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Identification of Polymorphic Loci between Donors and Recipient Parents for Marker Assisted Backcross Breeding in Rice (*Oryza sativa* L.)

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ABSTRACT

The study deals with three varieties; two blast resistance gene donors PTB21 (Thekkan) and PTB7 (Parambuvattan) and recurrent parent Uma (MO16). The three parental lines were screened using the 30 chosen microsatellite loci through polymerase chain reaction (PCR) carried out in a 25 µl reaction volume comprising 50 ng template DNA, 10 pmoles of each primer, 10 mM dNTPs, 10X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) and 1 U of Taq DNA polymerase. Of the 30 loci, 12 were found to be polymorphic for Uma and PTB21 while 17 markers were polymorphic for Uma and PTB7. The identified polymorphic loci can be effectively used in marker assisted backcross breeding programme for the speedy recovery of recurrent parent (Uma) genome.

Key words: *Molecular markers, Backcross breeding, Resistance, Genes, Blast.*

INTRODUCTION

Blast disease, caused by *Magnaporthe oryzae* is one of the most devastating diseases in rice and is a great threat to food security worldwide. During *kharif* season, the disease is prevalent throughout the rice growing areas in India including the southern states of Kerala, Tamil Nadu, Karnataka and Andhra Pradesh. Approximately 100 major blast resistance genes have been reported in rice and hence exploitation of host plant resistance can be employed effectively for the management of blast. With the advent of molecular markers it has become possible to tag individual genes responsible for resistance to different races of the pathogen and to identify multiple genes in the plant by indirectly selecting for the markers linked to resistant genes[2]. Marker Assisted Selection (MAS) has become an important tool for durable resistance screening, minimizing the time and cost to make progress in breeding programmes. MAS in rice is being practised for blast disease[1,4]. Speedy recovery of the recurrent parent genome in pyramided lines may be accomplished through background selection carried out using polymorphic SSR markers which can distinguish the recurrent parent and resistance gene donors[3].

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MATERIALS AND METHODS

Breeder seeds of the popular rice variety, Uma (MO16) was collected from Regional Research station KAU, Moncompu, Alleppy. The seeds of donor parents, PTB21 (*Pi2* gene) and PTB7 (*Pi1* and *Pikh* genes) were collected from Regional Agricultural Research station, Kerala Agricultural University, Pattambi, Palakkad, Kerala. Seeds of these varieties were germinated in glass bottles and DNA was isolated from the tender leaves using QIAGEN DNeasy plant mini kit. The quality and quantity of the DNA was assessed against 100 ng of undigested λ DNA. The genomic DNA was diluted to a concentration of 2 ng/µl and stored at 4°C as working solution while the stock DNA (undiluted) was stored at -20 ºC in aliquot.

A total of 30 microsatellite loci were chosen from the public domain (http://www.gramene.org). The loci with high recorded Polymorphism Information Content (PIC) were selected in such a way as to cover the rice genome as much as possible and spread over all the 12 chromosomes. The chromosome-wise distribution of loci chosen is as follows: chromosome 1 - 2 loci; chromosome 2 - 2 loci: chromosome 3 - 2 loci; chromosome 4 - 3 loci; chromosome 5 – 2 loci; chromosome 6 - 4 loci; chromosome 7 – 2 loci; chromosome 8 – 2 loci; chromosome 9 – 2 loci; chromosome 10 – 2 loci; chromosome 11- 5 loci; chromosome 12- 2 loci.

The three parental lines were screened using the 30 chosen microsatellite loci through Polymerase Chain Reaction (PCR) carried out in a 25 µl reaction volume comprising 50 ng template DNA, 10 pmoles of each primer, 10 mM dNTPs, 10X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) and 1 U of Taq DNA polymerase.

Amplifications were carried out in an Eppendorf master cycler nexus gradient PCR. Template DNA was initially denatured at 94°C for 5 min followed by 38 cycles of PCR amplification with the following parameters: a 30sec to 1 min denaturation at 94°C, a 30sec to 1 min annealing at 55°C, 72°C for 1 min of primer extension and final extension 5-7 min at 72°C. After completion of amplification, PCR products were stored at -20°C. The amplified product was electrophoretically resolved on a 2% agarose gel containing 0.5 µg/ml of ethidium bromide in 1x TBE buffer and visualized under SYNGENE G-Box F3 gel documentation unit.

RESULTS AND DISCUSSION

Of the 30 loci screened, 12 markers were found to be polymorphic for Uma and PTB21 (Thekkan) while 17 markers were polymorphic for Uma and PTB7 (Parambuvattan). The list of polymorphic markers and their chromosome location are given in Table 2. The annealing temperatures of all the polymorphic markers were standardised and found to be 55^0C. A total of 12 markers which were found to distinguish Uma and PTB21 can be effectively used for the speedy recovery of recurrent parent (Uma) genome through background selection in a marker assisted backcross breeding programme for transferring blast resistance gene *Pi2*. Plate 1 depicts the parental polymorphism shown by marker RM151. Uma and PTB7 can be distinguished by 17 polymorphic SSR markers and hence may be used for the rapid recovery of recurrent parent (Uma) genome through background selection in a marker assisted backcross breeding programme for transferring blast resistance genes *Pi1* and *Pikh*. Plate 2 shows the screening of parental lines with polymorphic marker RM21.

Table 1: Details of microsatellites used in the study

Sl.No	Loci	Chr.	Forward primer	Reverse primer	Tm (^{0}C)
1	RM151	1	GGCTGCTCATCAGCTGCATGCG	TCGGCAGTGGTAGAGTTTGATCTGC	55
2	RM595	1	CCTTGACCCTCCTCTTACTT	TCCTATCAAAATTTGGCAAC	55
3	RM485	2	CACACTTTCCAGTCCTCTCC	CATCTTCCTCTCTTCGGCAC	55
4	RM266	2	TAGTTTAACCAAGACTCTC	GGTTGAACCCAAATCTGCA	55
5	RM422	3	TTCAACCTGCATCCGCTC	CCATCCAAATCAGCAACAGC	55
6	RM545	3	CAATGGCAGAGACCCAAAAG	CTGGCATGTAACGACAGTGG	55
7	RM335	4	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG	55
8	RM567	4	ATCAGGGAAATCCTGAAGGG	GGAAGGAGCAATCACCACTG	55
9	RM551	4	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG	55
10	RM440	5	CATGCAACAACGTCACCTTC	ATGGTTGGTAGGCACCAAAG	55
11	RM592	5	TCTTTGGTATGAGGAACACC	AGAGATCCGGTTTGTTGTAA	55
12	RM204	6	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC	55
13	RM217	6	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC	55
14	RM541	6	TATAACCGACCTCAGTGCCC	CCTTACTCCCATGCCATGAG	55
15	RM585	6	CAGTCTTGCTCCGTTTGTTG	CTGTGACTGACTTGGTCATAGG	55
16	RM481	7	TAGCTAGCCGATTGAATGGC	CTCCACCTCCTATGTTGTTG	55
17	RM234	7	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	55
18	RM210	8	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG	55
19	RM547	8	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCCTCGTAGCG	55
20	RM410	9	GCTCAACGTTTCGTTCCTG	GAAGATGCGTAAAGTGAACGG	55
21	RM444	9	GCTCCACCTGCTTAAGCATC	TGAAGACCATGTTCTGCAGG	55
22	RM228	10	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC	55
23	RM333	10	GTACGACTACGAGTGTCACCAA	GTCTTCGCGATCACTCGC	55
24	RM21	11	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	55
25	RM187	11	CCAAGGGAAAGATGCGACAATTG	GTGGACGCTTTATATTATGGG	55
26	RM229	11	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT	55
27	RM260	11	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG	55
28	RM287	11	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	55
29	RM247	12	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	55
30	RM453	12	CGCATCTCTCTCCCTTATCG	CTCTCCTCCTCGTTGTCGTC	55

Table 2: Simple sequence repeats markers that are polymorphic between donors and recipient parent

Uma x PTB 21		Uma x PTB7	
Sl No	**Loci (Chro. No.)**	**Sl No**	**Loci (Chro. No)**
1	RM 422(3)	1	RM 567(4)
2	RM 595(1)	2	RM 551(4)
3	RM 333(10)	3	RM 595 (1)
4	RM 229(11)	4	RM 229(11)
5	RM 585(6)	5	RM 21(11)
6	RM 21(11)	6	RM 204(6)
7	RM 204(6)	7	RM 592(5)
8	RM 592(5)	8	RM 228(10)
9	RM 151(1)	9	RM 187(11)
10	RM 210(8)	10	RM 151(1)
11	RM 547(8)	11	RM 210(8)
12	RM 335(4)	12	RM 547(8)
	12 markers	13	RM 440(5)
		14	RM 545(3)
		15	RM 333(10)
		16	RM 585(6)
		17	RM 335(4)
			17 markers

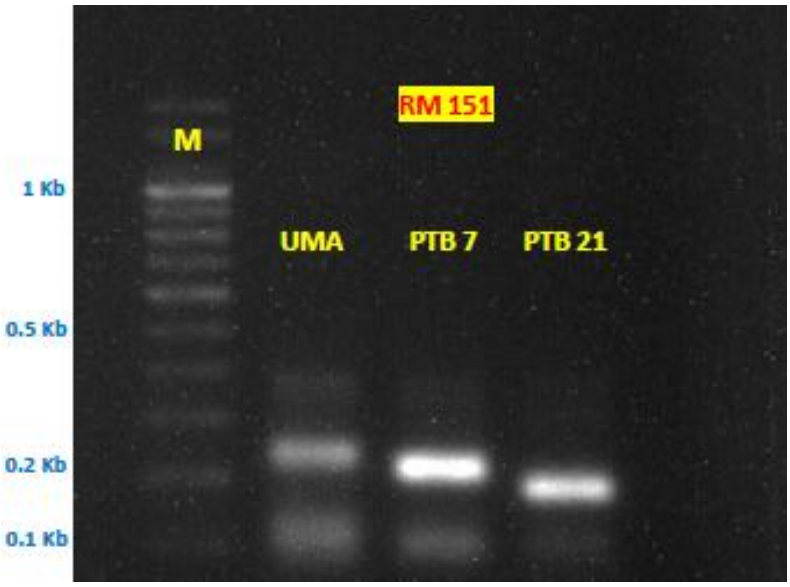

Plate 1: Parental polymorphism shown by marker RM151

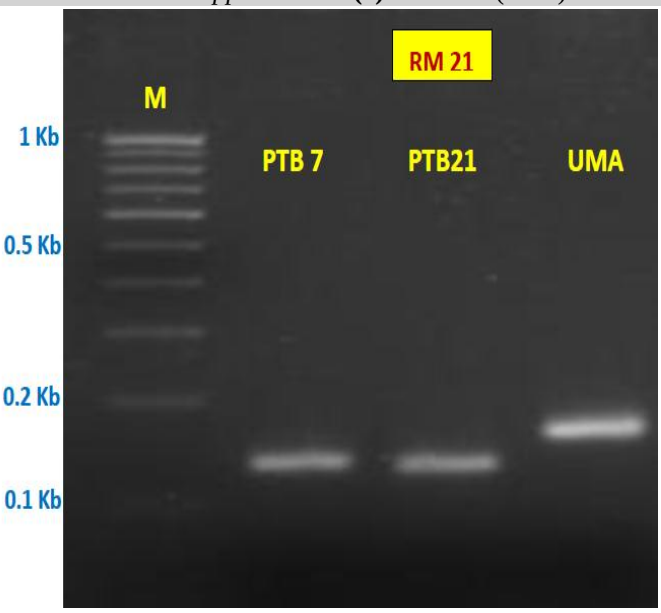

Plate 2: Screening of parental lines with polymorphic marker RM21

REFERENCES

1. Liu, G., Lu, G., Zeng, L. and Wang, G.L., Two broad-spectrum blast resistance genes, Pi9(t) and Pi2(t), are physically linked on rice chromosome 6. *Molecular Genetics and Genomics*, **267:** 472 – 480 (2002).
2. Saikumar, S., Varma, C.M.K., Nagesh, K., Thippeswamy, S. and Shenoy, V., Marker-assisted breeding of *Pi-1* and *Piz*-5 genes imparting resistance to rice blast in PRR78, restorer line of Pusa RH-10 basmati rice hybrid. *Plant Breeding*, **132:** 61- 69 (2012).
3. Suh, J.P., Jeung, J.U., Noh, T.H., Cho, Y.C., Park, S.H. and Park, H.S., Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. *Rice*, **6:** 5-10 (2013).
4. Toojinda, T., Tragoonrung, S., Vanavichit, A., Siangliw, J.L., Pa-In, N., Jantaboon, J., Siangliw, M. and Fukai, S., Molecular breeding for rainfed lowland rice in the Mekong region. *Plant Prod. Sci*. **8(3):** 330-333 (2005).