Identification of a new source of resistance in wild rice, *Oryza rufipogon* to bacterial blight of rice caused by Indian strains of *Xanthomonas oryzae* pv. *oryzae*

Rice (*Oryza sativa* L.) is the most important crop of India and is grown all over the country in diverse ecosystems. The productivity of rice is being affected by a number of biotic and abiotic stresses. Among the biotic stresses, bacterial leaf blight (BB) caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* is an important disease of rice that causes significant yield reductions worldwide. It is particularly destructive in Asian countries. In India, it is one of the major production constraints ever since it first occurred in epidemic proportions in Bihar and other neighbouring states of India[1] during 1975. Now, the disease is prevalent in almost all the states of the country[2] and in its severe form, is known to cause yield losses ranging from 74 to 81% in susceptible cultivars[3].

More than twenty-six genes conferring resistance to specific races or clusters of races of *X. oryzae* pv. *oryzae* have been identified[4–6] and have been incorporated into modern rice varieties. Among all the known genes for resistance to BB, *Xa21* is the dominant gene from a wild rice species (*O. longistaminata*) resistant to all 6 races of BB in the Philippines[7,8]. However this was broken down in India[9] and other countries (Nepal and Thailand) of Asia. Recently, Zhang *et al.*[6,10] reported a new gene for resistance to bacterial blight (BB) from wild species *O. rufipogon* which was also identified and mapped (to rice chromosome 11). They designated it as *Xa23*(*t*) (ref. 10). This new gene showed a high level of resistance to race 10 to which *Xa21* is highly susceptible at all growth stages. This report deals with the identification of a R-gene in Indian accessions of *O. rufipogon* which when analysed through sequence-tagged-site (STS) markers specific to *Xa21*, has shown similar DNA polymorphisms as that of *Xa23*(*t*) and also has shown high levels of resistance to more than 50 southern Indian strains of *X. oryzae* pv. *oryzae*[11]. These include strains which can also overcome *Xa21*. We consider this as a potential source of resistance to bacterial blight in India.

During 2000–03 we assembled more than 1,200 strains of *X. oryzae* pv. *oryzae* from infected rice materials collected from ten states of India. The collection locations included sites in Haryana, Punjab, Uttaranchal, Uttar Pradesh, Bihar, West Bengal, Andhra Pradesh, Kerala and Tamil Nadu. Two hundred and eight of the southern Indian strains from Tamil Nadu and Kerala were pathotyped in field plots at the Regional Agricultural Research Station (RARS) at Pattambi, Kerala on near-isogenic lines of IR24 rice developed at the International Rice Research Institute (IRRI) in the Philippines. These lines carry a single R-gene or their combinations in each and were obtained with import permit from the NBPGR, New Delhi. Standard procedures were followed to prepare the bacterial cell suspension which had 10^6 cfu/ml (pathogen inoculum)[12] and rice leaves of 45-day-old plants of the different nearly isogenic lines (NILs) were clip-inoculated with sterile scissors that were dipped in the inoculum[13]. Development of bacterial blight lesions was monitored and the lesion lengths were measured 14 days after the inoculation. Bacterial blight lesions < 3.5 indicated a resistant (R) disease reaction while lesions > 5.0, a susceptible (S) disease reaction[12]. Based on their virulence spectrum to different genes and gene combinations carried by the rice NILs, they were grouped into pathotypes (races).

On the basis of disease reactions of rice NILs, 21 virulence groups (pathotypes) were identified within 208 strains of *X. oryzae* pv. *oryzae* and these included strains which can infect rice NIL IRBB21 that carries the bacterial blight resistance gene, *Xa21* (data not presented).

In a fresh effort to locate possible new sources of resistance to *X. oryzae* pv. *oryzae*, wild rice plants, *Oryza malampuzhaensis* and *O. rufipogon* were collected from Topslip in Coimbatore district of Tamil Nadu and Panamaram in Wyanad district of Kerala, respectively. Their identification was confirmed by scientists at National

Table 1. Evaluation of wild rices *Oryza rufipogon* and *O. malampuzhaensis* for bacterial blight resistance with strains of *Xanthomonas oryzae* pv. *oryzae* (race 1, race 6, and southern Indian (SI) strains)

X. oryzae pv. *oryzae* strain	Disease reaction in *Oryza rufipogon*[1,2]	Disease reaction in *Oryza malampuzhaensis*
PXO61 (Race 1)	R	R
PXO99 (Race 6)	S	MR/MS[2]
XTNCm1.1 (from Southern India)	R	S
XTNCm1.3	R	S
XTNCm2.11	R	S
XTNTi1.6	R	S
XTNTi2.20	R	S
XTNTi2.11	R	S
XTNAi1.2	R	S
XTNAi2.13	R	S
XTNAi3.21	R	S
XTNAi3.29	R	S
XTNTr1.2	R	S
XTNTr1.8	R	S
XTNTr2.11	R	S
XTNTr3.22	R	S
XTNTy1.1	R	S
XTNTy1.10	R	S
XTNTy2.20	R	S
XTNTy3.27	R	S

[1]Disease reactions were scored from a minimum 3 to maximum 10 measurements.
[2]R refers to resistance disease reaction characterized by < 3.5 cm long bacterial blight (BB) lesions; S refers to susceptible reactions characterized by > 5 cm long BB lesions. MR/MS refers to moderately resistant or moderately susceptible type of a disease reaction.

Bureau of Plant Genetic Resources (NBPGR), Trichur, Kerala. These wild rice plants were maintained in pots in greenhouses at the Regional Agricultural Research Station, Pattambi, Kerala and University of Madras, Chennai. To determine their resistance to *X. oryzae* pv. *oryzae*, fully grown leaves of the wild rices were clip-inoculated with diagnostic *Xoo* strains *PXO61* (race 1 strain that detects the presence of *Xa4*), *PXO99* (race 6 strain that detects the presence of *Xa21*) and 50 strains of *Xoo* from southern India. Control plants of IR24 and IRBB21 (*Xa21*) and IRBB4 (*Xa4*) rices were also inoculated at the same time.

Results presented in Table 1 were observed disease reactions for the diagnostic *Xoo* strains (*PXO61, PXO99*) and for 20 of the 50 southern Indian strains of the pathogen. The disease data in response to artificial inoculations under highly conducive conditions indicate that *O. malampuzhaensis* is susceptible to *PXO*99 (indicating the absence of *Xa*21) and to all 50 pathogen strains from southern India. Such an observation about the susceptibility of *O. malampuzhaensis* to Indian strains of *X. oryzae* pv. *oryzae* has already been made by Devadath *et al.*[14]. However, there has been no assessment of bacterial blight resistance in *O. rufipogon*. Table 1 has disease data that show that it was resistant to *Xoo* strain *PXO*61 similar to *O. malampuzhaensis* and unlike *O. malampuzhaensis* showed uniform resistance to all 50 strains of the pathogen from southern India. It was susceptible to *PXO*99 indicating that it does not have *Xa*21. Its resistance to South Indian pathogen population also establishes that it has a major R-gene which is different from *Xa*21.

An effort was made to identify the bacterial blight resistance gene in *O. rufipogon* using a sequence tagged site (STS) marker using protocols described by the Chinese researchers[10].

Plant DNA from leaf tissues of *O. rufipogon* was extracted following the method of Tai and Tanksley[15]. To determine the polymorphism between IRBB21 and *O. rufipogon*, sequence tagged site (STS) marker (U1/I1) specific to the *Xa21* locus was used as this also will determine the tentative identification of the R-gene with reference to *Xa21*. The two primer sequences used were forward – U1 5′-CGATCGGTATAACAGCAAAAC-3′ and reverse-I1 5′-TAGCAACTGATTGCTTGG-3′. PCR amplification was performed in a 20-µl volume reaction mixture containing 50 ng template DNA, 50 ng of each primer, 0.05 mM dNTPs, 1X PCR buffer (10 mM Tris pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$, and 0.01 mg/ml gelatin) and 1 unit *Taq* DNA polymerase. PCR mixture was subjected to initial denaturing for 5 min at 94°C and then was subjected to 30 cycles of PCR (denaturation at 94°C for 30 s, annealing at 55°C, and 1 min extension at 72°C) followed by a final extension for 5 min at 72°C using a DNA thermal cycler (MJ Research, Watertown, MA, USA). To visualize the DNA, 10 µl of the PCR products was loaded onto a gel containing 1% agarose and 0.5X Tris-borate buffer (89 mM tris, pH 7.8; 89 mM boric acid; and 2 mM EDTA). Gel was run for 3 h at 125 V, stained with ethidium bromide and subsequently, gel documented.

The marker-assisted selection (MAS) with STS marker (U1/I1) specific to the *Xa21* locus when used to detect polymorphisms between IRBB21 and *O. rufipogon* showed banding profiles of IRBB21 (*Xa21*) (band at 1.4 kb) that were different from those observed in *O. rufipogon* (band at 1.3 kb) (Figure 1).

With such a diverse bacterial blight pathogen population prevailing in the country, search for a wild rice as a new source for BB resistance is an option that we have explored in this study. In previous studies, however, approaches such as pyramiding of BB resistance genes and creating transgenic rices have also been studied[10,16–18]. We assessed the usefulness of *Oryza rufipogon* for its BB resistance to Indian strains of *X. oryzae* pv. *oryzae*. *O. malamphuzhaensis* while another wild rice has already been examined for its BB resistance by researchers at the Central Rice Research Institute, Cuttack[14]. It was reported susceptible then[14] and has also been found susceptible now[11].

O. rufipogon was identified as a source of BB resistance in China[6]. These researchers identified the new R-gene for BB resistance tentatively as *Xa-23(t)*. We have collected local stocks of *O. rufipogon* available in Kerala forests in India and have observed that this wild rice is resistant to 50 Indian strains of *X. o.* pv. *oryzae* and also to Philippine race 1 (*PXO61*) (Table 1).

In further analysis of PCR-based polymorphisms, R-gene that contributes to BB resistance in *O. rufipogon* appears to be distinct from *Xa-21* (Figure 1). We have suggested that it is, perhaps, *Xa-23(t)* on the basis of the observed DNA polymorphisms and its pattern of resistance to strains of *X. o.* pv. *oryzae*. However, more careful research is required to identify the R-gene and more importantly, to make this R-gene available for BB resistance breeding efforts in India. Creating an indica introgression line would be the first step in this direction and we are initiating steps to achieve this.

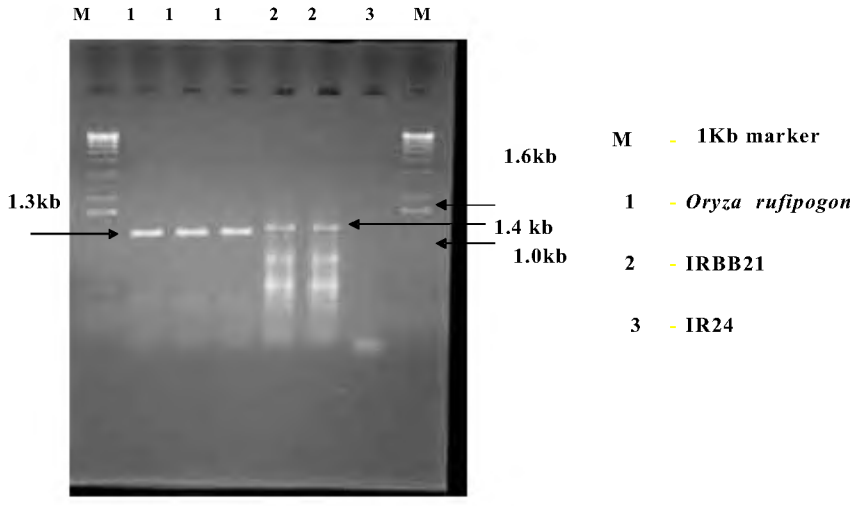

Figure 1. Detection of polymorphisms between IRBB21 (*Xa21*) and *O. rufipogon* by the use of a STS marker in PCR amplification.

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