e-planet **19** (2): 99-103 (December 2021)

Marker-assisted selection for bacterial blight resistance in rice

T. R. DAS[1*], S. K. PRADHAN[2] AND O. N. SINGH[2]

[1]ICAR- IARI Regional Station, Pusa, Samastipur, Bihar- 848125, India
[2]ICAR- National Rice Research Institute, Cuttack, Odisha-753006, India

**trdas.iari@gmail.com*

Date of receipt: 11.03.2021 Date of acceptance: 02.10.2021

ABSTRACT

Bacterial blight caused by *Xanthomonas oryzae pv. oryzae (Xoo*) is one of the destructive diseases of rice that causes a significant yield loss. The development and use of resistant genotypes against this disease are the most effective, eco-friendly, and economic ways to control production loss. The *xa5* is an important recessive bacterial blight resistant gene which is effective and important in rice breeding programs. In present study, BC_1F_1 populations of the cross between CRMAS-2232-85 (the donor parent of *xa5* gene) and *Jalamagna* (the recurrent parent) were screened for the bacterial blight resistant gene *xa5* by using molecular markers. Based on the amplification of resistance-specific bands, twenty-seven lines out of forty-six BC_1F_1 lines showed the presence of the *xa5* resistance gene. These gene introgression lines can be further utilized in the breeding programme for the development of bacterial blight resistant cultivars with higher yield potential which can be helpful to meet the rice production and productivity targets.

Key words: Bacterial blight resistance, marker-assisted selection, rice, *xa5*, *Xoo*

INTRODUCTION

Rice is the world's most important food crop and is a dietary staple food for more than 60% of the world population. In India, rice plays a major role in diet, economy, employment, culture, and history. It is the staple food for more than 65% of the Indian population contributing approximately 40% to the total food grain production, thereby, occupying a pivotal role in the food and livelihood security of people (Pathak et al*.,* 2018). Thus, to maintain self-sufficiency, India needs additional rice production of around two million tons annually to fulfill the target of around 140 million tons by 2030. One of the main causes of low productivity in India is the lack of suitable genotypes having resistance to different biotic and abiotic stresses with high yielding capacity. Among different biotic stresses, Bacterial blight (BB) is a seed-borne disease caused by gram-negative bacterium *Xanthomonas oryzae pv oryzae (Xoo*) and a severe threat to rice production in most of the rice growing countries (Srivastava and Rao, 1962; Hopkins et al., 1992; Verdier et al*.,* 2012). Generally, it causes yield loss ranging from 20-30%. In case of severe infection, this disease reduces crop yield up to 50-100% (Harikesh and Kausik, 2020). To prevent yield loss, the development of resistant varieties is suggested as the most effective method to control the disease without requiring collateral input from the farmer and having no environmental impact (Khush et al*.,* 1989; Gnanamanickam and Mew, 1992; Huang et al*.,* 1997; McDowell and Wofenden, 2003; Suh et al*.,* 2009; Kim et al*.,* 2015). To date, approximately 44 genes conferring resistance to BB have been identified from diverse sources (Kim, 2018). Among the different genes available few genes i.e. *xa5, xa8, xa13, xa21, xa33,* and *xa38* are known

to be effective under Indian conditions (Sundaram et al., 2018). The *xa5* is an important race-specific recessive resistant gene in rice breeding due to its broad resistance spectrum to most *Xoo* strains (Petpisit et al., 1977; Blair and Mc Couch, 1997; Sanchez et al., 2000; Singh et al., 2001; Huang et al., 2016). In the breeding method for disease and pest resistance at present, the segregating populations derived from crosses between the resistant sources and otherwise desirable and productive genotypes are selected either under natural disease or pest hotspots or under artificially created disease and pest nurseries or by infecting individual plants under controlled environments. These procedures are time-consuming and expensive and are prone to be ambiguous. With the use of molecular techniques, it would now be possible to hasten the transfer of desirable genes among varieties. The availability of comprehensive molecular linkage maps, tight linkage of target genes with molecular markers, and rapid development of polymerase chain reaction (PCR)-based DNA markers have facilitated the employment of marker-assisted selection (MAS) in rice breeding. In a backcrossing breeding programme these are very useful to increase the efficiency of selection. There are five main considerations for the use of DNA markers in MAS: reliability; quantity and quality of DNA required; technical procedure for marker assay; the level of polymorphism; and cost (Mackill and Ni, 2000; Mohler and Singrun, 2004; Collard and Mackill, 2008). Techniques that are particularly promising in marker assisting selection for desirable characters involve the use of molecular markers such as RAPD, RFLP, microsatellites, AFLP, and PCR-based DNA markers such as SCAR, Sequence Tagged Sites (STS), Cleaved Amplicon Polymorphisms (Jarvis et al., 1994). However, Sequence tagged site (STS) and simple sequence repeat (SSR) markers were commonly used to detect the genes for Bacterial blight in rice (Begum et al., 2007). Many varieties and improved lines have been developed through marker-assisted selection in rice (Sundaram et al., 2008; Ramalingam et al., 2017). Therefore, this study was taken at ICAR-National Rice Research Institute (NRRI), Cuttack to introgress bacterial blight resistance genes (*xa5*) into Jalamagna and screens the backcrossing materials (BC_1F_1) for this disease resistance gene using gene-specific markers and techniques.

Table 1. Details of markers used for bacterial blight resistance genes in marker assisted breeding

Marker	Resistance gene	Primer sequences used for gene detection		Reference
		Forward (5'-3')	Reverse(5'-3')	
*xa5*S	*xa5*	GTCTGGAATTTGCTCGCGTTCG	GAGCTCCAGCTCTCCAAATG	Sundaram et al. (2011)
*xa5*R	*xa5*	AGCTCGCCATTCAAGTTCTTGAG	TGACTTGGTTCTCCAAGGCTT	

MATERIALS AND METHODS

Plant materials and molecular markers

Forty-six selected progeny (BC_1F_1) of the cross between resistant parent, CRMAS-2232-85, and susceptible parent, *Jalamagna* were taken as test materials for detecting the BLB resistance gene, *xa5* using molecular markers i.e. *Xa5*S and *Xa5*R (Table 1). The donor parent CRMAS-2232-85 contained the resistance gene *xa5* in the background of mega variety Swarna and the recurrent parent *Jalamagna*, a highly popular variety of deepwater ecosystem of India, but highly susceptible to bacterial blight disease.

Isolation of plant genomic DNA

For isolation of DNA, fresh leaves were collected during morning hours as there will be no photosynthesis and no accumulation of photosynthatase. The leaves were immediately kept in ice after plucking and then immediately stored at -70°C till further use. The genomic DNA was isolated using the IRRI protocol (Zheng et al., 1995). The 0.5 g leaf tissue was grinded in 200 µl extraction buffer [Tris-HCl 50 mM (pH 8), EDTA 25 mM, NaCl 300 mM, SDS 1%]. After grinding, another 400 µl of DNA extraction buffer was added and transferred to a 1.5 ml tube containing another 400 µl. In order to remove proteins, 500

µl chloroform/isoamyl alcohol (24:1) was added to the tube. It was mixed well by inversion and centrifuged at 25°C and 10000 rpm for 3 minutes. The top aqueous phase was transferred to another 1.5 ml centrifuge tube and labeled. 800 µl of 100% ethanol was added to precipitate the DNA after mixing by gentle inversion. It was then centrifuged at 13000 rpm for 3 minutes at 4°C. Then the supernatant was decanted. The pellets were washed with 70% ethanol and the DNA was air dried at room temperature for at least 20 minutes (for the evaporation of residual ethanol). The DNA was re-suspended in 100 µl of TE Buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0) and stored at -20°C.

PCR amplification and profiling

The PCR reaction mixture contained 50 ng templates DNA, 5 picomole of each of the primers, 200 µM dNTPs, 1 X PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01 mg ml^{-1} gelatin) and 0.6 unit of Taq DNA polymerase in a volume of 20 µl. PCR amplification of the isolated DNA samples was performed in an AB system thermal cycler with *xa5* for identification or presence of different BLB resistant genes. The cycling condition was an initial denaturation of 94°C for 4 min, then for 35 cycles 94°C for 30 sec, 55°C for 1 min and 72°C for 1min concluding with a final extension of 72°C for 7 min. PCR products were analyzed with 6X gel loading dye by electrophoresis in ethidium bromide stained (0.5 µg ml^{-1}) 2.5% agarose gels. A 50 bp ladder molecular weight standard was used to estimate PCR fragment size. Gel images were analysed on a gel documentation system (SynGene).

RESULTS AND DISCUSSION

DNA isolation was made from 48 genotypes (46 progeny lines and their both parents) using leaves from plant seedling (plant of 8-12 days old) and molecular analysis was done using gene-specific primers. The amplification products seen as bands in gel electrophoresis are presented in Fig. 1. Out of these 46 BC_1F_1 lines, 27 genotypes (1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 21, 25, 28, 30, 33, 34, 35, 36, 37, 38, 40, 41 and 46) with a banding pattern identical for bacterial blight resistance gene specific bands at 170 bp to that in CRMAS-2232-85 were determined to be containing *xa-5*. The remaining 19 lines indicate that they were lack of *xa5* gene. This novel disease resistance *xa5* gene provides adult plant resistance and encodes the gamma subunit of transcription factor IIA (TFIIAγ), one of several general transcription factors responsible for accurate transcription by RNA polymerase II (Orphanides et al., 1996). Many earlier results on marker assisted backcrossing in rice for transfer of BB resistance gene such as *xa5* were reported for the development of bacterial blight resistance in rice (Shanti et al., 2001; Bharatkumar et al., 2008; Sundaram et al., 2008; Dokku et al., 2013; Nayak et al., 2015).

Bacterial blight in rice caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) is one of the most devastating diseases of rice. In extreme cases, BB disease severity becomes epidemic and causes significant yield losses. The most effective approach to combat BB is the use of resistant varieties. Thus, the transfer of a suitable resistance gene into a high yielding agronomically desired genotype through

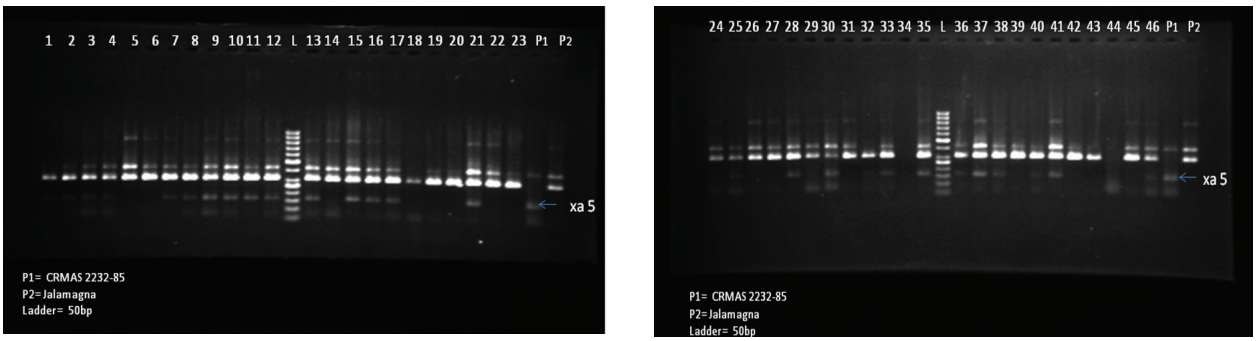

Fig. 1. Amplification of BC_1F_1 lines with *xa5* gene specific markers

marker assisted backcrossing method is necessary to develop the suitable resistance varieties as this method has three main advantages over conventional backcrossing. Firstly, DNA markers can be used for the efficient selection of the target locus. Secondly, the size of the donor chromosome segment containing the target locus can be minimized. Thirdly, the recovery of the recurrent parent can be accelerated by selecting backcross lines with a higher proportion of recurrent parent genome (Kabir et al., 2017). The effectiveness of *xa5* in preventing disease by strains of *Xanthomonas oryzae* pv *oryzae* (*Xoo*) is dependent on major transcription activation-like (TAL) effector gene and correlates with reduced expression of the Cognate S genes (Huang et al., 2016). Marker-assisted backcrossing using functional markers reduce the risk of false selection in recombination between the molecular marker and the gene of interest (Nayak et al*.,* 2015). In present investigation out of 46 selected BC_1F_1 progeny, 27 lines showed the presence of bacterial blight resistance *xa5* gene. These BB resistant lines could be helpful to utilize in the breeding programme for the development of bacterial blight resistant varieties that can be more popular in lowland and flood-prone ecosystems of the country where there are outbreaks of bacterial blight disease. Multiple stress resistant genes could be incorporated into a high yielding rice variety by gene pyramiding using the molecular markers and MAS techniques can increase the rice production.

ACKNOWLEDGEMENT

The authors are highly grateful to the Director, NRRI, Cuttack (Odisha) and Director, IARI, New Delhi for their valuable guidance, moral support and encouragement along with providing all necessary facilities during this research.

REFERENCES

Begum, S.N., Virk, P.S. and Veracruz, C.M. 2007. Introgression of effective bacterial blight resistance genes, fragrance genes and QTL mapping for aroma and other grain quality traits in Basmati-derived rice lines. In: *Proc. The 2nd International Conference on Bacterial Blight of Rice*, Nanjing, China. p. 63

Bharatkumar, S., Paulraj, R.S.D., Brindha, P.V., Kavitha, S. and Gnanamanickam, S. 2008. Improvement of bacterial blight resistance in rice cultivars ajayothi and IR50 via marker-assisted backcross breeding. *J Crop Improve* **21**: 101–116.

Blair, M.W. and McCouch, S.R. 1997. Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial leaf blight resistance gene *xa5*. *Theor. Appl. Genet.* **95**: 174-184.

Collard, B.C.Y. and Mackill, D.J. 2008. Marker-assisted selection: an approach for precision plant breeding in the 21st century. *Phil. Trans. Royal Soc. B Rev.* **363:** 557-572.

Dokku, P., Das, K.M. and 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**: 87–96.

Gnanamanickam, S. S. and Mew, T. W. 1992. An overview of bacterial blight disease of rice and strategies for its management. *Ann. Phytopathol. Soc. Jpn*. **58**: 380–385.

Harikesh and Kaushik, P. 2020. Impact of marker assisted breeding for bacterial blight resistance in rice: A review. *Plant Pathol. J.* **19**(2): 151-165.

Hopkins, C.M.,White, F.F., Choi, S.H., Guo, A. and Leach, J.E. 1992. Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*, *Mol. Plant-Microbe Interact.* **5**(6): 451-495.

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

Huang, S., Antony, G., Li, T., Liu, B., Obasa, K., Yang, B. and White, F.F. 2016. The broadly effective recessive resistance gene *xa5* of rice is a virulence effector-dependent quantitative trait for bacterial blight. *Plant J.* **86**: 186–194

Jarvis, P., Lister, C., Szabo, V. and Dean, C. 1994. Integration of CAPS markers into the RFLP map generated using recombinant inbred lines of *Arabidopsis thaliana* plant. *Mol. Biol*. **24**: 685–687.

Kabir, M.E., Iftekharuddaula, K.M., Khan, M.A.I., Mian, M.A.K and Ivy, N.A. 2017. Marker assisted introgression of bacterial blight resistant gene into submergence tolerance rice variety BRRI dhan 52. *Bangladesh J. Agril. Res.* **42**(3): 403-411

Khush, G. and Virmani, S. 1989. Breeding rice for disease resistance. In Russell, G. E. (ed.), *Progress in Plant Breeding*, *Vol. I*, Butterworths, London. pp. 239–279.

Kim, S.M. 2018. Identifcation of novel recessive gene *xa44(t)* conferring resistance to bacterial blight races in rice by QTL linkage analysis using an SNP chip. *Theor. Appl. Genet.* **131**: 2733–2743.

Kim, S.M., Suh, J.P., Quin, Y., Noh, T.H., Reinke, R.F. and 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.* **128**: 1933-1943.

Mackill, D.J. and Ni, J. 2000. Molecular mapping and marker assisted selection for major-gene traits in rice. In : *Proc. Fourth Int. Rice Genetics Symp*. (eds G. S. Khush, D. S. Brar and B. Hardy), International Rice Research Institute. Philippines. pp. 137–151.

McDowel, J.M. and Woffenden, B.J. 2003. Plant disease resistance genes: Recent insights and potential applications. *Trends Biotechnol.* **21**(4): 178-83.

Mohler, V. and Singrun, C. 2004. General considerations: marker-assisted selection. In: *Biotechnology in Agriculture and Forestry, vol. 55: Molecular Marker Systems* (eds, H. Lorz and G. Wenzel), Berlin, Germany: Springer. pp. 305–317.

Nayak, D.K., Pandit, E., Mohanty, S. Barik, D.P. and Pradhan, S.K. 2015. Marker-assisted selection in backcross progenies for transfer of bacterial leaf blight resistance genes in to popular lowland rice cultivars. *Oryza* **52**(3):163-172.

Orphanides, G., Lagrange, T. and Reinberg, D. 1996. The general transcription factors of RNA polymerase II. *Genes Dev*. **10**: 2657-2683.

Pathak, H., Samal, P. and Shahid, M. 2018. Revitalizing rice production system for enhancing productivity, profitability and climate resilience. In: *Rice Research for Enhancing Productivity, Profitability and Climate Resilience* (eds., H. Pathak, A.K. Nayak, M. Jena, O.N. Singh, P. Samal, S.G. Sharma), ICAR-National Rice Research Institute, Cuttack, Odisha, India. pp. 1-17.

Petpisit, V., Khush, G.S. and Kauffman, H.E. 1977. Inheritance of resistance to bacterial blight in rice. *Crop Sci*. **17**: 551–554.

Ramalingam, J., Savitha, P., Alagarasan, G., Saraswathi, R. and Chandrababu, R. 2017. Functional marker assisted improvement of stable cytoplasmic male sterile lines of rice for bacterial blight resistance**.** *Front. Plant Sci.* **8**: 1131.

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

Shanti, M.L., George, M.L.C., Vera Cruz, C.M., Bernardo, M.A., Nelson, R.J., Leung, H., Reddy, J.N. and Sridhar, R. 2001. Identification of resistance genes effective against bacterial leaf blight pathogen in eastern India. *Plant Dis*. **85**: 506–512.

Singh, S., Sidhu, J.S., Huang, N., Vikal, Y., Li, Z., Brar, D.S., Dhaliwal, H.S. and Khush, G.S. 2001. Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *xa21*) using marker-assisted selection into indica rice cultivar PR-106. *Theor. Appl. Genet.* **102**: 1011–1015.

Srivastava, D.N. and Rao, Y.P. 1963. Epidemic of bacterial blight disease of rice in North India. *Indian Phytopathol.* **16**: 393-394.

Suh, J.P., Noh, T.H., Kim, K.Y., Kim, J.J., Kim,Y.G. and Jena, K.K. 2009. Expression levels of three bacterial blight resistance genes against K3 a race of Korea by molecular and phenotype analysis in japonica rice (*O. sativa* L.). *J. Crop Sci. Biotechnol.* **12**:103-108.

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, ANGRAU, Hyderabad. pp. 154-182.

Sundaram, R.M., Madhav, M.S., Neeraja, C.N., Balachandran, S.M., Mangrauthia, S.K., Barbadikar K.B., Divya, P.S., SubbaRao, L.V. and Hariprasad, A.S. 2018. Multi-trait improvement in rice through marker-assisted breeding. *Oryza* **55**(spl): 24-37.

Sundaram, R.M., Vishnupriya, M.R., Biradar, S.K., Laha, G.S., Reddy, A.G., Rani, N.S., Sharma, 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.

Verdier, V., Cruz, C.V. and Leach, J.E. 2012. Controlling rice bacterial blight in Africa: Needs and prospectus. *J. Biotechnol.* **159**: 320-328.

Zheng, K., Subudhi, P.K., Domingo, J., Maopantay, G. and Huang, N. 1995. Rapid DNA isolation for marker assisted selection in rice breeding. *Rice Genet. Newslett.* **12**: 48.