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Validation of linked markers of bacterial leaf blight resistance genes in rice variety of Odisha (*Oryza sativa*)

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Abstract
The present investigation was carried out during Rabi season, 2017 at the Rice Research Station and Sinha molecular laboratory, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar. Two rice varieties *i.e.*, Pratikshya and Swarna MAS, were screened for bacterial leaf blight genes. To examine the presence of any blight resistance genes, phenotypic screening was done by using leaf clip inoculation method with isolates of *Xanthomonas oryzae* pv. *oryzae* (Xoo) for blight in field and genotyping for three major blight (*xa5, xa13* and *Xa21*) resistant genes was carried out by using gene linked markers RM164, RM230 and RM21 respectively. Both phenotypic screening and genotyping results showed that Pratikshya was highly susceptible, while Swarna MAS was resistant to bacterial leaf blight. This experiment is helpful for designing improvement of bacterial leaf blight resistant versions of rice by gene pyramiding with help of molecular markers.

Keywords: *Oryza sativa*, Bacterial leaf blight, Linked markers, Validation

Introduction
Rice being the oldest of the cereals in the world accounts for a significant contribution to the total food grain production in India. Rice is a staple food crop for more than half of the world's population. In the Indian subcontinent more than a quarter of the cultivated area is under the rice cultivation. India has 43.78 million hectares area under cultivation and 121.46 million tonnes production with an average productivity of 2.55 tonnes per hectare amid 2019-20 (Ministry of Agriculture and Farmer welfare). In Odisha, about 69% of the cultivated area comes under rice cropping and is the major crop, covering about 63% of the total area under food Grains. Among all the rice infecting diseases, bacterial leaf blight (BLB) is an important disease. Bacterial leaf blight disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* reduces rice yield in all rice-growing parts of the world. Depending upon the stage of the crop, cultivar susceptibility, and the environmental conditions, the estimated yield loss due to leaf blight under severe infection varies from 50 to 80% in tropical Asia (Khush; 1989) [7]. To control BLB no effective chemical control measures have been found (Devadath *et al*; 1989) [3], so deployment of resistant cultivars is the most economical and effective method to control the disease. Marker assisted selection (MAS) is as a new field in Plant Breeding. Gene pyramiding with help of MAS has emerged as hope for advancement of broad-spectrum resistance. Maximum no. of the presently cultivated popular Indian rice cultivars are facing the problems due to various biotic stresses. Pratikshya is a Mid late duration popular rice variety released by Odisha University of Agriculture and Technology (OUAT), Odisha, India. However, it is vulnerable to bacterial leaf blight (BLB) infection which is endemic disease to many rice growing parts of Odisha. So, for obtaining resistance in long run it is essential to opt for pyramiding multiple genes from Swarna MAS into Pratikshya genetic background. The present investigation has been done to validate and identify resistant and susceptible varieties to bacterial leaf blight of rice induced by *Xanthomonas oryzae* pv. *oryzae (Xoo)* inoculation and with help of molecular markers. Identified resistant plants further to be used for gene pyramiding.

Materials and Methods

Experimental materials

Two varieties of rice *viz*., Pratikshya and Swarna MAS are used as parent. Swarna MAS collected from NRRI, Cuttack. Swarna MAS possessing (*xa5, xa13 and Xa21*) was used as positive control as it has three BLB genes.

Screening for bacterial blight resistance

The isolates of *Xanthomonas oryzae* pv. *oryzae* (Xoo) was collected from Crop Protection Division, National Rice Research Institute, Cuttack. Xoo isolates were used to screen the parents for bacterial blight resistance under field conditions. Twenty-one days old seedlings were transplanted with spacing 20 × 20 cm. The recommended package of practices was followed to raise a healthy crop. In the evening two rice varieties were inoculated at maximum tillering stage with freshly prepared solution through the clip inoculation method of Kauffman *et al*. (1973) [6] and were screened for BLB after 14 days of inoculation. Severity of disease is assessed based on estimation of diseased leaf area. The disease score was also calculated as per IRRI standard evaluation system (IRRI-SES) scale (IRRI, 2013).

Table 1: Details of primers used for the validation of BLB genes in rice varieties

Linked gene	Marker name	Primer sequence
xa5	RM164	F: TCTTGCCCGTCACTGCAGATATCC
		R: gcagccctaatgctacaattcttc
xa13	RM230	F: gccagaccgtggatgttc
		R: caccgcagtcacttttcaag
Xa21	RM21	F: acagtattccgtaggcacgg
		R: gctccatgagggtggtagag

Marker assisted selection for BLB resistance

Pratikshya and Swarna MAS were screened for BLB resistance using gene linked markers. SSR markers RM164, RM230, and RM21 were used to identify *xa5*, *xa13* and *Xa21* genes, respectively (Table 1). PCR was set up using 1 U of Taq DNA polymerase and 10XPCR buffer in 10µl reaction volume with a thermal profile of 94 °C for 5 min (initial denaturation), followed by 35 cycles of denaturation at 94 °C for 30s, annealing at 50 °C for 1min, extension at 72 °C for 1 min and a final extension of 7 min at 72 °C. The amplified product was electrophoretically casted in 3% agarose gel containing 0.5 mg/ml of ethidium bromide in 0.5XTBE buffer and visualized under UV in a Gel-Doc system and photographed.

Results and Discussion

Genotyping of parents for blight resistance

Pratikshya and Swarna MAS were phenotypically screened for bacterial blight in main field. Rice varieties were screened for *xa5*, *xa13* and *Xa21* genes using RM164, RM230 and RM21, respectively. Swarna MAS showed resistant and Pratikshya showed susceptibility for all the three blight genes. For *xa5* gene the resistant parent Swarna MAS is having amplicon size of 240bp and susceptible parent Pratikshya are having amplicon size of 250bp. For *xa13* gene the resistant parent showed amplicon size of 280bp and susceptible parent are having amplicon size of 270bp.For *Xa21* gene, resistant parent showed amplicon size of 190bp and susceptible parent showed amplicon size of 180bp. (Fig. 1).

Discussion

Bacterial leaf blight is one of the major diseases affecting rice production and leads to significant yield loss (Mew; 1987) [9]. Therefore, development of host plant resistance is considered to be the best option for managing the diseases (Hulbert *et al*., 2001) [4]. Due to the highly variable nature of the pathogen, resistance conferred by single resistant genes has broken

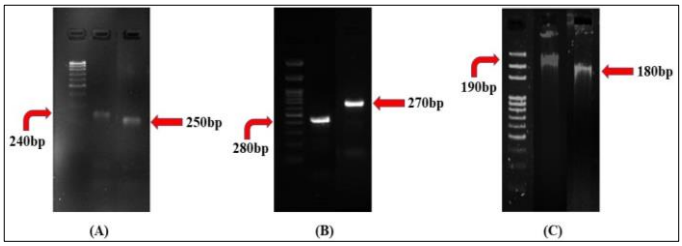

Fig 1: Gel photographs of parents indicating (A) presence of expected base pair specific band for xa5 (B) specific band for xa13 (C) specific band for Xa21. Lane 1 represents DNA ladders (100 bp); Lane 2: P1 - Swarna MAS; Lane 3: P2 - Pratikshya

down and only those varieties possessing multiple resistance genes are effective over the years and at multiple locations. In this context, for achieving durable resistance it is vital to pyramid multiple genes.

Pratikshya is one of the dominating varieties of Odisha. The two great qualities of Pratikshya are their yield and wide environmental adaptability, however the main constraint being susceptibility towards bacterial leaf blight. The present experiment was conducted to transfer valuable genes in rice variety Pratikshya for bacterial blight resistance using marker assisted selection and phenotypic selection. The molecular markers closely linked to the target traits were very useful for foreground selection. Three major resistance genes (*xa5*, *xa13* and *Xa21*) conferring resistance for BLB were selected for evaluation of these two varieties of rice. Several studies utilized these markers for improvement of popular varieties of rice. Ashiba *et al*., 2020 [2] assessed indigenous rice varieties for bacterial blight resistance using similar markers Kumar *et al*., 2020 [8] screened Samba Mashuri for bacterial leaf blight resistance genes xa5, xa13 and Xa21. Ahmed *et al*., 2021 [1] utilized molecular markers for the screening of black rice races of Manipur for blight resistance. Three major blight resistance genes (xa5, xa13 and Xa21) were introgressed into many genetic backgrounds which include Samba Mahsuri, Tellahamsa and JGL1798 (Sundaram *et al*., 2008; Jamaloddin *et al*., 2020 and Swathi *et al*., 2019) [10, 5, 11].

Conclusion

Bacterial leaf blight reduces the productivity of Pratikshya variety, efforts are needed to develop its resistant version by introgressing major BLB resistant genes through molecular breeding approach. The identified polymorphic foreground markers from the current experiment will be utilized for genotyping the backcross population carrying BLB genes.

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