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Short Communication

Identification of Indian rice germplasm lines with bacterial leaf blight (BLB) resistance genes

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Abstract

Bacterial leaf blight of rice, caused by *Xanthomonas oryzae* pv*. oryzae* is one of the major constraints limiting rice production and productivity in South East Asia, particularly in Japan, Philippines, Indonesia, China and India. No effective chemical control for the disease is available. It is therefore, important to identify lines resistant to blight, which can then be used as parental lines in breeding programmes. Gene-linked and gene-based molecular markers and well defined donors are available for most of these genes. In the present study, a total of 386 Indian rice germplasm lines including commercially cultivated Indian rice varieties were screened for the presence of four bacterial blight resistance genes i.e. *Xa4, xa5, xa13 and Xa21.* The rationale behind the study was to identify new hitherto unreported, rice lines carrying BLB resistance genes and studying the allelic distribution of these genes in the germplasm.

Key words: BLB, germplasm, MABB, molecular markers

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the major diseases of rice (*Oryza sativa* L.) in the world. In some areas of Asia it can reduce crop yield by 50-80% [1-3]. The most effective approach to combat BLB is using resistant varieties. It is essential to identify new sources of the BLB resistance genes which can be subsequently used in breeding BLB resistance in a desired background. Thirty seven BLB resistance genes have been identified hitherto [4]. A total of 386 Indian rice germplasm lines including landraces, farmer's varieties and released varieties were used in the study. These were screened for the presence of four bacterial blight resistance genes i.e., *Xa4, xa5, xa13 and Xa21* using molecular markers, RM1233, *xa5*-1F, *xa13-prom* and pTA248 [5], respectively (Fig. 1). The IRBB lines (IRBB4, IRBB5, IRBB13 and IRBB21) from IRRI were used as positive controls. The chromosomal location of genes, their respective markers and sequence of the primers used, their allelic variants, are given in Table 1. The distribution of genotypes in different categories with respect to each marker is presented in Table 2. The gene *Xa4* was the most widely distributed among the germplasm lines. A total of 81 genotypes were found to be positive for *Xa4*, 4 genotypes for *xa5*, two genotypes for *xa13* and two genotypes for *Xa21*. *Xa21* was found to be present in only two varieties (Pusa 1460 and Pusa 1463-02-1-1). The allelic frequencies for each of the genes is given in Fig. 2. Pusa 1460 is a bacterial leaf blight resistant line derived from Pusa Basmati 1 through marker assisted backcross breeding (MABB), with genes *xa13* and *Xa21* introgressed from IRBB 55 [6]. Pusa Basmati 1460 has been used as a donor for genes *xa13* and *Xa21* in some breeding programmes for BLB resistance [7]. The extremely rare presence of *Xa21* in the germplasm lines is in concordance with the fact that *Xa21* gene was introgressed from wild species *O*. *longistaminata*, an observation that has been earlier documented [8]. Two genotypes, Pusa 1460 (*xa13* and *Xa21*) and Pusa 1463-02-1-1 (*xa13* and *Xa21*) were shown to carry more than one BLB

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Table 1. Markers used for validation and number of genotypes in different allelic category

Gene	Donor	Ch	Marker	Primer sequence	Band sizes (bp)	No. of genotypes in different allelic category*					
						A1	A2	A3	A4	H	NA
Xa4	TKM6	11	RM 1233	F'- TTCGTTTTCCTTGGTTAGTG	R - 160	81	285	8	4	5	3
				R'- ATTGGCTCCTGAAGAAGG	S - < 160						
xa5	D2192	5	*xa5_1*	F'-CTCTACCGGAGGTCCACCATTG	R - 299	4	372	-	-	-	10
				R'-AGGAACAGCAACATTGCAAC	S - 199 + 100 (on dig. with *BsrI*)						
xa13	Long grain	8	*xa13-prom*#	F'-GGCCATGGCTCAGTGTTTAT	R - 250	2	380	-	-	4	-
				R'-GAGCTCCAGCTCTCCAAATG	S - 200						
Xa21	*O. longistaminata*	11	pTA248	F'-AGACGCGGAAGGGTGGTTCCCGGA	R - 950	2	208	150	10	-	16
				R'-AGACGCGGTAATCGAAGATGAAA	S - < 950						

*A1 is resistance (R) specific allele; A2, A3 and A4 indicates susceptibility (S) specific allele for respective markers; H: heterozygous status; NA: failure of amplification after repeated PCR, #Personal Communication with R M Sundaram, Senior Scientist, DRR, Hyderabad

Table 2. Genotypes positive for BLB resistance gene(s)

BLB	Variety
Xa4	PR120, K332, K429, JK201, JR503, Gangaball, Jai phulla, Thakurasuna, Nanu, Heerakani, Thakurbhog, Tulsiphoola, Dhusara, HUR-36, Karma Mahsuri, Bamleshwari, Naggar Dhan, HPR 2143, MTU 1010, CSR 10, Abhishek, Anjali, Hazari dhan, Kalinga 463, Kamlesh, Virendra, Sitwa dhan, Haldimuri , Jhulhat, Heera, Vanprabha, NDR 97, Ananda, Red trinaini, ADT 37, Govind, Rasi, Jaya, Pant Dhan 4, Narendra 359, Malviya dhan, Luni shree, HUR 36, Orugullu, Kavya, WGL 32100, Shiva, Erramallalu, WGL 23985, Varalu, MTU 7029, MTU 5249, MTU 5293, MTU 2067, MTU 4870, CT 1000-6-7-2M-5-1, Sew Maejan, Tai Pei 309, Ketaki joha, Zang Bhuman, Munna, Bela, Surendra, Khara Munga, Daya, Gajapati , Badami, Udyagiri, Rajeswari, Jajati, Pratap, Gouri, Bhubana, Birupa, Samanta, Surendra, Kharavela, Pratikshya, Jagannath, Manika, Ramachandi, Jagbandhu. (81 genotypes)
xa5	OYC183, PR113, CR143-2-2, Ajay. (4 genotypes)
xa13	MAS-946-1*, MAS-946*, PR120*, PAU201*, P1463-02-1-1, Pusa 1460. (6 genotypes including heterozygous lines)
Xa21	Pusa 1460, P1463-02-1-1. (2 genotypes)

*heterozygous lines

resistance genes. This investigation demonstrate the utility of molecular markers in identifying genes of agronomic importance. Marker assisted selection (MAS) can be suitably deployed in order to transfer these genes in a desired background. This is the first study which identifies potential sources of BLB resistance genes in Indian rice germplasm, which will facilitate the use of a diverse donor base in breeding for BLB resistance in rice helping alleviate the vulnerability of rice lines to an epidemic outbreak. It has been observed that the exploitation of only one gene results in significant shifts in the race frequency of Xoo [9]. In many areas of India, China and the Phillippines, rice varieties with only *Xa4* for defense against Xoo have become susceptible to the pathogen. One way to combat such a breakdown of BLB resistance is to pyramid multiple resistance genes into

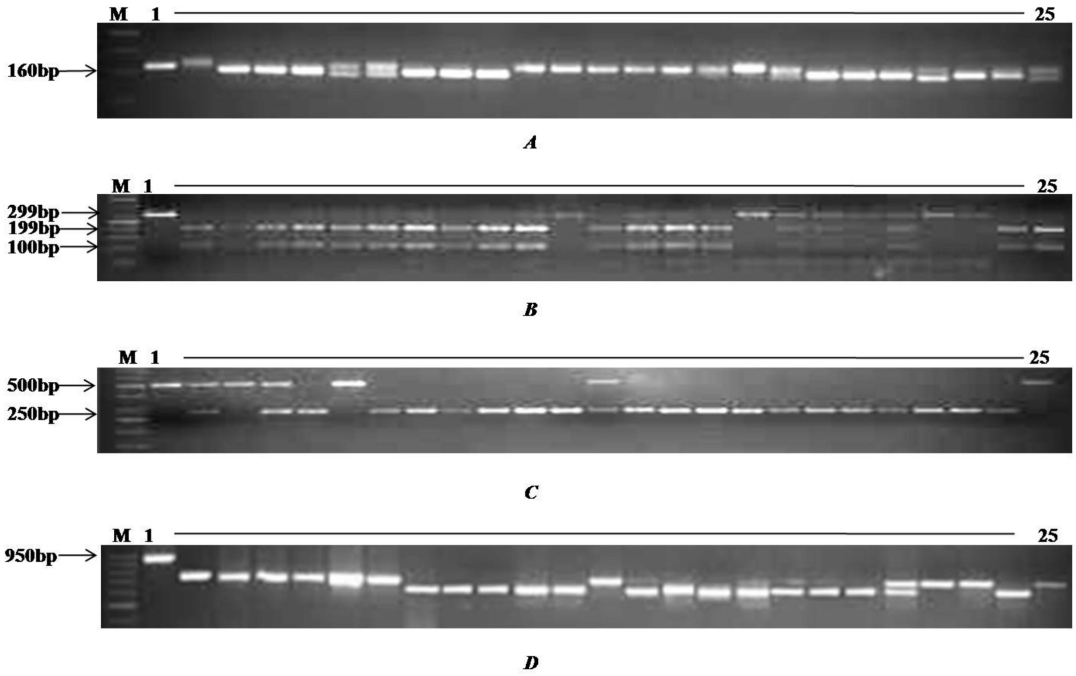

Fig. 1. Allelic profile of rice germplasm lines for BLB resistance genes using gene linked and gene based markers. (A) *Xa4* linked marker RM1233; (B) *xa5* gene based CAPS marker *xa5_1* & digestion with restriction enz. *BsrI*; (C) *xa13* gene based marker *xa13*-prom; (D) *Xa21* gene based marker pTA248; M: 50 bp ladder; 1: positive control (IRBB4, IRBB5, IRBB13 and IRBB21 in (A),(B),(C) and (D) respectively.) 2-25: germplasm lines

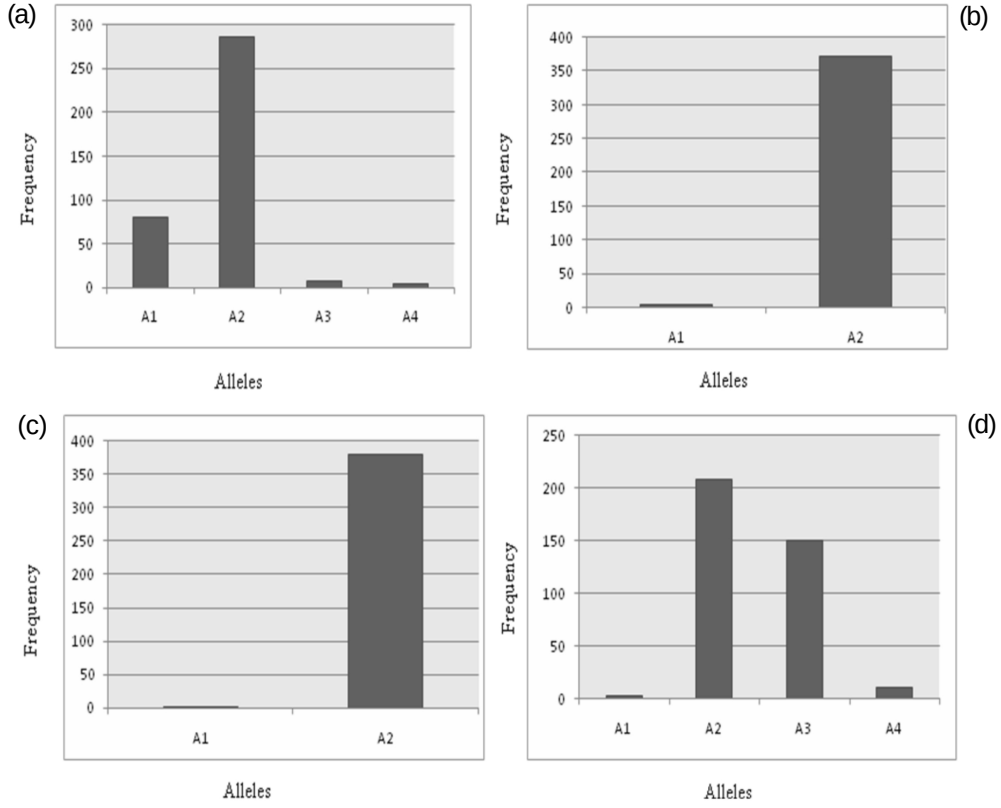

Fig. 2. Allelic distribution of BLB resistance genes in rice germplasm, A1 being the resistance specific allele; A2, A3 and A4 are the susceptibilty specific alleles; not amplified and heterozygous samples have not been considered, a) *Xa4* b) *xa5* c) *xa13* and d) *Xa21*

rice varieties in order to develop the broad-spectrum, durable resistance. Development of new molecular markers for each resistance gene, will enable the identification of plants with multiple genes.

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