



Research Note

Marker assisted selection of rice (*Oryza sativa* L.) genotypes for bacterial leaf blight disease resistance

K. Soumya² and P. Sindhumole^{1*}

¹Division of Plant Breeding & Genetics, Regional Agricultural Research Station, Pattambi

²Department of Biosciences, MES College, Marampally, Aluva, Kerala

Email: sindhumolp@gmail.com

(Received:19 Jul 2015; Accepted:21 May 2016)

Abstract

Majority of the cultivated rice (*Oryza sativa* L.) varieties are susceptible to Bacterial Leaf Blight disease (BLB), caused by *Xanthomonas oryzae* pv. *Oryzae*. Since effective chemical and biological methods are not available, the preferred strategy for disease management is through varietal resistance. To develop BLB resistant varieties, identifying resistance genes in the available germplasm is an urgent need. Molecular characterisation using microsatellite markers is a powerful tool for screening germplasm for resistance gene. Among the various resistance genes, *Xa5* is an important recessive bacterial blight resistant gene, which is effective and important in Asian rice breeding programmes. Hence main objective of the present study was marker assisted selection of rice germplasm accessions for *Xa5* gene, using RM122, a closely linked microsatellite marker. Forty traditional rice genotypes from the germplasm of Division of Plant Breeding and Genetics, Regional Agricultural Research Station, Pattambi, were initially screened in field for BLB resistance. Out of them, only twenty genotypes which had either no or less symptoms of BLB disease, were utilized for marker assisted selection. The PCR products were subjected to gel Agarose electrophoresis (1.5%). Among the thirteen genotypes which displayed marker specific bands during gel electrophoresis, five genotypes viz., Karuthakuruka, Kuruva, Kokkankoli, Kochuvithu (Thamarakulam) and Punjaparathu had no symptoms of BLB, indicating that BLB resistance in these genotypes is due to the gene *Xa5*. Hence these five resistant genotypes can be utilised as donors for Marker Assisted Breeding, to develop BLB resistant rice varieties.

Keywords

Rice, disease, gene, marker, *Xa5*, Bacterial leaf blight, microsatellite, resistance

Bacterial Leaf blight (BLB) disease, caused by *Xanthomonas oryzae* pv. *oryzae*, is known to occur in epidemic proportions in most of the rice (*Oryza sativa* L.) growing areas around the globe. The disease occurs in the host plant at the seedlings, vegetative and reproductive stages but infection at the tillering stage causes severe blighting of leaves resulting in yield loss (Shivalingaiah and Umesha, 2010). The chemical control of this disease is impractical during monsoon season and limited due to the concern over health hazards (Guvvala *et al.*, 2013). Therefore, the preferred strategy for BLB disease management is through resistant rice varieties (Naveed *et al.*, 2010).

In the case of BLB, more than thirty resistance genes had been identified and designated in a series from *Xa1* to *Xa32* (Shanti *et al.*, 2010), among which *Xa5* is an important recessive resistance gene. Marker assisted selection (MAS) using microsatellites or simple sequence repeats (SSRs) in combination with PCR has become a practical breeding method to screen the germplasm for identifying resistant varieties. SSR markers linked with BLB resistance in rice had been reported by many researchers like Wu and Tanksely (1993) and Chen *et al.* (1997). *Xa5* is an important BLB resistance gene located on

chromosome 5 and the SSR marker, RM122 is located 0.4 cM away from *Xa5* gene (Blair and McCouch, 1997).

Hence the objective of the present study was marker assisted selection of rice germplasm accessions for *Xa5* gene for BLB resistance using RM122 microsatellite marker.

Forty traditional rice accessions from the germplasm of Plant Breeding and Genetics Division, Regional Agricultural Research Station, Pattambi were initially subjected to field screening for BLB resistance during April 2013. Ten seeds each of the selected genotypes were germinated by top of the filter paper method. After fourth day, the seedlings were transplanted to disposable paper cups filled with soil. Three seedlings were planted per cup and they were irrigated daily. Natural occurrence of BLB disease was observed in these seedlings one week after planting onwards. Presence of BLB pathogen in seedlings of various genotypes with symptoms, was confirmed by ooze test.

Out of the total forty varieties, only twenty varieties which had either no or less symptoms of BLB disease, were used for marker assisted selection. The

selected genotypes were Karuthakuruka (T1), Kadamakudi Pokkali (T2), Pallipuram Pokkali (T3), Cheriya Oorpandy (T4), Karavala Kochuvithu (Shornad) (T5), Chuvanna IR8 - Thodupuzha (T6), Chettivirippu (T7), Kuruva (T8), Vyttila-Thuravoor (T9), Chettivirippu - Thuravoor (T10), Arupatham Kuruva (T11), Kochuvithu - Thamarakulam (T12), Gandhasala-I (T13), Punjaparathu (T14), Gandhasala-II (T15), PandiChempan (T16), Karuthamodan (T17), Jeerakasala - Pottisseri (T18), Kokkankoli (T19) and Pokkali (T20).

The molecular analysis was done using tender leaves of various genotypes by Plant Direct Gen Amp PCR kit method. PCR products were used for gel electrophoresis on 1.5% Agarose gel and the gel product was photographed using UV Transilluminator.

The PCR products exhibited polymorphism between the BLB resistant and susceptible genotypes. Fluorescent bands could be observed in thirteen samples for the specific marker RM 122, indicating the presence of *Xa5* gene (Plate1).

Rice genotypes which showed marker specific band for RM122 microsatellite marker, linked with *Xa5* gene, were T1 (Karuthakuruka), T2 (Kadamakudy Pokkali), T4 (Cheriya Oorpandy), T5 (Karavala Kochuvithu - Shornad), T6 (Chuvanna IR8 - Thodupuzha), T8 (Kuruva), T11 (Arupathamkuruva), T12 (Kochuvithu - Thamarakulam), T13 (Gandhasala-I), T14 (Punjaparathu), T16 (Pandi Chempan), T18 (Jeerakasala - Pottisseri) and T19 (Kokkankoli). Hence it can be inferred that *Xa5* gene is present in these thirteen rice genotypes. Specific bands were absent in DNA of the remaining seven genotypes indicating the absence *Xa5* gene in them.

Similar polymorphic survey of 88 rice lines was conducted earlier by Naveed *et al.* (2010), in which 45 rice lines along with IRBB-5 amplified 240 bp size fragments indicating the presence of *Xa5* gene.

Evaluation of BLB resistance in fourteen parental rice lines carrying multiple resistance gene was conducted by Swamy *et al.* (2006). The rice lines were screened for the presence of *Xa5*, *Xa13* and *Xa21* using linked DNA markers RM122 for *Xa5*, *RG136* for *Xa13* and *pTA248* for *Xa21* respectively. Two lines, MH2R and MH3R, showed the presence of *Xa5* gene, whereas all other lines were negative for the presence of the three resistance genes.

In a polymorphic survey of 34 rice cultivars, no amplicons specific to *Xa21* and *Xa13* alleles were

detected, showing the absence of these two genes in all the cultivars, while twenty cultivars along with resistant checks amplified 219 bp size fragments corresponding to the marker RM13, indicating the presence of *Xa5* (Singh *et al.*, 2015).

During the present study, the genotypes Karuthakuruka, Kuruva, Kokkankoli, Kochuvithu (Thamarakulam) and Punjaparathu showed no symptoms of BLB and they showed bands during gel electrophoresis, indicating that BLB resistance in these genotypes due to the gene *Xa5*. However, the genotypes Kadamakudi Pokkali, Cheriya Oorpandy, Karavala Kochuvithu (Shornad), Chuvanna IR8 (Thodupuzha), Arupatham Kuruva, Gandhasala-I, Pandi Chempan and Jeerakasala (Pottisseri), which showed the presence of band for *Xa5* gene, exhibited some symptoms of BLB also during subsequent growth stages. This may due to the breakdown of resistance to BLB by the particular *Xa5* gene, in these genotypes.

Thus, the presence of *Xa5* gene for BLB resistance could be confirmed in five BLB resistant rice genotypes *viz.*, Karuthakuruka, Kuruva, Kokkankoli, Kochuvithu (Thamarakulam) and Punjaparathu, with the help of RM 122 SSR marker. In future, these five rice genotypes can be utilised as donors for Marker Assisted Breeding (MAB) to develop BLB resistant rice varieties.

References

- Blair, M.W. and McCouch, S.R. 1997. Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial blight resistance gene *Xa5*. *Theor. Appl. Genet.*, **95**: 174-184.
- Chen, X., Temnykh, S., Xu, Y., Cho, Y.G. and McCouch, S.R. 1997. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor. Appl. Genet.*, **95**: 553-567.
- Guvvala, D., Lalitha, Pranitha, K., Vinay, S. and Lalitha, S.M. 2013. Improvement of resistance to bacterial blight through marker assisted backcross breeding and field validation in rice (*Oryza sativa*). *Res. J. of Biol.*, **1**: 82-83.
- Naveed, S.A., Babar, M., Arif, A., Zafar, Y., Sabar, M., Ali, I., Chragh, M. and Arif, M. 2010. Detection of bacterial blight resistant gene *Xa5* using linked marker approaches. *Afr. J. Biotechnol.*, **9**(24): 3549-3554.
- Shanti, M.L., Shenoy, V.V., Devi, G.L., Kumar, V.M.M., Premalatha, P., Kumar, G.N., Shashidhar, H.E., Zehr, U.B. and Freeman, W.H. 2010. Marker-Assisted Breeding for resistance to Bacterial Leaf Blight in popular cultivar and parental lines of hybrid rice. *J. Pl. Pathol.*, **92**(2): 495-501.



- Shivalingaiah and Umesha, S. 2011. Characterisation of *Xanthomonas oryzae* pv. *oryzae* from major rice growing regions of Karnataka. *Bioscan*, **6**(1): 5-10
- Singh, A.K., Nayak, R. and Singh, P.K. 2015. Identification of bacterial leaf blight resistance genes in rice (*Oryza sativa* L.). *Int. J. Sci. Nature*, **6**(2): 293-287
- Swamy, P., Ajay, N.P., Dodiya, P., Naik, V., Panchbhai, S.D., Usha, B.Z., Azhakanandam, K. and Bharat, R.C. 2006. Evaluation of bacterial blight resistance in rice lines carrying multiple resistance genes and *Xa21* transgenic lines. *Curr. Sci.*, **90**(6): 818-824
- Wu, K.S. and Tanksley, S.D. 1993. PFGE analysis of the rice genome: estimation of fragment sizes, organization of repetitive sequences and relationship between genetic and physical distances. *Plant Mol. Biol.*, **23**: 243-254

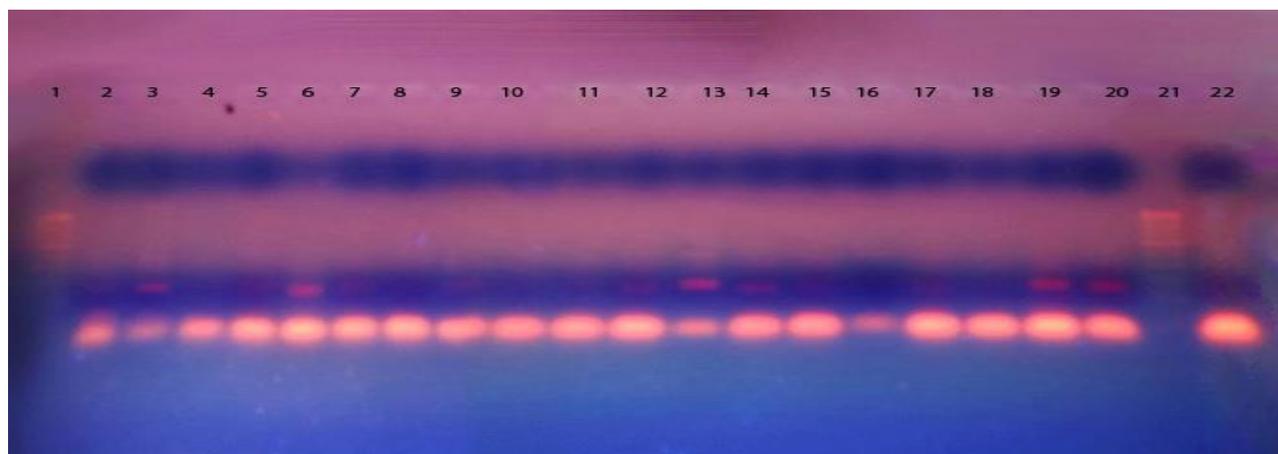


Plate1. Molecular characterisation of Rice genotypes using RM122

Lane 1 - 100bp Marker, 2 - T1, 3 - T2, 4 - T3, 5 - T4, 6 - T5, 7 - T6, 8 - T7, 9 - T8, 10 - T9, 11 - T10, 12 - T11, 13 - T12, 14 - T13, 15 - T14, 16 - T15, 17 - T16, 18 - T17, 19 - T18, 20 - T19, 21 - 100 bp Marker, 22 - T20