Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

Research Paper

Validation of gene targeted markers linked to the resistance genes viz., xa13, Xa21 and Pi54in Parents

Biotechnology

KEYWORDS : MTU1010, bacterial blight, blast, Validation and Resistance genes

K.Aruna Kumari Institute of Biotechnology, College of Agriculture, ANGRAU, Rajendranagar, Hyderabad

Ch. V. Durga Rani Institute of Biotechnology, College of Agriculture, ANGRAU, Rajendranagar, Hyderabad

ABSTRACT

MTU1010, the popular mega rice variety was used as a recurrent parent for improving the Bacterial blight (BB) and blast resistance. Improved Samba Mahsuri (ISM) and NLR145 were used as donors for source of resistance genes of BB and blast respectively. Before using the donor and recurrent parents in crossing programme for generation of breeding material, the parents must be validated for the presence of resistance genes using respective gene targeted markers.The results revealed that the STS marker pTA248 was linked to Xa21 whereas xa13 promoter linked to xa13 gene, the results also indicated that markers are RM206, Pi54 MAS were linked to Pi54 gene. These resistant and susceptible alleles shown in accordance with earlier reports

INTRODUCTION

Biotic stresses, such as diseases (Blast [caused by the fungus *Magnaporthe grisea*] and Bacterial leaf blight [caused by *Xanthomonas oryzae*pv. *Oryzae*] account for significant yield losses annually in rice. Resistance to these diseases controlled either by dominant or recessive major genes (*xa13, Xa21* and *Pi54*) by QTL (Alam and Cohen., 1998; Himabindu*et al*., 2010). To date, at least 38 BB resistance genes conferring host resistance against various stains of *Xoo* have been identified (Suh *et al*., 2013; Bhasin *et al*., 2012 and Natrajkumar *et al*., 2012). Among these, *xa13* and *Xa21* genes are highly effective against BB races of South and South asia (Khush *et al*., 1990).To date, a total of 100 rice blast resistance genes have been identified on the rice genome (Sharma *et al*., 2012). Among these, *Pi54 (*earlier it is *Pi-kh*) is a major dominant gene, which is highly effective against the pathogen populations in India (Sharma *et al*., 2002). The availability of such a large number of mapped resistance genes gives the possibility of integrating two or more of them into a genotype with Marker Assisted Selection (MAS). MAS have been applied for integrating different resistance genes into rice cultivars lacking the desired traits. Among the different resistance management strategies for BB and rice blast, a gene-pyramided rice cultivar with at least one dominant resistance gene each for conferring resistance against BB and rice blast can be considered as ideal.

Acharya N G Ranga Agricultural University (ANGRAU), Andhra Pradesh, India played an important role in Indian agriculture by releasing many popular varieties and hybrids in almost all crops. The popular rice varieties viz., Cottondora Sannalu (MTU1010), Samba Mahsuri (BPT5204), Swarna (MTU7029) and Vijetha (MTU1001) of ANGRAU have occupied more than 25% area in India. However, these high yielding fine grain varieties are showing susceptibility to major diseases (bacterial blight and blast) of rice. MTU1010 (Cottondora Sannalu), a short duration mega rice variety released by Andhra Pradesh Rice Research Institute (APRRI), Maruteru possessing BPH resistance with long slender grain occupied maximum area nearly 11lakhs hectares in Andhra Pradesh and gives 6-6.5tonnes/ha under good agronomic conditions particularly during *Rabi* season (APPRI, Maruteru). However, it is susceptible to bacterial blight (BB) and blast diseases. Hence there is an urgent need to improve this mega variety by incorporating resistance genes of bacterial blight and blast.

The popular rice variety, Samba Mahsuri (BPT5204) has been improved by introgression of *xa5, xa13* and *Xa21* genes from the donor, SS1113 and is released as ISM (Sundaram *et al*., 2008) and was used as one of the donor in the present study.

The rice cultivar 'Tetep' has been found to be resistant to most of the pathogenic races occurring in India (Padmanabhan *et al*., 1979). By using Tetep as a donor, a number of Nellore varieties were released from Agriculture Research Station, Nellore. NLR145 is one of Nellore variety resistant to blast released by ARS; Nellore was used as a donor for the blast resistance gene *Pi54*.

With this objective, an elite rice cultivar, Cottondora Sannalu (MTU1010), a mega variety released by ANGRAU,has been selected for improvement for BB and blast resistancethrough marker-assisted breeding. In the present study, before using the donors in crossing programme, the gene targeted DNA markers have been used effectively and validated to identify resistance alleles in donors and susceptible alleles in the recurrent parent MTU1010.

MATERIAL AND METHODS

Plant material: Three rice genotypes constituted the experimental material MTU1010, NLR 145 collected from ARI, Rice section, ANGRAU and ISM collected from DRR, Rajendranagar, Hyderabad.

DNA extraction and SSR analysis: Genomic DNA was extracted by modified CTAB method (Sambrook and Russel, 2001). 15-20 days rice leaves were extracted with DNA extraction buffer (2% CTAB, 100 mM Tris, 20 Mm EDTA, 1.4 M NaCl) preheated at 600C. The quality and quantity of extracted DNA was judged by comparing it with λ-DNA in Agarose gel electrophoresis. DNA quantification and purity was checked by measuring the O.D at 260 nm and 280 nm using a UV visible spectrophotometer. For marker assisted-multiple gene introgression, 4 gene specific primers were used for validation.

Polymerase chain reaction (PCR): The polymerase chain reaction was carried out in Eppendorf thermal cycler using 4 gene targeted primers. Then forward and reverse sequence of 4 gene targeted sequence shown in Table-1. The PCR reaction mix includes the following: DNA 10ng/µl; 10X buffer; 10mM dNTPs; 50 mM $MgCl_{2,1}$0µM each of forward and reverse primers. The PCR profile starts with Initial Denaturation 94° C for 5 min, Denaturation 94° C for 30 sec, primer annealing 55°C for 30 sec, extension 72°C for 1 min, final extension 72° C for 10 min, and cooling 4oC for α was included. These steps were repeated for 35 cycles for amplification of DNA. After completion of amplification, PCR products were stored at –20°C and the amplified products were analyzed by electrophoresis using 2% (for xa13 promoter and pTA248) and 3% (Pi54 MASand RM206)agarose gels. Ethidium bromide was added while pouring the gel so that the DNA fluoresces when gel was exposed to UV light. The DNA fragments was then visualized under UV Transilluminator and the banding pattern was observed and recorded using gel documentation unit (Gene flash) which was stored for further scoring and permanent records.

RESULTS AND DISCUSSION

With MTU1010, ISM and NLR145, initial screening was performed to determine the polymorphism in selected parents in comparison with original source materials viz., SS1113 donor for xa13 and Xa21) and Tetep (donor for Pi54). The PCR amplified products using pTA248,

Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

xa13 promoter, RM206 and Pi54 MAS revealed parental polymorphism. The amplified fragment from the ISM and SS1113 was about 500bp (Plate-1), while that from the susceptible parent, MTU1010 was about 250bp with xa13 promoter (Sundaram et al., 2011). The amplified fragment from ISM and SS1113 was about 950bp (Plate-2), while that from the susceptible parent MTU1010 was about 650bp (Ronald et al., 1992). PCR amplification with RM206 produced a resistant fragment size of 210bp in NLR145 and Tetep (Plate-3), while that of susceptible allele size of 220bp in MTU1010 (Sharma et al., 2005). PCR amplification with Pi54 MAS produced a resistant fragment size of 216bp (Plate-4), while that of susceptible size of 359bp (Ramkumar et al., 2011). Allele sizes of gene targeted markers shown in Table-2.

xa13-promois a functional marker, designed from promoter region of xa13 gene(Zhang et al. 1996) and it was more accurate than earlier RG136, CAPS marker is ~1.5 cM awayfrom xa13 gene.This major and dominant BB resistant gene Xa21 has been tagged and mapped with tightly linked PCR based marker pTA248 with a genetic distance of ~0.1cM (Ronald et al., 1992) and is an ideal choice for deployment in varietal development. Whereas Pi54gene belongs to the nucleotide binding site-leucine rich repeat class of disease resistance genes (Sharma et al., 2005). The transcriptional analysis of the Pi54 gene indicated that it is induced in response to pathogen attack. The candidate gene expression is induced after inoculation with the pathogen in both resistant and susceptible lines. However, the level of expression is lower in the susceptible as compared to the resistant genotypes (Sharma et al., 2005). The Pi54 gene also has been tagged and mapped with a tightly linked marker RM206, which is located at a genetic distance of ~ 0.7 cM (Sharma et al., 2004). Pi54MAS, a functional marker gives more accurate genotyping than the earlier reported SSR marker, RM206, since the newly developed functional marker is designed from the exonic region of the gene itself(Ramkumar et al., 2011).

The PCR-based DNA markers used in the present study (i.e. xa13-promo, pTA248 and *Pi54 MAS*) are tightly linked to *xa13, Xa21* and *Pi54* genes(Sundaram *et al*., 2011, Ronald *et al*., 1992 andRamkumar *et al*. 2011), respectively, and hence, we were able to identify the triple-positive plants preciselywithout any false positives at any stage of MABB. Advantages of using linked markers for selection of multiple genessimultaneously have been highlighted earlier by Hittalmani *et al*. (2000) and Hayashi *et al*. (2006) for blast screening.

Plate-1: Validation of xa13 gene in donor parent (ISM) with xa13 promoter

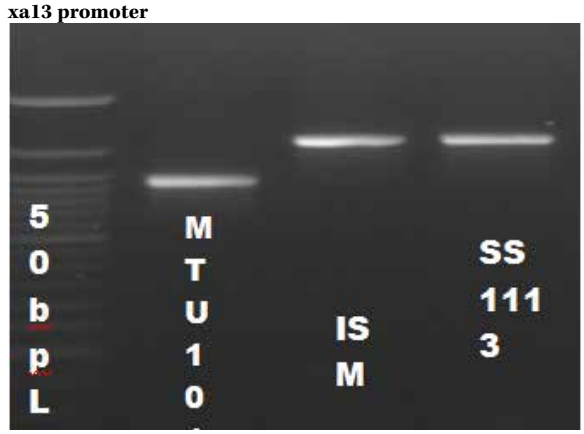


Plate-2: Validation of *Xa21* gene in donor parent (ISM) withpTA248

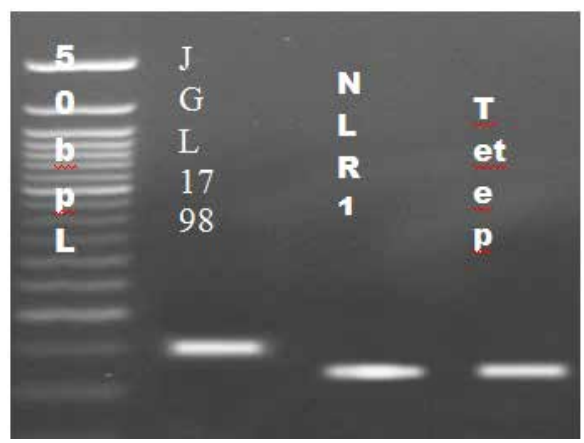
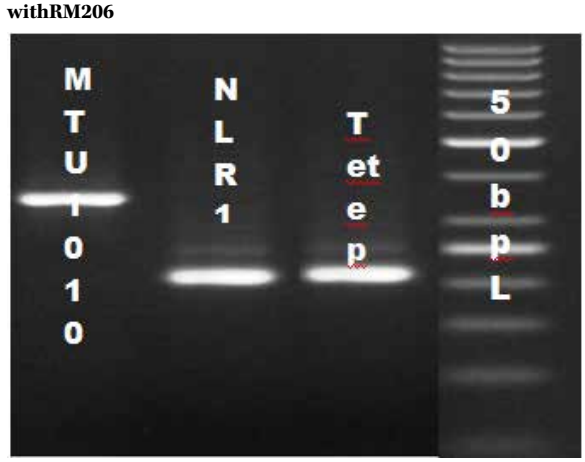


Plate-3: Validation of Pi54 gene in donor parent (NLR145) withRM206



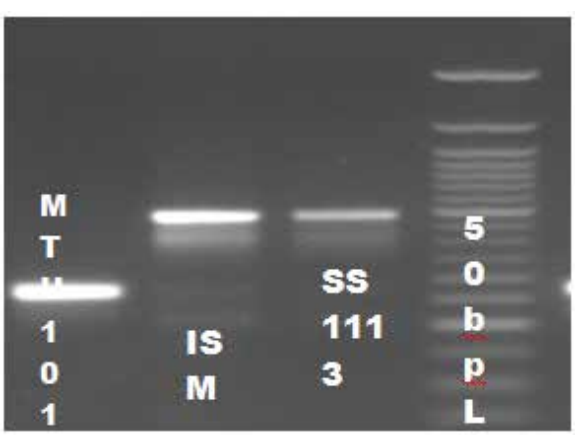


Plate-4: Validation of Pi54 gene in donor parent (NLR145) withPi54MAS

Gene	Marker	Chr	Sequence of the marker		Reference
			Order	Sequence	
xa13	xa13 promoter	8	F	TCCCAGAAAGCTACTACAGC	Sundaram et al. (2011)
			R	GCAGACTCCAGTTTGACTTC	
Xa21	pTA 248	11	F	AGACGCGGGAAGGGTGGTTCCCGGA	Ronald et al.(1992)
			R	AGACGCGGGTAATCGAAAGATGAAA	

Pi54	RM206	11	F	ATCGATCCGTATGGGTTCTAGC	Sharma et al. (2005)
			R	GTCCATGTAGCCAATCTTATGTGG	
	Pi54 MAS	11	F	CAATCTCCAAAGTTTTCAGG	Ramkumar et al. (2011)
			R	GCTTCAATCACTGCTAGACC	

Table-1: Sequence of gene targeted markers used for validation

Table-2: Allele sizes of gene linked markers

Gene	Markers	Resistant allele	Susceptible allele
xa13	xa13-prom	500bp	250bp
Xa21	PTA248	900bp	650bp
Pi54	RM206	210bp	220bp
Pi54	Pi54 MAS	216bp	359bp

REFERENCE

Alam, S.N and Cohen, M.B. 1998. Detection and analysis of QTLs for resistance to the brown plant hopper. Nilaparvathalugens, in a doubled-haploid rice population. Theoretical and Applied Genetics. 97: 1390-1379. | Bhasin, H., Bhatia, D.,Raghuvanshi, S., Lore, J.S.,Sahi, G.K., Kaur, B., Vikal, Y and Singh, K. 2012. New PCR-based sequence-tagged site marker for bacterial blight resistance gene Xa38 of rice. Mol Breed. 30:607-611. | Hayashi, K., Yoshida,H and Ashikawa, I. 2006. Development of PCRbasedallele-specific and InDel marker sets for nine rice blast resistancegenes. Theor. Appl. Genet. 113: 251-260. | Himabindu, K., Suneetha, K., Sama,V., Bentur, J. 2010. A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. Euphytica. 174(2): 179-187. | Hittalmani, S., Parco, A., Mew, T.V., Zeigler, R.S and Huang, N. 2000. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor Appl Genet. 100: 1121-1128. | Khush, G.S, Bacalanglo, E and Ogawa, T. 1990. A new gene for resistance to bacterial blight from O. longstaminata. Rice Genet News L: 121–2. | Natrajkumar, P., Sujatha, K., Laha, G.S., Srinivasarao, K., Mishra, B., Viraktamath, B.C., Hari, Y., Reddy, C.S., Balachandran, S.M., Ram, T., Sheshumadhav, M., Shobharani, N., Neeraja, C.N., Ashokreddy, G., Shaik, H and Sundaram, R.M. 2012. Identification and fine-mapping of Xa33, a novel gene for resistance to Xanthomonas oryzae pv. oryzae.Phytopathology 102:222–228 | Padmanabhan, S.Y. 1979. Blast resistance in India. In Rice Blast Workshop. International Rice Research Institute.,Philippinnes. pp43-61. | Ramkumar, G.,Srinivasa Rao, K.,Madhan Mohan, K.,Sudarshan, I.,Sivaranjani, A.K.P.,Gopala Krishna, K.,Neeraja, CN., Balachandran, SM., Sundaram, RM., Prasad, MS., Shobha Rani, N., Ram Prasad, A.M.,Virakmath, B.C and Madhav, M.S. 2011.Development and validation of functional marker targeting an In Del in the major rice blast disease resistance gene Pi54(Pikh).Molecular Breeding. 27:129-135 | Ronald, P.C., Albano, B., Tabien, R., Abenes, L., Wu, K and McCouch, S.R. 1992. Genetic and physical analysis of the rice bacterial blight disease resistance locus., Xa-21. Mol. Gen. Genet.236: 113–120 | Sambrook, J and Russel, D.W. 2001. Molecular cloning: A Laboratory Manual." Cold Spring harbor Laboratory Press, Cold Spring Harbor, New York. | Sharma, T.R., Chauhan, R.S., Singh, B.M., Paul, R., Sagar, V and Rathore, R. 2002. RAPD and pathotype analysis of Magnaporthe grisea population from North-western Himalayan region of India. J Phytopathol. 150:649–656 | Sharma, T.R.,Madhav, M.S., Singh, B.K.,Shanker, P., Jana, T.K.,Dalal, V.,Pandit, A., Singh, A.,Gaikwad, K.,Upreti, H.C. and Singh, N.K. 2005. High resolution mapping., cloning and molecular characterization of the Pikh gene of rice., which confers resistance to M. grisea. Mol. Genet. & Genomics. 274: 569-578. | Suh jung-pil.,Ji-Ung Jeung., Tae-Hwan Noh., Young-Chan Cho., So-Hyun Park., Hyun-Su Park.,Mun-Sik Shin Chung-Kon Kim and Kshirod, K Jena. 2013. 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. | Sundaram, R.M., Laha, G.S., Viraktamath, B.C., Sujatha, K., Natarajkumar, P., Hari, Y., Srinivasa Rao, K., Reddy, C.S., Balachandran, S.M., Madhav, M.S., Hajira, S.K., Rani N.S., Vishnupriya, M.R and Sonti, R.V. 2011. Marker Assisted Breeding For Development Of Bacterial Blight Resistant Rice. In: K. Muralidharan and E.A. Siddiq (eds.) Genomics and Crop Improvement: Relevance and Reservations, Institute of Biotechnology, Acharya NG Ranga Agricultural University, Hyderabad 500 030 India (pp: 154-182). | Sundaram, R.M.,Vishnupriya, M.R.,Biradar, S.K., Laha, G.S., Reddy, A.G., Rani, N.S.,Sarma, N.P and Sonti, R.V. 2008 Marker assisted introgression of bacterial blight resistance in Samba Mahsuri., an elite indica rice variety. Euphytica. 160: 411-422. | Zhang, G., Angeles, E.R., Abenes, M.L.P., Khush, G.S and Huang, N.1996. RAPD and RFLP mapping for the bacterial blight resistance gene xa-13 in rice. Theor Appl Genet 93:65–70. |