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Identification of rice genotypes with high resistance to bacterial leaf blight caused by *Xanthomonas oryzae* pv *oryzae**

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The global rice requirement in 2025 will be of the order of 800 million tonnes (Swaminathan 2006). Currently, the production is less than 600 million tonnes. The additional 200 million tonnes needed will have to be produced by increasing productivity/ha. The average productivity may have to go up to 8 tonnes/ha from the present about 5 tonnes (yield figures related to paddy or brown rice), which requires increase in productivity to at least 3 tonnes/ha from the present 2 tonnes/ha. One of the major reasons of low productivity is damage by insects-pests and diseases, which causes an annual loss of 10–15% to rice yield.

Bacterial leaf blight caused by gram-negative bacterium *Xanthomonas oryzae* pv *oryzae* (Ishiyama) Swins *et al*. (Xoo) is the second most important disease of rice after blast and most important bacterial disease in terms of economic loss. It causes an annual loss of 20–30% in Japan and 6–60% in India. This disease is now a serious constraint for rice production in the irrigated and lowland ecologies in all rice-growing countries. In the northern plains of India, the disease is a serious problem as rice is grown under irrigated and high fertilizer input conditions that are conducive to disease development.

Study on pathogenic variability as well as identification of resistance genes are two key objectives in resistance breeding. A common approach in differentiating pathogenecity identified Xoo isolates is to employ differential lines of near isogonics lines carrying different resistant genes. Five Xoo isolates, collected from Punjab and Haryana were characterized by employing such methods, which identified isolate 5 as most virulent (Satya *et al.* 2004, 2005). It has been observed that none of the designated resistance genes produce resistance to most isolates found in Punjab and Haryana. These genes are of little use in practical resistance breeding. The probability of identifying resistant sources is more in traditional rice cultivars and land races. Indian traditional rice germplasms may contain resistance genes that may be more suitable to counteract virulent Indian pathotypes more effectively than the resistance genes identified abroad. Considering this, 223 germplasms were tested against the most virulent and aggressive Xoo isolate for identifying new resistance sources.

A total of 223 traditional rice germplasm lines collected from Jharkhand and Bihar states through, NGO named Gene Campaign, Ranchi were grown at the farms Division of Genetics, Indian Agricultural Research Institute, New Delhi in randomized block design in 3 replications during rainy (*kharif)* 2005. The same lines were grown at CRRI Cuttack during winter *(rabi)* season of 2006. Each line was transplanted as a single row of 3 m length with a distance of 60 cm × 20 cm between rows and hill, respectively. Recommended agronomical practices were followed to raise the crop. These germplasms were artificially inoculated both the place with same isolate like Xoo and isolate are most virulent at both the places and isolate has done by clipping method. The bacterial suspensions were prepared from pure culture maintained in an artificial nutrient medium at 25°C.

The tip of leaf was clipped off by a scissor dipped in the inoculum solution. The scissor was kept horizontally. During the process of clipping, the inoculum was directly dropped on the cut surface. A minimum inoculum load of 10^{-10} cells/ml was kept as recommended by Mew 1987. Standard Evaluation System (SES) advocated by IRRI was used for scoring the reaction of infected leaves.

A set of 8 landraces which were found highly resistant

*Short note

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Table 1 Bacterial leaf blight resistant rice varieties received from Gene Campaign Ranchi screened at IARI, New Delhi during *kharif* 2006

Variety	Score in nursery	Score in field (transplanted)	Average
'Hardimuri'	2.3	1.25	1.8
'Kari Jiri'	2.5	1.46	1.98
'Bhatani'	2.1	1.5	1.8
'Sitwa Dhan'	2.2	1.35	1.8
'Swarna Gora'	2.5	1.6	2.05
'Sita Gora'	2.4	1.7	2.05
'Lamba Asari'	1.6	1.7	1.65
'Jhulat'	1.6	1.7	1.65
'IRBB 55'	1.8	1.5	1.65
'Pusa Basmati 1'	16.5	12.5	14.5

(Table 1), at both places Delhi and Cuttack were sown in *kharif* 2006, with two controls namely 'IRBB 55' (resistant variety to BLB) and 'Pusa Basmati 1' (susceptible variety to BLB). Inoculation was done in two stages first in nursery stage and secondly after transplanting in field, to recheck the resistance for bacterial leaf blight.

To support the results of test of allelism, screening of the concern genotypes with the respective marker was done for genes *Xa21 and xa13* by using polymerase chain reaction (PCR) amplification technique. PCR-based markers pTA 248 for *Xa21* and RG 136 for *xa13* were used to study the polymorphic relationships. Two sets of 8 genotypes were selected for the study of polymorphism.

Genomic DNA was extracted from the young leaves of the plant (after 60 days of transplanting) samples using the standard CTAB method, which is a modification of the methods, developed by Dellaporta *et al.* (1983) and Murray and Thompson (1980).

DNA markers linked to *Xa21* and *xa13* were based on published results as pTA 248 for *Xa21* at 0.1cM (F-AGACGCGGAAGGGTGGTTCCCGGA, R-GACGC-GGTAATCGAAAGATGAAA) and RG136 for *x13 at* 3.8 cM (F-TCCCAGAAAGCTACTACAGC, R-GCAGA-CTCCAGTTTGACTTC) (Joseph *et al.* 2004) were used for the analysis. The PCR reaction mixture were set up in 10 µl volume containing 25 ng template DNA, 25ng each of forward and reverse primers, 0.1 mM dNTPs, 1X PCR buffer (10 mM Tris, *p*H 8.0, 50 mM KCl and 50 mM ammoniums sulphate), 1.5 mM $MgCl_2$ and 0.2 unit Taq DNA polymerase. To obtain this concentration the following components were added and mixed in 0.2 ml thin walled sterile PCR tube in the order given below:

Sterilized distilled water : 6.79 µl
10X buffer : 1 µl
dNTP mix : 0.04 µl
(mixed equal volumes of 100 mM individual dNTPs)
Primer (forward) pF: 0.5 µl
Primer (reverse) pR: 0.5 µl
(the initial primer concentration was approx. 1 mg/ml, it was diluted to 50 ng/ml concentration)
Taq DNA polymerase (concentration. 3U/µl) : 0.167 µl
Template DNA (concentration 25ng/µl) : 1 µl

PCR was performed using a Perkin-Elmer, Model 9600, USA. The PCR cycling conditions were an initial denaturation of 94°C for 5 min., followed by 35 cycles of denaturation for 30 s at 94°C, 30 s annealing at 55°C, 60 s extension at 72°C; concluding with a final extension of 72°C for 5 min. The PCR products were electrophoretically resolved in 1% agarose gel in 1X TBE buffer at 55V, for 1 hour and 45 min. The amplified fragments were observed under UV light. While loading the PCR products, standard molecular weight marker was loaded in first well for determining the approximate sizes of the amplified fragments. The gel was later photographed, using polaroid photographic system. In the case of *xa13* gene, PCR amplification with RG 136 did not reveal any polymorphism among the 10 lines tested, hence CAPS was developed using restriction enzyme *Hinf-I.*

First, the PCR products were quantified to find out the quantity of restriction enzyme to be added for complete digestion. The amount of restriction enzyme needed was 1 U/mg of DNA. Only 2 µl of PCR products were used for restriction digestion. The total reaction volume was 10 µl. The reaction mixture consisted of 6.7 µl sterile distilled water, 1 µl of 10X restriction buffer supplied along with the restriction enzyme, 0.3 µl of restriction enzyme (10 U/µl) and 2 µl of PCR product. The reaction mixture was incubated overnight at 37°C. The DNA fragments were then separated through electrophoresis using 1.5% agarose gel.

Observations on diseases development were recorded 15 days after inoculation,. The average lesion length was recorded in cm (Table 2). Out of 223 germplasm lines screened 13 were resistant, 168 moderately resistant, and 27 moderately susceptible and 2 susceptible. Lines which showed resistant reaction were 'Bhathani' (2.1), 'Hhaainagora' (6.0), 'Hardimur' (1.25), 'Jhulat' (3.5), 'Khilbhojni' (6.0), 'Khodraphool' (5.5) 'Lalbhog' (5.5), 'Lamba-asari' (1.6), 'Nardha' (6.0), 'Sonpiya' (6.0), 'Sitwadhan' (2.2), 'Swarnagoda' (2.5) and 'Sitagora' (2.4). Among these Hardimuri showed minimum lesion length 1.2 cm and Chainagora, Khilbhojni, Nardha and Sondiya showed high lesion length (about 6.0 cm). Decision on the degree of resistance or susceptibility was taken on the basis of the following scale proposed by Ogawa (1993).

Bacterial blight reaction type	Score lesion length (cm)
Resistant (R)	<6.0
Moderate resistant (MR)	6.0–9.9 cm
Moderate susceptible (MS)	10–15
Susceptible (S)	>15.0

Table 2 Bacterial leaf blight score on the basis of lesion length in cm

Variety	Delhi (*Kharif*–2005)	Cuttack (*Rabi*–2005)	Variety	Delhi (*Kharif*–2005)	Cuttack (*Rabi*–2005)	Variety	Delhi (*Kharif*–2005)	Cuttack (*Rabi*–2005)	Variety	Delhi (*Kharif*–2005)	Cuttack (*Rabi*–2005)	*Variety*	Delhi (*Kharif*–2005)	Cuttack (*Rabi*–2005)
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
'Amma Dhoka'	9.5 MR	10.0 MS	'Charka Govind Bhog'	10.0 MS	11.5 MS	'Jhuna'	6.5 MR	7.0 MR	'Kalam Daani Choota'	7.0 MR	8.5 MR			
'Asamia'	7.0 MR	7.5 MR	'Charka Sonachur'	8.0 MR	7.5 MR	'Jhuller' (B)	7.0 MR	7.5 MR	'Kherka Kuchi' (B)	9.5 MR	9.0 MR	'Rutugora'	9.0 MR	8.5 MR
'Anjani'	8.0 MR	8.5 MR	'Chhot Pansera'	7.5 MR	8.5 MR	'Jhilli'	7.0 MR	7.5 MR	'Kidi Bot'	9.5 MR	10.5 MS	'Rani Kajar' (A)	7.0 MR	7.0 MR
'Asamia' (B)	6.5 MR	8.5 MR	'Charka Gora'	10.5 MS	11.5 MS	'Juhu' (B)	8.5 MR	8.0 MR	'Lolan Dhan'	6.5 MR	7.0 MR	'Rani Kesar'	8.0 MR	8.5 MR
'Arjun'	8.0 MR	8.5 MR	'Charka Rani'	7.5 MR	8.5 MR	'Jhulat'	3.5R	3.0 R	'Lal Bhog'	5.5R	8.0 MR	'Rupsri'	7.5 MR	9.0 MR
'Aamchhotka'	7.5 MR	8.0 MR	'Charka Kherka Kuchi'	8.5 MR	9.5 MR	'Khil Bhojni'	6.0R	8.5 MR	'Lal Mansaal'	8.5 MR	9.0 MR	'Ramdilal'	6.5 MR	7.0 MR
'Barka Mansuri'	8.5 MR	8.0 MR	'Chotka Suman'	8.5 MR	9.0 MR	'Khilbhosni'	6.5 MR	8.0 MR	'Lal Ki Sita'	6.5 MR	7.0 MR	'Rani Kajar' (B)	9.5 MR	10.5 MS
'Baraha Saal'	8.0 MR	10.0 MS	'Charka Nardha'	9.0 MR	10.5 MS	'Kalam Kathi' (A)	7.0 MR	9.5 MR	'Lalbhog' (B)	7.5 MR	8.5 MR	'Raz Bhokta'	6.5 MR	8.0 MR
'Bachcha Kalamdani'	9.0 MR	10.0 MS	'Doodh Kandar'	7.5 MR	8.0 MR	'Khodra Phool'	5.5R	6.5 MR	'Lal Mugdi'	9.5 MR	9.0 MR	'Rajsri'	6.5 MR	7.0 MR
'Baa S Buda'	9.0 MR	8.5 MR	'Dhan Phooly'	8.0 MR	7.0 MR	'Ketki'	9.0 MR	7.0 MR	'Lamba Asari'	1.6R	1.7R	'Sirhanthi'	6.5 MR	8.0 MR
'Barka Dhusri'	7.5 MR	7.5 MR	'Dhusri Safed'	15.5S	12.0 MS	'Karmu Saal'	9.0 MR	7.0 MR	'Lal Jari'	7.5 MR	8.5 MR	'Son Piya'	6.0 R	6.5 MR
'Bagh Panjaar'	7.0 MR	7.5 MR	'Dudha Raaes'	8.0 MR	9.5 MR	'Kala Zira'	7.5 MR	7.0 MR	'Lal Dhani Barka'	7.0 MR	8.5 MR	'Sur Guja'	7.5 MR	8.0 MR
'Badshah Bhog'	9.0 MR	8.5 MR	'Dhusria'	7.0 MR	8.0 MR	'Khir Beej'	7.0 MR	7.0 MR	'Lahi'	8.5 MR	10.5 MS	'Sona Mati'	12.5 MS	11.0 MS
'Barka Swarna'	10.0 MS	9.0 MR	'Dudh Kobi'	8.5 MR	9.0 MR	'Kankesaal'	7.5 MR	8.0 MR	'Lal Moti'	8.5 MR	9.5 MR	'Sira Hatti'	8.5 MR	7.5 MR
'Bhannjani'	7.0 MR	7.5 MR	'Dahia'(A)	7.5 MR	8.0 MR	'Kherka Kuchi' (A)	9.0 MR	8.5 MR	'Miri Mitti'	9.0 MR	7.0 MR	'Sorna'	9.5 MR	8.0 MR
'Boka Dhan'	11.0 MS	10.5 MS	'Dahia' (B)	7.0 MR	7.5 MR	'Khutuwa'	9.0 MR	8.5 MR	'Maina Thori'	8.0 MR	7.0 MR	'Sonagoti'	9.0 MR	10.2 MS
'Bhojni'	10.0 MS	8.5 MR	'Daani Gora'	9.0 MR	9.5 MR	'Kala Parwat'	8.0 MR	10.0 MS	'Makar Kalma'	7.0 MR	7.5 MR	'Sona Par'	9.0 MR	8.5 MR
'Budhnu Nanhia'	9.0 MR	10.5 MR	'Dudh Kandar' (B)	8.5 MR	9.5 MR	'Kera Kandhi'	7.0 MR	7.5 MR	'Mango Sar' (A)	8.0 MR	7.5 MR	'Sonachur'	8.5 MR	8 . 0
'Bhadwa Kalmdani'	9.0 MR	9.5 MR	'Dudhia'	8.5 MR	9.5 MR	'Karmi Dhan'	7.0 MR	8.0 MR	'Manjhla-Natta' (A)	8.5 MR	9.0 MR	'Sitwa Dhan'	2.2R	1.35R
'Bara Sitwa'	8.5 MR	9.0 MR	'Dehati Gora' (A)	9.0 MR	10.5 MS	'Kadwadhan'	11.0 MS	9.0 MR	'Maiya Dulari'	8.0 MR	7.5 MR	'Suga Thor'	9.0 MR	10.5 MS
'Bas Mansuri'	15.5S	12.5 MS	'Dehati Gora' (B)	9.5 MR	10.0 MS	'Karhaini'	6.5 MR	8.0 MR	'Mehra Dhan'	10.0 MS	8.5 MR	'Sir Pathi'	14.5 MS	13.5 MS

(Continued . . .)

(Table 2 Concluded . . .)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
'Barka Tilasaar'	9.0 MR	9.5 MR	'Ejaan'	7.5 MR	8.5 MR	'Kalam Daani'	6.5 MR	6.5 MR	'Mango Sar'(B)	9.0 MR	11.5 MS	'Sonam'	6.5 MR	7.0 MR
'Budhnu'	10.0 MS	9.5 MR	'Futua'	9.5 MR	10.5 MS	'Kherka Kuchi' (B)	7.0 MR	7.0 MR	'Manjhla-Natta' (B)	9.5 MR	8.5 MR	'Suleet'	9.0 MR	8.5 MR
'Bala Dhusri'	7.5 MR	8.5 MR	'Gutuwa'	10.5 MS	10.0 MS	'Kalam Kathi' (B)	7.5 MR	7.5 MR	'Motka-Dahia'	8.5 MR	8.0 MR	'Songa-Bas'	8.0 MR	10.0 MS
'Barka Sanam'	7.5 MR	8.5 MR	'Garib Saal'	11.5 MS	10.5 MS	'Kadwa Dhan' (B)	7.0 MR	8.0 MR	'Mahi Dhan'	7.5 MR	8.5 MR	'Sita'	7.5 MR	8.5 MR
'Badya'	10.0 MS	9.5 MR	'Gada Jhadi'	8.5 MR	7.5 MR	'Kanak'	8.0 MR	7.5 MR	'Mahsoori Natta'	6.5 MR	6.0R	'Sonachurlal'	10.6 MS	11.5 MS
'Bhathani'	2.1R	2.0R	'Gudna'	8.0 MR	8.5 MR	'Kanas Chaapa'	10.0 MS	8.5 MR	'Mehsuri'	7.5 MR	8.5 MR	'Sarna'	6.5 MR	7.5 MR
'Bala Joga'	8.5 MR	8.0 MR	'Guda'	9.5 MR	10.5 MS	'Katika'	9.0 MR	8.5 MR	'Nanhiya' (A)	8.0 MR	8.5 MR	'Sathi'	8.5 MR	9.5 MR
'Bala Gora'	8.5 MR	9.0 MR	'Garib Saal' (B)	8.5 MR	9.5 MR	'Kanak' (B)	15.5S	12.0 MS	'Nanhiya'(B)	9.5 MR	8.0 MR	'Sanamdhan'	7.0 MR	8.5 MR
'Bas' 370	8.0 MR	10.0 MS	'Has Kalma' (A)	13.0 MS	12.0 MS	'Karhaini' (B)	6.5 MR	7.0 MS	'Naditi Kaur'	7.5 MR	8.5 MR	'Sugandha'	6.5 MR	8.0 MR
'Burah Dhan'	9.5 MR	10.5 MS	'Has Kalma' (B)	8.5 MR	9.0 MR	'Karijiri'	3.5R	3.0R	'Nardha'	6.0R	8.5 MR	'Tarori-Basmati'	7.0 MR	8.0 MR
'Barka Kalma'	8.0 MR	9.0 MR	'Hazari Mahak'	6.5 MR	7.0 MR	'Khirdhat'	6.5 MR	7.0 MR	'Nambri Dhan' (A)	10.0 MS	11.5 MS	'Tulsi Maanjar'	8.0 MR	8.5 MR
'Banphool' (A)	8.0 MR	9.5 MR	'Hardi Muri'	1.25R	2.3R	'Khutura'	10.0 MS	9.5 MR	'Neta-Dhan'	6.5 MR	8.5 MR	'Thubka'	9.0 MR	9.5 MR
'Banphool' (B)	9.0 MR	9.5 MR	'Hans Kalma' (A)	7.5 MR	10.0 MS	'Kohra Phool Dhusri'	6.5 MR	6.0R	'Nanka-(A)	7.5 MR	8.5 MR	'Tulsi Ketki'	12.0 MS	11.0 MS
'Bahar'	11.5 MS	12.5 MS	'Hardi Murilal'	9.5 MR	10.5 MS	'Kanak' (B)	9.0 MR	10.5 MS	'Nanhiya Nanka'	7.5 MR	8.5 MR	'Tulsi Ketki' (B)	10.0 MS	9.5 MR
'Barka Dhan'	8.0 MR	8.5 MR	'Hans Kalma' (B)	8.0 MR	9.5 MR	'Kanke Saal' (B)	6.5 MR	8.0 MR	'Nardha-Ashoka'	8.0 MR	10.5 MS	'Tulsi Manjari'	7.0 MR	8.0 MR
'Bas Kuchi'	8.5 MR	9.5 MR	'Hazarek'	7.5 MR	8.5 MR	'Karhaini Chhota'	9.0 MR	10.2 MS	'Nambri-Dhan' (B)	6.5 MR	7.5 MR	'Tila Saar'	10.0 MS	10.5 MS
'Charin Lukia'	8.0 MR	8.5 MR	'Is Kalma'	7.5 MR	10.5 MS	'Khir Dhat' (B)	6.5 MR	6.0R	01 'Hajar' 01	9.5 MR	10.5 MS	'Tin Thoka'	9.5 MR	10.0 MS
'Chhotka Sitwa'	7.0 MR	6.5 MR	'I Jun'	10.0 MS	9.5 MR	'Khera'	9.5 MR	8.5 MR	'Pusa Basmati'–1	11.0 MS	11.5 MS	'Thupa Dhan'	8.5 MR	11.5 MS
'Chhotka Pansala'	7.0 MR	7.5 MR	'Jihul'	7.0 MR	6.5 MR	'Konhra Phool'	10.5 MS	11.5 MS	'Pusa'–2–21	7.0 MR	6.5 MR	'Ujla Basmati'	7.0 MR	6.5 MR
'Chhotka Dahia'	6.5 MR	8.0 MR	'Jhinga Saal'	8.5 MR	9.0 MR	'Kodowa'	8.5 MR	9.0 MR	'Pala Parbat'	10.2 MS	9.0 MR	'Yes Kalma'	7.5 MR	7.0 MR
'Chaanagora'	6.0R	8.0 MR	'Jonga'	6.5 MR	8.0 MR	'Kala Basmati'	8.0 MR	8.5 MR	'Panch–Saala'	8.5 MR	8.0 MR	'Swarna Gora'	2.5 R	1.6 R
'Chairai Nerhi' (A)	10.0 MS	11.0 MS	'Jhuller' (A)	8.5 MR	8.0 MR	'Kalam Daani' (B)	8.0 MR	9.0 MR	'Panjhali'	6.5 MR	8.5 MR	'Sita Gora'	2.4 R	1.7 R
'Chairai Nerhi' (B)	8.5 MR	7.0 MR	'Jagan Nath'	6.5 MR	9.0 MR	'Karanga'	9.0 MR	10.5 MS	'Ratgoli'	7.5 MR	8.0 MR			
'Charka Nanka'	9.5 MR	9.0 MR	'Juhu' (A)	7.5 MR	8.5 MR	'Karanga' (B)	11.5 MS	12.5 MS	'Roopasari'	7.0 MR	7.5 MR			

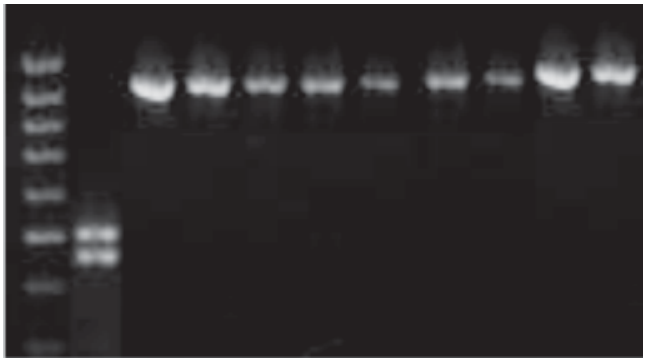

Fig 1 PCR amplification of resistant plant with the marker pTA248 linked to *Xa21*.
M: Gene ruler 100 bp ladder plus; Lane 1: IRBB55; ; Lane 2: PB-1; Lanes 3 Hardimuri,; Lane 4: Karijiri; Lane 5: Bhatani; Lane 6: Siwadhan; Lane 7: Swarna Gora; Lane 8 : SitaGora; Lane 9: Lambaasari; Lane 10: Jhulhat.

Fig 2 PCR amplification of resistant plant with the marker RH136-Hinf1 linked to xa13.
M: Gene ruler 100 bp ladder plus; Lane 1: IRBB55; ; Lane 2: PB-1; Lanes 3 Hardimuri,; Lane 4: Karijiri; Lane 5: Bhatani; Lane 6: Siwadhan; Lane 7: Swarna Gora; Lane 8 : SitaGora; Lane 9: Lambaasari; Lane 10: Jhulhat.

The lesion length of the 168 varieties which categorized as moderately resistant widely. 21 lines showed disease reaction closure to resistance type, ie 'Asamia' (6.5), 'Chhotkadahia' (6.5), 'Jonga' (6.5), 'Jhona' (6.5), 'Khilbhosni' (6.5), 'Karhaini' (6.5), 'Kalamdani' (6.5), 'Khirdat' (6.5), 'Karijiric' (B) (3.5), 'Kohraphool' (6.5), 'Kankesaal' (B) (6.5), 'Lalkisita' (6.5), 'Mahoorinaata' (6.5), 'Netadhan' (6.5), 'Nambridhan' (B) (6.5), 'Kanjhali' (6.5), 'Ramdilal' (6.5), 'Razbhokata' (6.5), 'Rajasri' (6.5), 'Sonam' (6.5), 'Sarna' (6.5), 'Sugandha' (6.5). while were closer to moderately susceptibility. 'Ammadhoka' (9.5), 'Burahdhan' (9.5), 'Chrkananka' (9.5), both 'Dehatigora', A and B (9.5), 'Futua' (9.5), 'Guda' (9.5), 'Hardimurilal' (9.5), 'Kherka' (9.5), 'Kharkakuchi' (B) (9.5), 'Idibot' (9.5), 'Lalmugdi' (9.5), 'Manjhlanaata' (B) (9.5), 'Nanhia' (B) (9.5), 01 'Hajari' (9.5), 'Ranikajjar' (B) (9.5), 'Tinthoka' (9.5), near to MS with lesion length (9.5) cm. There is significant difference between two MR type lines.

In reconfirmation for bacterial leaf blight highly (Table 1), it is clear that 8 varieties namely, 'Hardimuri', 'Karijiri', 'Bhatani', 'Sitwadhan', 'Swarnagoda', 'Sitagora', 'Lamba-asari' and 'Jhulat', show highly resistance for bacterial leaf blight in both stages nursery and after transplanting in field. Little variation in score is due to local environment effect. In nursery, plant is so delicate that infection affects more in comparision of after transplanting in filed. For test of allelism concerned genotypes were screened with the respective markers for *xa13* and *Xa21* genes. Markers were linked to *xa13* and *Xa21* genes. A set of 8 germplasms namely 'Hardi muri', 'Karijiri', 'Bhatani', 'Stwa dhan', 'Swarna gora', 'Sita gora', 'Lamba-asari' and 'Jhulat' found resistant during field inoculation were subjected to test of allelism with the help of linked molecular markers 2 checks, namely, 'Pusa Basmati 1' (susceptible) and 'IRBB 55' (resistant) were also included. 'IRBB 55', a line carrying 4 bacterial blight resistance genes *Xa13* and *Xa21* in homozygous condition. Molecular marker analysis with the help of linked molecular markers namely, *RG136-Hinf-I* and pTA248 were used for *xa13*, and *Xa21* gene. In case of *xa13* a product of 1100 bp amplified by polymerase chain reaction (PCR) in both resistant and susceptible parents using RG136 primer, which on digestion with *Hinf-I* restriction enzyme produces 2 fragment of 950 bp and 150 bp in susceptible plants and 3 fragments of 500 bp, 450 bp and 150 bp in resistant parent IRBB 55. In case of *Xa21*, fragment of 10 bp and 650 bp are amplified using PTA 248 primers in resistant and susceptible parents.

A set of 10 genotypes each was screened for polymorphism using PCR-based markers pTA248 for *Xa21* and RG 136 for *xa13*. For screening with 'RG136', the 10 genotypes included aromatic lines and non-aromatic lines. Among these only 'IRBB60' (control) having *xa13* and *Xa*21 genes and another 9 varieties ('Hardimuri', 'Karibiri', 'Bhatani', 'Sitwa dhan', 'Swarna Gora' and 'Sita Gura', 'Lama Assari', 'Jhulat') including 'Pusa Basmati 1' not having *xa13* and *Xa21* genes.

Amplification of genomic DNA constituting a set of 8 traditional germplasms and two checks was amplified with the help of marker with one resistant (IRBB 55) and susceptible (PB 1) checks. Using gene specific primer *RG 136-Hinf-I* and PTA 248 for *xa13* and *Xa21* gene respectively, all the resistant lines amplified corresponding to susceptible locus i.e. 950 bp and 150 bp in case of *RG 136-Hinf I* and 6 50 bp in case of PTA 248. This result suggested that none of line possessed *xa13and Xa21* genes. So, it is possible that these landraces may possess Xa4 or Xa5 gene. if they do not possess Xa4 and Xa5 gene then it is quite possible that there may be some new gene, which is to be done.

SUMMARY

Bacterial leaf blight, the second most devastating disease of rice causes substantial yield loss. Study of pathogenic variability and identification of resistance genes are key

factors in breeding against this disease. Bacterial leaf blight is now a serious constraint for rice production in the irrigated and lowland ecologies in all rice-growing countries. 223 germplasms collected from different regions of Bihar and Jharkhand and were screened against bacterial blight. The result showed that 8 land races namely 'Bhathani', 'Hardi Muril', 'Sitwadhan', 'Jhulat', 'Lamb Asari', 'Karijiri', 'Swarna Gora' and 'Sita Gora' were found to be highly resistant. All of above 8 landraces do not possess Xa13 and Xa21 genes which are also responsible for resistance to Bacterial leaf blight.

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