Available online at www.sciencedirect.com

ScienceDirect

Rice Science, 2016, 23(3): 144−151

A Single-Tube, Functional Marker-Based Multiplex PCR Assay for Simultaneous Detection of Major Bacterial Blight Resistance Genes *Xa21*, *xa13* and *xa5* in Rice

S. K. HAJIRA[#], R. M. SUNDARAM[#], G. S. LAHA, A. YUGANDER, S. M. BALACHANDRAN, B. C. VIRAKTAMATH, K. SUJATHA, C. H. BALACHIRANJEEVI, K. PRANATHI, M. ANILA, S. BHASKAR, V. ABHILASH, H. K. MAHADEVASWAMY, M. KOUSIK, T. DILIP KUMAR, G. HARIKA, G. REKHA

(*ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad 500030, India; [#] These authors contribute equally to this work*)

Abstract: In marker-assisted breeding for bacterial blight (BB) resistance in rice, three major resistance genes, viz., *Xa21*, *xa13* and *xa5*, are routinely deployed either singly or in combinations. As efficient and functional markers are yet to be developed for *xa13* and *xa5*, we have developed simple PCR-based functional markers for both the genes. For *xa13*, we designed a functional PCR-based marker, xa13-prom targeting the InDel polymorphism in the promoter of candidate gene *Os8N3* located on chromosome 8 of rice. With respect to *xa5*, a multiplex-PCR based functional marker system, named xa5FM, consisting of two sets of primer pairs targeting the 2-bp functional nucleotide polymorphism in the exon II of the gene *TFIIAγ5* (candidate for *xa5*), has been developed. Both xa13-prom and xa5FM can differentiate the resistant and susceptible alleles for *xa13* and *xa5*, respectively, in a co-dominant fashion. Using these two functional markers along with the already reported functional PCR-based marker for *Xa21* (pTA248), we designed a single-tube multiplex PCR based assay for simultaneous detection of all the three major resistance genes and demonstrated the utility of the multiplex marker system in a segregating population.

Key words: rice; bacterial blight; resistance; *xa5*; *xa13*; *Xa21*; functional marker; multiplex PCR

Rice is one of the most important food crops growing in various agro-climatic conditions throughout the world. Among the biotic stress affecting rice, bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major devastating disease that limit rice yields significantly across the world (Ou, 1985; Mew et al, 1993) and limits rice production up to 81% in countries like India (Kumar et al, 2012). Enhancement of host plant resistance is only a method available for management of BB and pyramiding of multiple disease resistance genes into elite varieties can provide durable and broad-spectrum resistance. Forty different resistance genes conferring host resistance to bacterial blight have been identified in rice so far (Kim et al, 2015).

Among the BB resistance genes identified so far, *Xa21*, originally introgressed from an accession of wild rice, *Oryza longistaminata* (Ronald et al, 1992; Song et al, 1995) and mapped on chromosome 11, is a major one conferring broad spectrum of resistance against many virulent isolates of the pathogen (like PX061, PXO86, PXO79 and DX020) collected from several countries including India (Wang et al, 1996; Sundaram et al, 2008). As a tightly linked, functional marker named pTA248 (Ronald et al, 1992) is available for *Xa21*, the gene has been successfully introgressed into several elite rice varieties and hybrid rice parental lines (Chen et al, 2001; Cao et al, 2003; Basavaraj et al, 2010; Hari et al, 2011, 2013; Shaik et al, 2014; Balachiranjeevi et al, 2015) either singly or in

Received: 11 September 2015; **Accepted:** 13 November 2015

Corresponding author: R. M. SUNDARAM (rms_28@rediffmail.com)

Peer review under responsibility of China National Rice Research Institute

http://dx.doi.org/10.1016/j.rsci.2015.11.004

combination with other major resistance genes like *Xa4*, *xa5* and *xa13* (Huang et al, 1997; Singh et al, 2001; Joseph et al, 2004; Sundaram et al, 2008, 2009; Perumalsamy et al, 2010; Pandey et al, 2013).

In addition to *Xa21*, another major resistance gene, *xa13*, discovered from rice variety BJ1 and mapped on the long arm of rice chromosome 8 (Ogawa et al, 1986; Khush and Angeles, 1999; Sanchez et al, 1999) has been previously and widely deployed in grouping along with *Xa21* (Huang et al, 1997; Singh et al, 2001; Sundaram et al, 2008, 2009; Rajpurohit et al, 2011). After fine-mapping (Sanchez et al, 1999), *xa13* has been cloned and a series of insertions and deletions (InDels) in the promoter region of the candidate gene *Os8N3* have been characterized to be responsible for functionality of the gene (Chu et al, 2006a). The expression of the dominant allele of the gene, i.e. *Xa13*, which encodes a sugar transporter, named *Os8N3*, is normally induced by compatible strains of *Xoo*, carrying the transcription activator-like (TAL) effector, *pthXo1*, which bind to the promoter of *Os8N3* to induce its expression (Chu et al, 2006b; Yuan et al, 2009). In rice genotypes carrying the recessive allele of the gene, i.e. *xa13*, *pthXo1* cannot bind to the promoter of *Os8N3* due to the InDels and hence cannot induce the rice sugar transporter to establish infection (Antony et al, 2010). A cleaved amplified polymorphic site (CAPS) marker, named RG136, located at a genetic distance of 3.8 cM has been widely deployed for marker-assisted selection of *xa13* (Huang et al, 1997; Singh et al, 2001; Joseph et al, 2004; Sundaram et al, 2008, 2009). However, routine utilization of the marker in marker-assisted breeding programs is cumbersome as it involves an additional step of restriction digestion in addition to PCR amplification. As *xa13* has been cloned and functional nucleotide polymorphism specific for the gene has been clearly identified, we developed four sets of primer pairs which target the InDels in the promoter of *Os8N3* as functional markers for *xa13* in this study.

Another major recessive resistance gene, *xa5* has also been used extensively in marker-assisted breeding programs, targeted towards improvement of BB resistance in previous varieties (Huang et al, 1997; Singh et al, 2001; Sundaram et al, 2008; Rajpurohit et al, 2011). The gene, originally identified from DZ192, provides a high degree of resistance to a wide range of *Xoo* races (Suh et al, 2013). *xa5* has been fine-mapped on chromosome 5 (Blair et al, 2003), cloned and characterized (Iyer and McCouch, 2004) to encode a small subunit of transcription factor IIAγ (*TFIIAγ*), possessing four exons. The resistant allele (i.e. *xa5*) has a 2-bp substitution in the second exon leading to a single amino acid change (valine to glutamiate) at position 39, thus disrupting the function of the transcription factor, culminating if resistance against *Xoo* in the rice varieties possessing *xa5* in homozygous condition. Even though a CAPS marker RG556, which is very close to the gene (Huang et al, 1997), has been widely used for marker-assisted transfer of *xa5* (Singh et al, 2001; Sundaram et al, 2008, 2009; Rajpurohit et al, 2011), it is based on restriction digestion and hence they are cumbersome to use and of limited utility for routine marker-assisted breeding programs. Recently, Ramkumar et al (2015) developed a PCR-based SNP marker for *xa5*, but it does not target the functional nucleotide polymorphism (i.e. 2-bp polymorphism in the exon II) of the candidate gene *TFIIAγ*. In the present study, we developed some multiplex-PCR based markers, which can differentiate resistant and susceptible allelic states unambiguously. In addition, we also developed a single tube, functional marker-based multiplex PCR assay for simultaneous detection of *Xa21*, *xa13* and *xa5* and demonstrated its utility in unambiguous detection of allelic status with respect to all the three major BB resistance genes either singly or in combination.

MATERIALS AND METHODS

Rice materials

Three separate mapping populations were developed and utilized in the present study. For validating the functional marker(s) specific for *xa13* and *xa5*, mapping populations consisting of 102 and 115 progeny tested F_2 plants derived from the crosses of IRBB13 × Samba Mahsuri and IRBB5 × Samba Mahsuri, respectively, were used. For validating the multiplex PCR based marker system targeting *Xa21*, *xa13* and *xa5*, a mapping population consisting of 340 F_2 lines derived from the cross of Improved Samba Mahsuri (possessing *Xa21*, *xa13* and *xa5*) × IR64 (devoid of any BB resistance gene) was used. In addition, TN1, Samba Mahsuri, Swarna, MTU1010 and IR64, were used as BB susceptible checks (as they are devoid of any BB resistance gene), while SS1113 (possessing *Xa21*, *xa13* and *xa5*), IRBB21 (possessing *Xa21*), IRBB13 (possessing *xa13*), IRBB5 (possessing *xa5*) along with Ajaya (possessing *xa5*; Sujatha et al, 2011) were used as resistant checks for checking the amplification pattern of the multiplex

Table 1. Sequences of the primers designed for *xa13*.

Primer name	Forward primer	Reverse primer
xa13FM1	TTGGAGACCCTCCACTTTTG	CCAAATGGCAACAGACACAC
xa13FM2	ACGTGTCATATTGCCCCTCA	GCTAGAGAGGAAGGCTTAAGTGC
xa13FM3	GGAGACCCTCCACTTTTGGT	TCTCCAAATGGCAACAGACA
xa13-prom	GGCCATGGCTCAGTGTTTAT	GAGCTCCAGCTCTCCAAATG

marker system.

Screening for BB resistance

A virulent isolate of the BB pathogen, viz., DXO22, which is incompatible with rice genotypes carrying *Xa21*, *xa13* and *xa5* either singly or in combinations, collected from the farm of Indian Institute of Rice Research at Rajendranagar, Hyderabad of India, was used for resistance screening following the leaf clipping technique of Kauffman et al (1973) as described in Laha et al (2009). Observations were taken both by measuring the lesion length and recording the disease score following the standard evalution scale (Anonymous, 2002).

Functional markers for BB resistance genes

For Xa21

The marker, pTA248, specific for the resistant allele of *Xa21* developed by Ronald et al (1992) was used as the functional marker for the gene (Perumalsamy et al, 2010; Salgotra et al, 2012).

For xa13

Based on InDel polymorphism in the promoter region of *Os8N3*, the candidate gene for *xa13* (Chu et al, 2006a), four sets of primer pairs (Table 1) were designed as candidate gene specific markers for the gene.

For xa5

A 2-bp polymorphism in the second exon of a gene encoding transcription factor 2A (TFIIA) was earlier characterized to be responsible for *xa5* conferred resistance (Iyer and McCouch, 2004). Targeting the 2-bp polymorphism, a set of four primers (one primer pair targeting both the resistant and susceptible alleles, another primer specific for the resistant allele and the other primer specific for the susceptible allele), as illustrated in Fig. 1, was designed and used.

Amplification of target genes through uniplex and multiplex PCR

PCR protocol for amplification of pTA248 as described in Sundaram et al (2008) was adopted. As regards to the newly designed functional markers for *xa13* and *xa5*, PCR was performed using 1 U of *Taq* DNA polymerase (Bangalore Genei, India), 50 ng template DNA, 5 pmol of each primer, 0.05 mmol/L dNTPs, and 1 × PCR buffer (Bangalore Genei, India) in 25 μL reaction with a thermal profile of 94 °C for 5 min (initial denaturation), followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for

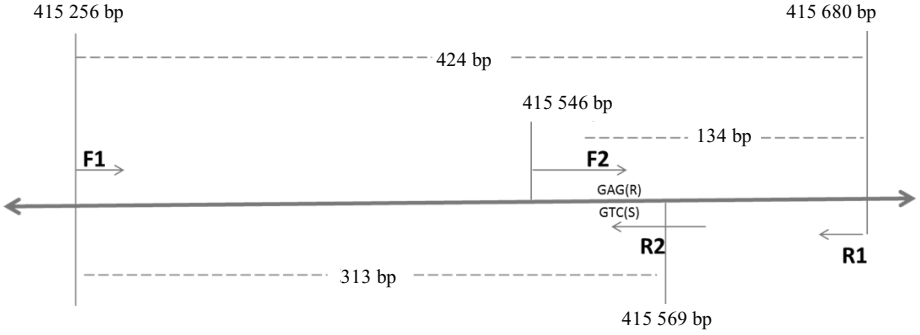

Fig. 1. Strategy for designing a functional PCR-based marker for *xa5*.

Targeting a 2-bp functional nucleotide polymorphism in exon II of the candidate gene encoding transcription factor IIA, a set of four primers were designed. F1 and R1 target a 424-bp region which is common to both resistant and susceptible alleles. The 3′ end of the primer F2 target the resistant allele (i.e. GAG) and the amplicon devided from F2 and R1 (amplified only in genotypes possessing the resistant allele, whether homozygous or heterozygous) is 134-bp long, while the 3′ end of the primer R2 targets the susceptible allele (i.e. GTC) and the amplicon devided from F2 and R1 (amplified only in genotypes possessing the susceptible allele, whether homozygous or heterozygous) is 313-bp long. Homozygous resistant genotypes amplify 424-bp and 134-bp fragments, homozygous susceptible genotypes amplify 424-bp and 313-bp fragments, while heterozygous individuals amplify all the three fragments.

Table 2. Functional primer sequences for *Xa21*, *xa13* and *xa5*.

Gene	Primer name	Primer sequence
Xa21	PTA248 F	AGACGCGGAAGGGTGGTTCCCGGA
	PTA248 R	AGACGCGGTAATCGAAAGATGAAA
xa13	xa13-prom F	GGCCATGGCTCAGTGTTTAT
	xa13-prom R	GAGCTCCAGCTCTCCAAATG
xa5	xa5FM-SF	GTCTGGAATTTGCTCGCGTTCG
	xa5FM-SR	TGGTAAAGTAGATACCTTATCAAACTGGA
	xa5FM-RF	AGCTCGCCATTCAAGTTCTTGAG
	xa5FM-RR	TGACTTGGTTCTCCAAGGCTT

30 s, extension at 72 °C for 1 min and a final extension of 7 min at 72 °C. The amplified products were electrophoretically resolved on 1.5% Seakem LE agarose gels (Lonza, USA), stained with ethidium bromide and visualized in a gel documentation system (Alpha Innotech, USA). However, with respect to the multiplex PCR assay for simultaneous detection of *Xa21*, *xa13* and *xa5*, the protocol was essentially same as described above, except that annealing was done at 57 °C and the amplified products were resolved in 2% agarose gels. The functional primer sequences for the three resistance genes are given in Table 2.

RESULTS

Functional marker for *xa13*

Among the four primer pairs designed based on InDel polymorphism in the promoter region of *Os8N3* (Table 1), three primer pairs did not show consistent amplification among resistant and susceptible genotypes and hence were not considered for further analysis. The fourth primer pair, xa13-prom showed good, robust polymorphism between the resistant and susceptible genotypes with an amplicon size of 450-bp in the resistant genotypes and a 220-bp amplicon in the susceptible genotypes, while heterozygous individuals amplified both the two fragments (Fig. 2). The polymorphic primer pair (xa13-prom) was then validated in the mapping population derived from the cross of IRBB13 × Samba Mahsuri. No recombination was observed among the F_2 population (data not shown). Thus, xa13-prom primer pair can be considered as an ideal functional marker for *xa13* gene and can be used in breeding programs.

Functional marker for *xa5*

Based on a 2-bp polymorphism in the second exon of a gene encoding transcription factor 2A (TFIIA) which is the candidate gene for *xa5* conferred resistance, four primers were designed as illustrated in Fig. 1. When the primer pairs were multiplex and amplified, the resistant genotypes were observed to amplify 424-bp (common fragment) and 134-bp (resistance specific fragment) fragments, while the susceptible genotypes amplified both 424-bp and 313-bp (susceptibility specific fragment) fragments, while heterozygous individuals amplified all the three fragments in a co-dominant fashion (Fig. 3). Further, the newly designed functional marker system was also able to clearly identify resistant, susceptible and heterozygous individuals in the mapping population derived from the cross of IRBB5 × Samba Mahsuri (data now shown). Thus, the functional marker system (xa5FM), based on multiplex-PCR can be considered as an ideal functional marker for *xa5*.

Multiplex-PCR based marker system for simultaneous detection of *Xa21*, *xa13* and *xa5*

Combining the functional markers specific for *Xa21* (pTA248), *xa5* (xa5FM) and *xa13* (xa13-prom), a multiplex PCR assay was developed. The marker

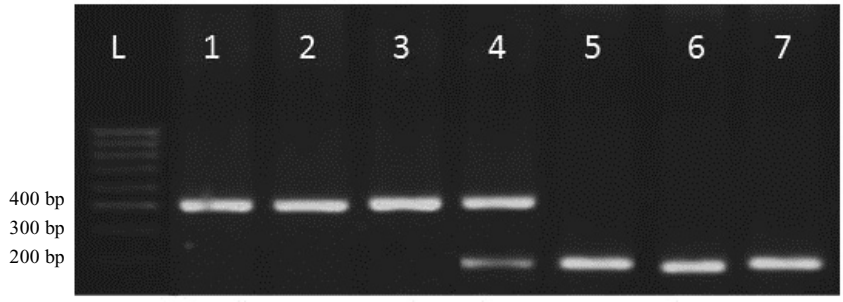

Fig. 2. Amplification pattern of polymorphic marker xa13-prom.

L, 100-bp ladder marker; Lane 1, IRBB13; Lane 2, Improved Samba Mahsuri; Lanes 5 to 7, F_1 plants in which the gene is in heterozygous condition (lane 4) and those which do not have the resistant allele of the gene.

Individuals homozygous for the resistant allele amplify a 450-bp fragment, while those which are homozygous susceptible for the gene amplify a 220-bp fragment and heterozygous individuals amplify both the two fragments in a co-dominant fashion.

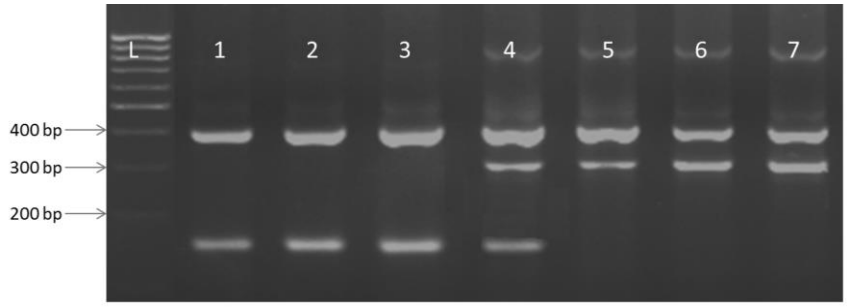

Fig. 3. Amplification pattern of polymorphic marker xa5FM.

The functional marker was analyzed in a set of rice genotypes which possess *xa5* (lanes 1 to 3). F_1 plants in which the gene is in heterozygous condition (lane 4) and those which do not have the resistant allele of the gene (lanes 5 to 7). Individuals homozygous for the resistant allele amplify 424-bp and 134-bp fragments, while those which are homozygous susceptible for the gene amplify 424-bp and 313-bp fragments and heterozygous individuals amplify all the three fragments in a co-dominant fashion. L, 100-bp ladder marker; Lane 1, IRBB5; Lane 2, Improved Samba Mahsuri; Lane 3, SS1113; Lane 4, F_1 of Improved Samba Mahsuri/IR64; Lane 5, IR64; Lane 6, TN1; Lane 7, Samba Mahsuri.

system was able to clearly distinguish resistant and susceptible parents carrying one or more of the three resistance genes (Fig. 4). Further, when the multiplex marker system was analyzed in the mapping population derived from the cross of Improved Samba Mahsuri × IR64, perfect co-segregation was observed between trait-phenotype and marker-genotype with respect to all the three resistance genes. Further, the marker system was able to clearly distinguish plants carrying homozygous alleles for each of the three resistance genes from those carrying heterozygous alleles (data not shown). Thus the multiplex-PCR based marker system for simultaneous detection of *Xa21*, *xa13* and *xa5* can be used in the breeding programs aimed at targeted introgression of either *Xa21* + *xa13*, *Xa21* + *xa5*, *xa13* + *xa5* or *Xa21* + *xa13* + *xa5*.

DISCUSSION

Biotic stress poses a major threat to crop productivity in all rice growing countries of the world. Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most devastating diseases that leads to severe yield and economic loss to farmers across the globe (Mew et al, 1993). In India, the extent of yield loss is upto 81% (Rao and Kauffman, 1971; Kumar et al, 2012). BB is particularly severe in the irrigated and rainfed lowland ecosystems (Mew, 1987), where high yielding varieties and hybrids are cultivated. As chemical control for BB is not effective (Devadath et al, 1989), deployment of host plant resistance is most effective, economical and environmentally safe option for management of BB

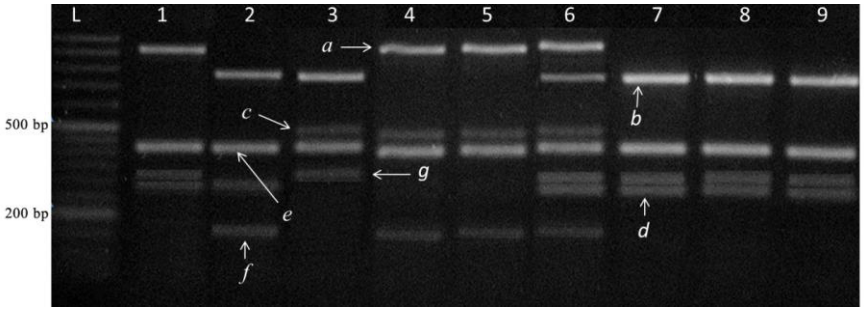

Fig. 4. Amplification pattern of PCR based multiplex marker system for simultaneous detection of *Xa21*, *xa13* and *xa5*.

L, 50-bp ladder marker separated on agarose gel to correlate the amplicon size; Lane 1, IRBB21 (possessing only *Xa21*); Lane 2, IRBB5 (possessing only *xa5*); Lane 3, IRBB13 (possessing only *xa13*); Lane 4, Improved Samba Mahsuri (possessing *Xa21*, *xa13* and *xa5*); Lane 5, SS1113 (possessing *Xa21*, *xa13* and *xa5*); Lane 6, F_1 plant of Improved Samba Mahsuri/Vandana (heterozygous for all the three genes); Lane 7, IR64; Lane 8, TN1; Lane 9, Samba Mahsur.

Lanes 7, 8 and 9 are the samples which are devoid of the resistant allele of all the three genes. Fragments *a* and *b* correspond to the resistant (950-bp) and susceptible alleles (660-bp) of *Xa21*, *c* and *d* correspond to resistant (450-bp) and susceptible alleles (220-bp) of *xa13*, while *f* and *g* correspond to the resistant (134-bp) and susceptible alleles (313-bp) of *xa5* with 424-bp common fragment to both resistant and susceptible individuals. The multiplex marker system can clearly distinguish plants possessing *Xa21*, *xa13* and *xa5* from those do not possess the genes and also those which possess the genes in heterozygous condition.

(Khush et al, 1989). At least 40 genes conferring resistance to BB have been identified in rice (Kumar et al, 2012; Kim et al, 2015). Even though many effective resistance genes have been identified against BB, unfortunately, it has been observed that resistance conferred by single resistance gene has broken down in many places (Yoshimura et al, 1995; Huang et al, 1997) and hence pyramiding two or more genes conferring resistance into a single genetic background has been advocated (Sundaram et al, 2008). As conventional breeding involving phenotype-based selection is cumbersome and many times impossible for pyramiding multiple BB resistance genes, marker-assisted gene pyramiding has been widely adopted (Huang et al, 1997; Singh et al, 2001; Joseph et al, 2004; Sundaram et al, 2009; Dokku et al, 2013). Among the resistance gene-combinations, which are highly suitable for deployment in India, the combination *Xa21* + *xa13* + *xa5* has been widely deployed previously as all the three genes have different mechanism of resistance (Chen et al, 2006a; Sundaram et al, 2008; Shaik et al, 2014) and gene-pyramid lines possessing *Xa21* + *xa13* + *xa5* have shown high levels of resistance against BB across several locations in India (DRR Progress Report, 2013, 2014; Sundaram et al, 2014).

Even though a simple PCR-based functional marker specific for *Xa21*, i.e. pTA248, which displays amplicon length polymorphism (ALP) is available (Ronald et al, 1992; Ramalingam et al, 2001; Salgotra et al, 2011), similar functional markers displaying ALP for *xa13* and *xa5* are not available so far. We developed the functional ALP markers, which can differentiate allelic states for both *xa13* and *xa5* in a co-dominant fashion and validated their utility. Even though a functional marker based on CAPS is available for *xa5* (Iyer and McCouch, 2007), the analysis involves two steps, viz., PCR amplification and restriction digestion using *Dra*I, and hence it is cumbersome to handle. Further the marker does not target the functional nucleotide polymorphism (i.e. a 2-bp difference in the sequence in the exon II of the candidate gene *TFIIA*γ) and hence the marker developed in this study will be of significant utility for analysis of accurate allelic state with respect to *xa5* in segregating populations. It is worthwhile to mention that adopting an similar approach, Ramkumar et al (2015) have developed a functional marker for *xa5*. However, the marker does not target the functional nucleotide polymorphism specific for *xa5* and hence the marker developed in this study will be of significant utility for analysis of accurate allelic state with respect to *xa5* in segregating populations.

It is pertinent to note that the gene-combination *Xa21* + *xa13* + *xa5* is widely deployed by many rice breeding groups (Huang et al, 1997; Sanchez et al, 2000; Singh et al, 2001; Joseph et al, 2004). Hence, it is worthwhile to deploy the functional markers specific for all the three genes through a single-tube multiplex PCR assay for simultaneous detection of *Xa21*, *xa13* and *xa5*, so that the cost and time involved in the assay can be significantly reduced. The multiplex-PCR based assay developed in this study, involving functional markers specific for *Xa21*, *xa13* and *xa5* clearly and unambiguously, identified the different allelic states for all the three resistance genes (Fig. 1). The utility of the multiplex-marker system was also validated in a segregating mapping population. We therefore assume that the marker system will be of significant utility in those gene-pyramiding programs targeting at simultaneous introgression of *Xa21* + *xa13* + *xa5*, *Xa21* + *xa13*, *Xa21* + *xa5* or *xa13* + *xa5*.

In conclusion, we developed functional markers for the major BB resistance genes, *xa13* and *xa5* targeting the functional nucleotide polymorphism in the candidate genes, developed a multiplex-marker system based on functional markers specific for *Xa21*, *xa13* and *xa5* and demonstrated its utility in simultaneous detection of the three major BB resistance genes.

ACKNOWLEDGEMENTS

The authors acknowledge the funding support provided by the Department of Biotechnology (DBT), Government of India (Grant Nos. BT/AB/FG-2 (PH-II)/2009 and BT/PR11705/AGR/02/646/2008). The authors also thank Project Director, ICAR-Indian Institute of Rice Research for providing all the necessary facilities.

REFERENCES

Anonymous. 2002. Standard Evaluation System for Rice. Manila, the Philippines: International Rice Research Institute: 56.

Antony G, Zhou J H, Huang S, Li T, Liu B, White F, Yang B. 2010. Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene *Os-11N3*. *Plant Cell*, **22**(11): 3864–3876.

Balachiranjeevi C H, Bhaskar N S, Abhilash V, Akanksha S, Viraktamath B C, Madhav M S, Hariprasad A S, Laha G S,

Prasad M S, Balachandran S M, Neeraja C N, Kumar M S, Senguttuvel P, Kemparaju K B, Bhadana V P, Ram T, Harika G, Swamy H K M, Hajira S K, Yugander A, Pranathi K, Anila M, Rekha G, Kousik M B V N, Dilip Kumar T, Swapnil R K, Giri A, Sundaram R M. 2015. Marker-assisted introgression of bacterial blight and blast resistance into DRR17B, an elite fine-grain type maintainer line of rice. *Mol Breeding*, **35**(7): 151.

Basavaraj S H, Singh V K, Singh A, Singh A, Singh A, Anand D, Yadav S, Ellur R K, Singh D, Krishnan S G, Nagarajan M, Mohapatra T, Prabhu K V, Singh A K. 2010. Marker assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. *Mol Breeding*, **26**(2): 293–305.

Blair M W, Garris A J, Iyer A S, Chapman B, Kresovich S, McCouch S R. 2003. High resolution genetic mapping and candidate gene identification at the *xa5* locus for bacterial blight resistance in rice (*Oryza sativa* L.). *Theor Appl Genet*, **107**(1): 62–73.

Cao L Y, Zhuang J Y, Yuan S J, Zhan X D, Zheng K L, Cheng S H. 2003. Hybrid rice resistant to bacterial leaf blight developed by marker assisted selection. *Rice Sci*, **11**(1): 68–70.

Chen S, Xu C J, Lin X H, Zhang Q Q. 2001. Improving bacterial blight resistance of '6078', an elite restorer line of hybrid rice, by molecular marker-assisted selection. *Plant Breeding*, **120**(2): 133–137.

Chen X W, Shang J J, Chen D X, Lei C L, Zou Y, Zhai W X, Liu G Z, Xu J C, Ling Z Z, Cao G, Ma B T, Wang Y P, Zhao X F, Li S G, Zhu L H. 2006. A β-lectin receptor kinase gene conferring rice blast resistance. *Plant J*, **46**(5): 794–804.

Chu Z H, Fu B Y, Yang H, Xu C G, Li Z K, Sanchez A, Park Y J, Bennetzen J L, Zhang Q F, Wang S P. 2006a. Targeting *xa13*, a recessive gene for bacterial blight resistance in rice. *Theor Appl Genet*, **112**(3): 455–461.

Chu Z H, Yuan M, Yao J L, Ge X J, Yuan B, Xu C G, Li X H, Fu B Y, Li Z K, Bennetzen J L, Zhang Q F, Wang S P. 2006b. Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes Dev*, **20**: 1250–1255.

Devadath S. 1989. Chemical control of bacterial blight of rice. *In*: International Rice Research Institute (IRRI). Bacterial Blight of Rice. Manila, the Philippines: IRRI: 89–98.

Dokku P, Das K M, Rao G J N. 2013. Pyramiding of four resistance genes of bacterial blight in Tapaswini, an elite rice cultivar, through marker-assisted selection. *Euphytica*, **192**(1): 87–96.

DRR Progress Report. 2013. All India Coordinated Rice Improvement Programme (ICAR). Directorate of Rice Research, Rajendranagar, Hyderabad, India.

DRR Progress Report. 2014. All India Coordinated Rice Improvement Programme (ICAR). Directorate of Rice Research, Rajendranagar, Hyderabad, India.

Hari Y, Srinivasarao K, Viraktamath B C, Hariprasad A S, Laha G S, Ahmed M, Natarajkumar P, Ramesha M S, Neeraja C N, Balachandran S M, Rani N S, Balaji Suresh P, Sujatha K, Pandey M, Ashok Reddy G, Madhav M S, Sundaram R M. 2011. Marker-assisted improvement of a stable restorer line KMR-3R and its derived hybrid KRH2 for bacterial blight resistance and grain quality. *Plant Breeding*, **130**(6): 608–616.

Hari Y, Srinivasarao K, Viraktamath B C, Hari Prasad A S, Laha G S, Ahmed M, Natarajkumar P, Sujatha K, Srinivas Prasad M, Pandey M, Ramesha M S, Neeraja C N, Balachandran S M, Rani N S, Kemparaju B, Mohan K M, Sama V S A K, Shaik H, Balachiranjeevi C, Pranathi K, Reddy G A, Madhav M S, Sundaram R M. 2013. Marker-assisted introgression of bacterial blight and blast resistance into IR58025B, an elite maintainer line of rice. *J Plant Breeding*, **132**(6): 586–594.

Huang N, Angeles E R, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J, Khush G S. 1997. Pyramiding of bacterial blight resistance genes in rice: Marker aided selection using RFLP and PCR. *Theor Appl Genet*, **95**(3): 313–320.

Iyer A S, McCouch S R. 2004. The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance. *Mol Plant Microbe Inter*, **17**(12): 1348–1354.

Iyer-Pascuzzi A S, McCouch S R. 2007. Functional markers for *xa5*-mediated resistance in rice (*Oryza sativa* L.). *Mol Breeding*, **19**(4): 291–296.

Joseph M, Gopalakrishnan S, Sharma R K, Singh V P, Singh A K, Singh N K, Mohapatra T. 2004. Combining bacterial blight resistance and basmati quality characteristics by phenotypic and molecular marker assisted selection in rice. *Mol Breeding*, **13**(4): 377–387.

Kauffman H E, Reddy A P K, Hsieh S P Y, Merca S D. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Dis Rep*, **57**(6): 537–541.

Khush G S, Mackill D J, Sidhu G S. 1989. Breeding rice for resistance to bacterial leaf blight. *In*: International Rice Research Institute (IRRI). Bacterial Blight of Rice. Manila, the Philippines: IRRI: 207–217.

Khush G S, Angeles E R. 1999. A new gene for resistance to race 6 of bacterial blight in rice, *Oryza sativa* L. *Rice Genet Newsl*, **16**: 92–93.

Kim S M, Suh J P, Qin Y, Noh T H, Reinke R F, Jena K K. 2015. Identification and fine-mapping of a new resistance gene, *Xa40*, conferring resistance to bacterial blight races in rice (*Oryza sativa* L.). *Theor Appl Genet*, **1238**(10): 1933–1943.

Kumar P N, Sujatha K, Laha G S, Srinivasa Rao K, Mishra B, Viraktamath B C, Hari Y, Reddy C S, Balachandran S M, Ram T, Sheshu Madhav M, Shobha Rani N, Neeraja C N, Ashok Reddy G, Shaik H, Sundaram R M. 2012. Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. *Phytopathology*, **102**(2): 222–228.

Laha G S, Reddy C S, Krishnaveni D, Sundaram R M, Srinivas P M, Ram T, Muralidharan K, Viraktamath B C. 2009. Bacterial blight of rice and its management. *In*: DRR Technical Bulletin No. 41. Hyderabad, India: Directorate of Rice Research: 37.

Mew T W. 1987. Current status and future prospects of research on bacterial blight of rice. *Annu Rev Phytopath*, **25**: 359–382.

Mew T W, Alvarez A M, Leach J E, Swings J. 1993. Focus on bacterial blight of rice. *Plant Dis*, **77**(1): 5–12.

Ogawa T, Yamamoto T. 1986. Inheritance of resistance to bacterial blight in rice. *In*: Rice Genetics. Manila, the Philippines:

International Rice Research Institute: 471–480.

Ou S H. 1985. Rice Diseases. 2nd edn. Kew: Surrey Commonwealth Mycological Institute: 380.

Pandey M K, Rani N S, Sundaram R M, Laha G S, Madhav M S, Srinivasa Rao K, Sudharshan I, Hari Y, Varaprasad G S, Subba Rao L V, Suneetha K, Sivaranjani A K P, Viraktamath B C. 2013. Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection. *Mol Breeding*, **31**(1): 239–246.

Perumalsamy S, Bharani M, Sudha M, Nagarajan P, Arul L, Saraswathi R, Balasubramanian P, Ramalingam J. 2010. Functional marker-assisted selection for bacterial leaf blight resistance genes in rice (*Oryza sativa* L.). *Plant Breeding*, **129**(4): 400–406.

Rajpurohit D, Kumar R, Kumar M, Paul P, Awasthi A, Basha P O, Puri A, Jhang T, Singh K, Dhaliwal H S. 2011. Pyramiding of two bacterial blight resistance and a semidwarfing gene in Type 3 Basmati using marker-assisted selection. *Euphytica*, **178**(1): 111–126.

Ramalingam J, Basharat H S, Zhang G. 2001. Polymorphism of DNA markers linked to bacterial blight resistance genes in useful rice germplasm. *Int Rice Res Notes*, **26**(2): 23–24.

Ramkumar G, Prahalada G D, Hechanova S L, Vinarao R, Jena K K. 2015. Development and validation of SNP-based functional codominant markers for two major disease resistance genes in rice (*O. sativa* L.). *Mol Breeding*, **35**: 129.

Rao P S, Kauffman H E. 1971. New Indian host of *Xanthomonas oryzae*, incitant of bacterial leaf blight of rice. *Curr Sci*, **40**: 271–272.

Ronald P C, Albano B, Tabien R, Abenes L, Wu K S, McCouch S, Tanksley S D. 1992. Genetic and physical analysis of the rice bacterial blight resistance locus, *Xa21*. *Mol General Genet*, **236**: 113–120.

Salgotra R K, Millwood R J, Agarwal S, Steward N C. 2011. High throughput functional marker assay for detection of *Xa/xa* and *fgr* genes in rice (*Oryza sativa* L.). *Electrophoresis*, **32**(16): 2216–2222.

Salgotra R K, Gupta B B, Millwood R J, Balasubramaniam M, Stewart J C N. 2012. Introgression of bacterial leaf blight resistance and aroma genes using functional marker-assisted selection in rice (*Oryza sativa* L.). *Euphytica*, **187**(3): 313–323.

Sanchez A C, Ilag L L, Yang D, Brar D S, Ausubel F, Khush G S, Yano M, Sasaki T, Li Z, Huang N. 1999. Genetic and physical mapping of *xa13*, a recessive bacterial blight resistance gene in rice. *Theor Appl Genet*, **98**(6): 1022–1028.

Sanchez A C, Brar D S, Huang N, Li Z, Khush G S. 2000. Sequence tagged site markers assisted selection for three bacterial blight resistance genes in rice. *Crop Sci*, **40**(3): 792–797.

Shaik H, Yugander A, Balachiranjeevi C H, Pranathi K, Anila M, Mahadevaswamy H K, Kousik B V N, Dilip Kumar T, Ashok Reddy G, Bhaskar S, Abhilash Kumar V, Harika G, Rekha G, Laha G S, Viraktamath B C, Balachandran S M, Neeraja C N, Sheshu Madhav M, Mangrauthia S K, Bhadana V P, Sundaram R M. 2014. Development of durable bacterial blight resistant lines of Samba Mahsuri possessing *Xa33*, *Xa21*, *Xa13 & Xa5*. *Progr Res*, **9**: 1224–1227.

Singh S, Sidhu J S, Huang N, Vikal Y, Li Z, Brar D S, Dhaliwal H S, Khush G S. 2001. Pyramiding three bacterial blight resistance genes (*xa-5*, *xa-13* and *Xa-21*) using marker-assisted selection into indica rice cultivar PR-106. *Theor Appl Genet*, **102**(6): 1011–1015.

Song W Y, Wang G L, Chen L L, Kim H S, Pi L Y, Holsten T, Gardner J, Wang B, Zhai W X, Zhu L H, Fauquet C, Ronald P. 1995. A receptor kinase like protein encoded by the rice disease resistance gene *Xa-21*. *Science*, **270**: 1804–1806.

Suh J P, Jeung J U, Noh T H, Cho Y C, Park S H, Park H S, Shin M S, Kim C K, Jena K K. 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.

Sujatha K, Natarajkumar P, Laha G S, Mishra B, Rao K S, Viraktamath B C, Kirti P B, Hari Y, Balachandran S M, Rajendrakumar P, Ram T, Hajira S K, Madhav M S, Neeraja C N, Sundaram R M. 2011. Inheritance of bacterial blight resistance in the rice cultivar Ajaya and high-resolution mapping of a major QTL associated with resistance. *Genet Res Cambr*, **93**(6): 397–408.

Sundaram R M, Vishnupriya M R, Biradar S K, Laha G S, Reddy A G, Rani N S, Sarma N P, Sonti R V. 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica*, **160**(3): 411–422.

Sundaram R M, Vishnupriya M R, Laha G S, Rani N S, Rao P S, Balachandran S M, Reddy G A, Sarma N P, Sonti R V. 2009. Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety by molecular marker assisted breeding. *Biotechnol J*, **4**(3): 400–407.

Sundaram R M, Madhav M S, Balachandran S M, Neeraja C N, Mangrauthia S K, Padmavathi G, Bhadana V P, Laha G S, Prasad M S, Krishnaveni D, Bentur J S, Padmakumar A P, Katti G, Jhansi Lakshmi V, Shobha Rani N, Viraktamath B C. 2014. Marker-Assisted Selection for Biotic Stress Resistance in Rice. Directorate of Rice Research, Rajendraganar, Hyderabad, Andhra Pradesh, India: 79.

Wang G L, Song W Y, Ruan D L, Sideris S, Ronald P C. 1996. The cloned gene, *Xa21*, confers resistance to multiple *Xanthomonas oryzae* pv. *Oryzae* isolates in transgenic plants. *Mol Plant Microbiol Inter*, **9**(9): 850–855.

Yoshimura S, Yoshimura A, Iwata N, McCouch S R, Abenes M L, Baraoidan M R, Mew T W, Nelson R J. 1995. Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. *Mol Breeding*, 1: 375–387.

Yuan Z, Gao S, Xue D W, Luo D, Li L T, Ding S Y, Yao X, Wilson Z A, Qian Q, Zhang D B. 2009. *Retarded Palea1* controls palea development and floral zygomorphy in rice. *Plant Physiol*, **149**(1): 235–244.

(Managing Editor: LI Guan)