International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 7(4): 777-785 December 2014
DOI Number: 10.5958/2230-732X.2014.01387.4

GENETICS AND PLANT BREEDING

Inheritance of resistance in indica rice cultivar HUR 4-3 against bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*)

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Paper No. 268 Received: 15 September, 2014 Accepted: 14 October, 2014 Published: 17 December, 2014

Abstract

The mode of inheritance of resistance to *Xanthomonas oryzae* pv. *oryzae* strain *BXO1* and *BX043 wild type* of bacterial leaf blight disease of rice was studied in six generations of crosses of cultivars HUR 4-3 into PB-1460. The resistance cultivars PB-1460 showed 4.54% disease severity, while susceptible cultivar HUR 4-3 showed 53.01% disease severity against *Xanthomonas oryzae* pv. *oryzae.* The area under the disease progress curve (AUDPC) of resistance cultivar was 65.61 which are significantly less than the susceptible cultivar 649.90. The F_1 plants were observed to be resistant with an average disease severity 08.87% and AUDPC 110.26. The F_2 populations were classified in to four distinct classes on their genotypic ratio of 9:3:3:1 and phenotypically these populations were grouped in two distinct classes resistant and susceptible with their ratio of 13:3, respectively. However, B_1 and B_2 populations were classified in to two distinct classes as resistant (Resistant/ moderately resistant) and susceptible (moderately susceptible/ susceptible) in the ratio of genotypic 1:1:1:1 and 1:1 and phenotypically 1:1 and 1:0, respectively. The disease resistance occurs in the population is mainly due to cumulative effects of dominant and recessive two resistant genes *i.e., Xa21* and *xa13*. Chi-square analysis of the population was confirm the inheritance of resistance with their value are 1.24 and 0.66 indicating that the observed data are in line with expected ratio and follow Mendelian pattern of inheritance of resistance to bacterial leaf blight in B_1 and B_2 generations and modification in the Mendelian ratio of inheritance in the F_2 populations, it showed inhibitory gene action *i.e.,* 13:3 that means dominant gene have cumulative effect of recessive gene.

Highlights

- Two popular rice cultivars *viz.,* HUR 4-3 and PB-1460 were used to study the inheritance of resistance for bacterial leaf blight disease.
- Disease resistance is governed by the synergistic effect of one dominant *Xa21* and one recessive gene *xa13,* and dominant gene has higher effect than the recessive gene.
- The resistance and susceptible reaction were observed in the progenies and it segregates in the ratio of 13 : 3 (Inhibitory gene action) in F_2, 1 : 1 (test cross ratio) in B_1 and 1 : 0 in B_2 generation.

Keywords: Bacterial leaf blight, disease severity, inheritance, inhibitory gene action, AUDPC

Rice (*Oryza sativa* L.) is the most widely consumed stable food crop of Poaceae family for a large part of the worlds human population, especially in Asia and over half of the global population depends on it for their feed (Sasaki, 2005 and Lal *et al.*, 2014). India, the second largest rice growing country has a production of 104.32 million tonnes and cultivation area of about 44.6 million hectares with an average productivity of 2.34 tonnes per hectare (Anonymous, 2013 and Rajasekar and Jeyakumar, 2014). It is, however, unfortunate that rice crop is threatened by considerable number of diseases (more than 40 diseases) of fungal, bacterial and viral origin, and that is one of the reasons for low yield of rice in the world including Asia, especially in India (Latif *et al*., 2011, Barnwal *et al.*, 2013 and Singh *et al.*, 2013a). The diseases may appear at any growth stage of the plant, attacking the seed sown, root system, foliage, stalk, leaf sheath, inflorescence and even the developing grain (Virmani and Siddiq, 1998).

and to a great extent, the conduciveness of the environment in which it occurs (Gnanamanickam *et al.* 1999, Singh *et al.*, 2013a and Barnwal *et al*., 2013).

Application of various chemicals to control the BLB is not an effective approach (Devadath, 1989). Therefore, exploitation of host plant resistance is considered the most effective, economical and environmentally safe measure for controlling BLB (Singh *et al*., 2013a and b). The most effective approach to control BLB is using resistant varieties. This is due to the fact that the presence of different pathogenic races subsequently breaks the resistance of rice cultivars. The several attempts have been made to identify and characterise BLB resistance genes. Globally, more than thirty-eight genes (25 dominant and 13 recessive) conferring resistance against various strains of *X. oryzae* pv. *oryzae* have been identified (Chen *et al.* 2011) from diverse sources. Major resistance genes, including *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21* have been incorporated into rice cultivars, in order to

Table 1. Parental description of *Indica* rice cultivar, its pedigree and features

Name of cultivars	Parentage and Year of release	Specific features of Indica rice cultivars
HUR 4-3	Mutant of Lanjhi (2009)	Semi dwarf 90 - 100 cm, 135-140 days to maturity, grain type – long grain slender, fine resistant to leaf roller and brown plant hopper, susceptible to bacterial leaf blight disease; yield: 55-58 q/ ha
PB-1460 (Improve Pusa Basmati-1)	Pusa Basmati-1 x IRBB 60 (2008)	Semi dwarf 95-110 cm, 130-135 days to maturity, basmati rice grain type: long grain slender, very fine grain shape, elongation ratio 1.5: 1.8, strong aroma present, resistant to bacterial leaf blight, tolerant to sheath blight and blast disease due to presence of *Xa21*, *xa13, qSBR 11.1* and *Pi54* genes; yield: 45-48 q/ ha

Bacterial leaf blight (BLB) of rice caused by gram negative bacteria *Xanthomonas oryzae* pv. *oryzae*, is one of the most destructive diseases throughout the world, occurs mostly during the wet season when water overflows in rice fields. Bacterial leaf blight (BLB) can cause yield loss by 20 - 50% and as high as 80% and even 100% under very severe conditions (Agarwal *et al.* 2005 and Singh *et al*., 2013b). 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

develop new resistant varieties (Perumalsamy *et al.*, 2010). However, the cultivars containing a single major resistance gene proved to be susceptible due to mutation in pathogen race. Recently, pyramiding of more than one major resistance gene has been proven to deliver durable resistance against BLB (Rajpurohit *et al.*, 2010). Therefore, this study was carried out to know the inheritance of bacterial leaf blight resistance in cultivar HUR 4-3, PB-1460 with their F_1's and its segregating generation against *Xanthomonas oryzae* pv. *oryzae*.

Table 2. Scale for bacterial leaf blight disease (Anonymous, 1996 and IRRI, 1996)

Infection %	Score	Host response
0 %	0	Highly resistant (HR)
> 1-10 %	1	Resistant (R)
> 10-30 %	3	Moderately resistant (MR)
> 30-50 %	5	Moderately susceptible (MS)
> 50-75 %	7	Susceptible (S)
> 75-100 %	9	Highly susceptible (HS)

Materials and Methods

Experimental site

The experiment was carried out during two consecutive wet (*Kharif*) season and one dry (*Off-season*) season of 2012-2013 to 2013-2014. The two wet (*Kharif*) seasons trials were taken during June, 15th to November, 15th of 2012-2013 and 2013-2014 at the Agricultural Research Farm of Institute of Agricultural Sciences, Banaras Hindu University, Varanasi in Northern Gangetic Alluvial Plain of India (83°03'0"E longitude; 25°18'0"N latitude and an altitude of 128.93 m above sea level). The experimental soil was Gangetic alluvial (Ustochrept) with pH 7.6. It was moderately fertile-being low in organic carbon (0.39%); available nitrogen (198.4 kg ha^{-1}); and medium in available phosphorus (15.7 kg ha^{-1}) and potassium (215.4 kg ha^{-1}). However, dry (*Off*-season) season trial was taken at Central Rice Research Institute, Cuttack, Odisha during December, 15th to May, 20th 2013 - 2014.

Raising of rice seedlings and creating population

The experimental material of *Indica* rice (Varietal details describe in table-1) staggered sown at seven days interval in nursery bed during 15th, 22th and 29th June, 2012-2013. Twenty five days old single seedlings were transplanted in crossing block during seven days interval in July, 2012-2013, at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, UP. The high yielding BLB susceptible cultivar HUR-4-3 (P_1) taken as female crossed with bacterial leaf blight resistance cultivar PB-1460(P_2) as male to produced five hundred seeds of F_1's. Only two hundred seeds of F_1 and both parents were sown at seven days interval in nursery bed during 15th, 22th and 29th December, 2012-2013 at Research Form of Central Rice Research Institute, Cuttack, Odisha. Forty days old single seedlings were transplanted in crossing block during seven days interval in January to February, 2013-2014. At flowering stage, fifty F_1 plants were selfed to produce approximately 1000 F_2 seeds and both the parents crossed with remaining F_1's (used as female) plants to generate 400-500 seeds of B_1 (F_1 with HUR 4-3 (P_1)) and B_2 (F_1 with PB-1460 (P_2)) backcross generation. Both recurrent and donor parents were again crossed within themself to produce F_1 (HUR 4-3 x PB-1460) seeds. Six generations, namely, P_1 (susceptible parent), P_2 (resistance parent), F_1, F_2, B_1 and B_2 were raised in nursery bed during 15th June 2013-14 during *Kharif-Season*. Twenty one days old single seedling were transplanted in complete randomized block design under three replications in separate plots of 3 meter length spaced at 20 cm apart and the distance between plant to plant (15 cm) was maintained at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Cultural Practices and Fertilizer Application

The experimental field was kept free from weeds by adopting manual weeding. The Fertilizers were applied @ 120 kg N, 60 kg P_2O_5, and 60 kg K_2O per hectare under well irrigated condition. Half of N and full dose of P_2O_5 and K_2O were used as basal in irrigated field after puddling. Remaining half of nitrogen was applied at a time of tillering as a top dressing. All the recommended cultural practices and plant protection measures (except bacterial leaf blight disease control) were followed for raising the healthy crop under irrigated conditions.

Inoculum preparation and Inoculation on rice plants

The cultures of *Xanthomonas oryzae* pv. *oryzae* (strain *BXO_1* and *BX043 wild type*) was obtained from Directorate of Rice Research, Hyderabad, India and sub cultured on peptone sucrose agar (PSA) medium (10 g l^{-1} Sucrose, 10 g l^{-1} Peptone, 1 g l^{-1} glutamic

Table 3. Comparison of per cent disease incidence (PDI) and Area Under Disease Progress Curve (AUDPC) on P_1, P_2, F_1, F_2, B_1 and B_2 progenies against *Xanthomonas oryzae* pv. *oryzae* strains *BXO1* and *BX043 wild type*

Six generations with population size	Plant classified in Resistant: Susceptible group	Per cent Disease incidence (%) at 7 days interval of Inoculation				AUDPC value	Disease Score at 28 DAI	Disease Score at 28 DAI
		7 DAI ± SD	14 DAI ± SD	21 DAI ± SD	28 DAI ± SD			
P_1 (HUR 4-3) 300 plants	300 S	10.56 ± 1.16	22.04 ± 2.02	38.57 ± 1.88	53.91 ± 1.82	649.90	7	S
P_2 (PB-1460) 300 plants	300 R	1.54 ± 0.32	2.26 ± 0.33	4.07 ± 0.85	4.54 ± 0.62	65.61	1	R
F_1s (200 plants)	196R : 4S	1.81 ± 0.37	4.67 ± 0.47	5.77 ± 0.50	8.81 ± 0.87	110.26	1	R
F_2's population 640 plants	113 R	1.62 ± 0.43	3.85 ± 0.70	4.90 ± 0.60	7.13 ± 0.53	91.89	1	R
	353 R/MR	6.08 ± 0.64	15.93 ± 1.18	16.51 ± 0.93	20.08 ± 1.23	318.68	3	MR
	55 MR	8.84 ± 1.30	19.43 ± 0.95	24.50 ± 2.18	25.10 ± 1.50	426.33	3	MR
	131 S	12.98 ± 2.13	22.07 ± 2.72	42.70 ± 1.48	55.98 ± 1.76	694.73	7	S
B_1 (F_1 x HUR 4-3) 300 plants	141 MR	7.75 ± 1.06	15.90 ± 0.66	20.42 ± 0.93	21.27 ± 1.12	355.76	3	R
	159 MS/S	10.86 ± 1.64	20.30 ± 1.77	33.17 ± 2.08	43.87 ± 1.16	565.80	5	MS
B_2 (F_1 x PB-1460) 300 plants	156 R	2.68 ± 1.02	4.03 ± 1.10	5.33 ± 1.15	6.89 ± 1.01	99.05	1	R
	144 MR	7.82 ± 1.18	16.93 ± 1.72	22.93 ± 1.37	27.60 ± 0.85	403.04	3	MR

SD: Standard deviation, DAI: Days after inoculation, R: Resistant, S: Susceptible, MR: Moderately resistant and MS: Moderately susceptible

acid, 0.5 g l^{-1} K_2HPO_4, 0.25 g l^{-1} $MgSO_4.7H_2O$, 16 g l^{-1} bacto-agar and maintained it at pH 7.2 - 7.4 with 40% NaOH) for 2–3 days at 28 ºC (Fahy and Persley, 1983). For pathogenicity test, leaf-clipping method was used for inoculation as described previously (Kauffman *et al.*, 1973) in the rice plants with *Xanthomonas oryzae* pv. *oryzae*. The test was conducted on fully developed leaves at the age of 55 days old rice plants after transplanting. The sterilised scissor dipped in bacterial suspension containing 10^9 cfu ml^{-1}, was used for inoculation. Approximately 15-20 leaves of all plants were grasped in one hand and the top 1-3 inches of leaves were clipped off, simultaneously.

Disease Scoring, observation recorded and data collection

Following inoculation, the plants were observed and note it after every 24 hours time interval, the appearance of disease symptoms (lesion length was measured) and disease incidence were recorded at 07, 14, 21 and 28 days after inoculation using a disease score index of 0 – 9 (IRRI, 1996). The disease scoring data were generated from disease score chart given in Table 2 in the Standard Evaluation System for rice (IRRI, 1996 and Anonymous, 1996) for disease appearance on 300 randomly selected plants from both parents, 200 plants from F_1 hybrids, 300 to 500 plants in segregating generations were used for hybrids and segregating generation to evaluate the response of host plant.

Statistical Analysis

The per cent disease incidence was calculated according to formula given by Gnanamanickam *et al*. (1999). The collected data for studied traits were pooled and standard statistical procedure (Singh and Chaudhary, 1995) and statistical software Windostat ver. 8.3 were applied for statistical analysis. χ^2 (chi-square) test for goodness-of-fit was used to study

Table 4. Inheritance of bacterial leaf blight resistance of six generation P_1, P_2, F_1, F_2, B_1 and B_2 progenies of *Indica* rice against pathogen *Xanthomonas oryzae* pv. *oryzae* strains *BX01* and *BX043 wild type*

Six generations with population size	PDI at 28 DAI with SD (%)	Host response at 28 DAI	No. of plants observed	Gene combination (*Xa21*: Dominant in nature and *xa21*: recessive in nature)	Genotypic ratio	χ^2 value	Phenotypic ratio (R : S)	χ^2 value
P_1 (HUR 4-3) 300 plants	53.91 ± 1.82	S	300	xa21xa21Xa13Xa13	-	NS	-	NS
P_2 (PB-1460) 300 plants	4.54 ± 0.62	R	300	Xa21Xa21xa13xa13	-	NS	-	NS
F_1s (200 plants)	8.81 ± 0.87	MR	194	***XA21****xa21Xa13xa13*	-	NS	-	NS
F_2's segregating population 640 plants	7.13 ± 0.53	R	113	***Xa21Xa21xa13xa13:Xa21****xa21****xa13xa13***	3	0.41	13 : 3 (509 : 131) R : S	0.23
	20.08 ± 1.23	MR	347	***Xa21Xa2****1Xa13Xa13:****Xa21Xa21****Xa13xa13 : **Xa21****xa21Xa13Xa13:****Xa21****xa21Xa21Xa13xa13*	9	0.47		
	25.10 ± 1.50	MR	49	*xa21xa21****xa13xa13***	1	2.03		
	55.98 ± 1.76	MS / S	131	*xa21xa21Xa13Xa13:xa21xa21Xa13xa13*	3	1.01		1.01
B_1 (F_1 x HUR 4-3) 300 plants	21.27 ± 1.12	R	143	***Xa21****xa21Xa13xa13:****Xa21****xa21Xa13Xa13*	1	0.33	1 : 1 (143:157) R : S	0.33
	43.87 ± 1.16	MS / S	157	*xa21xa21Xa13xa13:xa21xa21Xa13Xa13*	1	0.33		0.33
B_2 (F_1 x PB-1460) 300 plants	6.89 ± 1.01	R	158	***Xa21Xa21xa13xa13:Xa21Xa21****Xa13xa13*	1:1	NS	1 : 0 All resistant	NS
	27.60 ± 0.85	MR	142	***Xa21****xa21****xa13xa13:XA21****xa21Xa13xa13*	1:1			

PDI: Per cent disease incidence, SD: Standard deviation, DAI: Days after inoculation and gene (bold letters) showed resistance

Figure 1(a). HUR 4-3 a portion of normal field view, Bacterial leaf blight symptoms appeared in the field indicated by arrow.

(b) Comparison of lesion size pattern between leaves carrying either single gene, double gene or no gene of resistance

Figure 1 (c). Field plot inoculated with BLB pathogen (and arrow indicate appearance of lesion on the leaves) in cultivars HUR 4-3; (d) Panicle (F2 population) showing typical symptom of BLB after 21 days of inoculation. (e) Healthy plants of PB 1460 (un-inoculated) showing resistance against BLB under field condition. (f) B2 (H UR 4-3 x PB1460/ + PB1460) plant showing resistance against BLB after 21 days of inoculation

the Mendelian pattern of inheritance of resistance in bacterial leaf blight.

Results and Discussion

The parental lines with their segregating population were evaluated under field condition for their resistance to bacterial leaf blight (BLB) using artificial inoculation of two different isolates of *Xoo*. One of these isolates, called *BXO1*, belongs to pathotype Ib. It is widely distributed *Xoo* pathotype of *Xanthomonas species* in all over India (Yoshitola *et al.,* 1997). The *Indica* cultivars PB-1460 was resistance due to presence of resistance genes *Xa21* and *xa13* which showed 4.54 % disease severity, while HUR 4-3 susceptible due to absence of these genes and showed 53.91 % disease severity against *Xanthomonas oryzae* pv. *oryzae* under epiphytotic condition (Table 3). The initial symptoms of bacterial leaf blight *viz*. linear yellow to straw coloured stripes with wavy margins, generally on both edges of leaf, rarely on one edge was observed with variable intensities in cultivar HUR 4-3 (Figure 1a). These finding were good agreement with the earlier report by Kihupi *et al.* (2001) and Singh *et al.* (2013b). The dominant gene *Xa21* for bacterial leaf blight resistance was introgressed in the cultivar PB-1460 from the isogenic line IRBB 60, and in the isogenic line IRBB 60, *Xa21* gene was introgressed from the wild source *Oryza*

longistiminata (Kihupi *et al.*, 2001 and Sunderam *et al*., 2009).

The area under the disease progress curve of resistance cultivar (PB-1460) was 65.61 which are significantly less than the susceptible cultivar (HUR 4-3) 649.90. The F_1 plants were observed to be resistant to moderately resistant when screened with a virulent isolate of *Xanthomonas oryzae* pv. *oryzae* strains, *BXO1* and *BX043 wild type* with average per cent disease severity 08.81% and AUDPC 110.26. This indicates the possible involvement of dominance gene(s) in governing the resistance. The F_1 plants of cross HUR 4-3 x PB-1460 were selfed to produce F_2 mapping population, these F_2 mapping population individually scored and could be classified in to four distinct classes on the basis of their genotypic ratio of 9:3:3:1 with their gene combination (9 *Xa21Xa21Xa13Xa13/ Xa21Xa21Xa13xa13/ Xa21xa21Xa13Xa13/ Xa21xa21Xa13xa13* **:** *3 Xa21Xa21xa13xa13/ Xa21xa21xa13xa13* **:** *3 xa21xa21Xa13Xa13/ xa21xa21Xa13xa13* **:** *1 xa21xa21xa13xa13*) and two distinct classes on the basis of their phenotypically performance as resistant and susceptible with their ratio of 13:3, respectively (Table-4 and Figure 1b). Out of 640 F_2 plants, 503 plants were resistant and 131 plants showed susceptible reaction against BLB in the ratio of 13 : 3 with $\chi 2 = 1.24$, $P > 0.05$ indicating that observed data are in accordance with expected ratio. These results showed the *xa13 + Xa21* gene combination was preferred in parental and their segregating generations to exploit the synergistic effects of this combination in preventing BLB infection. Similar findings were also reported by Sidhu *et al.* (1978), Kihupi *et al*. (2001) and Natarajkumar *et al.* (2008).

In backcross population, three hundred plants of each backcross were screened against isolates of *Xanthomonas oryzae* pv. *oryzae.* In first backcross population B_1 (F_1 x HUR 4-3) and in second backcross B_2 (F_1 x PB-1460) populations were individually disease scored and grouped in two distinct classes (Figure 1d and e). The per cent disease severity (PDI) and AUDPC value of the both backcross populations were 21.27% to 43.87% and 6.89% to 27.60% and 355.76 to 565.80 and 99.05 to 403.04, respectively (table-3). These population were again classified as resistant / moderately resistant and moderately susceptible / susceptible group on the basis of their genotypic ratio and gene combination 1:1 (1 ***Xa21****xa21Xa13xa13/* ***Xa21****xa21Xa13Xa13****:*** 1 *xa21xa21Xa13xa13/ xa21xa21 Xa13Xa13*) and 1:1 (1 ***Xa21Xa21xa13xa13/ Xa21Xa21*** *Xa13xa13****:*** 1 ***Xa21****xa21****xa13xa13/ XA21****xa21Xa13xa13*), respectively. However, on the basis of phenotypic performance of both backcrosses they again classified in to diverse grouped *i.e.,* resistant and moderately susceptible/ Susceptible. B_1 revealed 1 (resistant) : 1 (susceptible) ratio, thereby indicating that the 143 plants have single dominant resistance gene, While, B_2 population were exhibited 1 (resistant) : 0 (susceptible) ratio, it indicates the resistance is governs by single dominant and recessive two resistance genes or sometimes it governs by only single dominant resistance gene (Figure 1f). These finding were in agreement with earlier report by Sundaram *et al.* (2009). Out of 300 plants of each backcross population, 143 resistant and 157 susceptible were found in B_1 generation and all are resistant were found in B_2 generation $\chi 2 = 0.66$, $P > 0.05$ and $\chi 2$ = Non Segregating (NS), $P > 0.05$, respectively indicating that observed data are in agreement with the expected ratio in backcross generation.

The F_2, B_1 and B_2 progenies were segregates in the ratio 13 : 3, 1 : 1 and 1 : 0 which is mainly due to cumulative or synergistic effects of both resistance genes *i.e.,* one dominant gene *Xa21* and *one recessive xa13* resistant genes. Interestingly, when we compared the level of bacterial leaf blight resistance of cultivar HUR 4-3 with PB-1460, it was observed that the latter showed a higher degree of resistance as compared to PB - 1460. This could be due to the effect of different genetic backgrounds of HUR 4-3 and PB-1460, which may be responsible for modulating the level of resistance.

Acknowledgements

The financial support for this study was provided by Ministry of Science and Technology, Department of Science and Technology, New Delhi, Government of India as a DST-INSPIRE Fellowship (INSPIRE Code:

IF-120215) for full-time doctoral (Ph. D.) degree programme at Banaras Hindu University, Varanasi, Uttar Pradesh, India.

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