Field screening of rice (*Oryza sativa* L.) genotypes for resistance to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) disease

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ABSTRACT
Rice is the most important crop in Nepal followed by maize and wheat in terms of area (1.4 million ha), production (5.15 million) and productivity (3.5 mt/ha). Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* is considered to be the most widespread and destructive disease of rice in both irrigated and rainfed environments in Nepal. Use of host plant resistance is the most feasible and economical way to combat this disease. However, breakdown of resistance of a variety often occurs after few years of release due to genetic adaptation of the pathogen. Regular screening of the genotypes is utmost for developing the resistant genotypes. Considering the point, this investigation effort has been made to screen the rice genotypes against BLB under natural field condition at Khajura, Banke during 2018 and 2019. In the study, plants were assessed by measuring disease severity (percentage of leaf diseased) and area under the disease progress curve (AUDPC). Differences in resistance among the rice genotypes were observed in both of the tested year. Among the screened 150 rice genotypes during 2018, 6 genotypes scored resistant (R), 81 moderately resistant (MR), 59 moderately susceptible (MS) and 4 susceptible (S) to BLB. Whereas, in 2019, among the tested 315 rice genotypes, none of the genotypes were resistant, 183 MR, 131 MS and 1 S to BLB disease. This showed that the tested genotypes were composed of different genetic background.

Keywords: Rice genotypes, bacterial leaf blight, screening, resistance, susceptible

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INTRODUCTION
Rice (*Oryza sativa* L.) is the world’s third most important cereal crop which occupies an area of 167 million ha with a productivity of 4.67 mt/ha globally (FAOSTAT, 2018). Rice is the number one staple food crop in Nepal and contributed significantly to livelihood of majority of people and to national economy (Shrestha *et al*., 2020). In Nepal, rice is the most important crop followed by maize and wheat in terms of area, production and consumption of Nepalese people. It occupies about 1.4 million ha of land with the total production of 5.15 million mt and the productivity of 3.5 mt/ha in Nepal (MoALD, 2018/19). It is grown extensively under a wide range of agro-ecological conditions from lowland in terai (60 m) to high mountain valley, river basin area and mountain slopes (3050 m) in Jumla, the highest altitude of rice growing location in the world (Paudel, 2011).
Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* is the most widespread and destructive diseases of rice in tropical rice-growing areas of Asia, Australia, United States, Latin America and Africa (Mew et al., 1993) and, in particular Nepal (Chaudhary et al., 2015). The disease was first found in Balaju in Kathmandu in 1965 (Khadka et al. 1968) and kresek stage (systemic infection of seedlings 2-3 weeks after transplanting) in 1975 (Adhikari & Shrestha 1989). In Nepal yield reduction due to BLB is from 5-60 % in Terai and mid-hills during hot and humid periods (Burlakoti and Khatri Chhetri, 2005). Use of resistant cultivars against the pathogen is the best non chemical method for its management. However, the resistant varieties become susceptible after few years of release due to development and dominance of virulence races in the pathogenic population (Mew et al., 1992; Adhikari et al., 1994). Breaking down of resistance of IRBB-21 rice line harboring dominant gene Xa21 by some of Xoo isolates from Japan, Nepal, Korea and India have been reported in earlier studies (Lin et al., 1996; Adhikari et al., 1999). Different BLB pathotypes are active in Nepalese rice field (Amagain, 2012). Therefore, continuous and rigorous screening of the rice genotypes against BLB is required before variety release. In this context several rice genotypes with different genetic background were evaluated resistance to Bacterial Leaf Blight at Regional Agricultural Research Station (RARS), Khajura, Nepalgunj during 2018 and 2019.

**MATERIALS AND METHODS**

**Collection of plant materials and experimental site**

National Rice Bacterial Blight Nursery (NRBBN) composed of 150 and 315 rice genotypes during 2018 and 2019, respectively sent from National Rice Research Program (NRRP), Hardinath, Dhanusa was screened at Regional Agricultural Research Station, Khajura (latitude. 28° 06” North, longitude 81° 37” East, 181 masl) under natural epiphytotic field condition. The rice variety Sabitri and TN1 were used as resistant and susceptible checks, respectively. The experiment was conducted on the field which had been cropped to winter wheat in the previous season. The site has an average annual rainfall of 1000-1500 mm.

**Collection of diseased materials**

Diseased plants affected with BLB were identified by specific symptoms i.e. with the appearance of yellow to white water-soaked stripes at the margins of leaves. The infected leaves were collected from the BLB infested field of Banke and Bardiya and preserved in the laboratory.

**Isolation, purification of pathogen and inoculation**

Infected rice leaves were cut into small pieces (5mm infected tissue and 5mm of adjacent healthy tissue) and were grinded in mortar and pestle and finally bacterial suspension was prepared. For inoculation of pathogen in plots, clip method (Kauffman et al., 1973) was used. In this method sterilized surgical scissors dipped in bacterial suspension was used for inoculation. For this purpose a pair of scissors was dipped in bacterial suspension. Selected leaves were grasped in one hand and the top 1-3 inches leaves were clipped off simultaneously. The plant infected by such pathogen was confirmed by symptoms observation i.e. yellow lesion on leaf surface.

**Screening of rice genotypes in field**

Seedlings of the test genotypes were raised in the dry raised seedbed conditions. Seeds were sown on line at an interval of 15 cm on having 50 cm width maintaining two rows per entry.
During the summer prior to transplanting, the site was moldboard plowed and disked twice to control weeds. Seedlings were uprooted at 25 days after sowing and transplanted to the puddled field with non-replicated design. Each rice cultivar was transplanted in two rows of 3 m long and spaced 20 cm apart. BLB susceptible genotype TN1 was transplanted around the whole trial plot to develop the inoculum pressure. The fertilizer rate was 120:30:30 kg NPK/ha. The experimental field was kept free from weeds by adopting manual weeding. The trial blocks were irrigated as and when needed. Other 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). Observations were recorded at the milky stage on the severity of bacterial leaf blight reaction on a 0-9 scale (Anon., 1996).

**Disease rating scale**

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

**RESULTS**

In the field screening, no rice cultivar was found immune to BLB disease during both of the tested year 2018 and 2019. Around 58% of the cultivars were moderately resistant to resistant to BLB in 2018. Whereas, none of the cultivars were resistant to BLB in 2019 and about 58% of the genotypes were moderately resistant to the disease. Genotypes were classified into five classes based on degree of reaction and genotypes falling in particular class are presented in table 1 to 5. Six genotypes in 2018 and none of the genotypes in 2019 were found resistant to BLB disease. The genotypes found resistant in 2018 were IR 108541:1-23-1-14-B-B ,IR 108541:1-23-1-14-B-B, IR 108541:1-70-1-21-B-B, IR 108541:6-29-1-9-B-B, IR 108541:6-29-3-3-B-B and IR 108541:6-36-1-20-B-B. Among 150 genotypes in 2018, 81 were moderately resistant, 59 were moderately susceptible and 4 genotypes were found susceptible along with susceptible check. In 2019, 183 genotypes were moderately resistant, 131 were moderately susceptible and 1 was found susceptible to the disease. There were no any highly susceptible genotypes to BLB in both the years.

![Figure 1: Rice genotypes showing different level of resistance to bacterial leaf blight during 2018 and 2019 at RARS, Khajura, Nepalgunj](image-url)
Table 1: Rice genotypes showing resistant response for bacterial leaf blight disease at RARS, Khajura, Nepalgunj in 2018 and 2019

| Experimental year | Resistant genotypes (Score=1) |
|-------------------|-------------------------------|
| 2018              | IR 108541:1-23-1-14-B-B, IR 108541:1-23-1-14-B-B, IR 108541:1-70-1-21-B-B, IR 108541:6-29-1-9-B-B, IR 108541:6-29-3-3-B-B and IR 108541:6-36-1-20-B-B |
| 2019              | None                          |

Table 2: Rice genotypes showing moderately resistant response for bacterial leaf blight disease at RARS, Khajura, Nepalgunj in 2018 and 2019

| Experimental year | Moderately resistant genotypes (Score=3) |
|-------------------|-----------------------------------------|
| 2018              | Sabitri(RC), IR 99993-B-B-RGA-1RGA-2RGA, IR 16L 1421, IR 16L 1637, GSR310, TP 30529, IR 16L 1829, TP 30566, TP 30535, TP 30539, IR 15L 1717, IR 24172, IR 14L 363, IR 106529-2-40-3-2-B, NR 2169-10-1-1-6-2-1-1, IR 2187-33-1-1-2-1, IR 15D 1031, TP 30528, TP 30588, IR 15L 1018, IR 30578, IR 14D 134, TP 30549, SVIN 188, SVIN 123, NR 2184-23-3-1-2-1, NR 2189-1-1-2-1, NR 2179-82-2-4-1-1-1, IR 2182-33-3-2-1-1-1, TP 29784, IR 99742-2-22-4-1-9-B, TP 30251, TP 30257, IR 99742-2-11-7-1-9-B, IR 99742-2-11-7-1-9-B, IR 90020-22-283-2-B-4, IR 14L 537, IR 14L 537, IR 97073-32-2-1-3, TP 30582, IR 98786-13-1-2-1, IR 98786-13-1-2-1, IR 15D 1031, IR 108541:6-36-3-19-B-B, IR 108541:6-36-3-19-B-B, IR 108541:12-27-1-3-B-B, IR 108541:12-27-1-3-B-B |
| 2019              | Sabitri(RC), IR 99993-B-B-RGA-1RGA-2RGA, IR 16L 1421, IR 16L 1637, GSR310, TP 30529, IR 16L 1829, TP 30566, TP 30535, TP 30539, IR 15L 1717, IR 24172, IR 14L 363, IR 106529-2-40-3-2-B, NR 2169-10-1-1-6-2-1-1, IR 2187-33-1-1-2-1, IR 15D 1031, TP 30528, TP 30588, IR 15L 1018, IR 30578, IR 14D 134, TP 30549, SVIN 188, SVIN 123, NR 2184-23-3-1-2-1, NR 2189-1-1-2-1, NR 2179-82-2-4-1-1-1, IR 2182-33-3-2-1-1-1, TP 29784, IR 99742-2-22-4-1-9-B, TP 30251, TP 30257, IR 99742-2-11-7-1-9-B, IR 99742-2-11-7-1-9-B, IR 90020-22-283-2-B-4, IR 14L 537, IR 14L 537, IR 97073-32-2-1-3, TP 30582, IR 98786-13-1-2-1, IR 98786-13-1-2-1, IR 15D 1031, IR 108541:6-36-3-19-B-B, IR 108541:6-36-3-19-B-B, IR 108541:12-27-1-3-B-B, IR 108541:12-27-1-3-B-B |
Table 3: Rice genotypes showing moderately susceptible response for bacterial leaf blight disease at RARS, Khajura, Nepalgunj in 2018 and 2019

| Experimental Year | Moderately susceptible genotypes (Score=5) |
|------------------|-----------------------------------------|
| 2018             | NR2181-15-1-1-6-1-1-1, IR 15D 110, HHZ 27-Y16-Y3-Y1, IR 2180-20-2-5-1-1-1-1, IR 95786-9-2-1-2, IR 108196-1-B-3-2-5, IR 2152-23-2-1-1-1-1-1, KALANAKAM, IR 2179-6-1-1-4-1-1, Lakka basmati, IR 2188-3-2-4-1-1, IR 2168-44-2-1-1-1-1-1, NR 2181-160-4-1-1-1-1-1, NR 2179-82-2-4-1-1-1-1-1, NR 2168-65-1-1-1-1-1-1-1-1, NR 2169-10-4-1-1-1-1-1-1, NR 2182-33-3-2-1-1-1, IR 13F 115, IR 102885-2-7-17-2, IR 06A 146, IR 2168-44-2-1-1-1-1-1-1-1, 2015 SA 10, IR 08L 201, IR 14L 546, IR 103575-76-1-1-B, Sukha dhan-4, HHZ25-DT9-Y1-Y9, IR14D 198, GSR 310, IR 95809-25-1-1-1, IR 14L 160, IR 14L 572, IR 14L 576, IR 103575-76-1-1-B, IR 98846-2-1-4-3, Radha-4, Hardinath-3, IR 82589-b-95-2, IR 82589-B-144-3, 97043-3-1-2, IR 12L 355, IR 82635-B-2-5-4, HHZ12-SAL2-Y3-Y2, IR97096-15-1-1-3, IR 13F 228, Radha-11, IR 2181-139-1-3-1-1-1-1, IR 103587-23-2-1-1-B, IR 15L 1745, IR 2168-44-2-1-1-1-1-1, IR 2179-82-2-4-1-1-1-1-1, IR 99784-255-78-2-3-1-2, IR 13F 228, Radha-13, IR 108541-6-36-3-9-B-B, IR 108541-8-66-1-4-B-B, IR 108541-8-66-2-12-B-B |
| 2019             | IR 15T 1133, IR 16L 1678, IR 103575-76-1-1-B, IR 103587-22-2-3-B, IR 2169-10-4-1-1-1-1-1, TP 356, IR 101465-5-25, IR 2179-112-22-24-1-8-1-5, NR 2184-6-1-1-4-1, NR 2193-6-3-1-1-1-1, NR 2179-82-2-4-1-1-1-1, NR 2187-33-3-2-4-1-1, TP 29766, TP 30583, IR 2152-23-1-2-1-1-1-1, IR 103575-76-1-1-B, IR 12L 353, IR 14L 560, IR 238, IR 98853-6-1-3-2, IR 2170-5-5-1-6-1-3-1-3, IR 106523-25-34-3-2-1-1-1, IR 2182-58-3-1-1-1, NR 2192-66-3-1-3-1, TP26777, NR 2182-31-1-2-1-1-1, NR 2188-13-3-4-1, NR2188-13-3-4-3-1, NR2168-44-2-1-1-2-2-1-1-1, NR2182-22-1-3-1-1-1, IR106523-25-34-3-1-B-45-1, NR2191-6-1-2-1-2-1, NR2188-13-3-2-5-1, NR2208-14-3-2-2-1, NR2187-6-2-2-1-1-1-1, PANT DHAN-2, NR2180-20-2-5-1-1-1-1-1-1, NR2187-6-2-2-1-1-1-1, SUGANDHIT DHAN 1, IR 16L 1753, IR 16L 1831, IR 17L-1323, IR 17L-1481, IR 17L-1544, IR 16L-1844, IR 16L-1591, IR 16L-1795, IR 17L-1420, IR 15T-1133, IR 14F-717, IR 14L-245, TP 30531, SR 253, SR 279, SR 224, SR 221, IR 16L 1708, IR 16L 1713, IR 16L 1743, IR 16L 1855, SR 209, SR 168, SR 123, SR 179, SR 188, SR 268, SR 074, SR 056, SR 079, NR 2210-11-2-2-1-1, NR 2193-6-3-1-1-1-1, NR 2192-62-1-1-2-1, NR 2184-9-2-1-3-1, SR 051, SR 055, SR 082, SR 172, SR 188, SR 141, SR 207, NR 2201-5-1-1-1-1-1, NR 2191-78-1-1-1-1-1, NR 2191-50-3-1-3-1-1, NR 2187-6-5-1-1-1-1, NR 2188-35-2-1-4-1, IR 16L 1678, IR 16L 1742, IR 16L 1591, IR 16L 1795, IR 16L 1855, IR 16L 1755, IR 16L 1753, IR 106523-25-34-3-2-2-1-1-3, IR 2200-8-1-1-2-1-1, NR 2192-16-1-1-1-1, NR 2192-7-1-1-1-1, NR 2191-6-2-1-2-1, NR 2189-1-1-1-2-1, NR 2184-50-1-1-1-2-1, NR 2191-172-2-1-1-1-1, SR 066, SR 102, SR 083, SR 056, SR 096, SR 071, SR 2189-11-4-1-2-1-1, SR 2189-9-1-1-1-1, SR 2188-8-2-1-2-1-1, SR 2187-33-2-3-4-1-1, IR 16L-1411, IR 12A 173, IR 15L-1505, IR 14L-261, IR 11A-151, IR 06-151, BH5-86FNR-11R-2-11, IR 101-152,
Table 4: Rice genotypes showing susceptible response for bacterial leaf blight disease at RARS, Khajura, Nepalgunj in 2018 and 2019

| Experimental year | Susceptible genotypes (Score=7)                                      |
|-------------------|---------------------------------------------------------------------|
| 2018              | Susceptible check (TN-1), Pant-2, Kalanuniya, Masuli                |
| 2019              | SVIN 207                                                            |

Table 5: Rice genotypes showing highly susceptible response for bacterial leaf blight disease at RARS, Khajura, Nepalgunj in 2018 and 2019

| Experimental year | Highly susceptible genotypes (Score=9) |
|-------------------|----------------------------------------|
| 2018              | None                                   |
| 2019              | None                                   |

DISCUSSION

Earlier many rice genotypes were evaluated for resistance to BLB at RARS, Khajura, sent from National Rice Research Program, Hardinath, Dhanusa, Nepal among which most of the rice genotypes were susceptible, but a few of them were considered resistant. During this research also most of the rice cultivars were susceptible, only few had reactions low enough to be considered resistant. Genotypes differed in disease reaction to BLB at RARS, Khajura during both the year. None of the genotypes were immune during both the year. Six genotypes (IR 108541:23-1-14-B, IR 108541:1-23-1-14-B-B, IR 108541:1-70-1-21-B-B, IR 108541:6-29-1-9-B-B, IR 108541:6-29-3-3-B-B and IR 108541:6-36-1-20-B-B) in 2018 and none of the genotypes in 2019 were found resistant to BLB disease. The different genetic background of rice lines used in this study showed different interaction to BLB. During 2018, the genotypes TN-1, Masuli, Kalanuniya and Pant-2 were found susceptible, whereas, during 2019, SVIN 207 was recorded as susceptible genotypes. The susceptible response of this germplasm might be due to absence of resistance genes against BLB. These genotypes showing resistant reaction might have certain resistant genes against the BLB disease. Kaushal et al. (1998), have also reported 9 accessions as resistant among the tested 167 wild rice accessions. Sabitri was found moderately resistant and the TN1 was found moderately susceptible. Similar to our findings Adhikari (2004) also reported Sabitri as a bacterial leaf blight resistant and Chaudhary et al., (2004) also found TN1 as susceptible to BLB. The present results are in line with various earlier reports for other locations in the country. The reaction of disease on susceptible check indicates that there was sufficient inoculum pressure in the field for disease development. The difference of genotypes in disease severity may be due to diversity in their genetic makeup.

CONCLUSION

From the present experiment, it can be concluded that 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 Nepal. The genotypes found moderately resistant could be used as the resistant source for developing bacterial leaf blight resistant varieties for various domains of Nepal. 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.
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Authors’ Contributions
B. Acharya and S. KC together conducted this experiment and worked together for analysis, result interpretation and manuscript development. Both the authors approved the final version of this manuscript.

Conflict of interest
The author declares no conflicts of interest regarding publication of this manuscript.

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