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Evaluation of rice (*Oryza sativa* L.) Pre-breeding genotypes for resistance to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) disease under field condition

Santanu Kumar Sahoo, Indra Deo, Himanshu Prashad, Amit Kumar and Shubham Kumawat

Abstract
The present investigation was conducted at the Norman E. Borlaug Crop Research Centre (NEBCRC), Govind Ballabh Pant University of Agriculture and Technology, Pantnagar during *Kharif* 2021, to investigate the genetic potential of 29 pre-breeding lines developed and maintained at GBPUA&T gene bank against bacterial leaf blight. Bacterial suspension from infected disease materials was prepared. Clip method of artificial inoculation was used. All the genotypes along with 3 checks were scored for BLB by using SES for Rice (IRRI, 1996). no genotype were found for BLB resistant. Six pre-breeding lines *viz.* CGIL 1303-3, CGIL 1065-3,CGIL 1382-1,CGIL 1072-3,CGIL 861-1 and CGIL 853-3 were found to be resistant to bacterial leaf blight having less than five percent infected area. 12 genotypes *viz.* CGIL 944-3,CGIL 644-2,CGIL 622-2,CGIL 865-3,CGIL 908-3,CGIL 919-3,CGIL 918-3,CGIL 801-3,CGIL 718-2,CGIL 856-3,CGIL 648-2 and CGIL 815-3showed moderately resistant to BLB. Five, four and two genotypes were found to be moderately susceptible, susceptible and highly susceptible, respectively.

Keywords: Pre-breeding lines, BLB, *Xanthomonas oryzae, Rice*

Introduction
Rice (*Oryza sativa* L.) is one of the important cereal crop which has the second largest production after wheat in the world. It occupies an area of 165.25 million hectares with a production of 516 million tones and productivity of 4.25 metric tonnes/ha in the global level (FAOSTAT, 2022) [4]. India ranks first in the area and second in the production after China. The area, production and productivity of rice are 47 million hectares, 132 million tones and 4.2 tons/ha, respectively (USDA, 2022). The yield plateau is not achieved in India due to several biotic and abiotic stress. Bacterial leaf blight (BLB) caused by the Gram-negative proteobacterium *Xanthomonas oryzae pv. oryzae* is one of the serious biotic stress in India which results decline in the productivity of rice upto 70% when susceptible variety is grown (Qian *et al*. 2013; Tian *et al*. 2014) [9, 11]. BLB attacks almost all the popular commercial rice strains (Duy *et al*. 2021) [3], and is one of the three main pests of rice, challenging both *Oryza sativa* (*Os*) subsp. *japonica* and *Oryza sativa* subsp. *indica* (Ji *et al*. 2016) [6]. The major symptoms are yellowish white or golden yellow marginal necrosis, drying of leaves back from tip and curling mid rib intact. This bacterial disease less affects adult plant but the quality of rice has declined.

Management of BLB is the challenging task as it poses a threat to global food security. There are many controlling measures adapted *viz.* chemical control, biological control, cultural practices and resistant variety. Chemical control, such as the use of antibiotics, has been restricted partly because of concerns over safety, practicality, and bacterial resistance. Biological control method using *Bacillus subtilis* UiTMB1 treatment in rice plant before being inoculated with BLB pathogen results less severe disease symptoms with low disease severity index of 3.43 compared to untreated rice plant with *Bacillus subtilis* UiTMB1 has high disease severity index of 8.4 (Ku *et al.*, 2020) [8]. The above methods for controlling BLB is not so potent which have several disadvantages. The best method for managing BLB is growing resistant variety.

Therefore, the present study was conducted to screen 29 pre-breeding lines which were developed and maintained at G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India for bacterial leaf blight resistance under field condition.

Materials and Methods

Collection of plant materials and experimental site

The experiment for present study consisted of 29 indigenously developed pre-breeding lines at G.B Pant University of Agriculture and Technology, Pantnagar. The field experiment was done at the Norman E. Borlaug Crop Research Centre of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. Field evaluation and phenotyping were carried out during *kharif*, 2021. Each genotype was sown in double row of length 2m and plant-plant and row-row spacing was maintained by 15 cm and 20 cm respectively. All the pre-breeding lines are presented in the Table 1.

Collection of diseased materials

Disease affected plants were identified by specific symptoms of yellow streaks on leaf margins. The diseased plants were collected from the sick field near Haldi, Pantnagar.

Isolation, purification of pathogen and inoculation

Infected rice leaves were cut into small pieces and were grinded in mortar and pestle. The bacterial suspension containing spores was prepared to inoculate the healthy plant by clip methods (Kauffman *et al.*, 1973) [7]. For this purpose, I used sterilized surgical scissors. The scissor was dipped in bacterial suspension and was used to cut top 1-3 inches leaves at seedling stage. The plant infected by such inoculums was confirmed by symptoms appearance i.e. yellow lesion on leaf surface.

Screening of rice genotypes in field

Seeds are **s**own in the nursery plot in the month of June in the double row of two meter. Seedlings were transplanted after 25 days of sowing. Seedling planted at a spacing of 15 cm plant to plant and row to row spacing maintained as 20 cm. The experimental field was kept free from weeds by adopting manual weeding. The trial blocks were irrigated as and when needed. Other standard agronomic practices were followed as per recommendation. Disease scoring was done at 1-9 scale after three weeks of inoculation. On the basis of disease scoring value, genotypes were classified into different categories according to their resistance level using standard IRRI procedure (IRRI, 1996) [5]. Observations were recorded at the milky stage on the severity of bacterial leaf blight reaction on a 0-9 scale (Anonymous, 1996) [2]. The standard IRRI score chart is presented in the Table 2.

Results and Discussion

In the field condition, no genotype were found for BLB resistant. Six pre-breeding lines *viz.* CGIL 1303-3, CGIL 1065-3,CGIL 1382-1,CGIL 1072-3,CGIL 861-1 and CGIL 853-3 were found to be resistant to bacterial leaf blight having less than five percent infected area. 12 genotypes *viz.* CGIL 944-3,CGIL 644-2,CGIL 622-2,CGIL 865-3,CGIL 908-3,CGIL 919-3,CGIL 918-3,CGIL 801-3,CGIL 718-2,CGIL 856-3,CGIL 648-2 and CGIL 815-3showed moderately resistant to BLB which scored three with 6-12% infection area. There are five genotypes having score 5 and infection area 13-25% exhibited moderately susceptible reaction to bacterial leaf blight. These genotypes are CGIL 936-1, CGIL 890-3, CGIL 96-2, CGIL NH 686 and NH 162. Only 4 pre-breeding lines (CGIL 374-3, CGIL 1429-1, CGIL 1391-1 and CGIL 754-3) were found to be susceptible whereas two genotypes named CGIL 803-1 and CGIL 267-3 showed highly susceptible to BLB. The similar result found by Acharya *et al.*, 2021 [1] and Sarawgi *et al.*, 2013 [10].

All the pre-breeding lines showed different disease reaction to bacterial leaf blight are presented in the Table 3. The number of genotypes exhibited different disease reaction to bacterial leaf is shown in the Figure 1. Out of 29 pre-breeding lines, more than 50% lines are resistant to bacterial leaf blight and these lines are source of resistant genes.

Table 1: List of 29 pre-breeding genotypes developed at GBPUA&T, Pantnagar

S. No	Genotypes	S. No	Genotypes
1	CGIL 936-1	16	CGIL 918-3
2	CGIL 1303-3	17	CGIL 801-3
3	CGIL 374-3	18	CGIL 803-1
4	CGIL 944-3	19	CGIL 267-3
5	CGIL 1065-3	20	CGIL 853-3
6	CGIL 644-2	21	CGIL 718-2
7	CGIL 622-2	22	CGIL 1419-1
8	CGIL 865-3	23	CGIL 856-3
9	CGIL 908-3	24	CGIL 1391-1
10	CGIL 1382-1	25	CGIL 805-3
11	CGIL 919-3	26	CGIL 648-2
12	CGIL 890-3	27	CGIL 74-3
13	CGIL 1072-3	28	NH 686
14	CGIL 861-1	29	NH 162
15	CGIL 96-2		

Table 2: IRRI-SES Scale, 1996 for BLB

Disease rating scale	Lesion area on leaf (%)	Category
0	0%	Immune
1	1-5%	Resistant
3	6-12%	Moderately Resistant
5	13-25%	Moderately Susceptible
7	26-50%	Susceptible
9	51-100%	Highly Susceptible

Table 3: Genotypes response to disease reaction BLB.

Disease Reaction	Genotypes (Pre-breeding lines)
Immune	Zero
Resistant	CGIL 1303-3, CGIL 1065-3,CGIL 1382-1,CGIL 1072-3,CGIL 861-1 and CGIL 853-3
Moderately Resistant	CGIL 944-3,CGIL 644-2,CGIL 622-2,CGIL 865-3,CGIL 908-3,CGIL 919-3,CGIL 918-3,CGIL 801-3,CGIL 718-2,CGIL 856-3,CGIL 648-2 and CGIL 815-3
Moderately Susceptible	CGIL 936-1, CGIL 890-3, CGIL 96-2, CGIL NH 686 and NH 162
Susceptible	CGIL 374-3, CGIL 1429-1, CGIL 1391-1 and CGIL 754-3
Highly Susceptible	CGIL 803-1 and CGIL 267-3

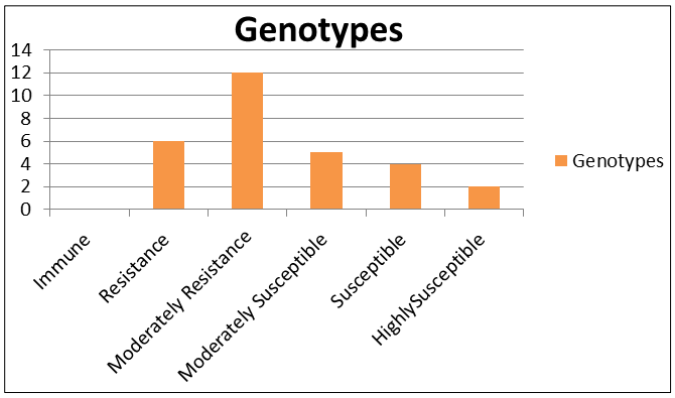

Fig 1: Rice genotypes showing different level of resistance to bacterial leaf blight

Conclusion

Due to different genetic background the genotypes varied significantly for bacterial leaf blight disease. Rice genotypes found resistant could be used as a donor source for developing bacterial leaf blight resistant variety in India. The genotypes found moderately resistant could be used as the resistant source for developing bacterial leaf blight resistant varieties for various domains of India. The pre-breeding lines are further evaluated through genotyping by using BLB SSR markers. These lines will used for finger printing to find out resistance loci which further could be used for gene pyramiding to cultivated high yielding varieties.

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