Biological Forum – An International Journal **14(2): 863-866(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Phenotypic Screening of Segregating Population for Bacterial Blight Resistance in Rice Variety Pratikshya (*Oryza sativa*)

M. Pradhan[1*], D.N. Bastia[1], K. C. Samal[2] and M.K. Mishra[3]
[1]Department of Plant Breeding and Genetics,
Odisha University of Agriculture and Technology, Bhubaneswar (Odisha), India.
[2]Department of Agricultural Biotechnology,
Odisha University of Agriculture and Technology, Bhubaneswar (Odisha), India.
[3]Department of Plant Pathology, College of Agriculture,
Odisha University of Agriculture and Technology, Bhubaneswar (Odisha), India.

(Corresponding author: M. Pradhan)*

(Received 16 March 2022, Accepted 07 May, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: 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 an endemic disease in many rice-growing areas of Odisha. The present experiment was carried out during the Kharif season, 2019 at the Rice Research Station, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar. Screening of 1598 plants of BC_2F_2 segregating population derived from Pratikshya × Swarna MAS was conducted against bacterial blight at field level through clip inoculation method at maximum tillering stage. Out of 1598 plants, 1412 plants were found to be resistant, while 186 plants showed susceptibility reaction. The above values showed the goodness of fit with an expected ratio of 55:9. The chi-square value for the expected ratio was found to be non-significant at a 1% level of significance. This ratio indicated the presence of three resistance genes in the plant of the BC_2F_2 segregating population. This investigation is helpful for screening and selection of bacterial leaf blight resistant versions of Pratikshya in marker-assisted pyramided generations by using *Xoo* inoculum.

Keywords: *Oryza sativa*, Bacterial leaf blight, *Xanthomonas oryzae*pv. *oryzae*, Leafclip inoculation method.

INTRODUCTION

Rice is the oldest cereal grain in the world. “Rice is life” for more than half of humanity. It has shaped the culture, diets, and economy of millions of people (Gnanamanickam *et al.*, 2009). In the Asian subcontinent, 90 percent of the world’s rice is grown and consumed. Rice 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 rice cultivation. India has 43.78 million hectares of area under cultivation and 121.46 million tonnes of production with average productivity of 2.55 tonnes per hectare from 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. The total area covering rice in Odisha is 3.9 million hectares, production is 9.63 million tonnes and productivity is 2.45 tonnes/ha in 2019-20 (Odisha Agricultural statistics, 2020).

Plant diseases play a role as one of the major limitations in rice cultivation. Among all the rice infesting diseases, bacterial leaf blight is an important disease. Depending upon the stage of the crop, cultivar susceptibility, and environmental conditions, bacterial leaf blight can cause severe yield losses of up to 50% (Mew, 1987). No effective chemical control measures have been found against bacterial blight (Devadath 1989), so the use of resistant varieties is the only way to protect the crop from bacterial blight.

Marker-assisted selection (MAS) has come into sight as a new field in Plant Breeding i.e., gene pyramiding for the advancement of broad-spectrum resistance abilities. Maximum no. of the presently cultivated popular Indian rice cultivars are facing problems due to various biotic and abiotic stresses. Pratikshya is a ruling rice variety of Odisha, India. However, it is susceptible to bacterial leaf blight (BLB) infection. In this circumstance, for attaining durable resistance it is essential to pyramid multiple genes. The present study has been undertaken to determine the level of resistance to bacterial leaf blight of rice induced by Xanthomonas oryzae pv. oryzae (*Xoo*) inocula to identify plants resistant to bacterial leaf blight in BC_2F_2 segregating population obtained from gene pyramiding of Pratikshya × Swarna MAS.

MATERIALS AND METHODS

Experimental materials are composed of two varieties of rice viz., Pratikshya and Swarna MAS. These two varieties were used to develop the backcross population (BC_2F_2 population) consisting of 1598 plants. Previously identified resistant parent 'Swarna MAS' was used as donor parent to transfer bacterial leaf blight resistance carrying xa5, xa13, and Xa21 genes to susceptible parent Pratikshya. Twenty-one days old seedlings were transplanted with a spacing of 20 × 20 cm. The recommended packages of practices were followed to raise a healthy crop.

Bacterial Culture and inoculation. The isolates of Xanthomonas oryzae pv. oryzae (*Xoo*) was procured from Crop Protection Division, National Rice Research Institute, Cuttack. From the stock culture, the isolate was sub-cultured and maintained at 4°C for further use. On slants of Wakimoto's medium for 3 days at 30°C cultures were grown (Table 1). By suspending the bacterial mass in sterilized distilled water inoculum was prepared and diluted to a concentration of about 109 cells/ml. The freshly prepared inoculum was utilized for inoculation.

Table 1: Composition of Wakimoto's media (1000 ml solution).

Sr. No.	Component	Quantity
1.	Peeled Potato	300g
2.	Sucrose	20g
3.	Peptone	5g
4.	Sodium dihydrogen phosphate	1.87g
5.	Calcium nitrate	0.5g
6.	Agar-Agar (Bacteriological grade)	17g
7.	Distilled water	1000 ml
8.	pH	6.8

The leaf clip inoculation method developed at the AllIndia Coordinated Rice Improvement Project (AICRIP) was implemented at the maximum tillering stage of the screening population (Kauffman *et al.*, 1973). The top of the fresh leaves of each plant was cut with a sterilized scissor dipped in freshly prepared bacterial solution as presented in Fig. 1 (A) early in the morning. Observations were noted 14-21 days after inoculation by the percentage of diseased leaf area (DLA) followed by the standard evaluation system (SES, IRRI, 2013). Noor *et al*. (2021) utilized followed this method for the screening of black rice races of Manipur for blight resistance. Scoring of the disease was done as shown in Table 2.

Table 2: Standard evaluation system (SES) for bacterial blight resistance in the field.

Disease score	% Leaf area infected/lesion length	Reaction
0	0%	Immune
1	1-5%	Highly resistant
3	6-12%	Resistant
5	13-25%	Moderately resistant
7	26-50%	Susceptible
9	51-100%	Highly susceptible

RESULT AND DISCUSSION

The bacterial leaf blight of rice is the most destructive affliction of cultivated rice. It is also a destructive disease in South and Southeast Asia. Depending on the severity of the infection, the loss may be as high as 50-80% (Khus, 1989). To minimize the severe attack and yield loss, some management strategies are needed through the development of BLB-resistant cultivars. However, none of the modern cultivars are completely resistant to this disease. Host plant resistance offers the most effective, economical, and environmentally safe option for the management of bacterial leaf blight (Khush *et al*., 1989).

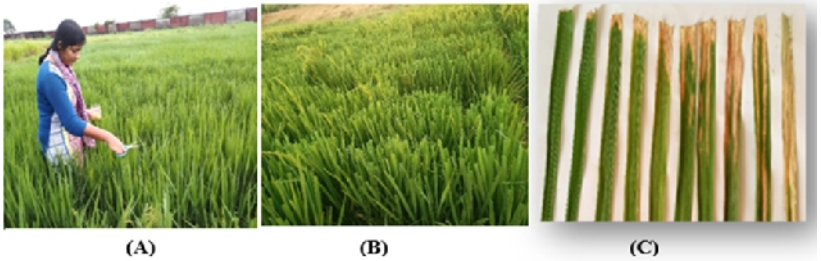

Fig. 1. A. Leaf clip inoculation *of Xanthomonas oryzae* pv. In experimental population and its screening at field level. B. Inocoluated BC_2F_2 population C. Gradual increase in disease severity.

To date, 46 bacterial leaf blight resistance genes have been identified and some of them have been incorporated into the modern popular high yielding varieties of rice (Pradhan *et al*., 2015; Chen *et al*., 2020; Chukwu *et al.*, 2019; Hsu *et al*., 2020). In the present study, the segregating backcross population has been evaluated for the presence of resistance genes in the plants. Out of 1598 plants, 1412 plants were found resistant 186 were susceptible. Bacterial leaf blight scores 1, 3, and 5 were considered as a resistant class while those having scores 7 and 9 with disease severity very much nearer to recurrent parent were considered as a susceptible class. These values indicated goodness of fit with an expected ratio of 55:9. The chi-square value for the expected value was obtained as non-significant at a 1% level of significance of probability (Table 3). This ratio indicated the presence of three resistance genes in the plant of the BC_2F_2 population. This can be explained by considering that bacterial blight resistance is governed by three genes xa5, xa13, and Xa21. So, the genotype and phenotype of a parent, F_1, and BC_2F_2 would be as follows:

Parent **Pratikshya** **Swarna MAS**
Genotype *Xa*5*Xa*5 *Xa*13*Xa*13 *xa*21*xa*21 *xa*5*xa*5 *xa*13*xa*13 *Xa*21*Xa*21
Phenotype Susceptible (S) Resistant (R)
F_1Genotype *Xa*5*xa*5 *Xa*13*xa*13 *Xa*21*xa*21
Phenotype Resistant

BC_2F_2	**Genotype Frequency**	**Phenotype**
*Xa*5_ *Xa*13_ *Xa*21_	27	Resistant
*Xa*5_ *Xa*13_ *xa*21*xa*21	9	Susceptible
*Xa*5_ *xa*13*xa*13 *Xa*21_	9	Resistant
*xa*5*xa*5 *xa*13_ *Xa*21_	9	Resistant
*Xa*5_ *xa*13*xa*13 *xa*21*xa*21	3	Resistant
*xa*5*xa*5 *Xa*13_ *xa*21*xa*21	3	Resistant
*xa*5*xa*5 *xa*13*xa*13 *Xa*21_	3	Resistant
*xa*5*xa*5 *xa*13*xa*13 *xa*21*xa*21	1	Resistant

Phenotypic ratio: 55 Resistant (R): 9 Susceptible (S

Table 3. The disease reaction of BC_2F_2 segregating population of Pratikshya x Swarna MAS against *Xanthomonas oryzae* pv. *oryzae*

Cross (BC_2F_2 population)	**Total no. of progeny**	**Observed no. of plants Resistant: Susceptible (55:9)**	**Expected no. of plants Resistant: Susceptible (55:9)**	**χ^2 value**	**P-value (0.01)**
Pratikshya × Swarna MAS	1598	1412:186	1373:225	2.309	6.635

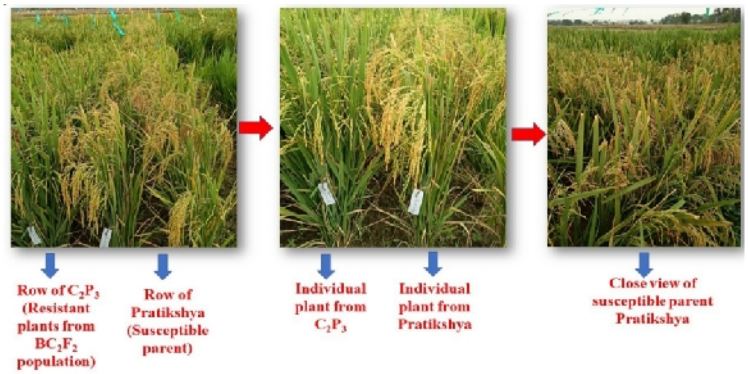

Fig. 2. The reaction of BC_2F_2 segregating population and Pratikshya against *Xanthomonas oryzae* pv.*Oryzae*.

Phenotypic data based on the leaf clip inoculation method separates the whole population into resistance and susceptible classes. Based on disease severity and similarity of agronomic characters with recipient parent Pratikshya, thirty-five superior plants were randomly selected from the BC_2F_2 population.

CONCLUSION

Phenotypic data based on the leaf clip inoculation method could not differentiate the plants having single resistant genes, two resistant genes, or all three resistant genes. It only separates the whole population into resistance and susceptible classes. Selected resistant plants were utilized for molecular studies in the BC_2F_3 population to trace the presence of a gene(s) (xa5, xa13, and Xa21) individually or in combinations.

REFERENCES

Chen, S., Wang, C., Yang, J., Chen, B., Wang, W., and Su, J. (2020). Identification of the novel bacterial blight resistance gene *Xa46(t)* by mapping and expression analysis of rice mutant H120. *Scientific Report*, *10*: 14642.

Chukwu, S. C., Rafii, M. Y., Ramlee, S. L., Ismail, S.L., Hasan, M. M., Oladosu, Y. A., Magaji, U.G., Akos, I. and Olalekan, K. K. (2019). Bacterial Leaf Blight Resistance in Rice: A Review of Conventional Breeding to Molecular Approach. *Molecular Biology Reports*, *10*, 1007.

Devadath, S. (1989). Chemical control of bacterial blight of rice. In bacterial blight of rice. International Rice Research Institute, Manila, Philippines, pp. 89-88.

Gnanamanickam, S. S., Pryiyadarasani, V., Narayanan, N. N., Vasudevan, P. and Kavitha, S. (1999). An overview of bacterial blight disease of rice and strategies for management. *Current Science*, *77*: 1435-1444.

Hsu, Y., Chiu, C., Yap, R., Tseng, Y., and Wu, Y. (2020). Pyramiding Bacterial Blight Resistance genes in Tainung82 for Broad-Spectrum Resistance Using Marker-Assisted Selection. *International Journal of Molecular Sciences*, *21*(4): 1281.

Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y. and Merca, S. D. (1973). An improved technique for evaluating the resistance of rice varieties to *Xanthomonas oryzae* pv.*Oryzae*. *Plant Disease Report*, *57*: 537- 541.

Khush, G. S., Mackill, D. J. and Sidhu, G. S. (1989). Breeding rice for resistance to bacterial blight. In: Bacterial blight of rice, IRRI, pp. 207-217.

Mew, T. W. (1987). Current status and prospects for research on bacteria blight of rice. *AnnualReview of Phytopathology*, *25*: 359-382.

Noor Ahmed, S., Madhav, M.S., Padma, V., and Satish, Y. (2021). Validation of Linked Markers of Blast and Blight Resistance Genes among Black Rice Accessions of North East India. *Biological Forum – An International Journal*, *13*(2): 201-207.

Pradhan, S. K., Nayak, D., Mohanty, S., Behera, L., Barik, S. R., Pandit, E., Lenka, S. and Anandam, A. (2015). Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deep-water rice variety, Jalmagna. *Rice*, *8*: 19.

Pradhan S. K., Nayak, D., Pandit, E., Barik, S., Mohanty, S.P., Anandan, A. and Reddy, J. (2015). Characterization of morpho-quality traits and validation of bacterial blight resistance in pyramided rice genotypes under various hotspots of India. *Australian Journal of Crop Science*, *9*: 127-134.

How to cite this article: M. Pradhan, D.N. Bastia, K. C. Samal and M.K. Mishra (2022). Phenotypic Screening of Segregating Population for Bacterial Blight Resistance in Rice Variety Pratikshya (*Oryza sativa*). *Biological Forum – An International Journal*, *14*(2): 863-866.