

Effects and possible causes of an unprecedented rice blast epidemic on the traditional farming system of Bhutan

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Abstract

In 1995, an unprecedented rice blast epidemic (caused by *Pyricularia grisea*) occurred in Bhutan in mostly traditional cultivars in the high (1800–2600 m) and some parts of mid altitude (1200–1800 m) agroecological zones. Possible causes of the outbreak such as introduction of a new pathogen race, unusual weather patterns and changes in agricultural practices were investigated and yield losses analysed. Weather data were examined for unusual patterns and farmers from the affected areas were interviewed to determine possible changes of cultural practices. Blast isolates were collected from various affected rice areas and analysed for variability of the pathogen population by MGR (*Magnaporthe grisea* repeat 586) fingerprinting. Rice yields declined parabolically with increasing disease with losses for some farmers being nearly 100%. Restriction fragment length polymorphism analysis using MGR fingerprints of 71 isolates resulted in 58 haplotypes that grouped into 13 lineages. Lineage BhR1 and BhR4 were represented by 22 and 13 isolates, respectively, whereas the other lineages were represented by only one to six isolates each. Haplotypic diversity both in the early and late season's collections was very high suggesting that the blast epidemic was not caused by a new introduction or race change of the pathogen. There was no drastic shift in agricultural practices or rice cultivars which could explain the blast outbreak. However, the weather data indicated that the climatic conditions in 1995 favoured blast and these unusual conditions, in combination with the ubiquitous presence of the pathogen, probably account for the epidemic. ©2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Rice is the principle crop and staple food in Bhutan and is grown mainly in river valleys from 100 to

2600 m altitude in irrigated terraced fields on an area of approximately 45,000 ha. More than 90% of the area under rice is planted with traditional cultivars or landraces and a typical farmer will grow more than one cultivar. The area under improved high yielding cultivars is limited because of the high inputs required to grow them, unacceptable grain colour, taste and poor yield of straw. Seedlings are raised in dry beds for more than 90 days in high altitudes (1800–2600 m) but in the mid elevations (1200–1800 m) both wet and dry bed nurseries for 40–45 days are common. Farm

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yard manure is applied and use of inorganic fertilisers and pesticides with the exception of herbicides, is minimal. The landraces are highly polymorphic within a field and average yield of local rice cultivars is in the range of 2–3 t/ha in the high altitudes and about 1–2 t/ha in the mid and low altitudes.

In 1984, the first systematic study of plant diseases in Bhutan was undertaken by Peregrine et al. (1984) and rice blast, caused by the fungus *Pyricularia grisea*, Cav. (Teleomorph *Magnaporthe grisea*) was recorded among many other diseases. The blast pathogen can affect all above ground parts of the rice plant at any growth stage. Infections of the stem nodes and neck or panicle at the reproductive stage can result in 100% losses in severely affected plants. Since 1984, low levels of disease have been noted predominantly in areas where irrigation water enters the fields and in shady wind protected areas. Regular localised outbreaks of seedling, neck or node blast are reported by extension agents in all the ecological zones and farmers sometimes even have to replant their seedbeds because of complete destruction of the seedlings. In high altitudes, blast is not observed in nurseries but can occur after tillering. Often, node and panicle blast are observed without previously visible leaf infections (Thinlay, 1998), but before 1994 it was never considered to be a problem.

In 1994, a locally severe blast outbreak was reported from a high valley (ca 2400 m) about 20 km west of the capital Thimphu in western Bhutan and in June, 1995, leaf blast was observed in nurseries in many mid-altitude rice growing areas. The first reports of severe node and neck blast were received from villages 40 km west of Thimphu in late August. Subsequent reports from other villages in mostly high altitude environments indicated that a significant epidemic was underway. The disease affected mostly the traditional cultivars, and was also reported from mid-altitude environments, although it was generally less severe. The Ministry of Agriculture (MoA) registered households with affected rice fields for the assessment of the disease severity and crop loss. At the time of the epidemic the yield loss was assumed to be directly proportional to the percentage of affected panicles in the fields (Ministry of Agriculture, 1995). However, this estimation is most likely not very accurate as yield loss relationships are not usually linear. This relatively large and severe epidemic

outbreak contradicts commonly held views that epidemics should not occur in mixed and/or traditional farming systems (Thurston, 1990, 1992).

One or more possible changes could account for the epidemic. Firstly, farmers could have changed their rice management practices. A shift to susceptible cultivars, widespread increase in nitrogen application (especially inorganic nitrogen), and increased planting densities are all factors known to increase blast incidence (Ou, 1985). Secondly, unusual weather, such as continuous cloud cover, rain and lower temperatures during the reproductive stage of blast can lead to epidemics (Kim, 1994). Thirdly, a virulence change or introduction of a new pathogen strain virulent on the traditional cultivars could render resistance in traditional cultivars ineffective. The blast pathogen genome carries multiple copies (40–60) of the middle repetitive DNA element MGR 586 = (*Magnaporthe grisea* repeat 586). Restriction enzyme digests of genomic DNA of the fungus produce DNA ‘fingerprints’ which may be used to infer the genetic relatedness among isolates. Those with >80% similar fingerprints have been considered to belong to one genetic ‘lineage’ (i.e. descent from a common ancestor), and to share a common genetic background (Levy et al., 1993; Chen et al., 1995). Isolates within a lineage share some of their virulence characteristics and these differ from isolates belonging to different lineages (Zeigler et al., 1995). This paper reports the results of studies to determine the losses from and the causes of this unusual outbreak.

2. Materials and methods

2.1. Crop loss assessment

Loss assessments were made in the Paro and Thimphu districts in western Bhutan in all the affected fields of farmers in the high, valleys (2200–2450 m). In each affected field, panicle and node blast incidence was assessed from three randomly selected areas, with each sample measuring 0.5 m × 0.5 m. The grain yield (unmilled) was determined after adjusting moisture content to 14–16%.

Because the range of neck/node blast incidences was variable among sites, all observations were first

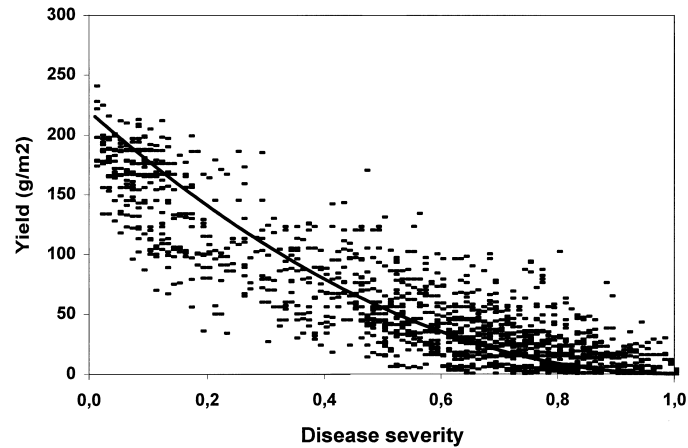


Fig. 1. Relationship between yield (g/m^2) and neck and node blast incidence. The fitted line is based on Eq. (1).

plotted in one graph (Fig. 1) and a trend line fitted manually using PLOTIT on a subset of data points. One hundred thirty eight data points were randomly selected for each of the five classes: $x = 0-0.2$, $>0.2-0.4$, $>0.4-0.6$, $>0.6-0.8$, $>0.8-1$ ($n = 690$). A good fit was obtained with the equation:

$$y = 220(1 - x)^2 \quad (1)$$

where y is the observed yield and x the neck/node blast incidence. The equation predicts zero yield at 100% disease incidence and at no disease the predicted yield is 2.2 t/ha across all sites. The fixed exponent 2 was chosen for regressions for each individual site because independent estimation of an exponent would have been very inaccurate in sites where disease incidence did not cover the whole range. Regressions were carried out with SAS (1988).

2.2. Weather data and agricultural practice

The available weather data from 1990 until 1996 for the Thimphu and Paro districts (courtesy of the Meteorology Department of the Ministry of Communication) consisted primarily of monthly means and did not correspond exactly to the areas where the blast outbreak was most severe. As weather conditions in Bhutan are extremely variable over very short distances because of topography, these data may not directly correspond to the affected areas. They probably give a good impression of overall year-to-year differences, however.

Data were analysed to examine and compare the maximum and minimum temperature, accumulated rainfall for each month, and the number of cloudy and rainy days during the rice season when available.

In addition, a small survey was conducted during the blast outbreak in 1995 in western Bhutan to assess farmers practices, their assessment of weather compared with earlier years and their experience with the disease. Fourteen farmers, both male and female ranging in age from 25 to 75 years were interviewed in late September and early October. Eleven of the farmers were from high elevations where blast was most severe, and three were from moderately affected mid-altitude regions. All the interviewed farmers were responsible for the management of their farms.

The open-ended interviews were conducted in the local language in an informal setting and covered the following topics: (1) Cultural practices and any changes in cultivars planted, fertilisation or irrigation regimes and pesticide applications; (2) Weather during the rice season; (3) The occurrence and severity of blast in previous years; and (4) Farmers views as to why the blast epidemic occurred in 1995.

2.3. Collection of isolates

Isolates were collected from infected leaves, panicles and nodes primarily from traditional cultivars. Leaf samples from nurseries were collected in June and July and infected panicles and nodes were col-

Table 1
Pyricularia grisea isolates collected from five districts in Bhutan in 1995

District ^a	Eco-zone	Disease level	Number of fields	Number of isolates
June/July (leaf samples from nurseries)				
Tongsa	humid subtropical	trace	5	5
Punakha	humid subtropical	high	5	7
Wangdi	dry subtropical	trace	3	5
Thimphu	warm temperate	trace	11	13
September/October (neck and node blast)				
Paro	warm temperate	high	13	15
Thimphu	warm temperate	high	10	14
Punakha	humid subtropical	low	2	3
Wangdi	dry subtropical	low	8	9
Total			57	71

^a Tongsa is located in central Bhutan (ca. 2000 m altitude), Punakha and Wangdi in western Bhutan, 1200–1800 m, and Paro and Thimphu in western Bhutan, 2200–2600 m.

lected during September and October 1995. Samples were stored in a freezer until pathogen isolation. Of the 71 isolates subjected to DNA fingerprinting, 30 were obtained from the leaf samples collected during June–July (Table 1). Samples from infected leaves collected in the nurseries from Central Bhutan (Tongsa) in June 1995, were included in the analysis as reference, since no epidemic occurred then.

2.4. Extraction of DNA

DNA was extracted from isolates from a single lesion. Tissue samples were incubated for 24 h at 28°C in a Petri dish lined with moist filter paper. Conidial masses were picked from sporulating lesions and streaked onto 4% water agar with 40 mg/l streptomycin again for 24 h at 28°C. Single germinating spores, or rarely, conidial masses, were picked and transferred directly into liquid Fries medium (Van Etten and Daly, 1980) supplemented with 0.5 g/l casein hydrolysate and onto prune agar slants for storage (per litre of de-ionised water: three prunes, 5 g lactose, 1 g yeast extract, 17 g agar bacto). The liquid cultures were incubated for 7 days; left stationary in the incubator at 28°C over night, and put on a shaker during the day with a hot air blower placed nearby.

Freshly harvested mycelium was ground in liquid nitrogen and total DNA extracted using the procedure for plants by Murray and Thompson (1980).

The DNA samples were brought to the International Rice Research Institute (IRRI) for digestion with restriction enzymes, Southern blotting and probing with MGR586. DNA samples (1.5–2 µg) were digested with EcoR1 following manufacturer's instructions (Boehringer Mannheim, Germany) and fractionated in 0.7% agarose-0.5x Tris-borate-EDTA gel at 2 V/cm for 24–26 h using a 18 cm × 30 cm gel moulder. Resolved fragments were transferred to Hybond-N+(Amersham, UK) using the alkali transfer procedure recommended by the manufacturer. The clone pCB586, which contains the repetitive element MGR586 (gift of J. Hamer and B. Valent, DuPont Co., Wilmington, DE), was used as a probe to generate DNA fingerprints. The probe was labelled with digoxigenin-11-dUTP as described in the Boehringer Mannheim non radioactive DNA labelling and detection Kit (Genius Kit; Boehringer Mannheim, Indianapolis, IN). Blots were hybridised with the digoxigenin-labelled probe, and signals detected using a chemiluminescent substrate for alkaline phosphatase (CSPD, Boehringer Mannheim, Germany or Tropix, Bedford, MA).

2.5. Phylogenetic analysis

The DNA fingerprints were visually examined to identify the various haplotypes, or unique banding patterns. The DNA bands of each haplotype were scored as binary data, with '1' and '0' indicating presence and absence of a band, respectively. The Dice coefficient of similarity [$F = 2N_{xy}/(N_x + N_y)$, where N_{xy} is the number of bands common to two haplotypes, and $(N_x + N_y)$ is the total number of bands for the two haplotypes] was calculated for all pairwise combinations of haplotypes using the WINDIST program (Yap and Nelson, 1996). A dendrogram was constructed based on the unweighted pair-group method for the arithmetic means (UPGMA) (Sneath and Sokal, 1973), using the SAHN clustering program in the NTSYS-pc package, version 1.70 (Rohlf, 1992). Bootstrap analysis was done using the computer program WINBOOT (Yap and Nelson, 1996) to assess the robustness of the groupings produced by cluster analysis. The phenograms were reconstructed 2,000 times by repeated sampling with replacement, and the frequency with which a particular grouping

was formed was used as a measure of the strength of the grouping (Felsenstein, 1985; Hedges, 1992). Distinct robust groups were considered to represent different phylogenetic lineages.

3. Results

3.1. Observations on disease incidence

The most severe outbreaks occurred in the high altitude areas of Thimphu and Paro (Table 2). Some fields were completely destroyed by neck and node blast. The incidence varied from 0 to 100% with an average of 55 and 66% in affected fields in Thimphu and Paro, respectively. The disease was most severe on the local traditional high altitude cultivars such as 'Janam', 'Zechum', 'Kuchum', and 'Dumja'. These cultivars were also quite susceptible in greenhouse tests (Thinlay, 1998). Punakha and Wangdi had some severely affected higher elevation areas, but were generally only slightly affected. The mean disease incidence in affected fields in these districts was less than 20%. Blast was recorded from other parts of the country but its occurrence was sporadic and the incidence was low.

The majority of fields surveyed in the Paro, Thimphu, Punakha and Wangdi districts were planted with traditional rice cultivars. Many of the cultivars are mixtures of many types and given 'cultivar' names by the farmers mainly based on grain colour such as white and red grains. A significant observation was that high altitude cultivars planted in the Wangdi district on the

Renewable Natural Resources Research Centre-Bajo (RNRRC-Bajo) experimental farm (Mid-altitude rice research station) were completely destroyed by node and panicle blast where as mid-altitude cultivars were only slightly affected in the same trial. In a neighbouring field, some pedigree lines and candidate cultivars developed for high altitude areas were free of significant blast indicating variation in resistance.

3.2. Yield loss analysis

The ranges of observed disease incidences and the number of fields assessed for blast incidence and yield varied among the different gewogs (administrative units) (Table 3).

With the exception of Mewang gewog in Thimphu, the regressions of yield on incidence were statistically highly significant and between 22 and 78% of the variation in yield could be explained by disease incidence (Table 3).

The yield predictions for individual sites corresponded with the authors' general assessment of the site fertilities except for Tshento in Paro. There, the predicted yields were higher than expected based on the general knowledge of the site. For comparison the estimated mean yields for these areas by the Ministry of Agriculture (MoA) based on data collected by extension agents are included in (Table 3). The MoA data have been put together in 1995 by the Land Use Planning Project of the MoA and the Danish Development Corporation DANIDA. The MoA estimates were generally higher than the estimates based on

Table 2
Blast affected areas and yield loss in Bhutan in 1995

Agroecological zone	District	Rice area (ha) ^a	Estimated production (t) ^a	Area affected (ha)	Mean percent incidence ^b	Yield loss (t) ^c	Yield loss (%)
High altitude	Thimphu	1662	6509	200	55	219	3.36
	Paro	2420	7406	308	66	663	8.95
Mid-altitude	Punakha	3342	13800	54	20	66	0.48
	Wangdi	4064	15673	73	19	142	0.91
Subtotal		11488	42988	635		1090	
	Others	33598	54889	112	^d	^d	^d
Total		45086	97877	747		1090	

^a Source: LUPP (1995).

^b The mean incidence refers to the mean in affected fields only.

^c As estimated by the MoA.

^d Indicates that blast infection in those districts was low and not assessed for severity and yield losses.

Table 3

Estimated yield losses in Bhutan in 1995 due to rice blast in different gewogs of varying fertility in the high altitude districts of Paro and Thimphu

Gewog ^a	Elevation ^b	Fertility ^c	Range of disease incidence min/max	Number of fields assessed	Predicted yield (t/ha) ^d	R ^{2e}	MoA estimate (t/ha) ^f
Paro							
Shari	l	fertile	37/100	138	3.01	0.64	2.41
Naja	m	fertile	10/96	26	2.70	0.78	3.05
Tshento	h–l	variable	21/100	171	2.06	0.43	2.41
Horrey	m	fertile	27/86	31	1.90	0.38	3.37
Shaba	l	fertile	10/100	104	1.84	0.71	3.76
Lango	l	fertile	26/91	173	1.84	0.38	2.48
Wangchang	l	variable	13/99	199	1.54	0.54	3.95
Dogar	h–l	poor	19/100	31	1.29	0.49	4.00
Dotey	h–l	poor	11/95	129	1.07	0.46	2.39
Thimphu							
Chang	l	moderate	19/98	49	2.34	0.66	2.66
Geynekha	h–m	moderate	29/100	27	2.16	0.26	2.68
Kawang	m	moderate	28/93	115	2.12	0.22	2.66
Mewang	l	variable	16/100	174	2.06	0.09	2.66
Toep	h–l	variable	2/24	295	1.92	0.43	2.66

^a Gewog is an administrative unit consisting of several villages.

^b h: High (ca 2400–2600 m); m: Medium (ca 2200–2400 m); l: Low (ca 2000–2200 m).

^c Based on personal perception.

^d Predicted yield at no disease calculated based on Eq. (1).

^e R² are regressions of yield on incidence based on Eq. (1). All regressions had *P*-values <0.001 except for Mewang gewog (not significant).

^f Yield estimates for the gewogs provided by the Ministry of Agriculture based on data from LUPP (see text for details).

Eq. (1) and especially in the Paro district discrepancies were sometimes more than two-fold.

3.3. Weather data and agricultural practices

Although the available weather data were not exactly from areas where the blast outbreak was the most severe together with the farmers' survey they allow for the comparison of the general environmental conditions during the rice season in Paro and Thimphu from 1990 to 1996. In both districts, there were more cloudy and rainy days in 1995 in August and in Paro also in September (Fig. 2a, b, only data for Thimphu are shown) with somewhat more rainfall than usual in August. Also, the temperature conditions in August and September favoured dew formation in Paro and Thimphu with high differences between minimum and maximum temperature (Fig. 2c). In addition, during the early part of the rice season (May in Paro and May–July in Thimphu) rainfall was very low leading to drought conditions in the seed beds and there were

prolonged dew periods that are known to favour blast (Fig. 2d).

Confirming this impression, 10 out of 14 farmers thought that there was more than the usual amount of rainfall during the season of 1995. However, perceptions as to rainfall distribution varied considerably. Although farmers from the mid-altitude found the distribution normal, seven out of 10 farmers from the high altitudes reported unusual patterns (for more detail see Thinlay, 1998).

All farmers interviewed had used the same transplanting and irrigation practices and planted the usual traditional cultivars, except for one farmer who had shifted to IR64 for its higher yield. His field had no or a very low level of blast. The use of farm yard manure was no different from usual and there was a general consensus that, in general, highland rice farmers do not use inorganic fertilisers. Adjacent fields with and without herbicide (Butachlor) applications had similar levels of blast (personal observation). Blast is known to farmers as a harmless phenomenon in their fields and two farmers had seen blast outbreaks before but

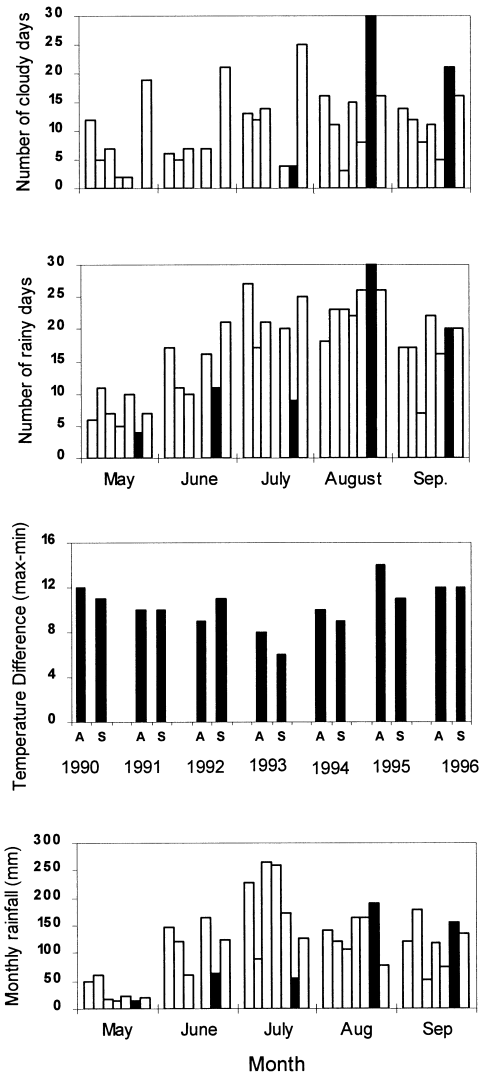


Fig. 2. (a) Number of cloudy days, (b) number of rainy days, (c) difference between maximum and minimum temperature in August (A) and September (S) and (d) total monthly rainfall from May to September during 1990–1996 in Thimphu. The weather data do not exactly correspond with the places where the blast disease epidemic was the most severe, but they indicate the general weather conditions during the epidemic year (shown with black column). Missing columns are due to missing data.

never as severe as in 1995. Elderly farmers mentioned that this was the first time in over 60 years of rice cultivation that they had seen a blast epidemic. Most farmers felt that, since the blast epidemic was a sort of penance for bad deeds or a natural cycle of bad luck,

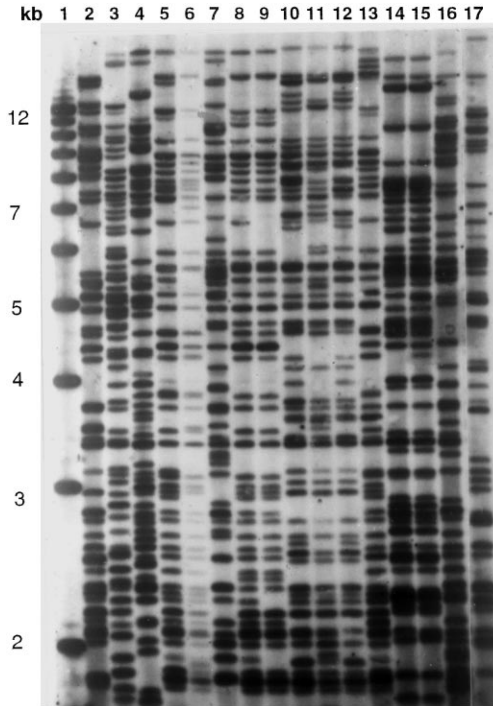


Fig. 3. MGR586-DNA fingerprints of *P. grisea* isolates from fields in the districts of Paro, Thimphu, Punakha, Wangdi and Tongsa. Lanes are: 1 = 1 kb ladder, 2 = BhR6, 3 = BhR11, 4 = BhR12, 5 = BhR1-1, 6 = BhR1-2, 7 = BhR7, 8 = BhR1-3, 9 = BhR1-4, 10 = BhR4-1, 11 = BhR4-2, 12 = BhR4-3, 13 = BhR2, 14 = BhR13-1, 15 = BhR13-2, 16 = BhR8, 17 = BhR10 (see Fig. 4 for similarities of lineages).

they were grateful that it was the rice crop that had sickened and died and not their children.

3.4. Genetic diversity of the fungal population

DNA fingerprinting of the 71 *P. grisea* isolates obtained during the blast epidemic year in Bhutan revealed a very diverse blast population with 58 different haplotypes. For each isolate, there were 49–73 (average 59) bands hybridising to the MGR586 probe (Fig. 3) ranging from 1 to 23 kb in size as is expected of rice blast isolates (Hamer et al., 1989). Based on UPGMA and bootstrap analysis, the 58 haplotypes formed 13 robust clusters at 75% similarity (bootstrap values >0.99). Each cluster was considered as phylogenetic group or lineage (Fig. 4) (Talbot et al., 1993; Chen et al., 1995). The largest number of haplotypes

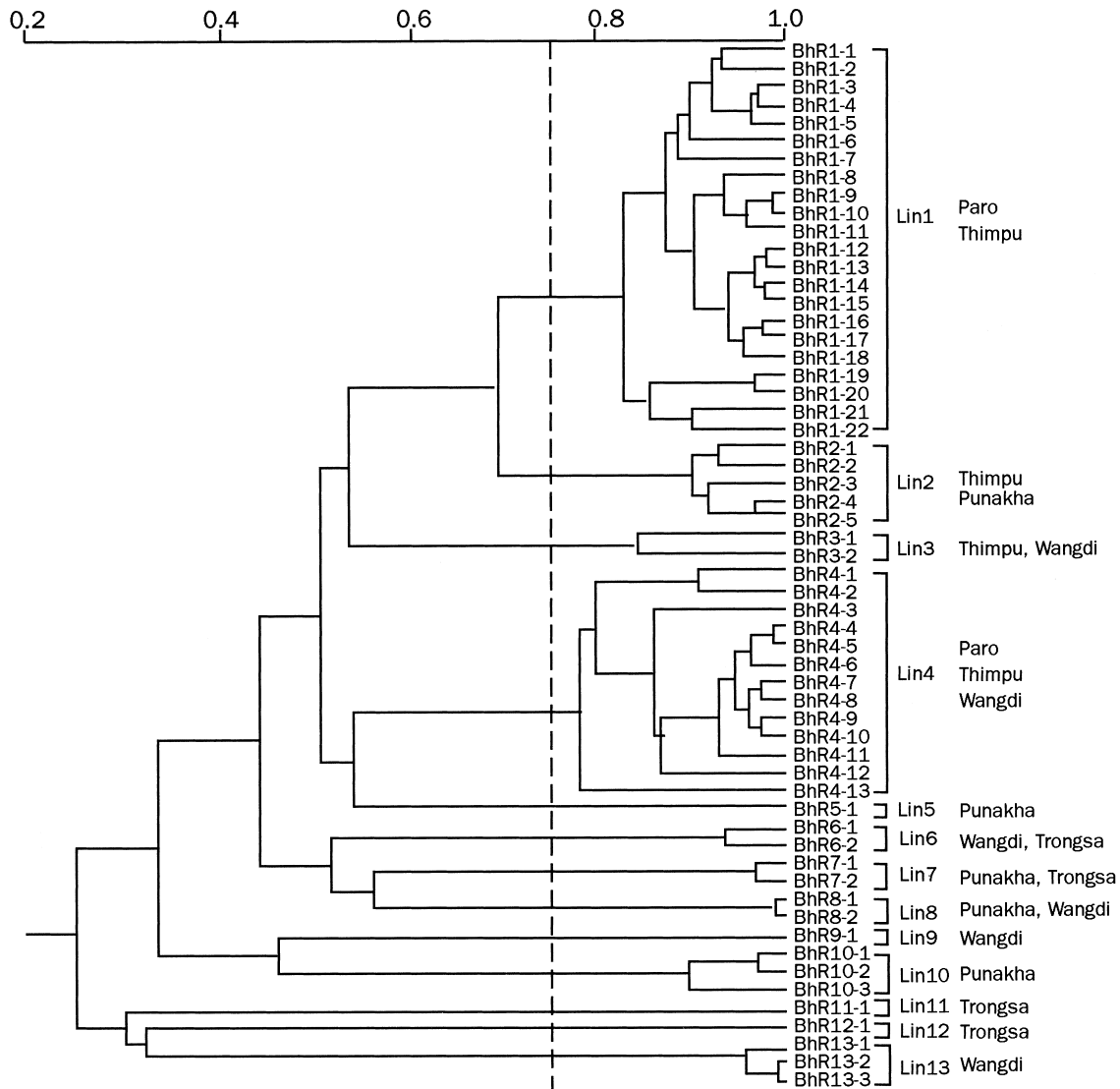


Fig. 4. Phylogenetic tree for *P. grisea* isolates collected in Bhutan in 1995. The phenogram was derived from restriction fragment length polymorphism band data, based on the hybridisation of EcoRI-digested DNA with the multilocus element MGR586.

belonged to lineages BhR1 and BhR4 which comprised 36 and 21% of the collection, respectively. In contrast, the other lineages were represented by only one to six haplotypes each (Table 4). Lineage one and four isolates were found in both high altitude environments Thimphu and Paro, and, late in the season, a few lineage four isolates were recovered from Wangdi. Also, late in the season, there were a few lineage two and three isolates recovered from Thimphu whereas

all other lineages were only found in Punakha, Wangdi and Trongsa with most lineages being found in only one of the sites either early or late in the season. Lineages one and four increased in the collection from 43 to 68% from the early to the late season. This was most likely an artefact of collection because no isolates were collected from Paro early. Thimphu and Paro districts were the most heavily infected and most late season isolates (70%) were collected from there.

Table 4

Distribution of lineages and haplotypes, as defined by MGR586, of *P. grisea* isolates collected in Bhutan during a blast epidemic in 1995

BhR lineage	Total number of isolates per lineage (%)	Number of isolates per lineage and district collected early/late ^a in the season					Number of isolates per lineage early/ late season
		Paro	Thimphu	Punakha	Wangdi	Tongsa	
1	26 (36.3)	–/13	7/6				7/19
2	6 (8.5)		0/3	3/0			3/3
3	2 (2.8)		0/1		0/1		0/2
4	15 (21.1)	–/2	6/4		0/3		6/9
5	1 (1.4)			1/0			1/0
6	3 (4.2)				0/1	2/–	2/1
7	3 (4.2)			2/0		1/–	3/0
8	4 (5.6)			1/0	0/3		1/3
9	2 (2.8)				1/1		1/1
10	3 (4.2)			0/3			0/3
11	1 (1.4)					1/–	1/0
12	1 (1.4)					1/–	1/0
13	4 (5.6)				4/0		4/0
Total		0/15	13/14	7/3	5/9	5/0	30/41

^a Numbers for isolates collected early and late in the season are separated by a slash. No isolates were collected from Paro early in the season because blast did not occur in seed beds or in young seedlings. Also none collected from Tongsa late in the season because no epidemic occurred. See footnote of Table 1 for location and altitudes of districts.

Bhutanese lineages were unique when compared with lineages from the Philippines (Chen et al., 1995) and the Himalayas of Uttar Pradesh, India, (Kumar et al., 1999), two sources of recent rice germplasm introduction. However, no comparisons were possible with blast populations from Nepal, another source of varietal introduction, since no Nepalese populations have been fingerprinted.

Total haplotypic diversity as well as haplotypic diversity for all districts both in early and late season collections were very high. The ratio of isolate to haplotype was 1.22:1. Only two haplotypes, BhR1-17 and BhR4-4, were found in two districts each, Paro and Thimphu and two haplotypes, BhR4-4 and BhR9-1, were found early and late in the season.

Within lineage one, band differences between haplotypes averaged 13, ranging from 1 to 27, producing two subclusters within the lineage (Fig. 4). Similarly, three subclusters with band differences ranging from 17 to 26 were identified within lineage four. It is impossible to infer such information from the other lineages because too few isolates representing each lineage were collected. The subclusters in lineages one and four suggest haplotypic divergence and also suggest that these are old lineages.

4. Discussion

The 1995 blast outbreak was the first officially recorded disease epidemic in rice in Bhutan. Even the majority of farmers interviewed during the epidemic season did not remember having seen such an outbreak in their rice fields previously, although some of them confirmed that blast in rice has been there all the time but at low, insignificant levels.

No changes were reported in nitrogen management and irrigation water supply cultivars or other management practices most likely to predispose rice crops to the blast disease. Also, there was no evidence of higher incidence of blast in fields where Butachlor was used to control weeds, and the authors are not aware of any reports in the literature of relationships between Butachlor use and blast incidence. The currently used cultivars are highly susceptible to *P. grisea*. However, considering that these cultivars have been planted for many years in those highland environments, their susceptibility per se cannot explain the epidemic.

On the basis of haplotype diversity in the more common lineages one and four, it appears that these lineages have been present for a long time. MGR586 is a high copy number (45–70) resolvable

RFLP fragments) and active transposable element (Hamer et al., 1989) that has been found to be distributed throughout the *P. grisea* genome. Studies throughout the world have confirmed that virtually all rice-infecting strains carry a high MGR586 copy number, whereas high copy number is rare in non-rice infecting strains (Shull and Hamer, 1994). It has proved to be useful in determining genetic relatedness of strains as the RFLP fingerprints offer an assessment of the distribution of the element and the EcoRI restriction enzyme binding sites across all the chromosomes of the fungus. Similarity of fingerprints therefore is consistent with similar genetic backgrounds, or relatedness. Recovery of similar fingerprints over many years and across great distances (Levy et al., 1993) suggests that, although capable of transposition, the element is sufficiently stable to allow tracking of related strains over several years. Given the predominance of asexual reproduction in the field, isolates with similar fingerprints are inferred to constitute members of clonally-derived lineages.

Differences in MGR586 banding patterns among closely related strains could also reflect genetic recombination as a result of a sexual cross between closely related strains. MGR586 fingerprints could diverge in very old lineages through accumulated transposition events, and such continuous variation would be indistinguishable from a sexually recombining population. However, in an unpublished study only a single mating type was detected among Bhutanese isolates used in this study (Borromeo, Leung and Zeigler, unpublished). Therefore, sexual recombination is not a likely explanation for the observed MGR586 fingerprint diversity within putative lineages in Bhutan. Nonetheless in future studies more stable markers may provide data with less ambiguity.

The isolates were collected from high and mid altitude areas only and isolates from the eastern and southern regions where rice is also grown widely are not represented. Lineage four which dominated together with lineage one the high altitude areas was found in the late season in Wangdi. This could be the result of regular transfer of high altitude germplasm from the Thimphu and Paro area to Wangdi for breeding and varietal testing purposes. Thus, inoculum could easily have been distributed that way. The lineage did not dominate in either location, however, making it an unlikely cause of the epidemic.

The remaining explanation that could account for this epidemic and for which the data are somewhat supporting is unusual weather. The blast pathogen requires ambient temperatures between 10 and 15–28°C and relative humidity greater than 93% and long dew periods for at least 15–16 h to infect plants and cause widespread destruction (Ou, 1985). In 1995, compared to other years, the weather conditions were favourable for a blast epidemic. In addition to the unusually wet August and September months compared to past years, a number of farmers from the high altitudes also reported drought in the rice nurseries which increases susceptibility to blast.

The yield loss analysis of blast affected fields in Paro and Thimphu indicated, not surprisingly, that yield loss was significantly related to the level of disease. The relationship, however, was not linear as had been assumed in a preliminary analysis by the Bhutanese Ministry of Agriculture (MoA). This can be explained by the ability of surviving plants to compensate for lost plants because of less competition from affected neighbouring plants. Torres and Teng (1993) reported that both leaf and panicle blast had significant negative effects on yield. However, the effect was not the same for all the fields, suggesting that factors other than disease such as soil fertility, micro-environmental conditions, and variation in measurement also influence the ultimate crop yield estimates. The discrepancies between the MoA yield estimates and the present estimates are large especially for some gewogs in the Paro district. This could partly be the result of differences in the number of samples taken and also because the estimation of yields for the MoA samples are usually taken from relatively high yielding areas. It could also be a result of the rice cultivars involved in the estimation of yield. Mostly traditional cultivars were affected by blast and thus sampled for the yield loss analysis. However, high yielding improved cultivars were not affected by blast but certainly were sampled by the MoA.

The epidemiology of blast in the temperate rice environments of Bhutan is not fully understood. For instance, neck and node blast were observed in the high altitudes with no noticeable earlier presence of leaf blast and very low to no seedling blast. Similar observations were also made in the subsequent years (Thinlay, 1998). Most likely, these outbreaks are the result of latent infections originating from infested seed as

was shown to occur in the high altitude rice growing regions of Nepal (Manandhar et al., 1998). Blast was observed in the most affected high altitude sites already in 1994 at relatively high levels (Thinlay personal observation). This could have been the source of seed borne inoculum.

Another factor contributing to neck and node blast susceptibility could be the anatomy of many traditional cultivars in Bhutan which have upright leaves. With such a leaf arrangement it is possible that fungal spores on the lamina joints are washed down and deposited on flag leaf sheaths and infect emerging panicles and nodes. It is also possible that in Bhutan rice growing environments other sources of blast inoculum, e.g., rice straw and grasses may be important for neck and node infections.

Blast populations in the mid altitudes possess a wider spectrum of virulence than in the high altitudes (Thinlay et al., 1999) thus exerting strong selective pressure for resistance already in the seedling stage. Correspondingly, mid altitude cultivars possess more effective resistance than altitude cultivars (Thinlay, 1998).

To deal with possible epidemics in the future, the Ministry of Agriculture and district agricultural officials drew up an action plan. The farmers were advised to improve their nursery techniques by seed treatment with the systemic fungicide Tricyclazole which can eliminate seed borne inoculum and protect the seedlings for at least 30–40 days after treatment even under the cold seedbed conditions prevailing in Bhutan (Zeigler et al. unpublished). It was also advised to locate nurseries where blast is less likely to occur, i.e., in open well-aerated areas away from streams. The plan also includes proper disposal of rice straw and stubble and training of extension agents and farmers in the identification of blast symptoms.

Except for the weather, it was not possible to attribute the cause of the blast epidemic in 1995 to a single probable factor as there is no conclusive evidence to demonstrate a change in the local pathogen population. There is a need to develop a pest and disease surveillance system with proper meteorological data gathering equipment and trained staff. The establishment of a special fund could help deal with unforeseen pest/disease outbreaks and other natural calamities.

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