An overview of bacterial blight disease of rice and strategies for its management

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This is a status paper on a destructive rice disease – bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). We review the occurrence and spread of this disease, the taxonomy and classification of the pathogen and strategies for disease management. Studies on pathogen variation have revealed that breeding with single major genes for resistance, may be ineffective due to resistance breakdown. Thus, pyramiding of resistance genes appears to be a good option for disease management. Evidence from our laboratory suggests that BB of rice can be managed through carefully selected biological control agents which can be used by themselves or can work in combination with single major genes for BB resistance (e.g. *Xa4*). We also present the view that elite indica rice cultivars can be constructed through traditional breeding and transgenic approaches to incorporate pyramids of blast and BB resistance genes to effectively manage these two devastating rice production constraints and to sustain rice yields in India.

RICE is perhaps the most widely cultivated food crop world over, whose production is constrained by diseases of fungal, bacterial and viral origin. Bacterial blight (BB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the oldest known diseases and was first noticed by the farmers of Japan in 1884 (ref. 1). Subsequently, its incidence has been reported from different parts of Asia, northern Australia, Africa and USA.

The disease is known to occur in epidemic proportions in many parts of the world, incurring severe crop loss of up to 50%. Crop loss assessment studies have revealed that this disease reduces grain yield to varying levels, depending on the stage of the crop, degree of cultivar susceptibility and to a great extent, the conduciveness of the environment in which it occurs.

The severity and significance of damages caused by infection have necessitated the development of strategies to control and manage the disease, so as to reduce crop loss and to avert an epidemic. Though the use of Bordeaux mixture, antibiotics and other copper and mercurial compounds were resorted to in the early fifties, environmentally safe and stable chemical control agents rendering control at very low concentrations are yet to be developed. Today, the exploitation of host resistance appears to be the only reliable method of disease management. The identification and characterization of major genes for qualitative resistance and polygenic factors controlling quantitative resistance have contributed a great deal to the success in breeding resistant cultivars. Recent research in our laboratory has provided considerable evidence that the deployment of bacterial antagonists to *Xoo* might be an effective strategy, bringing about disease suppression by biological control (unpublished data). This review aims at updating our present state of knowledge about different aspects of BB, its causative organism and more importantly, the various strategies that may prove effective in reducing disease severity and consequently in improving rice production in India and elsewhere.

The disease

BB is a vascular disease resulting in a systemic infection[2] that produces tannish-grey to white lesions along the veins. Symptoms are observed at the tillering stage, disease incidence increases with plant growth, peaking at the flowering stage[3]. Kresek is the more destructive manifestation of the disease, wherein the leaves of the entire plant turn pale yellow and wilt during the seedling to the early tillering stage, resulting in a partial or total crop failure. Plants less than 21 days old are the most susceptible and temperatures between 28 and 34°C favour kresek development[4,5]. BB is characteristic of yellow lesions with wavy margins on leaf blades that may extend to the sheath (Figure 1). These lesions acquire a whitish straw colour over a period of time. The occurrence of a bacterial ooze from infected leaves has been observed in warm and humid climates, which contributes to the spread of this disease. Though leaf blight does occur at all growth stages, it is most common from maximum tillering until maturity. While damage is extensive when kresek precedes BB, post flowering infections have very little effect on grain yield. However, when infection occurs during panicle initiation or subsequently during stages that precede flowering, a severe impairment of grain development and a consequent increase in sterility was observed.

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The pathogen

The pathogen is a yellow, slime-producing, motile, gram-negative rod with a polar flagellum and enters the host normally through wounds or natural openings. It reaches the vascular tissue, particularly the xylem, from where it multiplies and spreads throughout the plant. Recent advances in understanding of the principles underlying the interaction between the pathogen and its host, leading to either a compatible or an incompatible disease reaction has been reviewed[6].

A number of modern approaches to bacterial taxonomy, classification and nomenclature appear promising especially with the blight pathogen. In 1908, Takaishi found bacterial masses in dew drops of rice leaves but he did not name the organism. Bokura in 1911 isolated a bacterium, and after a study of its morphology and physiology, the bacterium was named *Bacillus oryzae* Hori and Bokura. Ishiyama[7] studied the disease further and renamed the bacterium *Pseudomonas oryzae* Iyeda and Ishiyama according to Migula's system. It was later transferred to *Bacterium oryzae* and subsequently to *Xanthomonas oryzae* (the name used for the last 40 years). According to the revision of the International Code of Nomenclature of Bacteria the committee on taxonomy of phytopathogenic bacteria of the International Society of Plant Pathology adopted the name *Xanthomonas campestris* pv. *oryzae Dye*[2,7]. In 1990, the pathogen was elevated to a species status and was named *Xanthomonas oryzae* pv. *oryzae*[8].

Programmes for resistance breeding and disease control of BB in rice depend on reliable identification and classification of the bacterial pathotypes. Morphological, physiological and biochemical characters do not reveal differences to delineate pathogenicity/virulence grouping of Indian isolates[9]. Five virulence groups of the BB pathogen have been identified based on infection responses elicited on rice lines containing combinations of 2, 3 and 4 major *R* genes. However, the difference in virulence was not sufficient in magnitude for classification into pathogenic races[10]. Classical pathotyping which uses a set of differential rice cultivars is laborious and time consuming. Therefore, a serological classification of Indian strains of the rice BB pathogen was carried out using monoclonal antibodies (Mabs)[11,12]. A panel of 6 Mabs (specific to *Xoo*) was reacted to 70 Indian strains of the pathogen and six serogroups were identified (Table 1, Figure 2). Serogroup I had 51 strains and the remaining 19 strains formed 4 new serogroups IIa, IIb, V and VII which have not been known among the global populations of the BB pathogen[13]. These results suggest that pathotypes may not relate to the serological diversity observed in the pathogen.

The use of RFLP as a reliable tool for understanding the population biology and structure of *Xoo* has been well documented[14–19]. A repetitive DNA sequence, pJEL101 isolated from the genome of *Xoo* was used to assess its genetic variability and population structure[15,18,19]. Also,

Table 1. Serological classification of Indian strains of *Xoo* with monoclonal antibodies (Mabs)

	Presence or absence of Indian strain in given serogroup					
Mab	I	IIa	IIb	V	VI	VII
X1	+	+	+	+	+	+
Xco-1	+	+	+	–	–	–
Xco-2	+	–	–	+	–	–
Xco-5	–	–	–	–	–	–
Xco-T	–	–	–	–	+	–
G4-7	–	+	–	–	–	+
Strains (no.)	51	3	2	1	2	11

+, Strain reacts with the given MAb; –, Strain does not react with the given Mab.

Figure 1. Symptoms of bacterial leaf blight in rice.

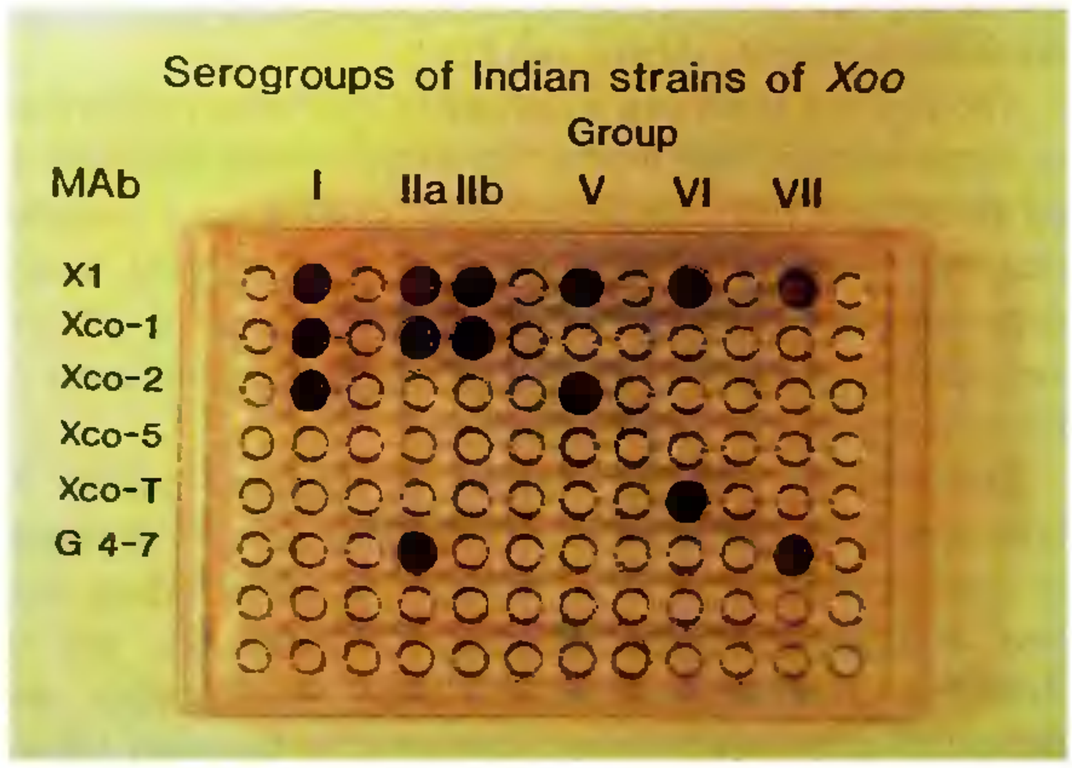

Figure 2. Serological classification of Indian strains of *Xoo*. A panel of six monoclonal bodies was used to serogroup 105 strains of *Xoo* collected from major rice-growing regions of India into six serological groups[13].

the repetitive elements IS*1113* and IS*1112* hybridized with large number of bands and served as informative probes[19]. One of the avirulence genes, *avrXa10*, has also been used as a probe to differentiate *Xoo* strains from the Philippines[20,21]. The probe hybridized with 9 to 23 different sized DNA fragments in the genome of a strain. In RFLP analysis, 9 haplotypes were detected by probe pJEL101 and 5 haplotypes were detected by *avrXa10* probe among the Indian strains. The study also identified 5 distinct genetic clusters from among the Asian collection of strains from China, India, Indonesia, Korea, Malaysia, Nepal and the Philippines. The Indian strains formed cluster No. 5 along with Nepalese, Malaysian and Indonesian strains[15]. Whether the haplotypes identified from such fingerprints correspond to the pathotypes distinguished through virulence analysis remains to be established[19]. However, in a recent attempt to fingerprint the pathogen population in India using the *avrXa10* and IS*1112* probes, the strains appear to be closely related to each other, belonging to a single lineage. Pathotyping analysis of these strains revealed that the strains in this lineage belong to the pathotype 1b (ref. 22).

DNA fingerprinting makes use of the presence of microsatellites (2–10 bp) and minisatellites (10–40 bp) which are repeated in tandem and dispersed in the genome[23–25]. These along with *avr* gene probe and repeat clone pBS 101, were used to generate DNA fingerprints of different pathotypes of *Xoo* from the Indian subcontinent[26]. Cluster analysis based on hybridization patterns using all the above probes showed five groups at 56% similarity[24] (Figure 3).

Analysis of genetic diversity of different groups of bacteria through rep-PCR has also been carried out by several workers[27–31]. An attempt to compare the efficiency of RFLP and rep-PCR in detecting the variation in the pathogen population, revealed that BOX primers used in rep-PCR detected the least polymorphisms and REP primers detected the most. The use of data from rep-PCR with two primer sets (ERIC and REP) and RFLP with one probe (IS*1113*) allowed higher level of detection than either probe/primer set or technique alone[32].

rep-PCR has several distinct advantages over RFLP for analysis of large populations in being a simpler, less expensive and less time consuming technique. However, in both cases, the resolving power is dependent on the type of primer or probe used[32].

Mode of infection and transmission of the pathogen

Successful infection of a host plant by a bacterium involves the movement of the bacterium towards the host, contact between the two, penetration of the host by the bacterium and proliferation of the bacterium inside the host immediately following entrance. In the case of BB disease, the pathogen chiefly enters through hydathodes as suggested by electron microscopic studies[33,34]

Wounds on rice leaves are also favourable avenues for entry of the pathogen. The infection seems more successful in the case of entry of the pathogen through wound sites than natural openings. However, new wounds are more conducive to infection than old wounds. Also, Kiryu *et al.*[35] demonstrated that abundant inoculum results in higher percentage of infection.

Transmission of BB pathogen

Irrigation water is considered to contribute to the spread of this disease over large areas of cultivated land, as it carries the bacterial ooze that drop into rice field water. However, the role of water as a primary mode of transmission has been disputed as the pathogen survives only for 15 days in field water[36,37].

The BB pathogen is seed-borne, although the extent to which it is transmitted through the seed has been questioned[38]. A PCR assay for amplification of *Xoo* DNA using primers derived from a repetitive mobile element IS*1113* could not detect the pathogen DNA from seeds collected from infected plants[39].

The controversy over seed transmission of the BB pathogen has resulted due to the fact that sowing seeds from a diseased field into a disease-free field did not always lead to a disease outbreak and most of the experimental evidences for seed transmission were obtained from the bacteriophage technique and not from the direct

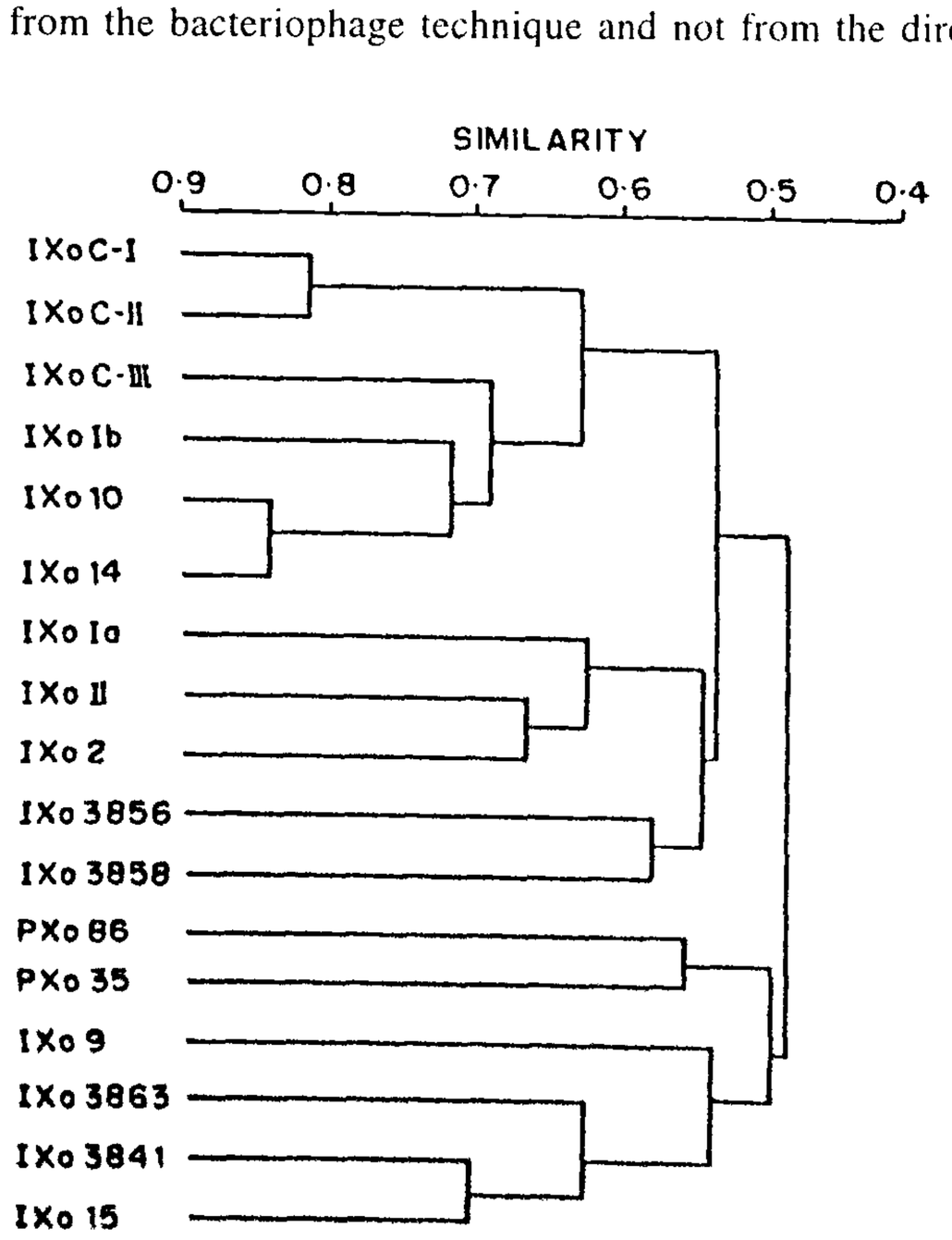

Figure 3. Dendrogram of 15 Indian strains based on similarity index (X_D) of the hybridization patterns generated with probes $(TG)_{10}$, pV47, *avrXa10* and pBS101 (ref. 26).

isolation method. Moreover, the seed-borne transmission of infection either in the nursery or in the field has not been positively proved[36,40].

Detection of *Xoo*

Several indirect and direct methods aid in identification of the pathogen. The phage technique involves the incubation of seed samples with a species phage. The increase in phage number by plaque count would detect the presence of bacterium[38].

Serological methods also serve as sensitive tools for detection of the pathogen. Gnanamanickam *et al.*[41] demonstrated the detection of *Xoo* in rice seeds inoculated with the pathogen using enzyme-linked immuno sorbent assay (ELISA), whereby the bacterial colonies that reacted positively to monoclonal antibodies specific to the bacterium were examined by direct immunofluorescence (IF). Though ELISA and IF provide conclusive evidence for the presence of the pathogen, neither techniques are sensitive enough to detect low numbers of the pathogen, which necessitates enrichment[42].

Molecular probes, on the other hand, facilitate detection of even low numbers of the pathogen through PCR analyses[43]. However, a serious limitation to their use is that they fail to distinguish live cells from dead ones.

Disease management

The severity of losses incurred due to the disease necessitates development of strategies that are ecology-conscious and cost effective. BB disease management centers around methods that reduce the initial inoculum and subsequent development of the pathogen on host plants and this can be accomplished through chemical protection, host plant resistance, and biological control.

Chemical control

An ideal agent for chemical control will be one that functions at low concentration by either killing or inhibiting the multiplication of the pathogen by blocking an important metabolic pathway. It should also readily translocate and be stable in the plant system and cause minimal damage to the environment. Attempts to control BB through chemicals like Bordeaux mixture with or without sugar, copper–soap mixture, copper–mercury fungicides were made. Spraying copper oxychloride[44] and streptomycin solution at short intervals was recommended to control this disease[45]. Chlorinating irrigation water with stable bleaching powder was also reported to be effective in minimizing the disease[46]. Synthetic organic bactericides such as nickel dimethyl dithiocarbamate, dithianone, phenazine and phenazine N-oxide were also recommended[47]. Spraying techlofthalam was more useful than soil application and it translocated readily and inhibited bacterial multiplication in rice plants[48–50]. Seed treatment with hot water at 57°C for 10 min or disinfecting with mercury compounds was suggested earlier to eradicate seed-borne inoculum[1]. A foliar spray of cowdung extract (20 g/l) was also reported to suppress BB development[51]. Soil conditions are also known to have a pronounced effect on the development of BB disease. Plants grown in soil containing potash levels greater than 183 ppm are more BB resistant[52]. Similarly, supplements of phosphorous fertilizers resulted in a BB incidence of 5 on a 0–9 scale, but brought about reduction in the number of diseased tillers[53]. Also, plants that received nitrogen supplements at tillering, showed high vigour and produced kresek-free tillers[54].

However, effective and economical chemical control has yet to be developed for this disease. This may be because the pathogen population is highly variable in its sensitivity to the antibiotics used for control. The existence and development of drug-resistant strains also pose serious problems in formulating fool-proof control agents. Also, reliable forecasting systems are essential to determine the proper time of application of these chemicals for effective control.

Biological control

Biological control for BB disease has not received much attention, though there have been reports of bacterial antagonists against the pathogen[55]. Breeding for resistance to BB with single major genes has often proved unsuccessful, as their long-term use results in sub-populations of the pathogen that overcome these resistance genes. Also, pathogen variation has hindered the development of suitable chemicals as agents of control. In this light, biological control appears to be a suitable eco-friendly strategy, for disease control and management. Recent studies from our laboratory have identified rice-associated rhizosphere bacteria antagonistic to *Xoo* from *in vitro* assays (Figure 4). These organisms were able to cause a

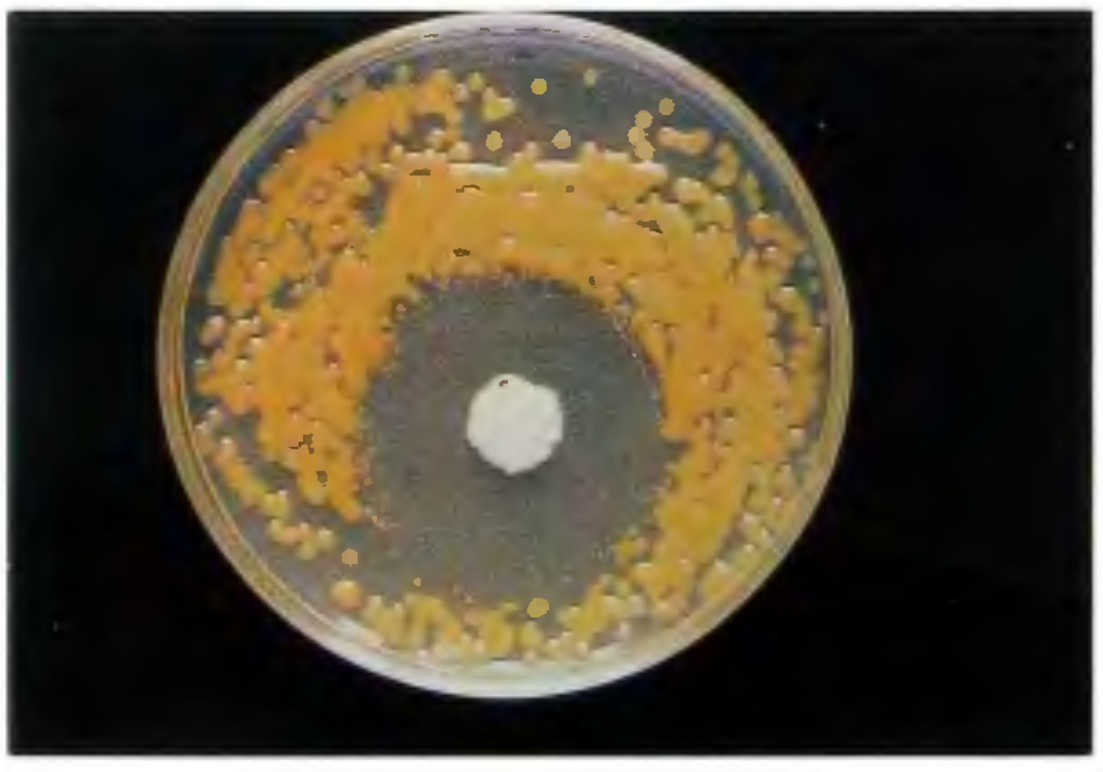

Figure 4. Laboratory assay showing inhibition of *Xoo* by bacterial antagonists.

Table 2. Laboratory and field assays for suppression of *Xoo*

Bacterial isolate	Plate assay	Field assay				
	Zone of inhibition (cm)	Mean lesion[a] length (cm)	Error mean square value	Observed difference in lesion length (cm)[b]	LSD value	
					$\alpha = 0.05$	$\alpha = 0.01$
Mon# 2–16	0.90	6.00	8.07	7.02	1.20*	1.59
Vyl 19	0.80	6.91	9.92	6.11	1.40*	1.87*
M1	1.40	7.91	14.00	5.11	1.81*	2.44*
AL23	1.30	8.03	10.82	4.99	1.47*	2.00*
Cal 9	1.50	8.50	6.03	4.55	0.99*	1.31*
Alp 18	0.60	8.73	15.36	4.29	1.94*	2.61*
Pat 8	1.00	9.08	7.32	3.94	1.12*	1.49*
M 16	1.50	9.56	18.31	3.46	2.22*	3.00*
NEL 16	1.40	10.56	11.61	2.46	1.56*	2.09*
M 11	1.80	10.58	14.42	2.44	1.85*	2.50**
VyI 18	1.10	10.64	17.63	2.38	2.16*	2.91**
Mon# 2–17	1.00	10.67	8.35	2.35	1.23*	1.63*
M 9	2.00	10.70	13.22	2.32	1.72*	2.30*
VyII 17	0.50	10.92	9.15	2.10	1.31*	1.74*
Mon 13	2.00	10.96	13.60	2.06	1.76*	2.35**
M 3	0.70	10.99	11.61	2.03	1.56*	2.09**
M 13	0.50	11.10	14.88	1.92	1.80*	2.56**
Mon 5	2.50	11.80	11.32	1.22	1.50**	2.01**
F 1	1.00	9.08	15.34	3.94	1.94*	2.62*
Control	–	13.02	13.60	–	–	–

a, Mean of normalized values;
b, Observed difference in lesion length [Mean lesion length of (control – treatment)];
*, Reduction in lesion length significant at α level of significance;
**, Reduction in lesion length not significant at α level of significance.

significant disease suppression in field assays suggesting that these strains can possibly be used as agents of biocontrol (Table 2, Figure 5).

A series of experiments to evaluate the performance of *Pseudomonas putida* strain V14i (a proven biocontrol agent against the sheath blight pathogen *Rhizoctonia solani*) in suppressing BB disease was also conducted. The results of these experiments have shown that among the different ways of application of the bacterial biocontrol agent, its application as a foliar spray offered maximum suppression of BB disease severity[56]. A direct correlation was also observed between the endophytic survival of *P. putida* in rice tissues and the extent of disease suppression (Table 3 and 4, Figure 6) indicating that biological control may be a strategy worth pursuing for the management of BB disease.

Figure 5. Suppression of leaf blight of rice by bacterial antagonists in field assays. *a*, Control; *b*, Treated.

Host resistance

In the absence of effective chemical or other control agents against the BB pathogen, host resistance has gained enormous importance in controlling this disease. In rice, the genetics of resistance to several pathogens has been well characterized. Resistance of rice plants towards *Xoo* at different growth stages varies according to host genotypes as seedling resistance (at seedling stage) and adult plant resistance (at adult stage but susceptible at seedling stage)[2]. In response to a pathogen, the host plant expresses various degrees of resistance which is usually classified into two categories namely qualitative resistance and quantitative resistance. Qualitative resistance is

generally controlled by major genes while quantitative resistance is controlled by polygenic factors.

Qualitative resistance: Qualitative resistance is the resistance conferred by a single major gene which may be dominant or recessive. Till now, about twenty-one major BB resistance genes have been identified[57] and some of them have been listed in Table 5.

More recently, a locus for resistance to BB was transferred from the wild species *Oryza longistaminata* to the cultivated rice line IR24 generating the introgression line IRBB21 (ref. 58). This locus, *Xa21*, was found to confer resistance to all known *Xoo* races in India and Philippines[58,59]. Recently, a gene at this locus has been cloned (*Xa21*)[60] which is the member of a small multi-gene family of kinases. Most of these family members are linked, suggesting that *Xa21* is part of a complex locus[61,62]. The structure of *Xa21* represents a previously uncharacterized class of cloned resistance genes. The deduced amino acid sequence of *Xa21* encodes a receptor kinase-like protein carrying leucine-rich repeats (LRR) in the putative extracellular domain, a single pass trans-membrane domain, and a serine–threonine kinase intra-cellular domain[62].

Earlier studies have determined if the multi-isolate resistance observed for line IRBB21 was due to a single gene or multiple genes at the *Xa21* locus. For example, the locus may encode a single gene product *Xa21*, that specifies resistance to multiple pathogen isolates, or the locus may be composed of a cluster of tightly linked genes, each of which recognizes a unique isolate-specific determinant. Wang *et al.*[60] reported that transgenic plants expressing the cloned *Xa21* gene conferred multi-isolate resistance to 29 diverse isolates from eight countries. However, recent studies have shown that Nepalese strains were virulent on *R* gene *Xa21* present in rice line IRBB21 (ref. 63). We have also observed a sub-population of *Xoo* virulent to rice line IRBB21 from a pathogen population isolated from a BB epidemic that occurred in 1998 in Kerala[64] (Table 6, Figure 7). Therefore, the usefulness of *Xa21* to the Indian subcontinent and South Asia may be in doubt. While, the near isogenic line IRBB21 carrying *Xa21* was susceptible to this sub-population, rice line NH56 carrying four *R* genes[65], (*Xa4* + *xa5* + *xa13* + *Xa21*) was found to be resistant[64] (Figure 7).

Quantitative resistance: Quantitative resistance, also known as horizontal resistance is a low-level resistance that

Table 3. Endophytic presence of *Pseudomonas putida Pp*V14i in IR24 rice leaves after application of bacteria

	Mean log cfu/cm of IR24 rice leaves recovered after (days)		
Treatment	1	5	10
Infiltration	ND*	3.2[a]	2.06
Foliar spray	2.73	1.74	4.25
Root dip	ND	ND	ND

*. Not detected; a, Each figure is a mean of 3 replicates.

Table 4. Suppression of BB in IR24 rice plants by treatment with *Pseudomonas putida* strain V14i

	Bacterial leaf blight	
Treatment	Per cent disease incidence[a]	Per cent disease control[b]
Infiltration		
*Pp*V14i	55.63	44.37
Water (control)	98.52	1.48
Root dip		
*Pp*V14i	48.40	51.60
cmc + water (control)	89.10	10.90
Foliar spray		
*Pp*V14i	22.88	77.12
cmc + water (control)	86.73	13.27

a, % disease incidence = $\frac{\text{Total lesion length}}{\text{Total leaf length}} \times 100$;

b, % disease control = 100 – % disease incidence;
cmc, carboxymethylcellulose.

Figure 6. Suppression of bacterial leaf blight in IR24 rice plants by foliar spray application of *Pseudomonas putida* V14i.

generally shows no pathogen race specificity[66,67]. This type of resistance gained interest because it can prevent the breakdown of varietal resistance in a breeding programme.

Quantitative resistance of rice varieties to BB is complicated for genetic analysis because of their continuous variation with no distinct classes in a segregating population. Washio *et al.*[68] were the first to report the slow lesion-developing type of resistance (quantitative) in Japan which was controlled by polygenes.

Many workers have also identified varieties showing polygenic resistance to BB[69–72]. Inspite of the voluminous data available, more research is needed to understand the nature of quantitative resistance to BB.

Table 5. Some genes identified for BB resistance

Gene identified	Cultivar analysed	Note	Reference
Xa1	Kogyoku Koganemaru Pi No.1	Chromosome 11	82
Xa2	Rantai Emas 2	Linked with *Xa1* (2–16%)	82
Xa3	Wase Aikoku 3 Java 14 Koentoelan Nagomasari	Adult resistance	83
Xa4[a]	IR22 Sigadis	Seedling resistance	84
Xa4[b]	Semora Mangga	Adult resistance	84
xa5	IR 1545 – 339 RP 291 – 7	Seedling resistance	85
Xa6	Malagkit Sungsong Zenith, etc.		86
Xa7	Dz 78 Dv 85 Dv 86	Adult resistance	87
xa8	P1231129		87
xa9	Khao Lay Nhay Sateng	Linked with *Xa6* (5.9%)	88
Xa10	CAS209	Linked with *Xa4* (27.6 ± 0.2) chromosome 5 Seedling resistance	89
Xa11	IR944-102-2-3-RP9-3		90
Xa12 *(Xakg)*	Kogyoku Java 14	Linked with *Xa1* (2%)	91
xa13	IR666 99-5-5-4-2	Chromosome 8	92
Xa14	Taichung Native 1		93
xa15	M41		93
Xa16	IR24		93
Xa17	Asominori		93
Xa18	Toyonishiki		93
xa19	XM5		93
xa20	XM6		93
Xa21	*Oryza longistaminata*	Chromosome 11	94

Genetic transformation

Genetic transformation of rice offers numerous important opportunities for the improvement of existing elite varieties and development of new cultivars. A major advantage of genetic engineering is that it allows breeders to rapidly develop new varieties by the introduction of cloned genes into commercial varieties. Zhang *et al.*[73] have recently reported the regeneration of transgenic fertile plants from four elite indica varieties in group 1, viz. IR64, IR72, Minghui63 and BG90-2 carrying bacterial blight resistance gene *Xa21*. This was found to impart significantly improved resistance to the BB pathogen, and this resistance was shown to be stably inherited in subsequent generations. Wang *et al.*[60] transformed japonica rice variety T309 with cloned *Xa21*, and its resistance spectrum was similar to that of the donor line IRBB21. However, T309 is no longer cultivated, and no new commercial rice cultivars with *Xa21* have been released. Recently, Tu *et al.*[74] reported that the transformation of elite indica rice variety IR72 with *Xa21* gene confers resistance to the BB pathogen and this resistance was shown to be stably inherited in subsequent generations.

Future directions

In future, management of BB in rice will have to be accomplished not by any single strategy or management practice[75]. We discuss here, a combination of more than one major strategy to alleviate this problem.

Biological control

The use of biocontrol agents to bring about BB suppression remains to be explored in detail. It is important to establish beyond doubt, the mechanism of control, so that these biological agents may be deployed and used efficiently. We plan to characterize the mechanism involved in disease suppression by bacterial antagonists and combine this trait with that of *Pseudomonas fluorescens* strain 7–14, whose ability to suppress both blast and

Table 6. Pathogenicity test for *Xoo* strains obtained from a BB epidemic in Kerala (Nethouse study, RARS, Pattambi)

Rice line	Resistance gene(s)	Disease reaction* R	MR	S
IR24	–	–	–	100
IRBB21	*Xa21*	6	88	6
NI156	*Xa4*, *xa5*, *xa13*, *Xa21*	100	–	–

*, According to SES scale 1988; R, Resistant; MR, Moderately resistant; S, Susceptible.

sheath blight of rice has been well established[76–78]. This proposed research work, if successful, will result in an improved strain or strains effective against the three most important rice pathogens, viz. *Xoo*, *Magnaporthe grisea* and *Rhizoctonia solani*.

Although pyramiding genes for resistance is a worthwhile effort, several major genes are expressed stage-specifically, conditioning adult plant resistance. Therefore, the deployment of biocontrol agents at the seedling stage may prevent early infection, resulting in increased levels of disease suppression. We plan to evaluate the integration of these two major approaches by combining host resistance and biocontrol for management of BB in rice.

Pyramiding of genes for resistance

Employing host resistance has been widely resorted to for bringing about control of the disease. Many major and minor genes have been identified and near isogenic lines (NILs) have been developed with a single major gene in a susceptible genomic background (IR24), to analyse virulence characteristics of the existing pathogen population. A gene-for-gene relationship was found in the interaction of the pathogen with every major resistance gene[2,79].

In some areas, although a single gene confers resistance to the existing pathogen population, the large-scale use of this gene results in the breakdown of resistance. To delay such breakdown, pyramiding of more than one resistance gene was found to be effective and an attempt to combine *R* genes available in NILs (14 lines containing a pair of *R* genes among *Xa1*, *xa13*, *Xa4*, *xa5*, *Xa7*, *Xa10* and *Xa11*) were selected[80]. Also, the *R* genes (*Xa4*, *xa5*, *xa13* and *Xa21*) were combined to result in a pyramid line NH56 (ref. 65). In rice breeding programmes at International Rice Research Institute (IRRI) and in national rice improvement programmes in the Philippines, Indonesia and India resistance genes *Xa4*, *xa5*, *Xa7* and *Xa21* are being transferred to commercially important rice varieties[81]. Likewise, in our laboratory, efforts are under way to incorporate a pyramid of four genes into a local high-yielding but BB prone cultivar, 'Jyothi' grown extensively in Kerala. In inoculation assays, this four-gene pyramid NH56 with resistance genes *Xa4*, *xa5*, *xa13* and *Xa21* was found to be effective in controlling existing pathogen populations. The resistant progeny will be selected by means of marker-aided selection (MAS) and through pathogenicity tests.

Figure 7. Bacterial blight reactions to South Indian strains of *Xoo* in rice cultivars IRBB21 (*Xa21*) and NH56 (*Xa4* + *xa5* + *xa13* + *Xa21*).

Genetic transformation

It has been suggested that biotechnology can contribute to the agronomic improvement of rice, when used in combination with traditional or in conventional breeding methods, which will make it possible to achieve the required increase in crop production and protection against various pathogens of rice. We propose to obtain multiple resistance in the elite indica rice variety IR50 for two important diseases of rice – blast and blight. In order to achieve this objective, we have planned to use blast resistant lines of IR50 (developed through our work) introgressed with *Pi2* gene for blast resistance as the starting material for genetic transformation with BB resistance gene *Xa21*. This approach combines both the traditional breeding and the transgenic approach for deriving elite rice cultivars with multiple resistance to pathogens.

Concluding remarks

How important is BB to rice cultivation in India today? This question is difficult to answer. The disease is very destructive in Punjab, Haryana, Bihar and Uttar Pradesh where it occurs regularly. It occurred in an epidemic form during 1998 in Palghat district of Kerala and destroyed the rice harvest. The pathogen population we obtained from this epidemic yielded 200 strains of *Xoo* which had the ability to overcome the resistance afforded by single major genes for BB resistance, such as *Xa4* and *Xa21* (Figure 7). This observation questions the value of single *R* genes, including *Xa21*, which was hailed to resist most of global strains of the BB pathogen. Therefore, the deployment of a pyramid of BB resistance genes[65] (Figure 7) discussed here appears to be the right strategy for management of BB in future.

A systematic search for biocontrol agents that would bring about significant BB suppression in field conditions

is necessary for establishment of biological control as a suitable strategy for resource-poor rice farmers. We have presented some evidence that carefully selected bacterial antagonists of the rice-associated group of bacteria provide substantial suppression of BB. Bacterial treatments when combined with the protection afforded by single *R* genes, whose expression appears limited to different stages of the rice crop, enhances the level of protection (Figures 4–6). This integration of two major approaches should be thoroughly tested for BB management in India.

Finally, there are exciting possibilities for building elite indica rice varieties which will have multiple resistances to rice pathogens such as blast and BB. We have initiated some work in this direction by mobilizing *Pi2* gene for blast resistance into blast-susceptible IR50, and then have planned to use this blast-resistant IR50 for transformation with the BB resistance gene *Xa21* (cloned gene) through a biolistic method (in collaboration with IRRI, Manila, Philippines). In spite of our fears expressed about the usefulness of *Xa21* for India, specifically for Kerala[64], transgenic rice plants with the cloned *Xa21* gene appear to be an attractive prospect and may be useful for other target regions of India. More genes may have to be added in future to make the resistance to blast and BB more durable.

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ACKNOWLEDGEMENTS. We acknowledge the generous funding received from Rockefeller Foundation under the International Rice Biotechnology Programme. We thank scientists at the RARS, Pattambi, Kerala for assistance and for providing us with field space. We thank Prof. D. Lalithakumari, Director, CAS in Botany for providing research facilities and encouragement. P.V. thanks CSIR for the award of JRF.

Received 17 May 1999; revised accepted 10 September 1999