, as follows.

2.1: Isolation of M. albescens:

A single lesion was taken from each leaf sample, and cut into small pieces (approx. $1mm^2$). These were surface sterilised for 10 seconds in ethanol, then for 90 seconds in 10 % sodium hypochlorite (bleach) solution. After three rinses in sterile distilled water, the leaf pieces were placed on 2% water agar plates and incubated in the dark at 25°C for 24 hours. Single hyphal tips emerging from these leaf pieces were excised and transferred to potato dextrose agar and maintained at 25°C for a further 2-3 days. Isolates were then identified on the basis of morphological characteristics, followed, in selected cases, by re-inoculation onto rice leaves for symptom expression. At least three isolations were made from each lesion.

2.2: Isolation of M. grisea:

More than four hundred and fifty rice blast samples were collected from various screening sites. Blast lesions less than 1.0 cm in size were surface sterilised in 1% sodium hypochlorite for 1.5 min followed by 3 rinses in sterile water before placing the pieces on moist filter paper in a Petri dish at 25°C for 24 h to induce sporulation.

For preparing a field isolate, *M. grisea* spores were collected under a binocular dissecting microscope and their identity confirmed by light microscopy. Spores were placed on Oat Meal Agar (OMA) plates containing Aureomycin (50 mg l^{-1}) and incubated at 25°C for about 7-10 days with 12 h light/12 h dark cycle. To prepare mono-conidial cultures, a small quantity of spores was harvested on a sterile moistened needle tip and a suspension prepared in a droplet of sterile distilled water. The suspension was streaked across a Petri dish containing 4% (w/v) Water Agar in a 'W' pattern. After 24 h incubation at 25ºC, agar fragment bearing a single conidium was transferred to a fresh OMA plate. For preservation, several small sterile filter paper discs (5 mm) were aseptically placed in front of the actively growing edge of colonies. Filter paper discs were overgrown with *M. grisea* mycelium and spores (7-10 days) were removed, dried in a dessicator and stored at -20°C (Valent *et al*., 1991). Details of the *Magnaporthe grisea* isolates used in this study are given in Appendix 3, Table 1.

3. Molecular and pathotypical analyses of the pathogens:

DNA was extracted from each of the pathogen isolates, and subjected to molecular analyses, as described below, to determine the genetic variability of the pathogen populations within and across the trial sites under investigation. Key isolates were selected from each of the sites, and bio-assays used to determine their pathogenicity against a range of rice cultivars, as outlined below.

3.1: M. grisea pathotypical analysis:

Pathotyping of *M. grisea* isolates was undertaken in the UK by virulence spectrum analysis on the eight cultivars of the international rice differential set - Raminad Strain 3, Zenith, NP-125, Usen, Dular, Kanto 51, Sha-tiao-tsao and Caloro and two susceptible checks B40 and CO39, replicated three times. Ten seedlings of each differential host were grown to the three-to-four leaf stage (18-21 days after planting) under greenhouse conditions in plastic trays using John Innes No. 2 compost and spray inoculated (using a badger air brush) with 30 ml aqueous conidial suspension (10^5 conidia ml^{-1}) containing gelatin (0.1%). After experiencing significant problems in ensuring spore production from oatmeal agar cultures, this was replaced by an artificial medium which gave markedly more consistent results. Controls included plants sprayed with the gelatin solution. The trays were placed in polythene bags for

two days to maintain high humidity and were maintained in growth chambers with a 12 h photoperiod and set at 25ºC.

Host responses were scored 7 days after inoculation 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 was recorded as a compatible (S) reaction. Pathotype designation was based on the nomenclature of Ling and Ou (1969).

3.2: M. grisea molecular analyses:

M. grisea cultures were grown in 2 X yeast extract glucose liquid medium (Hamer and Givan, 1990). DNA was extracted from freeze dried and ground mycelial powder following the CTAB (hexadecyltrimethyl-ammonium bromide) method (Valent *et al* 1986; Hamer and Givan, 1990). For DNA fingerprinting, *Eco* RI digested DNA blots were hybridised with the MGR586 probe (obtained from Dr. John Hamer, Purdue University, USA; Hamer *et al*., 1989). MGR586 was labelled with ^{32}P using a random labelling system from Amersham, UK and the fingerprints were obtained by standard autoradiography. Nucleotide sequences of the ribosomal DNA internal transcribed spacer (ITS) regions 1 and 2 were determined on an ABI automated sequencer using the Big-dye kit and protocols from ABI. ITS 1 and 2 regions were amplified by PCR using ITS1ext (5' GTAACAAGGTTTCCGTAGGTG '3) and ITS4ext (5' TTCTTTTCCTCCGCTTA-TTGATATGC '3) primers and sequenced using the primers ITS1, ITS2, ITS3 and ITS4 (White et al., 1990). RAPD-PCR was performed using primers A1, A3, A7, A11, A13, B4, B6 and B10 (Operon technologies, USA) and PAP2 (5' TACAACGAGG '3) and PAP3 (5' TGGATTGGTC '3) following standard protocols. MGR586 fingerprints and RAPD-PCR profiles were subjected to cluster analysis and dendrograms were generated by the UPGMA using Genstat 5 (Lawes Agricultural Trust, Harpenden, UK). DNA sequences were edited, assembled and analysed using DNASTAR (DNAstar Inc., USA) and GCG (University of Wisconsin, USA) packages.

3.3: M. albescens pathotypical analysis:

This was conducted using the method of Bonman *et al*.(1990), whereby detached rice leaf segments were inoculated with a 3mm plug of fungal mycelium, aseptically removed from the leading edge of a colony grown on potato dextrose agar (PDA). Inoculated leaves were incubated in a moist chamber at 25^{o}C for 3 days, under a 12hr light:12hr dark cycle. Lesion length was then used to determine the aggressiveness of the *M. albescens* strain on that cultivar. Strains were tested against two sets of rice cultivars. First the set of blast international differentials that were used to characterise the *M. grisea* strains (see 3.1 above) were used to test selected *M. albescens* strains. This was to permit comparison of the behaviour of these cultivars under the two pathogens. Secondly, the same *M. albescens* strains were tested against a set of W. African cultivars such as Moroberekan, a blast-resistant line used in the WARDA breeding programmes.

3.4: M. albescens molecular analyses:

Initially this involved restriction digestion of a polymerase chain reaction (PCR)-amplified region of the genome, specifically the internal transcribed spacer (ITS) region of the genomic ribosomal DNA (rDNA). This region was targeted, since it contains conserved regions, permitting PCR amplification with the 'universal' primers ITS-1 (White *et al.*, 1990) and R635 (Liu and Sinclair, 1993), and the variable ITS1 and ITS2 regions. However, only one of the eight restriction nucleases tested revealed a detectable level of variability in this region. Sequencing of the amplified region from selected isolates confirmed the remarkably low level of variability (Turner, 1999: EMBL Nucleotide Sequence Database, Accession numbers AJ132505, AJ132506, AJ132507, AJ132508 and AJ132509 – see Appendix 5 for details). Consequently, the adjacent but larger (c4.5Kb) intergenic spacer (IGS) region was amplified through PCR, and again subjected to restriction digest fingerprint analysis for variability. This second region revealed sufficient variation to permit comparison of populations from the various trial sites, as described below. Additionally, a subset of isolates were selected, on the basis of maximum variability of IGS restriction fingerprint and geographic origin, for random amplification polymorphic DNA (RAPD) PCR analysis, which is more indicative of variability across the whole genome, rather than within one specific region of the genome. Results of these analyses are presented below (Output 5).

Outputs

Output 1: Key blast screening sites identified and characterised.

In association with the Crops Research Institute (CRI) and the Savanna Agricultural Research Institute (SARI) in Ghana, and with WARDA, surveys have been conducted across Ghana and Nigeria to assess the significance of *M. albescens* and *M. grisea* at rice trial sites and in nearby farmers' fields. Trial sites in Ghana have included those on experimental stations at Kumasi (CRI) and Tamale (SARI), and in the field in the Volta and Western regions, these latter having been established under the DFID Plant Science Research Programme project on farmer-participatory evaluation of rice varieties. Sites in Nigeria have comprised WARDA trial sites at Amakama, Uyo and Ikenne Remo. Samples were collected and disease severity and incidence noted. A high incidence of brown spot (*Bipolaris oryzae*) was also observed in many farm sites across Ghana. Key sites in terms of Ghanaian priorities remain those concerned with farmer-participatory variety evaluation - project outputs relating to the blast populations at these sites will be of significant value to the Ghana-based NARS. Additionally, WARDA is developing key sites at Mbe and Man in Côte d' Ivoire, and also Banfora and Farako-Ba in Burkina Faso as part of the IPM-TF activities. At WARDAs request, additional samples of the blast pathogen from these sites were also characterised, under add-on funding (in 1999) as outlined under *Additional Output (b)* below.

In Burkina Faso, Farako-Ba, Banfora and Sideradougou rank as the sites, in that order, with high blast pathogen diversity. At Farako-Ba, all major lineages (BF-1 to BF-6), and 12 pathotypes representing six international groups (IA, IB, IC, IF, IG and IH) were recorded. At Banfora, five lineages (BF-1, 3, 4, 6 and 7), and six pathotypes representing four international groups (IA, IC, IF and IG) were recorded. At Sideradougou, four lineages (BF-2, 4, 5 and 7), and six pathotypes representing three groups (IA, IC and IF) were recorded (Appendix 4, Figure B1). Farako-Ba and Banfora with all the major lineages recorded so far and a wide range of virulence diversities are key sites suitable for blast resistance screening in Burkina Faso. However, in terms of further monitoring of lineage shifts, Sideradougou is important as the major lineage BF-1 has not been recorded so far at this site and it would be interesting to test whether BF-1 appears at this site.

In Côte d Ivoire, Man displayed high blast pathogen diversity with all five lineages recorded (CD-1 to CD-5) and seven pathotypes representing four international groups (IA, IB, IC and ID) and is a key site suitable for blast resistance screening. Two major lineages (CD-1 and 2) and two pathotype groups (IC and ID) were recorded at Korhogo. Mbe site showed low pathogen diversity and even the dominant lineage CD-1was not recorded (Appendix 4, Figure B2).

In Ghana, Hohoe with two lineages (GH-1 and 2) and four pathotype groups (IA, IB, IC and IG) and Bolgatanga with two lineages (GH-1 and 3) and three pathotype groups (IA, IB and IC) are key blast resistance screening sites (Appendix 4, Figure B3). At Tamale, although none of three lineages were recorded, several ungrouped genotypes were identified and whether any of these develop into dominant lineage(s) merits further investigation. In Nigeria, the dominant lineage NI-1 was present at Badeggi, Oyo and Uyo and all three sites displayed overlapping pathotype groups (Appendix 4, Figure B4) and appear suitable for blast resistance screening. However, further characterisation of *M. grisea* population is necessary to provide improved assessment of blast pathogen diversity at these sites. Man and Mbe sites in Côte d Ivoire (WARDA) as well as Bolgatanga and Hohoe sites (NARS – CRI and SARI) in Ghana are being used for follow on activities in R7552.

M. albescens was found to be present at all sites sampled, although the pathogen populations were observed to be exerting only a low disease pressure at the time of sampling. The characterisation of the molecular and pathogenic traits of *M. albescens* (Outputs 2 and 5) shows that the *M. albescens* populations were, in fact, uniformly distributed variability across the sites. This work therefore confirmed that the presently recommended site selection criteria will be suitable for future resistance screening trials. Further work is recommended, under a follow-on phase, to quantify the threat posed to new rice lines in Ghana by the brown spot pathogen, *Bipolaris oryzae*.

Output 2: Description of genetic organisation and diversity of blast and scald in terms of pathotypes or other relevant descriptors, in relation to location and rice ecosystem.

The genetic organisation and diversity of the scald and blast pathogens were determined across the targeted screening sites (see Appendix 2 for publications and reports).

2.1: Genetic diversity and organisation of M. albescens

As discussed above (Activity 3.4), restriction digest fingerprinting of the ITS region of the ribosomal DNA, a technique that is commonly used to examine molecular variability in fungal species, revealed a remarkably low level of variability within this region for *M. albescens* isolates, even when comparing isolates from material collected across widely divergent geographic origins (i.e. West Africa, Colombia and the Philippines). Sequencing of selected isolates from these locations indicated that, out of a PCR product of approximately 1200bp, only 4 base variations could be detected, one in the ITS1 variable region, three in the ITS2 variable region (see Appendix 5 for published EMBL database entries). The sequence alignment mapping the four variable bases detected is shown in figure 2. As a consequence of the low level of variability within the rDNA shown by this work, a much larger fragment from the nearby IGS region was amplified, and subjected to restriction fingerprint analysis, to yield the dendrogram shown in figure 3 below.

Despite the large number of isolates examined (295), and the large size (4.5Kb) of the PCR-amplified region used to generate these fingerprints, figure 3 shows that a remarkable degree of uniformity was again observed, with 101 isolates (i.e. 34% of the isolates fingerprinted) yielding identical fingerprints. No significant correlation was observed between isolate fingerprint grouping and either geographic or host origin.

The variability within the IGS region that was observed, however, did permit some assessment of the *M. albescens* populations within and between the trial sites in Nigeria and Ghana, and permitted comparison with isolates from Colombia and the Philippines. Although isolates taken from the same sites show some tendency to cluster together, indicating relatedness, this is not a universal observation. Isolates showing very similar or even identical fingerprints were observed from across the sites, indicating that the *M. albescens* populations across the sites examined are not significantly different in terms of their genetic composition.

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CIAT-7:  AGTTAACAACTCTCCAAACCATGTGAACTTACCACTGTTGCCTCGGTGGTTGGC
S-12.1:  -------AACTCTCCAAACCATGTGAACTTACCACTGTTGCCTCGGTGGTTGGC
S-11.1:  ---------CTCTCCAAACCATGTGAACTTACCACTGTTGCCTCGGTGGTTGGC
S-1.1:   ----------TCTCCAAACCATGTGAACTTACCACTGTTGCCTCGGTGGTTGGC
CIAT-11: --------------CAAACCATGTGAACTTACCACTGTTGCCTCGGTGGTTGGC 40

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CIAT-7:  GCTCCTCCCTCTCCGAAAGGGGCGCCGCCGCCGGCGGACAAACTAAACTCTTGTCAACTTTGTCAAATCTGA
S-12.1:  GCTCCTCCCTCTCTGAAAGGGGCGCCGCCGCCGGCGGACAAACTAAACTCTTGTCAACTTTGTCAAATCTGA
S-11.1:  GCTCCTCCCTCTCTGAAAGGGGCGCCGCCGCCGGCGGACAAACTAAACTCTTGTCAACTTTGTCAAATCTGA
S-1.1:   GCTCCTCCCTCTCTGAAAGGGGCGCCGCCGCCGGCGGACAAACTAAACTCTTGTCAACTTTGTCAAATCTGA
CIAT-11: GCTCCTCCCTCTCTGAAAGGGGCGCCGCCGCCGGCGGACAAACTAAACTCTTGTCAACTTTGTCAAATCTGA 132


CIAT-7: ATCTAAACTAAGAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
S-12.1: ATCTAAACTAAGAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
S-11.1: ATCTAAACTAAGAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
S-1.1:  ATCTAAACTAAGAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
CIAT-11:ATCTAAACTAAGAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG 204


CIAT-7: AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATTAG
S-12.1: AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATTAG
S-11.1: AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATTAG
S-1.1:  AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATTAG
CIAT-11:AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATTAG 276


CIAT-7: TATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTGTTGGGAGACTGCGCTA
S-12.1: TATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTGTTGGGAGACTGCGCTA
S-11.1: TATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTGTTGGGAGACTGCGCTA
S-1.1:  TATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTGTTGGGAGACTGCGCTA
CIAT-11:TATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTGTTGGGAGACTGCGCTA 348

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CIAT-7: AACCGCAGCTCCTCAAAACCAGTGGCGGAGTC-CTCTGTGCTCTGAGCGTAGTAATTCTCTATCTCGCTTGTA
S-12.1: AACCGCAGCTCCTCAAAACCAGTGGCGGAGTC-CTCTGTGCTCTGAGCGTAGTAATTCTCTATCTCGCTTGTA
S-11.1: AACCGCAGCTCCTCAAAACCAGTGGCGGAGTCGCTCTGTGCTCTGAGCGTAGTAATTCTCTATCTCGCTTGTA
S-1.1:  AACCGCAGCTCCTCAAAACCAGTGGCGGAGTCGCTCTGTGCTCTGAGCGTAGTAATTCTCTATCTCGCTTGTA
CIAT-11:AACCGCAGCTCCTCAAAACCAGTGGCGGAGTC-CTCTGTGCTCTGAGCGTAGTAATTCTCTATCTCGCTTGTA 420

              *                        *
CIAT-7: TGAACGCAGTGGTCGACGGCCATAAACCGCACCTCTCCCCCTCCAGGGATTGGGCACCTTTT
S-12.1: TGAACGCAGTGGTCGACGGCCATAAACCGCGCCTCTCCCCCTCCAGGGATTGGGCACCTTTT
S-11.1: TGAACGCAGTGGTCGACGGCCATAAACCGCACCTCTCCCCCTCCAGGGATTGGGCACCTTTT
S-1.1:  TGAACGCAGTGGTCGACGGCCATAAACCGCACCTCTCCCCCTCCAGGGATTGGGCACCTTTT
CIAT-11:TGAACGCAGCGGTCGACGGCCATAAACCGCGCCTCTCCCCCTCCAGGGATTGGGCACCTTTT 482
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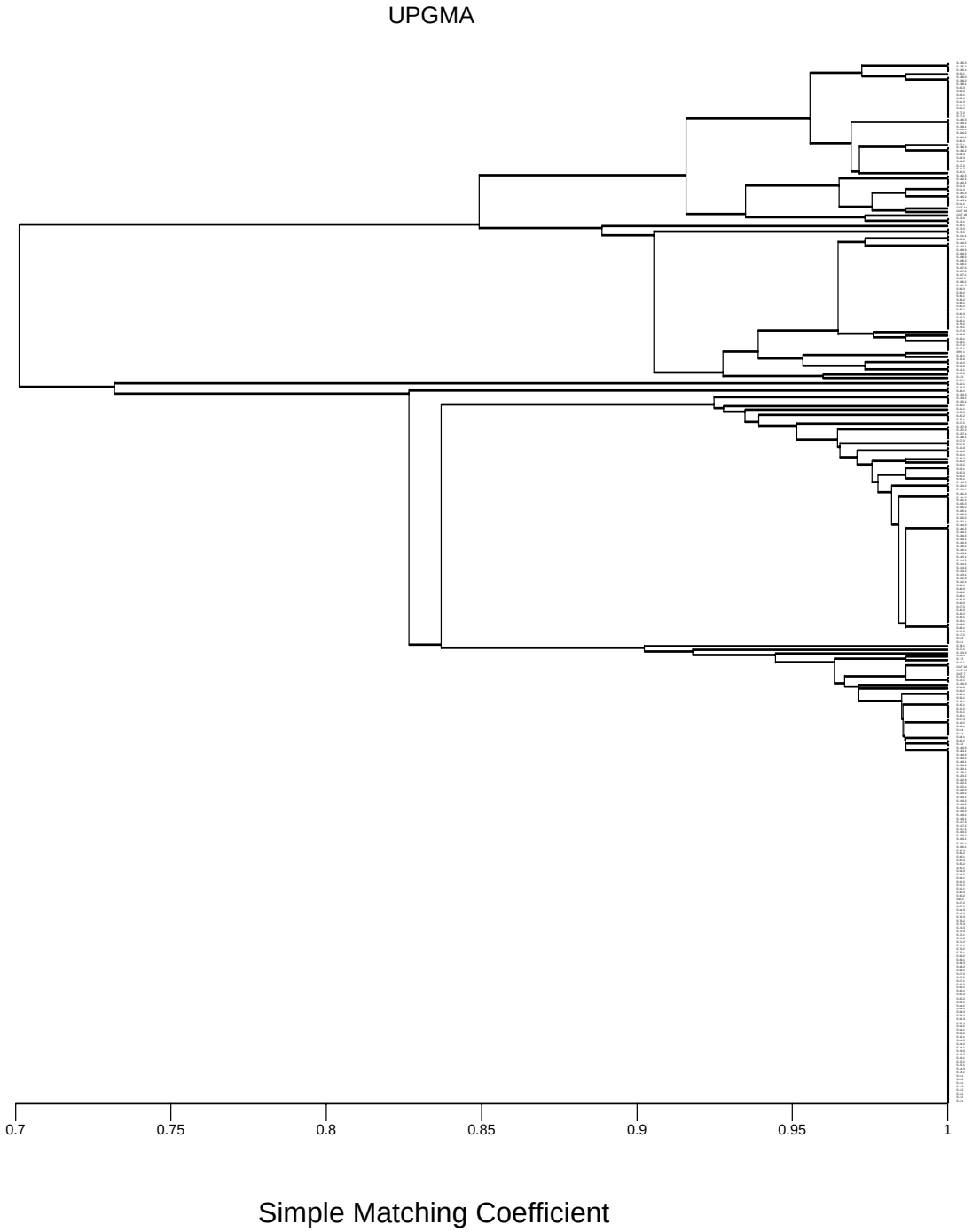
Figure 2: Alignment of ITS-1, 5.8S and ITS-2 sequences from five *M. albescens* isolates (CIAT-7, CIAT-11, S-1.1, S-11.1 and S-12.1). Sequences were aligned using Clustal method with weighted residue weight table, under Winstar Megalign™ software. Points of variation between the sequences are highlighted.

Figure 3: Cluster analysis, using Unweighted Pair Group Method Analysis (MVSP^{TM} software), of restriction digest fingerprints of the IGS region of genomic ribosomal DNA from *M. albescens* isolates.



Given the high proportion of identical fingerprints noted, even for the 4.5Kb IGS region, it was decided to also use RAPD-PCR to examine the molecular variability across the whole genome, using a sub-set of isolates selected to represent all of the trial sites, the non-African reference isolates and all of the major clusters of isolates identified from the IGS restriction fingerprinting. The results of this analysis are show in figure 4 below.

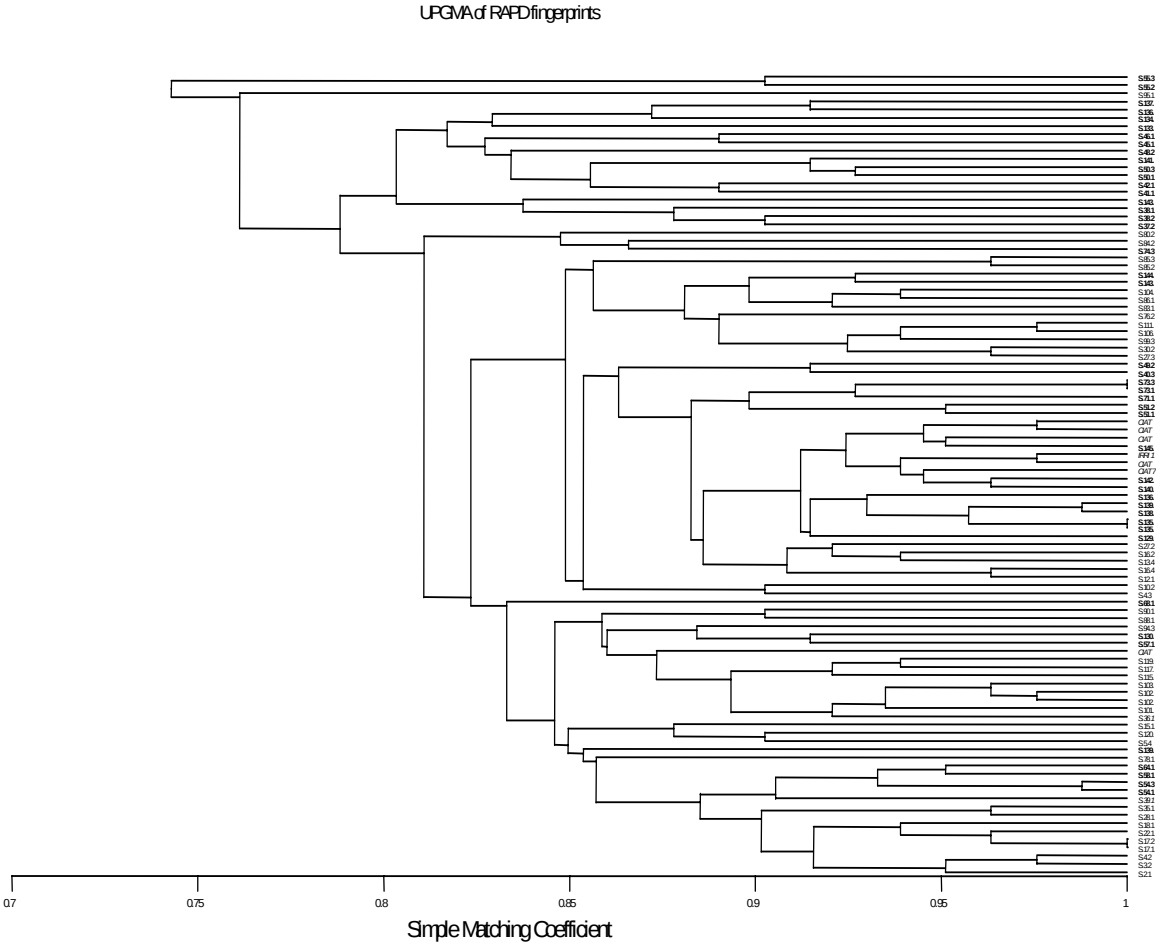


Figure 4: Dendrogram showing clustering of *M. albescens* isolates, determined using Unweighted Pair Group Method Analysis (MVSP^{TM} software), on the basis of RAPD-PCR fingerprints. Isolates from Ghana are indicated in bold face, isolates from Nigeria in normal face, and isolates from other locations (Côte d' Ivoire, Togo, Colombia or the Philippines) are indicated in italics.

As with the restriction digest fingerprints, RAPD fingerprints showed no clear and consistent correlation of clustering with geographic origin, or source cultivar.

Interestingly, although most isolates taken from a single lesion appeared identical under both RAPD-PCR and restriction digest fingerprinting, this was not always the case. A number of samples (for example samples 38, 50, 51, 55 and 143 from Ghana, and samples 4 and 85 from Nigeria) yielded genetically distinct isolates from a single lesion. Histological examination of some leaf samples also revealed the presence of sexual reproductive structures (pycnidia), although it was not possible to confirm that these were *M. albescens* structures through ascospore isolation and germination. Their presence, however, in conjunction with the detection of genetically distinct *M. albescens* strains within single lesions, is indicative of naturally occurring sexual reproduction within *M. albescens* populations in the field, in both Ghana and Nigeria.

No single screening site examined showed an obviously richer or poorer level of molecular variability in its *M. albescens* population compared to the other sites. This, combined with the pathotypical observations noted below, suggests that *M. albescens* populations across the region are relatively uniform, possibly facilitated by the spread of favourable genetic traits through sexual recombination. Since *M. albescens* populations across the region show a high degree of genetic and phenotypic uniformity, rice varieties showing a good level of disease resistance or tolerance at any of the trial sites examined, should be broadly applicable across the region, subject to the influence of local climatic and cultural conditions.

2.2: Diversity and distribution of genetic groups/lineages of Magnaporthe grisea in Burkina Faso, Côte d'Ivoire, Ghana and Nigeria

Based on the similarity of the MGR586 fingerprint patterns obtained for sixty-four *M. grisea* isolates from Burkina Faso (from 22 rice cultivars; some wild rice and non-rice hosts) seven lineages, designated BF-1 to BF-7 were identified (Appendix 4, Figure B5). These isolates were collected in 1996 and 1997 growing seasons and were from two main screening sites, Farako-Ba and Banfora and three other sites, Sideradougou, Vallée du Kou and Labola. BF-1 was the dominant lineage including approximately 30% of the isolates collected and comprised isolates collected in both seasons. BF-1 was present at four of the five sites sampled and on a range of hosts, for e.g. ten rice cultivars/lines (including a local variety), non-rice hosts (*Paspalum*, *Brachiaria*) and wild rice (*O. longistaminata*). However, the bulk of isolates in lineage BF-1 originated from the Farako-Ba site from eight cultivars. Lineage BF-2 comprised 20% of isolates from seven cultivars collected in 1996 mainly from Farako-Ba and was also present at Sideradougou. About 10% of the isolates from Burkina Faso belonged to lineage BF-3, which was recorded from both Farako-Ba and Banfora. Lineage BF-4 comprised four isolates collected from three different rice cultivars from Farako-Ba and two isolates from *O. longistaminata* in Banfora and Sideradougou. One of the isolates, S572, was from wild rice that was growing close to cultivar Usen. Lineages BF-5 and BF-6 comprised five and four isolates, respectively. BF-5 was present at Farako-Ba and Sideradougou, whist BF-6 was recorded from Farako-Ba and Banfora. Two *M. grisea* isolates from *O. longistaminata* and *Setaria* from Banfora and Sideradougou, respectively formed lineage BF-7 (Appendix 3, Table 2). Distribution pattern of the various lineages at the Burkina Faso sites is shown in Appendix 4, Figure B1. Percentage similarity of MGR586 fingerprint profiles of *M. grisea* isolates representing various lineages from Burkina Faso is shown in Appendix 3, Table 9.

M. grisea isolates from Man, M'be, Korhogo and Sakassou sites in Côte d'Ivoire were grouped into five lineages designated CD-1 to CD-5 (Appendix 4, Figure B6). CD-1 isolates were pathogenic to at least eighteen different rice cultivars. CD-1 comprised 50% of *M. grisea* isolates from Côte d' Ivoire and was present at Man, Korhogo and Sakassou sites. Lineage CD-2 consisted of five isolates from four different cultivars and was recorded from Man, Mbe and Korhogo. Lineages CD-3, 4 and 5 comprised five, three and two isolates, respectively and were only recorded from the Man site (Appendix 3, Table 3). Other *M. grisea* isolates from Côte d'Ivoire showed low similarity values (Appendix 3, Table 10) to the above lineages and could form distinct lineages. Some of the lineages appear to be common to Burkina Faso and Côte d' Ivoire and is being further investigated in R7552.

In Ghana, *M. grisea* isolates collected from screening sites at Bolgatanga, Hohoe, Kwadaso and Nyankpala (Tamale) grouped into three distinct lineages designated as GH-1, GH-2 and GH-3 (Appendix 4, Figure B7). Lineage GH-1 was the major lineage comprising 50% of the isolates sampled and was present in the North and the South at the Bolgatanga, Hohoe and Kwadaso sites. GH-1 isolates were pathogenic to at least 13 rice cultivars some of which could have common genetic background for example Tox -related lines. Lineage GH-2 comprised six isolates from six different cultivars and was recorded only from Hohoe. Lineage GH-3 was present only at Bolgatanga and represented two *M. grisea* isolates from red rice (Appendix 3, Table 4). Percentage similarity of MGR586 fingerprint profiles of *M. grisea* isolates from Ghana is shown in Appendix 3, Table 11. Some of the Ghanaian *M. grisea* isolates derived from different sites and cultivars did not readily fit into the above lineages and could represent additional lineages. An *M. grisea* isolate from rice in Kwadaso produced an 'atypical' fingerprint pattern with only four MGR586 hybridising bands. This may reflect the selection of international varieties and gives justification to the development of an appropriate set of tester varieties for use in West Africa, to determine heterogeneity within the region so that appropriate resistance breeding strategies can be developed.

In Nigeria, 21 *M. grisea* isolates produced typical MGR586 fingerprints (e.g. Appendix 4, Figure B9) and 20 formed a common lineage NI-1. One isolate, B58 from Badeggi was quite distinct and showed less than 40% similarity to all other isolates and was designated lineage NI-2 (Appendix 3, Table 12; Appendix 4, Figure B8). Two Nigerian *M. grisea* isolates obtained from rice showed atypical fingerprint patterns with only 7-9 MGR586 hybridising bands. The majority of isolates in lineage NI-1 were from Oyo and several from Tox-related cultivars (Appendix 3, Table 5). NI-1 was present at all three sites sampled, namely Badeggi, Uyo and Oyo. Some isolates in lineage NI-1 from Badeggi appear to be closely related in their fingerprint pattern to isolates in lineage GH-1 from Ghana and CD-1 from Côte d'Ivoire and could belong to the same lineage.

2.3: Diversity and distribution of Magnaporthe grisea pathotypes in Burkina Faso, Côte d'Ivoire, Ghana and Nigeria

The virulence spectrum of representative isolates of some of the *M. grisea* lineages prevalent in Burkina Faso, Côte d'Ivoire, Ghana and Nigeria was determined (Appendix 3, Table 6). In Burkina Faso, 23 pathotypes from 16 different cultivars were identified mainly from Banfora, Farako-Ba, Sideradougou and Vallée du Kou sites. The most frequently observed pathotype group (around 40% of the sampling) was IC. Some of the pathotypes, e.g. IC-1, IC-9, IC-25, IF-1, IG-1 and IG-2 were represented by 2-3 different isolates each. Up to 12 different pathotypes were present at the Farako-Ba site (Appendix 3, Table 6). *M. grisea* isolates S520, S576, S387, S389, S528, S529-2 and S567-1 from wild rice (*O. longistaminata*) at Banfora, Sideradougou and Vallée du Kou, were found to be pathogenic on B40 and CO39 as well as the international differentials. Similarly, isolates collected from non-rice hosts *Paspalum* (S326) and *Setaria* (S508) were observed to be pathogenic (pathotypes IA-105 and IF-2, respectively) when tested on the international differentials. This suggests that the weedy rices and weeds could serve as alternate hosts/inoculum reservoirs in the West African rice ecologies and merit further investigation. Weeds have been identified as a major constraint in the entire continuum of rice production ecologies in West Africa and the potential of some of these hosts to serve as inoculum reservoirs to the blast pathogen *M. grisea* further emphasises the need to develop integrated management strategies.

In Côte d'Ivoire 11 pathotypes were identified from four sites Man, M'be, Korhogo and Sakassou and these were pathogenic to at least nine different rice cultivars/breeding lines (Appendix 3, Table 6). IA and ID (30% each) were the most frequently occurring pathotype groups. One site, Man, expressed pathogen populations with diverse virulence characteristics and seven different pathotypes belonging to four different groups IA, IB, IC and ID were identified.

In Ghana, 21 pathotypes were detected from the four main sites Bolgatanga, Hohoe, Kwadaso and Nyankpala (Tamale). These pathotypes were identified from 17 rice cultivars/lines, four unknown rice cultivars and a weedy rice (Appendix 3, Table 6). In most instances, each isolate showed a distinct virulence spectrum defined by a particular pathotype. The most frequently observed pathotype groups were IB and IC (50% and 33% of the isolates, respectively).

In Nigeria, 16 pathotypes were observed from three sites Badeggi, Oyo and Uyo and IA and IB were the most prominent pathotype groups (30% each) and each of the pathotypes was represented by only one isolate (Appendix 3, Table 6). Two isolates, B12 and IKR10/2 recovered from rice were non-pathogenic on susceptible cultivars B40 and CO39 and also on the international differentials.

Output 3: Molecular profiles for improved blast identification and pathotype diagnosis.

Molecular analyses have led to a good understanding of the diversity and spatial distribution of the blast pathogen lineages at the rice screening sites in Nigeria, Ghana, Côte d' Ivoire and Burkina Faso (Output 2). Analysis of the virulence characteristics of representative isolates has enabled the identification of the pathotypes prevalent at these sites. Combined analysis of the lineage-pathotype data provides some understanding of the lineage-pathotype relationships (Appendix 3, Table 7). In Nigeria, lineage NI-1 that was present at all three sites surveyed represented 12 pathotypes. Although these pathotypes originated from 12 different cultivars, a number of these isolates showed close virulence patterns, for example, pathotypes IA-8 and IA-65; IB-1, IB-3 and IB-42; ID-6 and ID-16**.** *M. grisea* isolates originating from the same site showed lesser degrees of genetic diversity, although their virulence patterns varied. For instance, seven isolates collected from Oyo from different cultivars

expressed seven different pathotypes, but all belonged to lineage NI-1 with very high MGR586 fingerprint similarity (Appendix 4, Figure B9).

In Ghana, lineages GH-1, GH-2 and GH-3 represented seven, five and one pathotypes, respectively, and the lineage/pathotype relationships showed a similar trend to that in Nigeria. A degree of correlation was observed between the lineages and site of collection, as most isolates from Hohoe fell into lineages GH-1 or GH-2. Pathotypes represented in lineage GH-1 were mostly IB group and some of these were closely related (for example IB-1, IB-2 and IB-7) 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 (for example IA-1 and IA-2).

Eleven pathotypes identified in Burkina Faso were represented by lineages BF-1, BF-2, BF-5, and BF-7. The lineage/pathotype relationships observed in Burkina Faso were more complex compared to Nigeria and Ghana. Lineage BF-1 represented diverse pathotypes IA-105, IB-64 and IC-13, which originated from diverse hosts and different sites. Some isolates in lineage BF-2, however, expressed either closely related pathotypes (e.g. IC-25 and IC-27) or the same pathotype (IC-9 from cultivars K3 and Yashiro-mochi at the Farako-Ba site). In Côte d'Ivoire, pathotypes IA-13, IB-45 and ID-14 identified from Man, belonged to CD-1, indicating high virulence diversity in this lineage.

Results obtained so far generally suggest that each lineage comprised isolates that infected different cultivars and contained a range of pathotypes. The degree of pathotype diversity varied between lineages with a limited number of pathotypes belonging to some lineages, whilst other lineages expressed a much broader range of pathotypes (for instance 12 pathotypes in lineage NI-1). In some cases, pathotypes belonging to a lineage expressed relatively similar virulence spectra. The baseline data generated paves the way for understanding the virulence diversities of the blast lineages in the region, which is essential to test the lineage-exclusion model for breeding and deployment of resistance (being further addressed in R7552).

To further characterise the atypical *M. grisea* isolates (Appendix 3, Table 8), along with a limited number of reference isolates form rice and non-rice hosts, additional markers, specifically RAPD-PCR (Appendix 4, Figure B10) and ITS sequence analysis (Appendix 4, Figure B11) were used. Based on rDNA-ITS 1 nucleotide sequence data, the *M. grisea* isolates analysed were divided into 2 broad groups. Group 1 included *M. grisea* isolates from a range of hosts with varying fingerprint patterns indicating their close relationship. Two of the 'atypical' rice isolates, B12 and IKR10/2, from Nigeria (with nine and seven MGR586 hybridising bands, respectively) formed part of the group 2 and were closely related to an isolate from Pearl millet (Appendix 4, Figure B11). Sequence alignments are shown below in Figures 5-7. The matrix of divergence/homology is shown in Appendix 3, Table 13. Combined application of the molecular and pathogenicity tests has revealed that non-rice pathogenic *M. grisea* isolates are present on rice varieties as well as on some weeds and even more importantly weedy rices and some of the weeds harbour *M. grisea* isolates pathogenic to rice (Output 2, pathotype diversity). The epidemiological significance of these isolates, and their potential role in pathogen evolution, merit further investigation.

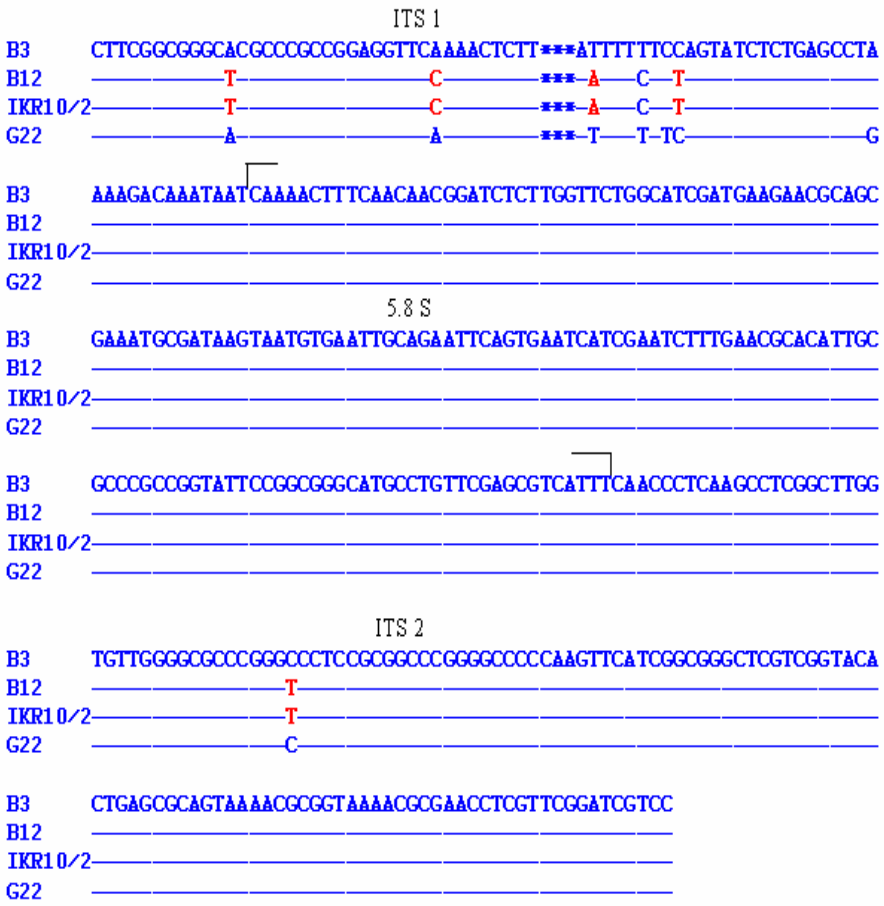


Figure 5: Aligned DNA sequences showing the variable regions of ribosomal DNA Internal Transcribed Spacers 1 and 2 of four *Magnaporthe grisea* isolates

The Internal Transcribed Spacer 1 region had a greater degree (11%) of intraspecific divergence than the Internal Transcribed Spacer 2 region (2%). Multiple sequence alignment was obtained using the alignment subroutines on CLUSTAL W (Thompson *et al*., 1994). B3, typical rice isolate (more than 30 MGR586-hybridising bands); B12 and IKR10/2, 'atypical' rice isolates (9 and 7 MGR586-hybridising bands respectively); G22, *Eleusine coracana* (a single MGR586-hybridising band).

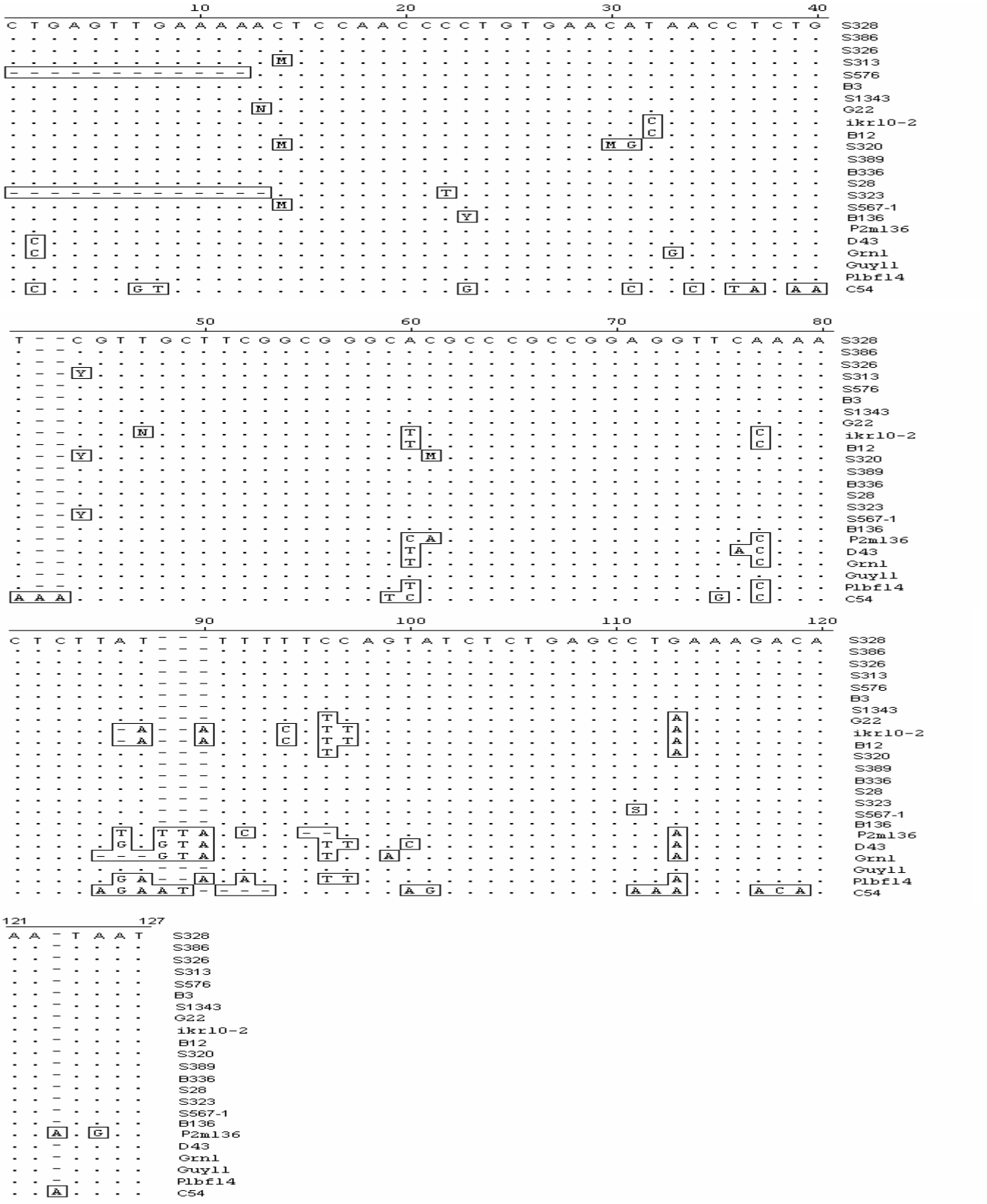


Figure 6: Aligned ITS 1 sequence of a range of *Magnaporthe grisea* isolates. Multiple sequence alignment was obtained using the alignment subroutines on CLUSTAL W (Thompson *et al*., 1994).The alignment resulted in 127 characters, of which 35.4 % were variable. Isolates analysed include atypical isolates, typical rice and non-rice isolates as described in Table 8 (Appendix 3). Ambiguous/unresolved bases are shown in International Base Code, where N = A/C/G/T; M = A/C; Y = C/T and S = C/G. In the multiple alignment, dots (.) indicate bases identical to S328; dashes (-) indicate gaps introduced to achieve optimal alignment. Isolate hosts are: S328, S326, S323 (Paspalum); S386, S576, S389, S528, S567-1, B136 (*O. longistaminata*); S313 (Rottbelia); B3, ikr10-2, S336, guy 11 (Rice); S1343 (unknown weed); G22 (*Eleusine corocana*); S320 (Bracharia); P2ml36, Plbf114 (Pearl millet); D43 (Digitaria); Grn1 (Ginger) and C54 (Cyperus).

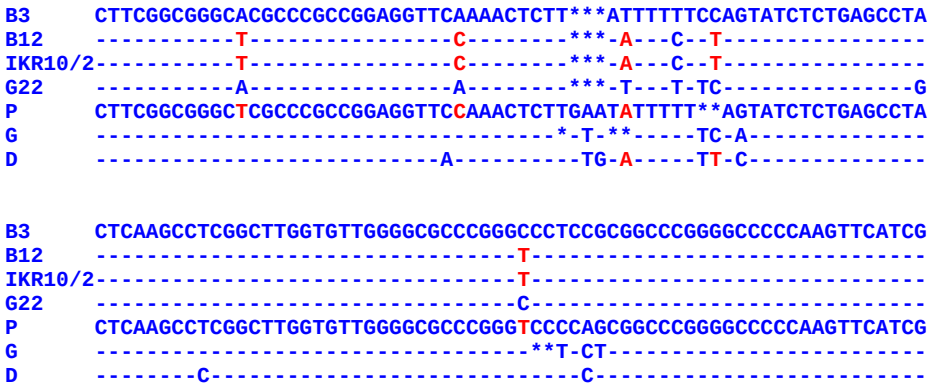


Figure 7: Aligned DNA sequences showing the variable regions of ribosomal DNA Internal Transcribed Spacers 1 and 2 of *Magnaporthe grisea* isolates Multiple sequence alignment was obtained using the alignment subroutines on CLUSTAL W (Thompson *et al*., 1994). B3, typical rice isolate (more than 30 MGR586-hybridising bands); B12 and IKR10/2, 'atypical' rice isolates (9 and 7 MGR586-hybridising bands respectively); G22, *Eleusine coracana* (a single MGR586-hybridising band); P, G and D refer to M. grisea isolates from Pennisetum, Digitaria and Ginger (unpublished data provided by Dr. M.H. Lebrun, INRA, France).

Output 4: Report on varietal performance in the context of the range of blast and scald variability at the screening sites, and the other pathogens that may present a threat to these varieties.

Preliminary data on varietal performance under scald infection indicated that, while no variety exhibited complete resistance to this pathogen, a range of tolerance levels could be observed. Preliminary findings indicated that the current range of rice germplasm held in W. Africa contains useful levels of field resistance which may beneficially be incorporated into future breeding programs. Analysis of scald molecular variability across the sites examined indicates that similar pathogen population structures are present across the region, suggesting that tolerant varieties are likely to be viable over a broad geographic range. The most tolerant varieties observed, out of the eighteen varieties tested (see Figures 10 and 11, Output 5 below), were WAB.56.104 (blast resistant), WAB.56.125 (blast resistant) and OB.677 (blast susceptible). All of these are lines developed by WARDA, and held as part of their current germplasm collection. Interestingly, some of the more blast-resistant lines such as Moroberekan, CG 20 and TOG 5810, show significantly higher ($p=0.01$) susceptibility to the scald pathogen, suggesting that these lines are less suitable donors of blast resistance traits for material to be disseminated in regions that have a history of leaf scald epidemics, such as Southern Nigeria. More positively, the demonstration of above-average resistance to *M. albescens* in other blast-resistant lines (WAB.56.104 and WAB.56.125) shows that resistance to both diseases can be successfully incorporated into varieties bred for local growing conditions.

Of the other fungal pathogens observed during this project, the brown-spot pathogen *Bipolaris oryzae* has been identified through this research as currently presenting an additional serious constraint to rice production, particularly under the low-input cropping systems prevalent in Ghana. This pathogen has also been identified as a cause for concern by Ghana NARS scientists at the CPP workshop on African rice crop protection, held at NRI 8-10 December (see Appendix 7). A particular issue identified here was the problem faced by counterparts in West African NARS in distinguishing between symptoms of the very frequently-encountered early stage brown spot infection and blast spots under field conditions, particularly in blast-resistant cultivars. This proved a significant constraint to successful visual assessment on different cultivars and in many cases it only proved possible to confirm the diagnosis through subsequent culturing. Field disease scores were therefore generally agreed to be suspect, as the leaf spots exist in the form of a complex, and visual expression of blast varies greatly between cultivars.

After the first year of experimentation, the farmer-participatory variety selection process eliminated all the highly blast-susceptible varieties. The knock-on effect of this was that, with the exception of check

varieties, subsequent field studies were all with cultivars showing partial resistance to the races present at each site. Field measurement thus became of dubious value in distinguishing between sites as the macro-scale differences seen were incremental rather than highly apparent.

Output 5: Set of rice differential germplasm suitable for scald pathogenicity assessment.

Analyses of the interactions between rice cultivars and isolates of *M. albescens* indicate that this pathogen does not comprise distinct pathotypical groupings which can be distinguished by a differential cultivar set, despite the observation of limited variation in the aggressiveness of selected isolates (see figures 8 and 9 below). A few isolates were able to induce lesions of above-average (e.g. S.46.3) or below-average (S.118.3) length, but variation was not generally statistically significant. It is also evident that the full range of *M. albescens* strain virulence was represented at each of the six trial sites examined. On the basis of pathogen aggression, therefore, none of the sites is better suited, or worse suited, to use for scald-resistance screening of rice germplasm. Reports from WARDA and NARS staff indicated a relatively low scald disease severity, but constant incidence, from all six sites, and surrounding farmers' fields. Again, this indicates that these sites are equally suitable for resistance screening with respect to this particular rice disease.

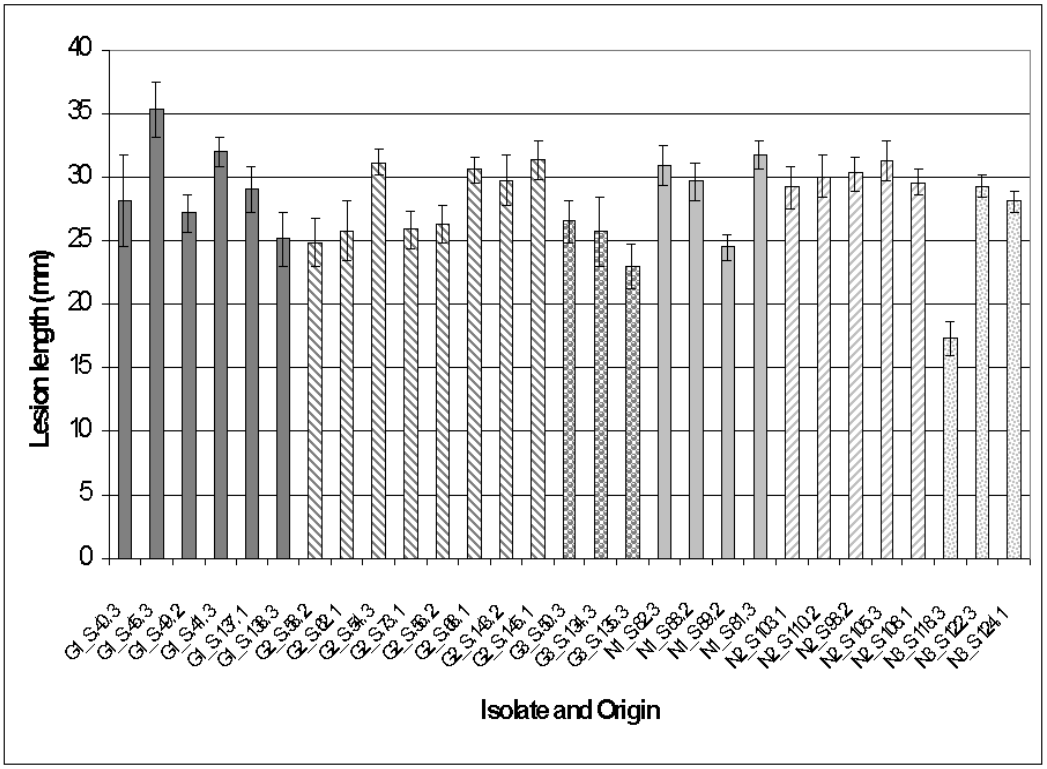


Figure 8: Aggressiveness of selected *M. albescens* isolates, in terms of mean lesion length (mm), +/- 99% confidence limits, as determined across the international blast differential set of rice cultivars. Isolate prefixes indicate origin as follows: G1 = Ghana site 1, Nyankpala; G2 = Ghana site 2, Hohoe; G3 = Ghana site 3, Tamale; N1 = Nigeria site 1, Oyo State; N2 = Nigeria site 2, Abia State; N3 = Nigeria site 3, Akwa Ibom State.

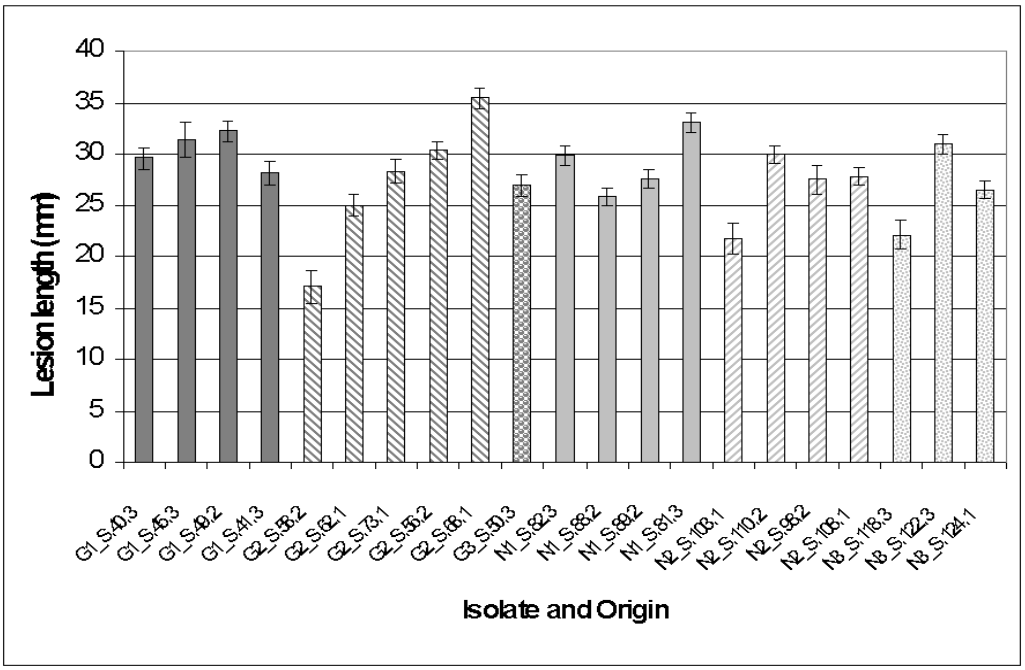


Figure 9: Aggressiveness of selected *M. albescens* isolates, in terms of mean lesion length (mm), +/- 99% confidence limits, as determined across selected West African rice cultivars. Isolate prefixes indicate origin as follows: G1 = Ghana site 1, Nyankpala; G2 = Ghana site 2, Hohoe; G3 = Ghana site 3, Tamale; N1 = Nigeria site 1, Oyo State; N2 = Nigeria site 2, Abia State; N3 = Nigeria site 3, Akwa Ibom State.

Some statistically significant differences in the tolerance of various rice cultivars to *M. albescens* were observed (see figures 10 and 11 below). Significance was determined through analyses of variance using Minitab software. Unlike other resistance-screening tests reported within the literature, the data presented here is quantitative, and based on tests using a range of isolates collected from a number of different locations. As noted earlier, previous work has yielded conflicting results, probably due to use of too narrow an isolate range. From figure 10, it is evident that, while *M. albescens* induces lesion development in all of the international set of blast differential cultivars, significantly (p=0.01) larger lesions developed on Dular than on any of the other cultivars. Of the other cultivars within this set, Caloro, Co 39, Kanto 51 and Raminad Strain 3 developed the smallest lesions.

When West African cultivars, supplied by WARDA, were tested against a total of 21 *M. albescens* isolates, all again proved at least partially susceptible to *M. albescens*, as evidenced by lesion development. However, three cultivars, WAB 56 125, WAB 56 104 and OB 677 showed significantly (p=0.01) reduced lesion development (see Figure 11), suggesting that these lines are of potential use as a source of improved scald tolerance in future breeding programmes. It should also be noted that some of the W. African lines favoured for their blast resistance traits, such as Moroberekan, are more susceptible to leaf scald disease, as evidenced by the formation of larger-than-average lesions (see Figure 11). These lines should therefore be treated with caution as blast resistance donors for the development of material intended for release in areas with a history of periodic severe scald outbreaks (e.g. southern Nigeria).

The significant (p=0.01) variation in the rate of lesion development between rice cultivars inoculated with *M. albescens* demonstrated by the present study indicates the importance of evaluation of quantitative resistance traits. It is therefore concluded that future resistance breeding programs need to focus on improving quantitative or field resistance traits, as opposed to qualitative resistance traits, in order to control rice leaf scald disease. Our findings also indicate that suitable blast-resistant donor lines with above-average leaf scald disease tolerance are extant within the WARDA-held germplasm set, and therefore already available for development.

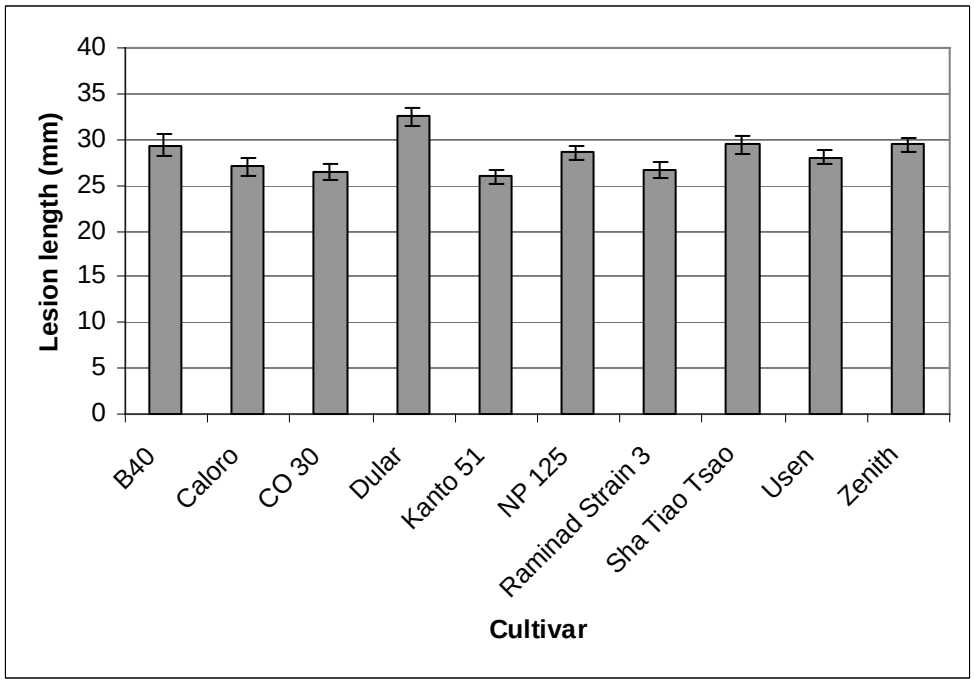


Figure 10: Mean lesion length, +/- 99% confidence limits, resulting from inoculation of the international blast differential set of rice cultivars with selected isolates of *M. albescens*.

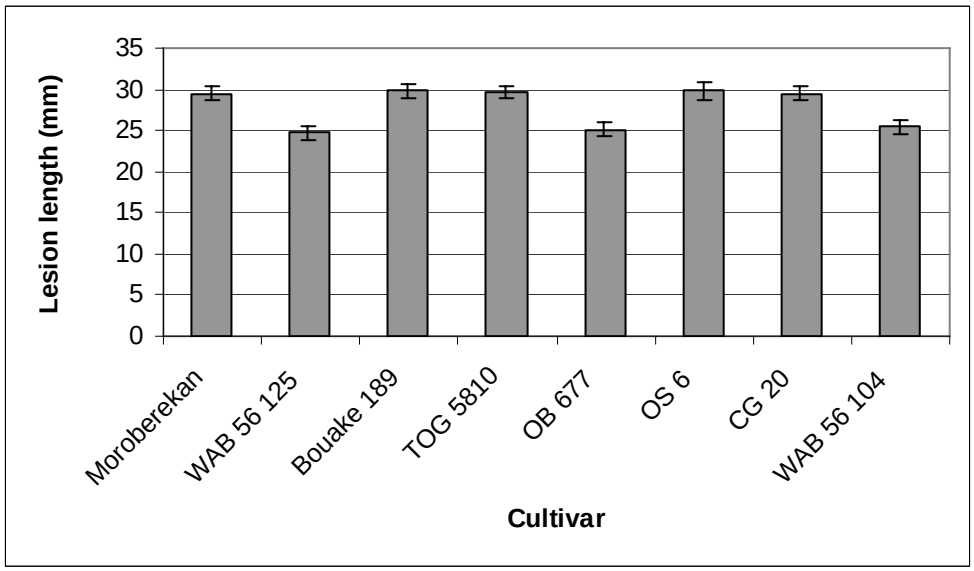


Figure 11: Mean lesion length, +/- 99% confidence limits, resulting from inoculation of West African rice cultivars with selected isolates of *M. albescens*.

Output 6: Description of direct and indirect interactions between blast and scald in vitro and in vivo.
Tests have shown that there is no direct interaction between *M. albescens* and *M. grisea*, when these two pathogens are grown *in vitro*, whether on glass slides or on potato dextrose agar or rice extract agar. Neither fungal species inhibited or excluded the growth of the other species, nor were they observed to suppress or promote sporulation of adjacent fungal colonies. The hyphae of these two species were observed to grow into areas occupied by the other species with no detectable change in growth pattern, even when subject to direct physical contact with each other.

Co-inoculation of the two species onto detached rice leaves, in a modification of the method of Bonman *et al.* (1990), did not reveal any change in lesion development rate from that observed under solitary inoculation. However, growth of *M. grisea* under these conditions was negligible, even in the control samples (with *M. grisea* as sole inoculant), suggesting a loss of *M. grisea* isolate viability. The lack of observable change in *M. albescens* lesion development cannot, therefore, be taken as conclusive evidence of a lack of plant-mediated interaction. However, it has been noted that dual infection by *M. grisea* and *M. albescens* is not uncommon in the field, in areas where both pathogens are active. This is suggestive, at least, that no significant negative plant-mediated interaction (i.e. induction of effective systemic resistance) occurs.

Output 7: African plant pathologist qualified in appropriate techniques to PhD level.
Mr. Jack Chipili, Plant Pathologist in the Ministry of Agriculture, Food and Fisheries, Government of the Republic of Zambia completed his Ph. D. thesis 'Characterisation of populations of *Magnaporthe grisea*, the rice blast fungus, in some of the West African countries'. As part of the thesis work incorporating the project activities, Jack has gained expertise in the application of molecular as well as pathological tools to characterise the rice blast pathogen diversity. Jack is continuing to work on the rice blast pathosystem in the follow-up phase 'Strategies for development and deployment of durable blast resistance in West Africa' (R7552).

Output 8: WARDA scientist trained in blast molecular biological protocols, with UK project staff.
Dr. Y. Séré, the Principal Plant Pathologist from WARDA, completed a research and training attachment to the UK between March and June 1998, participating in the molecular analysis of *M. grisea* isolates. Dr. Séré provided approximately 160 additional isolates, collected from various WARDA sites in Côte d' Ivoire and Burkina Faso. Molecular analyses (MGR 586 fingerprinting and ITS sequencing) of 60 of these isolates were initiated during Dr. Séré's attachment at HRI. Fingerprinting of the remaining isolates has been completed by the project Ph.D. student, Mr. Chipili. Results of the fingerprint profile analysis have subsequently been provided to WARDA through Dr. Séré, along with data on the pathotypical diversity of the blast pathogen. Pathotyping of these additional isolates was conducted in participation with Exeter University, under 1998 add-on funding (see Additional Output (b) below).

Additional outputs:
a) add-on in 1997:
Additional funding (£10 000), split between the three R6738 subcontracts, was obtained to;

i) bring Dr. S. K. Nutsugah, from the Savanna Agricultural Research Institute in Ghana, to the UK for 3 weeks of training in the project methodologies and for detailed discussion of current and future project activities. The objective of this visit was to facilitate uptake of project outputs, by providing Dr. Nutsugah with hands-on experience, and theoretical understanding, of the analyses being developed and applied under R6738. One week was spent with each of the three UK partner institutes, as follows:

NRI: Dr. Nutsugah received training, with the molecular diagnostics group, in the application of PCR-based analysis of the molecular variability of the rice leaf scald pathogen, and in the *in vitro* bioassaying of *M. albescens* virulence on a range of rice cultivars. On his return to Ghana, Dr. Nutsugah replicated the bioassay protocol at SARI, using selected Ghanaian isolates and four local rice cultivars. Seed for these four cultivars were also forwarded to NRI for further testing using this protocol.

CABI Bioscience: Dr. Nutsugah was provided with background training in the methodology, use and applications of PCR, including hands-on training in the

laboratory. This general introduction provided him with the background to undertake pathogen-specific investigations at NRI and HRI in subsequent weeks.

HRI: Dr. Nutsugah participated in the application of a range of molecular tools for fungal diagnostics, notably MGR 586 fingerprint analysis and ITS sequence analysis of the rice blast pathogen, *M. grisea*. The use of molecular biology software in the analysis of sequence data and fingerprint data was also demonstrated. Dr. Nutsugah will also be provided with a set of MGR 586-characterised isolates originating from Ghana to set up varietal screening of chosen local cultivars, as part of R7552.

ii) The remaining funding was provided to WARDA, through CABI Bioscience (contract ZA0065) to enable surveying of additional field sites in Nigeria in 1998. Unfortunately, the WARDA IPM Taskforce did not undertake the planned survey due to logistical problems for the newly-appointed WARDA pathologist, Dr. Séré. The funds are currently held at WARDA and they plan to use these to support an additional survey of disease occurrence across sites in Nigeria as a follow-on study..

b) add-on in 1998:

WARDA, through Dr. Y. Séré, requested that an additional 160 isolates of *M. grisea* be put through molecular and pathotypical characterisation. These isolates came from sites in Côte d' Ivoire and Burkina Faso. Molecular characterisation was initiated as part of Dr. Séré's training attachment to the UK (see output 8 above), and completed by the project Ph.D. student at HRI. Add-on funds (£17 697) were obtained to conduct pathotypical analysis of representative isolates from this set, at the University of Exeter, using the international rice differentials for blast. The virulence spectrum of fifty five *Magnaporthe grisea* isolates was determined and the pathotype designations assigned according to the nomenclature of Ling and Ou (1969). More information on *M. grisea* pathotype diversity is provided under Output 2.

Contribution of Outputs

The outputs described above will contribute to the alleviation of poverty in West Africa, by facilitating achievement of the CPP purpose '*yields of rice-based systems increased by application of environmentally-benign pest control*'. By providing information on the blast and scald pathogen populations at selected sites, and identifying those sites best suited for use in future resistance-screening programmes, the outputs from this project improve the capacity of WARDA and NARS scientists and breeders to develop appropriate disease-resistant rice varieties, and to deploy them in a manner that will promote the durability of this resistance in the field. West African rice lines exhibiting useful levels of resistance to both pathogens have been identified as potentially useful donor lines for further resistance breeding work. The project outputs also provide baseline data that will permit WARDA pathologists to monitor changes in the pathogen populations over time, an essential activity for ongoing efforts to minimise crop losses due to these diseases in the region. Outputs of the blast component are applicable in three WARDA projects; 1.1. Sustainable intensification of lowland rice-based ecosystems; 1.2. Stabilisation of upland rice-based systems under shortened fallow; and 2.1. Improvement of resource efficiency in irrigated rice-based systems. They feed into programmes of the WARDA-NARS IPM and Breeding Task Forces, as well as assisting varietal selection for the INGER-Africa rice screening programmes in each country

Additionally, the brown spot pathogen, *Bipolaris oryzae*, has been identified as the primary potential disease threat to blast- and scald-resistant material intended for release under the low-input systems of Ghana. Further investigation of the threat posed by *B. oryzae*, and of optimal farmer-accessible methods for its control, is recommended.

a. What further market studies need to be done ?

The results of the recent CPP Rice research uptake and adoption study will be particularly pertinent in establishing subsequent uptake pathways and needs to enable adoption through WARDA and the NARS.

b. *How will the outputs be made available to intended users ?*
Some dissemination has already been achieved through publication in peer reviewed journals, provision of progress reports to staff at the target institutions (WARDA and associated NARS), and through bringing WARDA and NARS scientists to the UK for periods of research and training with the UK-based partners in this project. Through Dr. Y. *Séré*, data on the blast pathogen diversity have been provided to WARDA, for uptake into their on-going projects on blast management. Further dissemination will be achieved through distribution of FTR materials to the target institutions, and involvement of WARDA and NARS staff in the preparation of further papers for publication in peer reviewed journals. WARDA and NARS staff will also participate in a DFID-funded follow-on phase to the current project, R 7552 'Strategies for development and deployment of durable blast resistance in West Africa'. Promotion of technology and local capability development to enable long-term local monitoring of the pathogen populations are planned under the follow-on phase to this project, R7552.

c. *What further stages will be needed to develop, test and establish manufacture of a product ?*
No further work is required to assess the W. African *M. albescens* populations. Target institutions can now incorporate the *M. albescens*-related outputs into their rice breeding and screening programmes, and germplasm dissemination strategies. A follow-on phase to the current project has been approved by DFID's Crop Protection Programme; R 7552 'Strategies for development and deployment of durable blast resistance in West Africa'.

d. *How and by whom will the further stages be carried out and paid for ?*
Incorporation of the *M. albescens*-related outputs into the target institutions' rice breeding and screening programmes, and germplasm dissemination strategies, will fall within the scope of these institutions' normal operations, and thus be covered by their normal funding routes. The blast-related follow-on project, R7552, is funded by DFID's Crop Protection Programme.

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