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

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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

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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