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Screening of Rice Genotypes for Resistance Against Bacterial Blight Disease

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Abstract

A study was undertaken at V. C. Farm, Mandya, Karnataka, India during 2020 to screen the genotypes against BLB under natural field conditions. Host Plant resistance is an important component of an integrated management program for this disease. Among the 102 rice genotypes screened under natural epiphytotic condition at Zonal Agricultural Station, V. C. Farm, Mandya, none of them were found immune against bacterial leaf blight. So, in the study, plants were assessed by measuring disease severity (% of leaf diseased) and area under the disease progress curve (AUDPC). The pathogenicity of Xoo was tested on IRRI rice cultivars, inoculation was conducted at the maximum tillering stage, and the lesion length was measured after 14 days of inoculation. An attempt was made to phenotypically characterize a set of 102 Genotypes from IRRI for BLB resistance by artificially inoculaton using clipping method. Out of the 102 genotypes and two checks tested, it was observed that five entries viz., IRGC 125853, IRGC 126264, IRGC 132357, IRGC 122088 IRGC 125658 and Improved Samba Mahsuri were highly resistant with score of 1. Only one entry (IRGC 125754) was resistant with score of 3, 33 lines being moderately resistant with score of 5 and 43 lines were susceptible with score of 7. 20 lines and also the susceptible check Jyothi-PTB 39, had the highest susceptibility with a phenotypic score of 9.

Keywords: Bacterial leaf blight, genotypes, phenotypic screening, resistance

1. Introduction

Rice (*Oryza sativa* L.) is a key staple food crop consumed by more than half of the world's population (Sharma et al., 2012), occupying nearly one-fifth of the total arable land area under cereal cultivation and is cultivated in diverse agro-climatic ecosystems (Chakravarthi and Naravaneni, 2006). The population may increase to 9 billion by the end of 2050 and food production is sufficient to meet the requirements of only 60% of the population (Anonymous, 2018). China is the leading producer of rice (142.3 mt) followed by India (110.4 mt) (Anonymous, 2018), thereby playing a major role in meeting the rice demand. In India, rice is shared by 48% of total food grain production and it is the main source of income to many people for meeting their daily requirements (Kiruthikadevi et al., 2020). It provides 21% of the energy and 15% of the protein requirements of human beings (Kennedy et al., 2002). Rice provides 75% of the calories and 55% of the proteins in the average daily diet of the people of Bangladesh (Bhuiyan et al., 2002). By the year 2025, 21% increase in production over that year 2000 of will be needed (Bhuiyan et al., 2002). To achieve a substantial improvement in grain yield in a limited period, a 'second green revolution' based on advanced plant biotechnology and plant genomics is needed (Conway and Toenniessen, 2000).

Among biotic stress, pest and disease problems cause major yield loss in rice production. Among the diseases, BLB causes potential yield loss in rainfed lowland areas that constitutes around 16 mha of rice growing states in India, of which a greater fragment falls under the eastern region of the country, mostly accounts for lower productivity (Ismail et al., 2013). Bacterial leaf blight (BLB) is one of the serious threats for rice worldwide caused by *Xanthomonas oryzae* pv. oryzae (Xoo) (Sundaram et al., 2014), it belongs to the family Xanthomonadaceae in the Gammaproteo bacteria (Natrajkumar et al., 2012).

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, 2011). Host plant resistance considered as important and effective strategy

for controlling the dreadful disease (Lu-sheng et al., 2005, Tang et al., 2002). It is critical to explore and identify the new resistant resources to control the changeful races (Xia et al., 2012). Since, the chemical control is not effective, the utilization of resistant varieties carrying resistance genes have been considered to be the most effective way to control the disease (Nino-Lui et al., 2006, Guvvala et al., 2013).

The pathogen causing BLB is seed-borne and hence the seed borne infection acts as a primary source of inoculum and leads to extremely high field incidence. Bacterial leaf blight early symptoms appear on the leaf blades at tillering stage, starting from lower plant parts and then reaching to above ones (Goto, 1992, Cha, 1982). In most damaging cases, yellow to white stripes are seen just inside the margins of the leaf blades, turning yellow and at the end result in mortality of leaf tissues (Ou, 1985). The wilt syndrome occurs from the seedling to the early tillering stage in which the leaves of infected plants wilt, roll up, and turn into yellow to straw-colour, wither and finally result in drying up of the entire plant (Naqvi, 2019). Therefore, in the present study an effort was made to phenotypically screen a set of lines from IRRI for BLB by artificial inoculation with *Xanthomonas oryzae* pv. oryzae.

2. Materials and Methods

2.1. Collection of plant materials and experimental site

The International Rice Research Institute (IRRI) in the Philippines provided a set of 102 rice lines. IRG's International Rice Germplasm Collection (IRGC) has more than 120,000 accessions from 129 countries throughout the world, of which 102 lines were phenotypically evaluated for blight resistance at ZARS, V. C. Farm, Mandya in *kharif*, 2020.

2.2. Plant material

The list of genotypes involved in the present study and checks used are furnished in Table 3. While the rice variety, Improved Samba Mahsuri was used as a resistant check, a red rice variety Jyothi (PTB 39) was used as a susceptible check.

2.3. Isolation, purification of pathogen and inoculation

Infected rice leaves were cut into small pieces (5 mm infected tissue and 5mm of adjacent healthy tissue) and were grinded in mortar and pestle and finally bacterial suspension was prepared. The virulent culture of *Xanthomonas oryzae* pv oryzae was obtained from Division of Rice Pathology, All India Co-ordinated Research Project (Rice), Zonal Agricultural Research Station, V. C. Farm, Mandya. The culture was grown in 100 ml nutrient broth with shaking at 80 rpm at 30˚C for 48 h was used for inoculation.

The bacterial suspension was adjusted to concentrations of approximately 10^9 cfu ml^{-1} by adding sterilized distilled water prior to inoculation. Seedlings were transplanted to the field after 25 days of sowing, with the spacing of 15 cm between plants and 20 cm between rows. The leaves of rice plants, aged about 50–55 days, were inoculated with the bacteria in plots and clip method (Kauffman et al., 1973) was used. In this procedure, inoculation was accomplished using sterilised surgical scissors dipped in bacterial suspension. A pair of scissors dipped in bacterial suspension was used for this. Selected leaves were held in one hand, and the top 1"–3" of leaves were trimmed off at the same time. The plant infected by pathogen was confirmed by symptoms observation i.e. yellow lesion on leaf surface. The disease reaction on inoculated plants was recorded 15 days post inoculation by measuring affected leaf area.

2.4. Screening of rice genotypes in field

Seedlings of the test genotypes were transplanted and raised in field following Randomized block design with three replications, Plants (45 days old) were artificially inoculated following a leaf clipping method (Kauffman et al., 1973). 15–20 fully expanded leaves were clip-inoculated with sterilized scissors dipped in a bacterial suspension. Disease scoring was done at 1–9 scale, 15 days after inoculation. The observations were recorded and scoring was done following Standard Evaluation System (SES of 2013) scale of rice for BLB (Table 1), developed by International Rice Research Institute. Briefly, the lesion length was recorded in centimeter using measuring scale and grouping was done using SES scale.

3. Results and Discussion

Bacterial Leaf Blight of rice has been reported in several parts of the world with high incidence and severity therefore, strategies adopted to particular environment must be developed to avoid possible epidemics. Among these many different control strategies, host-plant resistance is an important control measure. BLB is highly dependent on favorable environmental conditions (high temperature and rainfall) for its establishment and also depends on mechanical means for its spread (Yang, 2010, Goto et al., 1955).

Genotypes were classified into five classes based on degree of reaction as presented in Table 1. The 102 rice genotypes were screened for leaf blight resistance under open field conditions artificial inoculation. Pictorial representation of response of genotypes to Bacterial Leaf Blight disease reaction has been depicted in Figure 1. In the field screening, no rice cultivar was found immune to BLB disease. Among the entries, 5 entries *viz.,* IRGC 125853, IRGC 126264, IRGC 132357, IRGC 122088,

Table 1: Standard Evaluation System (SES of 2013) scale of Rice for BLB

Disease Score	Lesion size (% of leaf length)	Disease reaction
1	>1–10	Highly Resistant
3	>11–30	Resistant
5	>31–50	Moderately Resistant
7	>51–75	Moderately Susceptible
9	>76–100	Susceptible

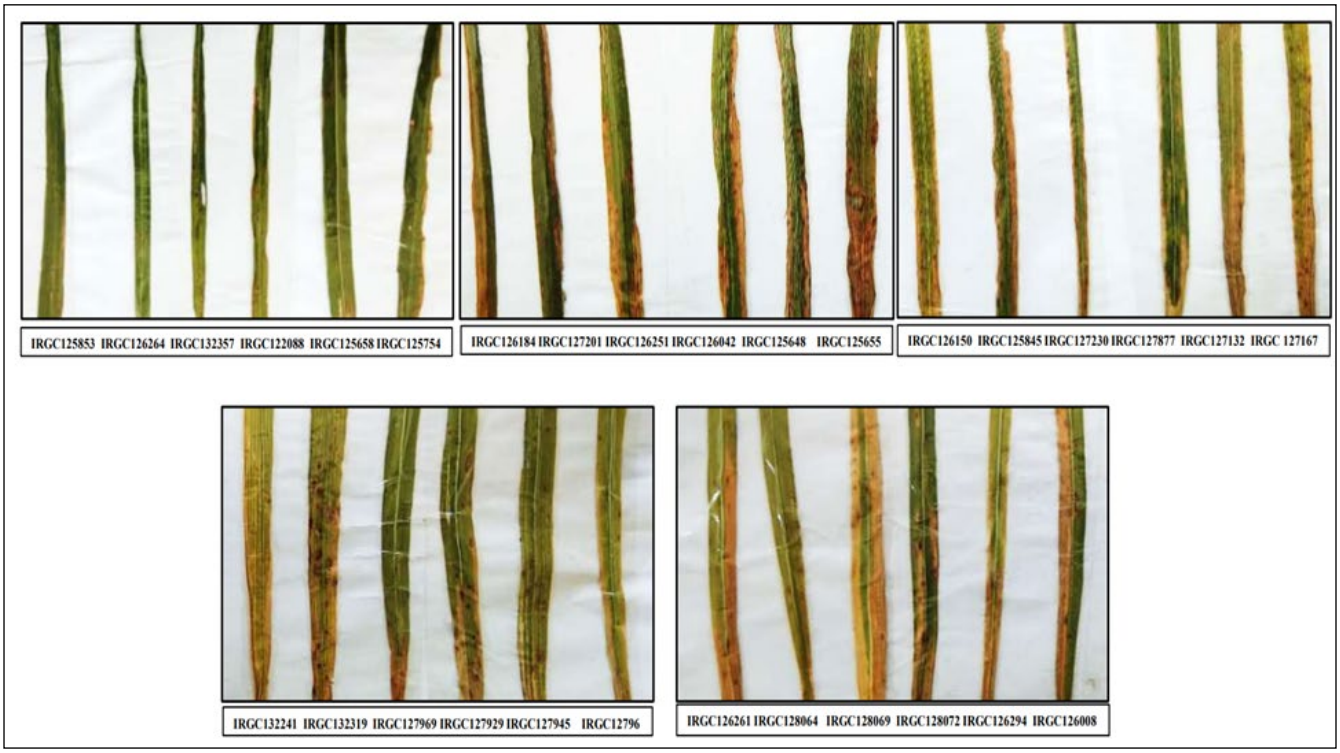

Figure 1: Pictorial representation of response of genotypes to Bacterial Leaf Blight disease reaction

IRGC 125658 were highly resistant with 1–10% of diseased leaf area along with the resistant check Improved Samba Mahsuri. Only one entry i.e., IRGC 125754 was resistant with 11–30% of diseased leaf area. 33 entries were Moderately Resistant with 31–50% diseased leaf area, on the other hand 43 lines were Moderately Susceptible with 51–75% of disease leaf area. Compared to all only 20 lines were susceptible with disease leaf area covering 76–100% along with susceptible check Jyothi (PTB 39), depicted in Table 2. Thimmegowda et al., 2011 screened 71 genotypes under natural epiphytotic condition and observed genotypes showing reaction from resistant to highly susceptible disease reaction, similar studies were also conducted by khan et al., 2010 and Ashwini et al., 2021. Similar to our findings Adhikari (2004) and Chaudhary et al., (2004) also found various resistant and susceptible disease reaction to BLB. The present results are in line with various earlier reports, the reaction of disease on susceptible check indicates that there was sufficient inoculums pressure in the field for disease development.

Singh and Borah (2000) also screened sixty local upland rice cultivars in Assam and reported that only one variety i.e. Chingdar was found to be resistant. Zuo et al., (2009) mentioned that the resistance levels of Zhongbaiyou 1 and Teyou 338 are as high as YSBR1, a rice line that has been identified with high resistance to sheath blight. Yadav et al., 2015 evaluated forty rice germplasm lines including 8 wild, 4 land races, 26 cultivated, and 2 advanced breeding lines for their reaction to sheath blight.

3.1. Disease assessment and statistical analysis

Disease scoring was done at weekly intervals after inoculation at different growth stages. Area Under Disease Progress Curve (AUDPC) was calculated for quantitative disease resistance assessment using the following formula (Das et al., 1992).

$$AUDPC=\sum_{i=1}^{n-1}[(xi+1+xi)/2]\ (ti+1-ti) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Where xi = disease severity on the ith date, ti = date on which the disease was scored (i^{th} day), n = number of dates on which disease was scored. AUDPC measures the amount of disease as well as rate of progress, and unit less.

On the basis of area under disease progress curve (AUDPC), all the genotypes were divided into different categories. These were: (I) moderately resistant (MR; AUDPC=237.22–291.67); (II) moderately susceptible (MS; AUDPC=295.56–350.00) and (III) Susceptible (S; AUDPC=357.78–618.33). So according to these 42 lines were moderately resistant, 15 lines were moderately susceptible and 45 lines were Susceptible.

Disease progress curves were drawn for disease developing in experimental plots at various time interval, infection rates were calculated for all lines. Effectiveness of bioagents against different plant diseases was reported by several workers (Elmer and McGovern, 2004; Verma and Dohroo, 2005 and Daghman et al., 2006) by the assessment of infection rate and AUDPC.

Among the selected top 10 genotypes resistant to blight

Table 2: Response of genotypes to Bacterial Leaf Blight at ZARS, Mandya during *kharif* 2020

Disease Score	% of leaf area diseased	No. of entries	Genotypes
1	>1–10	5	IRGC 125853, IRGC 126264, IRGC 132357, IRGC 122088 IRGC 125658 and Improved Samba Mahsuri
3	>11–30	1	IRGC 125754
5	>31–50	33	IRGC 126184, IRGC 127201, IRGC 126251, IRGC 126042, IRGC 125648, IRGC 125655, IRGC 126150, IRGC 125845, IRGC 127230, IRGC 127877, IRGC 127132, IRGC 127167, IRGC 132241, IRGC 132319, IRGC 127969, IRGC 127929, IRGC 127945, IRGC 127965, IRGC 128146, IRGC 120921, IRGC 127196, IRGC 127740, IRGC 127979, IRGC 125965, IRGC 127576, IRGC 127177, IRGC 127121, IRGC 125749, IRGC 127212, IRGC 125840, IRGC 125906, IRGC 128098, IRGC 121342
7	>51–75	43	IRGC 126261, IRGC 128064, IRGC 128069, IRGC 128072, IRGC 126294, IRGC 126008, IRGC 126011, IRGC 126000, IRGC 125869, IRGC 125815, IRGC 126158, IRGC 125636, IRGC 126175, IRGC 126280, IRGC 122181, IRGC 127160, IRGC 127544, IRGC 127535, IRGC 127107, IRGC 127128, IRGC 127932, IRGC 127972, IRGC 127131, IRGC 128121, IRGC 127952, IRGC 131967, IRGC 132320, IRGC 127960, IRGC 127963, IRGC 127159, IRGC 128229, IRGC 127968, IRGC 128095, IRGC 127885, IRGC 127632, IRGC 127319, IRGC 127850, IRGC 127171, IRGC 132279, IRGC 125813, IRGC 125913, IRGC 128205, IRGC 125739
9	>76–100	20	IRGC 128327, IRGC 127981, IRGC 128092, IRGC 132308, IRGC 127379, IRGC 125868, IRGC 125627, IRGC 125637, IRGC 127647, IRGC 127209, IRGC 127667, IRGC 127163, IRGC 127168, IRGC 128090, IRGC 127953, IRGC 127936, IRGC 127158, IRGC 127484, IRGC 126003, IRGC 125818 and Jyothi (PTB 39)

disease, IRGC126264 was resistant with AUDPC value 108. AUDPC value of genotypes IRGC125853, IRGC132357, IRGC122088, IRGC126658 showed a score of 162, whereas for IRGC127969, IRGC120921, IRGC127196 and IRGC 127212 also, AUPDC value of was180. IRGC125754 showed increased disease rate after 34 DAS, with AUPDC value of 189. The AUPDC value of test entries has been depicted in Table 3.

Domestication and modern breeding has reduced genetic diversity of crop plants (Tanksley and McCouch, 1997) by replacing landraces and traditional farmer cultivars with modern, high yielding varieties. New varieties are constantly needed to meet consumer demands and for protection of

Table 3: Disease severity and AUPDC Value of genotypes against leaf blight

SL. No.	Accession	Disease score	AUPDC value	SL. No.	Accession	Disease score	AUPDC value
1.	IRGC 126261	7	324	52	IRGC 128121	7	378
2.	IRGC 126184	5	270	53	IRGC 127167	5	270
3.	IRGC 125754	4	189	54	IRGC 127952	7	378
4.	IRGC 128327	9	432	55	IRGC 127953	9	396
5.	IRGC 127981	9	432	56	IRGC 132241	5	270
6.	IRGC 128064	7	378	57	IRGC 132319	5	216
7.	IRGC 128069	7	288	58	IRGC 131967	7	378
8.	IRGC 128072	7	378	59	IRGC 132320	7	324
9.	IRGC 128092	9	432	60	IRGC 127969	5	180
10.	IRGC 132308	9	486	61	IRGC 127929	5	270
11.	IRGC 127201	5	270	62	IRGC 127936	9	396
12.	IRGC 127379	9	486	63	IRGC 127945	5	270
13.	IRGC 125868	9	432	64	IRGC 127960	7	324

Table 3: Continue...

SL. No.	Accession	Disease score	AUPDC value	SL. No.	Accession	Disease score	AUPDC value
14.	IRGC 125853	3	162	65	IRGC 127963	7	324
15.	IRGC 126294	7	324	66	IRGC 127158	9	432
16.	IRGC 126251	5	270	67	IRGC 127159	7	378
17.	IRGC 126008	7	324	68	IRGC 127965	5	270
18.	IRGC 125627	9	432	69	IRGC 128229	7	324
19.	IRGC 126042	5	270	70	IRGC 127968	7	378
20.	IRGC 126264	3	108	71	IRGC 128095	7	378
21.	IRGC 126011	7	324	72	IRGC 128146	5	216
22.	IRGC 126000	7	378	73	IRGC 120921	5	180
23.	IRGC 125648	5	216	74	IRGC 127196	5	180
24.	IRGC 125869	7	360	75	IRGC 127740	5	216
25.	IRGC 125815	7	324	76	IRGC 132357	3	162
26.	IRGC 125655	5	270	77	IRGC 127885	7	324
27.	IRGC 125637	9	486	78	IRGC 127979	5	270
28.	IRGC 126158	7	288	79	IRGC 127632	7	324
29.	IRGC 126150	5	216	80	IRGC 127484	9	486
30.	IRGC 125845	5	216	81	IRGC 127319	7	324
31.	IRGC 125636	7	378	82	IRGC 122088	3	162
32.	IRGC 126175	7	378	83	IRGC 125965	5	270
33.	IRGC 126280	7	378	84	IRGC 126003	9	432
34.	IRGC 122181	7	324	85	IRGC 127576	5	270
35.	IRGC 127647	9	432	86	IRGC 127850	7	378
36.	IRGC 127209	9	432	87	IRGC 127177	5	270
37.	IRGC 127160	7	324	88	IRGC 127121	5	216
38.	IRGC 127230	5	270	89	IRGC 127171	7	378
39.	IRGC 127877	5	270	90	IRGC 132279	7	288
40.	IRGC 127667	9	486	91	IRGC 125813	7	378
41.	IRGC 127544	7	378	92	IRGC 125749	5	270
42.	IRGC 127535	7	378	93	IRGC 127212	5	180
43.	IRGC 127107	7	360	94	IRGC 125840	5	270
44.	IRGC 127163	9	486	95	IRGC 125906	5	216
45.	IRGC 127168	9	486	96	IRGC 128098	5	270
46.	IRGC 127128	7	378	97	IRGC 125913	7	360
47.	IRGC 127932	7	324	98	IRGC 125658	3	162
48.	IRGC 127132	5	270	99	IRGC 121342	5	216
49.	IRGC 127972	7	378	100	IRGC 128205	7	378
50.	IRGC 127131	7	378	101	IRGC 125739	7	378
51.	IRGC 128090	9	432	102	IRGC 125818	9	486

crops against highly unpredictable biotic and abiotic stresses that are encountered in agricultural systems. These seed collections represent a wide range of genetic diversity that is critical for maintaining and enhancing the yield potential and other quality traits, because they can provide new sources of resistance and tolerance to various stresses. BLB of rice is known to cause severe losses when comes in epidemic form. The severity of the diseases varied among different rice growing zones because of the crop age. It has been found that some of the varieties show severe symptoms during vegetative stage and others show aggressiveness near maturity. Similar results were also reported in Korea by Cha et al. (1982) who observed maximum incidence in young age plants.

Adaptation of pathogens and susceptibility to other stresses are continuous threats to existing elite crop varieties. Although there are demonstrated and valuable contributions of crop diversity to counter these threats, there is still a great potential hidden in available landraces, cultivars and wild species that remains under-explored. Large numbers of probably redundantly stored gene bank accessions and missing genotype × phenotype information make it difficult for modern breeding programs to select a feasible number of accessions for scoring traits of interest. The management of the pathogen is mainly dependent on the use of toxic fungicides, which are not only harmful for the environment, but also leads to development of resistance and new strains in the pathogen. This makes the problem even more critical than solving it. So there is need to screen number of rice genotypes against bacterial blight.

4. Conclusion

Some of the resistant lines viz., IRGC125853, IRGC126264, IRGC132357, IRGC122088 and IRGC125658 found in this study could be used in hybridization programs for varietal improvement against the BLB. From the present experiment, the varieties which have shown different disease reaction than the previous studies need to be tested further in different locations which will help in the confirmation of their resistant levels. These resistant genotypes could be utilized to develop bacterial leaf blight resistant rice varieties with desirable characters using conventional breeding or marker assisted selection and backcrossing in future.

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