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EVALUATION OF WILD RICE INTROGRESSION LINES FOR BROAD-SPECTRUM RESISTANCE TO BACTERIAL BLIGHT OF RICE

T. Dilip Kumar, Pragya Sinha, S. K. Hajira, S.K. Mastanbee, M.B.V.N. Kousik, K. Chaitra, M. Anila, P. Prashanth, R.M. Sundaram and G.S. Laha*

ICAR-Indian Institute of Rice Research, Rajendranagar Hyderabad-500 030, Telangana, India
*Corresponding author E-mail: lahags66@gamil.com
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ABSTRACT

Bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* has become a major threat to rice cultivation in irrigated and rainfed lowland ecosystem. In the absence of reliable chemical control strategies, deployment of resistant rice varieties is the most dependable and economical mean of managing the disease. Wild rices are the reservoir of novel genes against many biotic and abiotic stresses. In the present study, 44 introgression lines (ILs) from different accession of 9 species of wild rice were evaluated for their resistance to bacterial blight (BB) of rice. Three ILs viz., HWR 23 (IR 75084-15-3-B-B; derived from *O. officinalis*), HWR-36 (IR75083-49-25-9-5-B-B-B; derived from *O. longistaminata*) and HWR-42 (IR 75085-35-5-2-B; derived from *O. officinalis*) showed high level of resistance under field condition consecutively for two seasons against local strain (IX-020) of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Another 7 ILs showed moderate to good level of resistance and rest showed susceptible reaction. Two ILs viz., HWR-36 and HWR-42 which showed high level of BB resistance were screened against 20 diverse strains of *Xoo* in glasshouse. These two selected ILs showed broad-spectrum resistance to 10-11 *Xoo* strains. Genotyping with gene-specific marker of 4 major BB resistance genes, viz., *Xa21*, *xa13*, *xa5* and *Xa38* revealed absence of these genes in these two ILs. Based on phenotyping and genotyping data, it can be assumed that these two ILs may possess new BB resistance source.

Keywords **:** Rice, *Xanthomonas oryzae* pv. *oryzae*, Bacterial blight, Resistance

Introduction

Rice remains the most important food crop in most of Asia and parts of Latin America and Africa (Laha, 2024). The crop has immensely influenced the cultures, traditions, food habits, economic status and overall life style of millions of Asian and it is truly 'Rice is life' in Asia. More than 65% Indian population depends on rice as their primary food (Pathak *et al.*, 2020). Though diversity in food consumption has increased, demand for main staples like rice and wheat has constantly increased. Rice production faces several complex challenges like declining agricultural land, deteriorating soil health, depleting water resources, apparent changes in climatic conditions, increased incidences of several biotic and abiotic stresses and above all growing disinterest in agriculture among the farmers. Among several biotic stresses in rice, bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) remained a major one. Analysis of survey data of last 40 years indicated that the disease has not only increased in its intensity in different geographical regions, it has spread to newer areas (Laha *et al.*, 2023). Yield loss ranging from 2-74% have been reported by several workers in India depending on varieties affected, growing season, climatic conditions, stages of infection and extent of nitrogen fertilizer application. Due to limited success in chemical control of the disease, increased focus has been given in host plant resistance for the management of the disease. Development and deployment of resistant cultivars offers the best solution to combat the disease as it is economically feasible and environmentally friendly. Genetic analysis of diverse germplasm resulted in identification of 48 BB resistance genes (Laha, 2024). However, the effectiveness of these genes varies from region to region due to variation in virulence profile of the pathogen strains. Bacterial blight pathogen is highly dynamic in nature which is evident by the continuous appearance of new and more virulent forms of the pathogen overcoming the resistance offered by individual resistance genes. Therefore, there is need of continuous search for new and novel sources of

resistance to this dreaded pathogen. In this paper, we report the broad-spectrum resistance of selected rice lines having introgression from diverse wild rice species.

Materials and Methods

Plant Materials and planting scheme

A set of Forty-four wild rice introgression lines (ILs) derived from different species of *Oryza* viz., *O. glaberrima*, *O. officinalis*, *O. longistaminata*, *O. rufipogon*, *O. minuta*, *O. brachyantha*, *O. granulata*, *O. australiensis* and *O. latifolia*, in the genetic background of IR64, *O. sativa,* NPT (new plant type) line, IR31917-45-3-2, and IR69502-6-SRN-3-UBN-1-B were obtained from the International Rice Research Institute (IRRI), The Philippines. Details of these ILs are presented in Table 1. These lines were multiplied at experimental fields of ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad. These ILs were evaluated for their reaction to bacterial blight of rice along with resistant check Improved Samba Mahsuri (possessing 3 bacterial blight resistance genes, *xa5*, *xa13* and *Xa21*) and susceptible check TN1. Initially, the ILs were evaluated under field condition consecutively for 2 seasons (Wet seasons of 2018 and 2019). For field screening, seedlings of ILs were grown in raised seed beds and 25-days old seedlings of each IL were transplanted in 3 rows of 2-meter length with a spacing of 15 x 10 cm. Plants at the maximum tillering stage were used for resistance screening against the bacterial blight (BB) pathogen. Promising ILs which showed high level of BB resistance in both the season were selected and further evaluated for broad-spectrum BB-resistance with multiple bacterial blight pathogen strains under controlled conditions in glasshouse. The selected promising ILs along with susceptible checks (TN1 and Samba Mahsuri) and resistant check (Improved Samba Mahsuri) were directly sown in lines in plastic trays (60 x 40 x 7 cm). The trays were filled with a mixture of field soil (3 parts) and farm yard manure (1 part). The trays were fertilized with N @ 2.4 g/tray (both as basal and top dressing) and P_2O_5 @ 1.2 g/tray (as basal). The trays were irrigated as and when required. Care was taken to raise healthy plants free from any biotic and abiotic stresses. Plants at maximum tillering stage (40-45 days old) were then used for resistance evaluation with diverse *Xoo* strains. A single tray having all the promising ILs and checks was used for inoculation with a single *Xoo* starin.

Disease phenotyping

Initial field screening of the ILs was carried out using a local virulent strain (IX-020) of the bacterial blight pathogen. Screening of selected promising ILs for broad-spectrum BB resistance under controlled glasshouse conditions was carried out with 20 diverse *Xoo* strains collected from different geographical regions of India. These isolates belonged to different pathotypes (Yugander *et al.*, 2017; 2021), viz., IX-007 (Jorhat, Assam), IX-015 (Aduthurai, Tamil Nadu), IX-020 (Hyderabad, Telangana), IX-027 (Chinsurah, West Bengal), IX-052 (Malan, Himachal Pradesh), IX-071 (Sultanpur, Uttar Pradesh), IX-091 (Raichur, Karnataka), IX-123 (Cuttack, Odisha), IX-132 (Gorakhpur, Uttar Pradesh), IX-181 (Faizabad, Uttar Pradesh), IX-200 (Pantnagar, Uttarakhand), IX-206 (Arundhatinagar, Tripura), IX-207 (Arundhatinagar, Tripura), IX-209 (Arundhatinagar, Tripura), IX-212 (Raipur, Chhattishgarh), IX-216 (Kaul, Haryana), IX-226 (Ludhiana, Punjab), IX-246 (Kashipur, Uttarakhand), IX-248 (Nandyal, Andhrapradesh) and IX-266 (Srivelapally, Kurnool, Andhra Pradesh).

The *Xoo* strains were grown on modified Wakimoto's Agar (MWA) plates and incubated at 28°C for 72-94 hours (Figure 1). Fresh growth of each bacterial isolate was then harvested using a sterile cell scraper and a bacterial suspension was made with sterile distilled water with a final concentration of 10^8-10^9 cfu/ml. The plants at the maximum tillering stage were then clip-inoculated by cutting top 1-2 cm of 8-10 fully expanded leaves of each hill with a sterile scissor dipped in bacterial suspension (Kauffman *et al.*, 1973). Care was taken to inoculate green and healthy leaves free from any biotic and abiotic symptoms. In glasshouse, controlled conditions were maintained with cooling air pad and sprinkler system. Observations were made 15 days after inoculation both by measuring the lesion length and recording the disease score following the standard evaluation system for rice (IRRI, 2014).

Genotyping of selected ILs

Selected promising ILs which showed broad-spectrum BB resistance were screened for the presence of selected major BB resistance genes viz., *xa5*, *xa13*, *Xa21* and *Xa38*. Leaf samples were collected from the selected ILs and positive check (DRR Dhan 53 possessing four major BB resistance genes *xa5*, *xa13*, *Xa21* and *Xa38*) and negative check Samba Mahsuri. DNA extraction was performed using the CTAB method as described by Doyle and Doyle (1987). PCR detection of the above-mentioned BB resistance genes was carried out following (Sundaram *et al.*, 2008 and Bhasin *et al.*, 2012).

Results and Discussion

Forty-four ILs derived from different accessions of 9 wild rice species were evaluated for their reaction to bacterial blight of rice under field conditions

consecutively for 2 years. BB reaction of these ILs was consistent in both the years and the results are presented in Table 1. There was a wide variation in the level of resistance among the ILs. Distribution of the reactions of the ILs is presented in Figure 2. The reactions varied from highly resistant (lesion length 0.1 cm; SES disease score 1) in HWR-36 to highly susceptible (lesion length 20.4 cm; SES disease score 9) in HWR 4. Susceptible checks Samba Mahsuri and TN1 showed highly susceptible reaction with a disease score of 9 while resistant check Improved Samba Mahsuri showed a highly resistant reaction with a disease score of 1. Out of 44 ILs, 34 ILs showed susceptible to highly susceptible reaction with a SES disease score of 7-9. Seven accessions showed moderate to good level of resistance with a disease score of 3-5 (Table 1). Three accessions HWR-23 (IR 75084-15-3-B-B; NPT x *O. officinalis*), HWR-36 (IR75083-49-25-9-5-B-B-B; NPT x *O. longistaminata*) and HWR-42 (IR 75085-35-5-2-B; NPT x *O. officinalis*) showed highly resistant reaction to bacterial blight of rice with a disease score of 1 (lesion length ranging from 0.1-0.3 cm) (Figure 3). Accessions of different wild rice species provide an important source of many useful resistance genes to several biotic stresses in rice (Brar and Khush, 1997). However, bacterial blight pathogen is highly variable in nature and several research reports have revealed the existence of different pathotypes in this pathogen (Mishra *et al.*, 2013; Mondal *et al.*, 2014; Yugander *et al.*, 2017). Studies have also shown that several pathotypes of the pathogen can be present in a particular rice growing region (Yugander *et al.*, 2017) making highly complex nature of the disease. As a result, the effectiveness of individual BB resistance gene varies in different geographical region depending on the pathogen population structure (Laha, 2024). Therefore, it is pertinent to evaluate the spectrum of resistance of the selected promising ILs to multiple strains of the pathogen.

Three accessions viz., HWR-23 (derived from *O. officinalis*), HWR-36 (derived from *O. longistaminata*) and HWR-42 (derived from *O. officinalis*) showed high level of BB resistance in field condition. Two ILs, HWR-36 and HWR-42 were further evaluated in highly conducive glasshouse condition with 20 different *Xoo* strains for studying their broad-spectrum resistance. These *Xoo* strains were isolated from different rice growing regions of India and differed in their virulence profile (Yugander et al., 2017; 2021). The ILs showed varied reaction to different *Xoo* isolates. Both the ILs showed high level of BB resistance to 10-11 *Xoo* strains (Table 2; Figure 4) indicating broad-spectrum resistance in these two ILs. Two ILs showed similar reaction pattern to the *Xoo* strains tested except to strain IX-091 where HWR-36 showed resistant reaction while HWR-42 showed susceptible reaction (Table 2). The resistant check Improved Samba Mahsuri (possessing three BB resistance genes viz., *xa5*, *xa13* and *Xa21*) showed highly resistant reaction to all the *Xoo* strains while susceptible check Samba Mahsuri showed susceptible to highly susceptible reaction to all the *Xoo* strains (Table 2). Development and cultivation of disease resistant varieties is the most effective way of managing the pathogen infection. Broad spectrum resistance is very important in resistance breeding as it provides resistance against multiple pathotypes /races of the pathogen making the resistance more durable (Li *et al.*, 2020). Identification and exploitation of genetic factors that determines broad-spectrum resistance can help in turning a disease susceptible variety into a more durable disease resistant variety. Increased emphasis has been given in identification of rice broad-spectrum resistance genes which could be typical R gene or defense-related gene or quantitative trait loci (QTL) which can provide resistance to multiple races of a pathogen (Liu *et al.*, 2021). Application of suitable molecular biology and functional biology can help in deciphering the broad-spectrum resistance in these ILs and their use in resistance breeding.

Genotyping of selected ILs

Molecular screening of the promising resistant germplasm with gene-specific markers for the presence or absence of major BB resistance genes is an important step to confirm the novelty of the BB resistance (Sinha *et al.*, 2021). Both the promising ILs, viz., HWR-36 and HWR-42 were screened for the presence of 4 major BB resistance genes using gene-linked markers (Table 3). These genes were selected as they provide good to high level of resistance to Indian *Xoo* population and they have been used in BB resistance breeding program in India. Repeated PCR analysis with gene-linked markers could not detect the presence of these 4 major BB resistance genes in these two selected BB resistant ILs indicating that they may possess new BB resistance genes or QTLs (Table 3; Figure 5). Molecular genotyping for detection of resistance genes in promising germplasm offers a fast and confirmatory test of the uniqueness of the germplasm.

Although 48 BB resistance genes have been identified from diverse sources, all genes are not equally effective in all geographical regions because of variation in pathogen population structure. Some of the BB resistance genes have totally become ineffective against Indian *Xoo* population. Therefore, continuous

search for new or novel sources of BB resistance is required. Species of wild rice are reservoir of important resistance genes against many biotic stresses of rice (Brar and Khush, 1997). Based on the results of the resistance screening of the selected ILs with multiple *Xoo* strains and molecular genotyping, it can be assumed that these ILs (HWR-36 and HWR-42) could possess novel BB resistance. Further analysis of tagging and mapping of this novel gene/QTL will help to identify this new/novel BB resistance in these selected ILs and can be used in BB resistance breeding program.

Table 1: Reaction of different wild rice introgression lines to bacterial blight of rice under field conditions in Kharif-2018 and 2019

IL Code	IL Designation	Mean lesion length (in cm) ± SE/SES Score		Reaction
		2018	2019	
HWR-1	IR83784-5-28-B	12.3±0.6 (7)	9.3±0.3 (7)	S
HWR-2	IR71031-10-16-B	10.6±0.6 (7)	12.3±0.4 (7)	S
HWR-3	IR71031-9-7-B	12.7±0.7 (7)	13.4±0.5 (7)	S
HWR-4	IR 75862-129-24-B-45-B	18.4±0.4 (9)	20.4±0.7 (9)	HS
HWR-5	IR75862-134-3-B-30-B-B	17.5±0.4 (9)	18.6±0.5 (9)	HS
HWR-6	IR 65482-4-136-2-2-B	6.2±0.2 (5)	5.9±0.4 (5)	MR
HWR-7	IR 65482-7-216-1-2-B	5.8±0.3 (5)	5.8±0.2 (5)	MR
HWR-8	IR 54751-1-2-44-15-2-3-B	10.6±0.7 (7)	10.9±0.4 (7)	S
HWR-9	IR 75862-208-5-5-3-B	16.2±0.3 (9)	17.1±0.3 (9)	HS
HWR-10	IR 65483-118-25-31-7-1-5-B	10.3±0.6 (7)	8.9±0.3 (7)	S
HWR-11	IR 65483-111-5-9-2-11-B	11.4±0.7 (7)	11.4±0.5 (7)	S
HWR-12	IR 54751-2-41-10-5-1-B	13.5±0.6 (7)	13.2±0.4 (7)	S
HWR-13	IR 75085-35-2-B-B-B	6.0±0.4 (5)	6.3±0.5 (5)	MR
HWR-14	IR 65483-104-13-13-22-1-B	12.4±0.4 (7)	13.1±0.4 (7)	S
HWR-15	IR 75870-5-8-5-B-5-B	11.1±0.8 (7)	11.7±0.5 (7)	S
HWR-16	IR73382-80-9-3-13-2-2-1-3-B	11.8±0.7 (7)	11.8±0.2 (7)	S
HWR-17	IR 75870-5-8-5-B-2-B	11.4±0.4 (7)	11.8±0.2 (7)	S
HWR-18	IR 77981-19-7-B	8.7±0.3 (7)	9.4±0.4 (7)	S
HWR-19	IR 73382-80-9-14-14-1-3-2-3	10.8±0.7 (7)	10.6±0.3 (7)	S
HWR-20	IR 75870-5-8-5-B-1-B	10.2±0.6 (7)	10.9±0.4 (7)	S
HWR-21	IR 71033-121-15-B	11.6±0.7 (7)	11.5±0.4 (7)	S
HWR-22	IR 75863-30-5-B-B-B	11.5±0.8 (7)	11.5±0.4 (7)	S
HWR-23	IR 75084-15-3-B-B	0.2±0.1 (1)	0.2±0.1 (1)	HR
HWR-24	IR73382-7-12-1-1-3-B	12.5±0.3 (7)	12.8±0.2 (7)	S
HWR-25	IR 75862-115-22-B-16-B-B	15.2±0.4 (9)	16.6±0.4 (9)	HS
HWR-26	IR73382-85-9-1-2-3-B-1-B	10.7±0.6 (7)	11.7±0.6 (7)	S
HWR-27	IR 73681-11-4-1-2-4-1-B	11.5±0.6 (7)	11.9±0.3 (7)	S
HWR-28	IR73680-11-10-2-1-3-B-1-B	11.4±0.4 (7)	11.4±0.4 (7)	S
HWR-29	IR75870-8-1-2-B-6-2-1-B	12.8±0.5 (7)	13.1±0.4 (7)	S
HWR-30	IR80351-25-B-27-B	10.0±0.9 (7)	10.0±0.2 (7)	S
HWR-31	IR77384-12-17-3-18-2-B	14.6±0.9 (7)	13.3±0.5 (7)	S
HWR-32	IR80340-23-B-13-2-B-B	12.1±0.4 (7)	13.0±0.4 (7)	S
HWR-33	IR75870-8-1-2-B-6-1-1-B	13.5±0.2 (7)	13.8±0.4 (7)	S
HWR-34	IR77390-6-1-18-2-5	10.3±0.5 (7)	10.6±0.3 (7)	S
HWR-35	IR 75083-73-12-11-1-B-B	14.9±0.4 (7)	14.2±0.8 (7)	S
HWR-36	IR75083-49-25-9-5-B-B-B	0.2±0.0 (1)	0.1±0.0(1)	HR
HWR-37	IR 75083-31-28-13-4-10-B	11.2±0.3 (7)	10.8±0.4 (7)	S
HWR-38	IR 77981-19-3-6-2-B	3.8±0.3 (3)	3.5±0.2 (3)	R
HWR-39	IR77390-6-2-18-2-B	10.6±0.6 (7)	11.3±0.5 (7)	S
HWR-40	IR 77981-34-34-B	4.5±0.3 (3)	3.5±0.2 (3)	R
HWR-41	IR73382-7-12-1-9-3-B	13.4±0.5 (7)	13.7±0.2 (7)	S
HWR-42	IR 75085-35-5-2-B	0.3±0.1 (1)	0.3±0.1(1)	HR
HWR-43	IR75084-74-8-B-B-B	4.2±0.3 (3)	3.8±0.2 (3)	R
HWR-44	IR75864-68-3-B	4.3±0.4 (3)	3.8±0.2 (3)	R
ISM	Improved Samba Mahsuri	0.1±0.0 (1)	0.1±0.0 (1)	HR
BPT	Samba Mahsuri	14.8±0.4 (9)	14.1±0.2 (9)	S
TN1	TN1	23.9±1.3 (9)	27.3 ± 1.5(9)	HS

HR-Highly Resistant, R-Resistant, MR-Moderately resistant, S-Susceptible, HS-Highly susceptible.

Table 2: Reaction of selected ILs to multiple hyper-virulent strains of *Xanthomonas oryzae* pv. *oryzae*

Xoo strains	Mean lesion length (in cm) ± SE			
	HWR-36 (IR75083-49-25-9-5-B-B-B)	HWR-42 (IR 75085-35-5-2-B)	Improved Samba Mashuri (ISM)	Samba Mashuri (SM)
IX-007	0.2±0.2 (1)	2.28±1.1 (1)	0.14±0.0 (1)	7.44±0.3 (7)
IX-015	7.34±1.0 (7)	9.66±0.9 (9)	0.26±0.1 (1)	9.9±0.6 (9)
IX-020	0.2±0.2 (1)	1.7±1 (1)	0.4±0.1 (1)	6.8±0.9 (7)
IX-027	6.04±0.5 (7)	9.4±0.9 (9)	0.14±0.0 (1)	8.08±0.5 (7)
IX-052	8.3±0.3 (7)	8.4±0.2 (7)	0.2±0.0 (1)	8.9±0.2 (7)
IX-071	6.4±0.6 (7)	8.72±1.0 (7)	1.66±0.4 (1)	8.8±0.8 (7)
IX-091	0.8±0.1 (1)	8.8±0.3 (7)	0.2±0.0 (1)	9.5±0.2 (9)
IX-123	0.6±0.1 (1)	0.8±0.1 (1)	0.7±0.0 (1)	10.1±0.1 (9)
IX-132	2.3±0.2 (1)	2.1±0.2 (1)	0.2±0.0 (1)	7.6±0.1 (7)
IX-181	3.8±0.6 (3)	5.9±0.6 (5)	0.8±0.1 (1)	7.9±0.2 (7)
IX-200	8.74±0.4 (9)	8.9±0.7 (7)	1.34±0.2 (1)	12.3±0.6 (9)
IX-206	1.6±0.4 (1)	1±0.4 (1)	0.14±0.1 (1)	7.8±0.7 (7)
IX-207	0.1±0.0 (1)	3.6±0.2 (3)	0.4±0.1 (1)	7.8±0.2 (7)
IX-209	0.3±0.1 (1)	0.3±0.1 (1)	0.5±0.0 (1)	8.6±0.1 (7)
IX-212	0.74±0.2 (1)	1.34±0.3 (1)	0.34±0.1 (1)	8.84±0.7 (7)
IX-216	0.1±0.0 (1)	0.2±0.0 (1)	0.1±0.0 (1)	7.2±0.2 (7)
IX-226	5.7±0.1 (5)	5.6±0.1 (5)	0.2±0.0 (1)	7.4±0.2 (7)
IX-246	9.2±0.2 (9)	9.8±0.2 (9)	0.2±0.0 (1)	7.3±0.2 (7)
IX-248	8±0.2 (7)	9.1±0.3 (9)	0.6±0.1 (1)	9.4±0.2 (9)
IX-266	9.3±0.7 (9)	8.4±0.1 (7)	0.2±0.0 (1)	9.3±0.2 (9)

Table 3 : Molecular screening of promising ILs with gene-linked markers

Gene	Primer name	Primer sequence	Reference	Presence/absence of the target gene			
				HWR-36	HWR-42	SM	DRR Dhan 53
Xa21	PTA 248	F: AGACGCGGAAGGGTGGTTCCCGGA R: AGACGCGGTAATCGAAAGATGAAA	Ronald *et al*., 1992	-ve	-ve	-ve	+ve
xa13	xa13-prom	F: GGCCATGGCTCAGTGTTTAT R: GAGCTCCAGCTCTCCAAATG	Hajira *et al*., 2016	-ve	-ve	-ve	+ve
xa5	xa5FM-SF	SF: GTCTGGAATTTGCTCGCGTTCG SR: TGGTAAAGTAGATACCTTATCAAACTGGA RF: AGCTCGCCATTCAAGTTCTTGAG RR: TGACTTGGTTCTCCAAGGCTT	Hajira *et al*., 2016	-ve	-ve	-ve	+ve
Xa38	Os04g53050-1	F: TCTTCTATTGCTAACATTGGTG R: TCGCATTCATTTTCAGAG	Bhasin *et al*., 2012	-ve	-ve	-ve	+ve

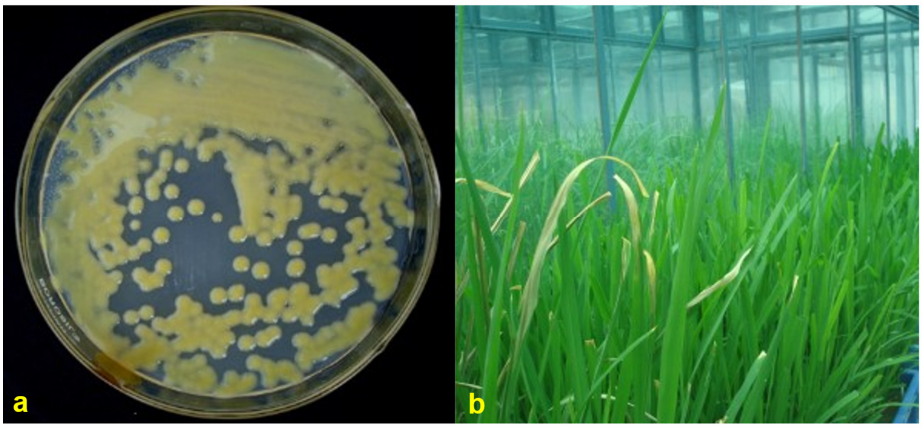

Fig. 1: Pure culture of *Xanthomonas oryzae* pv. *oryzae* (a) and symptoms of bacterial blight of rice on a susceptible variety (b)

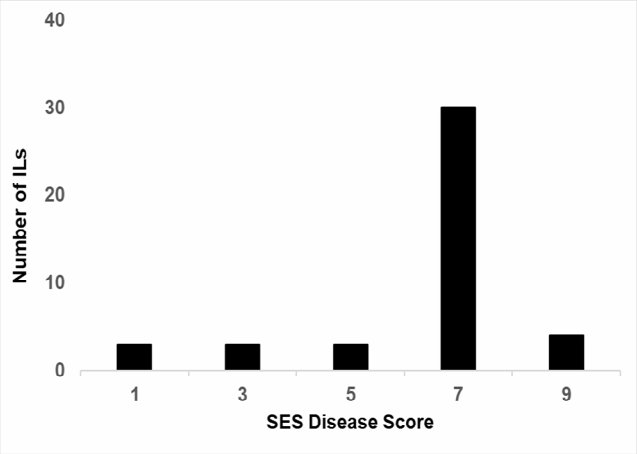

Fig. 2: Distribution of bacterial blight disease reaction scores (*Xoo* strain IX-020) of 44 wild rice introgression accession based on 2 years of screening in field condition

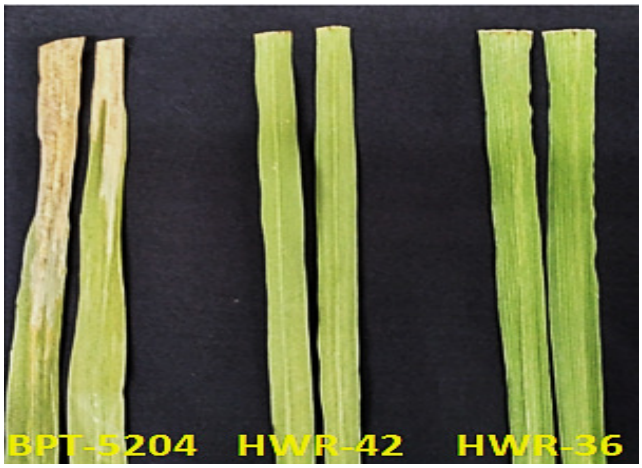

Fig. 3: Reaction of HWR 42 and HWR 36 to bacterial blight of rice (Strain IX-020) compared to susceptible check Samba Mahsuri

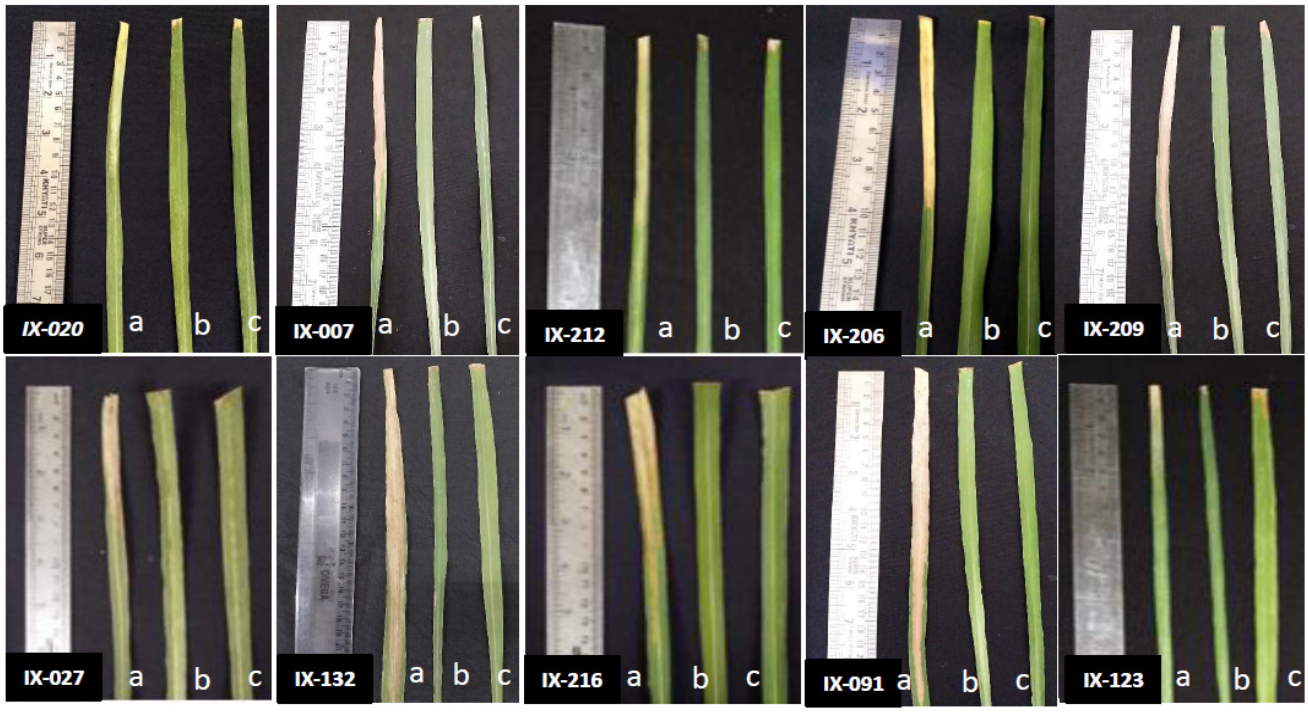

Fig. 4: Reaction of selected ILs (b: HWR-36 and c: HWR-42) to multiple strains of *Xanthomonas oryzae* pv. *oryzae*

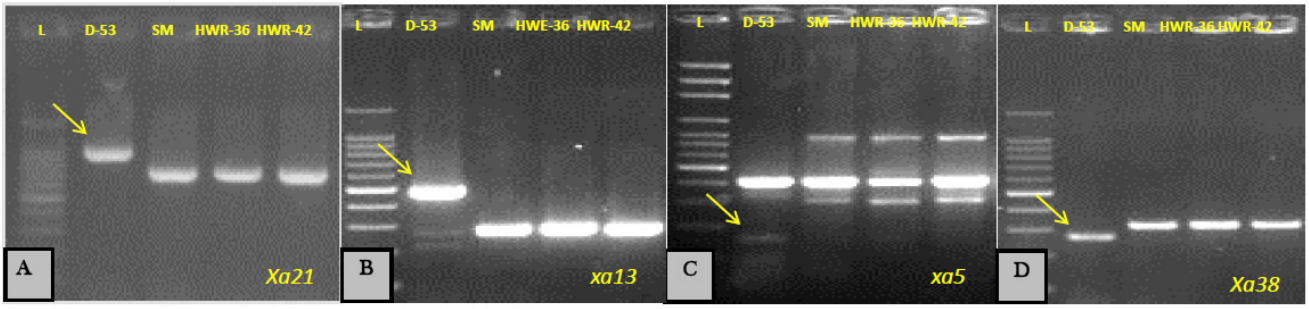

Fig. 5: Genotyping of selected ILs with gene specific markers for the detection of major BB resistance genes *Xa21*, *xa13*, *xa5* and *Xa38*: Legends: L -100 bp Ladder marker, D-53: DRR Dhan-53 (Positive check), SM: Samba Mahsuri (negative check). The arrow mark represents the resistant allele with respect to the corresponding genes. A. *Xa21*-Resistant allele 950bp and susceptible allele 660bp; B. *xa13*-Resistant allele 450bp and susceptible allele 220bp; C. *xa5*-Resistant allele 134 bp and susceptible allele 313bp and common fragment of 424bp and D. *Xa38*-Resistant allele 260bp and susceptible allele 320bp.

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