ORIGINAL RESEARCH ARTICLE

Marker Assisted Screening of Nepalese Rice for Bacterial Leaf Blight (BLB) Resistance

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Abstract

Bacterial Leaf Blight (BLB) is the most important yield limiting factor in Nepalese rice. BLB resistance rice varieties are highly demanding in the country. Breeding efforts for developing disease resistant depends on availability and use of resistant gene donors. Nepalese rice landraces could be the source of resistant gene. Therefore, ninety six Nepalese rice accessions were screened using eight Simple Sequence Repeats (SSR) markers and one Sequence Tagged Sites (STS) marker for presence and absence of BLB resistance gene. We have detected BLB resistance gene Xa-10 on five accessions, Xa-13 on six accessions, Xa-7 on 23 accessions, Xa-3 and Xa-4 on 52 accessions, Xa-5 on 25 accessions, Xa-8 on 30 rice accessions. No any rice accessions tested have Xa-21. Similarly, 17 rice accessions showed three and more than three BLB resistance genes. Presence of Xa-13 on susceptible check variety CNTRL-85033 confirmed that this resistant gene is not working in Nepalese rice field. Therefore, Nepal need to pyramide the BLB resistant genes for durable resistance.

Keywords: Bacterial Leaf Blight, Simple Sequence Repeats, resistant gene, Nepalese rice, Marker Assisted Screening

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Introduction

Bacterial Leaf Blight (BLB) is one of the productions limiting biotic stresses in rice. It is caused by Xanthomonas oryzae pv. oryzae (Xoo). It can reduce the yield up to 50% [1] and in Nepal; it reduced the yield from 5-60 % in Terai and mid-hills during hot and humid periods [2].

Twenty four genes conferring resistance to BLB have been identified through classical genetic analysis [3] and 10 out of 24 genes have been mapped using Restriction Fragment Length Polymorphism (RFLP), Rapid Amplified Polymorphic DNA (RAPD) and microsatellite markers [4-7]. Among them, 6 genes are recessive in nature [1]. Similarly, two new genes Xa-22 in rice variety Zha-Chang-long [8] and Xa-23 in Oryza rufipogon [9] were identified and mapped on different chromosomes. Most common BLB resistant gene used in rice breeding and BLB screening worldwide are Xa-1, Xa-2, Xa-3, Xa-4, Xa-5, Xa-7, Xa-8, Xa-10, Xa-11, Xa-13, Xa-14 and Xa-21 [10].

BLB resistance rice varieties are highly demanded worldwide. However, the continuous evolution of pathogenic races leading to the breakdown of resistance in many improved varieties [9].

Thus, success of resistance breeding program depends on the availability of the resistant donors. Similarly, pyramiding different resistant genes in a single rice variety will increase the resistance. However, two or more resistance gene pyramiding in a single variety is easy through molecular marker assisted selection

(MAS), and identification for the presence and absence of particular gene in a variety for MAS as donor and recipient parent through molecular marker assisted screening is very fast, reliable and cheaper. Therefore, this study was carried out to identify the accessions within Nepalese gene pool with the potential of BLB resistance genes.

Methods

Germplasm Collection

Seventy Nepalese rice accessions (NPGR No.s) collected from Terai region of Nepal were obtained from National Plant Genetic Resources Centre (NPGRC); and five breeding lines (NR series) and released rice varieties (Chandannath-1, Chandannath-3, Chhomrong, Macchapuchre-3, Manjushree-2, Palung-2 and Taichung-176) from Agriculture Botany Division of Nepal Agricultural Research Council (NARC). Similarly, rice varieties IR-64, Sabitri and Masuli, and breeding line CNTRL-85033; Terai rice landraces Bagari, Bhatti, Karma, Lal Tenger and Parewa Pankha; and Hill rice landraces Belkuti, Gerneli, Jumli Marshi and Seto Anadi were collected from different parts of Nepal (**Table 1**).

Molecular Marker and check variety

Eight SSR markers and one STS marker (pTA248) for the presence and absence of BLB resistance gene. Molecular markers are selected based on their linkage with particular BLB resistance gene (**Table 2**). IR-64 and CNTRL-85033 were used as resistant and susceptible check respectively.

DNA extraction, PCR reaction and data analysis

Genomic DNA of rice accessions was prepared using modified CTAB method as described by Sul and Korban [11]. Each PCR reaction was conducted with100ng of genomic DNA, 1 µM of each primer and 7.5 µl of 2x GoTaqGreen PCR Master Mix (Promega Corporation, Madison, WI, USA). PCR mixture was amplified in MJ Research PTC-100TM Programmable Thermal Controller (MJ Research, Inc, Watertown, MA, USA) with the following temperature regimes: initial denaturation for 2 min at 95oC followed by 33 cycles of 95oC for 30 sec, annealing as per primer for 1 min, extension at 72oC for 2 min and final extension at 72oC for 7 min followed by holding at 4oC as described on **Table 2** and Gramene [12].

Amplified PCR products were separated in 2% analytical grade agarose gel at 100V for 1H. Gels were stained with 0.1 µg/ml ethidium bromide (Promega Corporation, Madison, WI, USA) and then visualized under UV transilluminator gel documentation system (Wilber Lourmat, Marne-La-Valleen, France) using 1 µg guide size DNA ladder (Genetix, Biotech Asia Pvt. Ltd.). The presence and absence of particular band size was scored for screening disease resistance genes.

Results and Discussion

Different resistance genes were identified in Nepalese rice germplasm as defined by different molecular markers (**Table 3**). We identified BLB resistance gene Xa-10 on two accessions, Xa-13 on six accessions, Xa-7 on 23 accessions, Xa-10 on five accessions, Xa-3 and Xa-4 on 52 rice accessions (**Figure 2**), Xa-5 on 25 accessions, Xa-8 on 30 rice accessions. No any rice accessions have Xa-21 (**Figure 1**). Similarly, 17 rice accessions showed three and more than three BLB resistance genes (**Table 4**).

Table 1. Nepalese rice accessions used for screening BLB resistance using simple sequence repeats marker

2555	3110	3267	3510	3995	8511	8556	Bagari	Karma	Palung-2
2573	3118	3270	3975	3996	8516	8557	Belkuti	Lal Tenger	Parewa Pankha
2586	3124	3277	3978	7319	8518	8558	Bhatti	Macchapuchre-3	Pokhreli Masino
2587	3129	3344	3979	7364	8524	8559	ChandanNath-1	Manjushree-2	Sabitri
2588	3131	3348	3987	8303	8535	8562	ChandanNath-3	Masuli	Seto Anadi
2590	3134	3471	3989	8305	8539	8567	Chhomrong	NR10490-89-3-2-1	Taichung-176
2591	3138	3487	3990	8335	8546	8570	CNTRL-85033	NR10528-B2-21-3-1	
3050	3142	3488	3992	8506	8551	8571	Gerneli	NR10548-B-22-2	
3057	3175	3508	3993	8508	8553	8807	IR-64	NR10591-B-B-3-3	
3107	3231	3509	3994	8509	8554	Aanga (Hills)	Jumli Marshi	NR10676-B-B-4-3-1	

Note: Name as numbers without any alphabet denote the Nepalese Plant Genetic Resource (NPGR) number.

Table 2. Molecular markers used to identify presence of BLB resistance gene in Nepalese rice gene pool

Marker Name	Sequence-F [5'... ... 3']	Sequence-R [5'... ... 3']	Annealing Temperature, °C	BLB resistance gene	PCR product size, bp	Chromosome No.	References
pTA248	AGACGCGGAAGGGT GGTTCCCGGA	AGACGCGGTAATCGAA AGATGAAA	55	*Xa-21*	1000	11	[13]
RM21	ACAGTATTCCGTAGG CACGG	GCTCCATGAGGGTGGT AGAG	55	*Xa-5*	152-186	11	[14]
RM167	GATCCAGCGTGAGG AACACGT	AGTCCGACCACAAGGT GCGTTGTC	55	*Xa-3/4/10*	128	11	[13]
RM206	CCCATGCGTTTAACT ATTCT	CGTTCCATCGATCCGTA TGG	55	*Xa-10*	147	11	[13]
RM224	ATCGATCGATCTTCA CGAGG	TGCTATAAAAGGCATTC GGG	55	*Xa-3/4*	124-155	11	[13]
RM230	GCCAGACCGTGGATG TTC	CACCGCAGTCACTTTTC AAG	55	*Xa-13*	257	8	[13]
RM251	GAATGGCAATGGCG CTAG	ATGCGGTTCAAGATTCG ATC	55	*Xa-7/8*	111-141	3	[13]
RM263	CCCAGGCTAGCTCAT GAACC	GCTACGTTTGAGCTACC ACG	55	*Xa-8*	148-189	2	[13]
RM390	CCCTTGTTTCAGTGG CTCAG	CCAAGATCAAGAACAG CAGGAATC	55	*Xa-5*	169-176	5	[15]

Table 3. Nepalese rice germplasm with different BLB resistant gene identified using different molecular markers
Note: combination on parenthesis is respective SSR markers used to detect particular gene.

Xa-10 (RM206)	*Xa-3/4/10* (RM167)	*Xa-13* (RM230)	*Xa-5* (RM390)	(*Xa-5*) RM21	*Xa-8* (RM263)	*Xa-7/8* (RM251)	*Xa-3/4* (RM224)	
7364	3134	8807	7364	3987	3277	3270	3344	8508
8554	8553	7319	8535	8335	3488	3277	3508	8509
	8554	Sabitri	3131	3050	3509	3344	3509	8511
	8558	Masuli	8539	3057	3510	3471	3510	3110
	Jumli Marshi	CNTRL-85033	3134	8506	3975	3487	3975	8518
		Jumli Marshi	8546	8508	3979	3488	3978	8524
			3138	8509	3990	3508	3979	3118
			8551	8511	7364	3509	3987	8539
			3142	3107	8535	3510	3989	3134
			8553	8518	8539	3975	2555	8546
			8556	8524	3134	3978	3992	3138
				3129	8546	3979	3993	8551
				IR-64	3138	3990	3994	3142
				Sabitri	8551	2573	3995	8553
				Masuli	8553	8518	3996	8554
					8554	3118	2573	8556
					8557	8535	2586	8557
					8559	3131	2587	8558
						8539	2588	8567
						3138	8303	8570
						8558	8305	8571
						8559	2590	8807
						8562	8335	3175
							2591	7319
							3057	Masuli

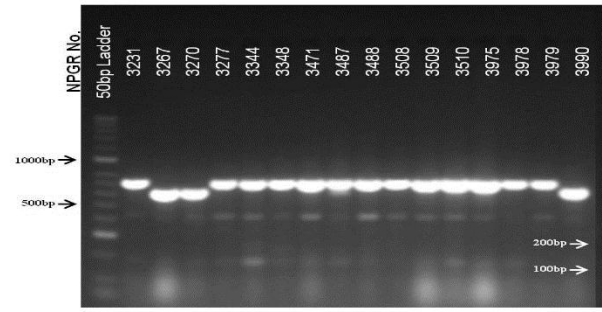

Figure 1. Amplification of STS marker pTA248 that don't show presence of Xa-21 (1000bp) on any Nepalese rice accessions

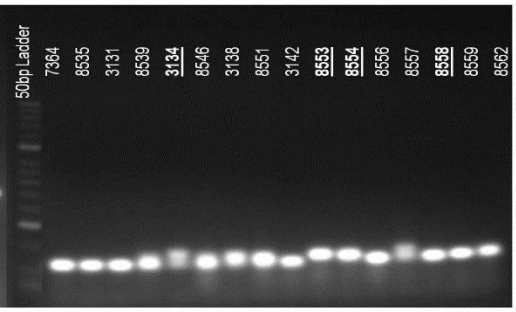

Figure 2. Amplification of SSR marker RM167 showing presence of Xa-3/4/10 (128bp) on different Nepalese rice accessions (underlined)

Table 4. Nepalese rice germplasm having three and more bacterial leaf blight (BLB) resistance gene detected by molecular markers

NPGR No.	*Xa-3, Xa-4* (RM167, RM224)	*Xa-5* (RM21, RM390)	*Xa-7* (RM251)	*Xa-8* (RM251, RM263)	*Xa-10* (RM167, RM206)	*Xa-13* (RM230)
3509	1	0	1	1	0	0
3510	1	0	1	1	0	0
3975	1	0	1	1	0	0
3979	1	0	1	1	0	0
8518	1	1	1	1	0	0
7364	0	1	0	1	1	0
8535	0	1	1	1	0	0
3131	0	1	1	1	0	0
8539	1	1	1	1	0	0
3134	1	1	0	1	1	0
8546	1	1	0	1	0	0
3138	1	1	1	1	0	0
8551	1	1	0	1	0	0
8553	1	1	0	1	1	0
8554	1	0	0	1	1	0
8558	1	0	1	1	1	0
Masuli	1	1	0	0	0	1

Note: 1=present, 0=absent

Nepalese rice accessions lack the Xa-21 genes which is more important for controlling BLB epidemics throughout the world. Kameshwara Rao [16] reviewed and noted that Xa-21 gene is transferred from O. longistaminata and integrated into some IRRI developed rice varieties. Lacks of such varieties in our study may be the result for this. Similarly, our susceptible check 'CNTRL-85033' showed the presence of Xa-13 but Amgai [17] reported that it was heavily infected by BLB at Nepalese rice field. This may be due to the difference on BLB isolated found in Nepal which may break the resistance reaction developed by Xa-13 gene. Resistant check IR-64 showed the presence of Xa-5 indicating that it is effective for Nepalese BLB pathogen.

Conclusion

Nepalese rice landraces contains many marker alleles for different BLB resistant genes. The rice landraces with effective resistant gene can be used as donor parent for MAS. However, for the enhancement of resistance in Nepalese rice with absence and/or ineffective resistance gene can be done by transferring broad spectrum resistance gene like Xa-21. BLB resistance is also affected by multiple resistance genes and their interaction [17]. Therefore, pyramiding BLB resistance gene on Nepalese rice variety is most important for durable resistance with BLB.

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