Response of rice genotypes to bacterial leaf streak caused by *Xanthomonas oryzae* pv. *oryzicola*, an emerging threat in Nepal

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**Abstract**

Bacterial leaf streak (BLS) is one of the devastating diseases of rice in Asia, Northern Australia, and West Africa that leads to up to 32% yield losses. Previously, BLS was rare in Nepal, but it has become more common in recent years, and it is supposed to cause significant yield losses. However, studies on various aspects of BLS of rice, such as yield loss assessment, management strategy, germplasm evaluation, etc., have not been done in Nepal. Therefore, realizing the fact that the identification of resistant genotypes is a cost-effective and efficient approach to managing crop diseases; seventy-six rice genotypes were evaluated, along with resistant (Sabitri) and a susceptible (TN1) checks (usually used for bacterial leaf blight) under artificial epiphytotic conditions at the National Wheat Research Program (NWRP), Bhairahawa, Nepal, in the year 2018 and 2019. This study identified thirteen resistant and fourteen moderately resistant genotypes based on the mean percentage of disease severity over two years. The resistant genotypes viz., IR 108196-1-B-B-3-2-5, IR 10A 134, NR 2168-44-2-1-1-1-2-1-1, B 11598C-TB-2-1-7, IR 14D 198, IR 96279-39-3-1-2, IR 103587-22-2-3-B, BP 9474C-1-1-B, IR 10L 185, IR 15L 1735, IR 106529-20-40-3-2-B, IR 15D 1031 and IR 108541:12-27-1-3-B-B could be used as resistance sources in the breeding programs. Furthermore, resistant genotypes with high yield potential after evaluation across different environments could be released as BLS resistant varieties in Nepal. This study may be the first effort to identify resistant rice genotypes, particularly against BLS, which is an emerging potential threat to rice production in Nepal.

**Keywords:** Evaluation, rice genotypes, *Xanthomonas oryzae* pv. *oryzicola*, disease severity, Nepal

1 Introduction

Rice (*Oryza sativa* L, *O. glaberrima*) is a staple food for more than half of the world’s population and one of the oldest domesticated crops (Ainsworth, 2008; Jiang et al., 2020). It is the most important cereal and staple meal in Nepal, providing 50 percent of the total calories required by 30 million people (Bastnet BMS, 2017). Rice was grown on 14, 58, 918 ha and produced 55, 50, 878 tons, establishing 3.8 t ha⁻¹ productivity (MoALD, 2020). However, Nepal imported around 1.2 million tons with a value of NRs 48.16 billion in the fiscal year 2020/21, which showed that, on an average, 6.85 million tons of rice is needed to meet the demand of the Nepalese people (TEPC, 2020). Rice production is substantially affected by diseases, among which bacterial leaf streak (BLS) is a
destructive disease caused by *Xanthomonas oryzae* pv. *oryzicola*, prevalent in tropical and subtropical Asia, northern Australia, and West Africa (Nino-Liu et al., 2006). The increased prevalence of the BLS across Asia and Africa is due to an expansion in the areas covered by hybrid rice cultivars or growing susceptible cultivars, as well as climate change (Gonzalez et al., 2007; Wonni et al., 2011). Earlier, BLS was uncommon, but its increased incidence in the last few years has led farmers to use pesticides to manage this disease in Nepal. Due to a minor rice disease, studies on different aspects of BLS viz., germplasm evaluation, yield loss assessments, management strategies, and pathogen virulence, have not been done yet. It leads to 8-32% yield abatement depending on rice variety, growth stage, geographical location, and favorable conditions (Liu et al., 2014).

*X. oryzae* pv. *oryzicola* (Xoc) is a Gram-negative, rod-shaped bacterium with cell lengths ranging from 0.7-2.0 µm and widths ranging from 0.4-0.7 µm. The colony of Xoc on solid media is round, convex, mucoid, and yellow in color due to the production of xanthomonadin, which is a characteristic feature of the genus (Bradbury, 1984). Xoc enters the leaf through stomata or wounds, multiplies in the substomatal cavity, and colonizes the parenchyma’s intercellular spaces, but never invades the xylem (Ou, 1973). A characteristic symptom of BLS in rice is a translucent and yellow streak on the leaf surface (Jiang et al., 2020). It spreads when exudates on the leaf surface fall into the field water or are disseminated by wind, rain, insects, or other factors (Mew, 1993; Nyvall, 1999). As the disease progresses, the diseased leaves turn greyish white and eventually die (Nino-Liu et al., 2006).

Control measures for BLS have not been studied much. It is expected that many of the control methods used for bacterial leaf blight of rice could be effective. Cultural methods, viz., seed treatment with hot water or antibiotics, field sanitation, and use of a recommended dose of fertilizer are practiced for bacterial leaf blight (BLB) management in some Asian countries, are also suggested for BLS management (Nino-Liu et al., 2006). Moreover, chemicals such as probenazole, tecloftalam, phenazine oxide, and nickel dimethyl dithiocarbamate are used for BLB management in temperate regions (Mizukami and Wakimoto, 1969; Goto, 1992), whereas no effective bactericides are available on the market to control BLS and BLB in Asia (Ou, 1973; Lee et al., 2003). Seeds treated with *Bacillus subtilis* / *P. putida*, and foliar application of *Pseudomonas fluorescens* or *P. putida* strain V14i are effective against BLS and are recommended for BLS management (Johri et al., 2003; Vasudevan et al., 2002).

Host resistance is the best way to control BLS among several disease control methods, although it is limited to quantitative resistance (Sheng et al., 2005; Tang et al., 2000). Despite many attempts, no major R gene providing BLS resistance in rice has been discovered. Nonetheless, a few studies have identified and mapped QTLs imparting BLS resistance, with qBlsr5a having a considerable influence (Xie et al., 2014). The introduction of a non-host R gene, Rxo1, originally obtained from maize, into transgenic rice produced a high level of resistance to BLS (Zhao et al., 2005).

Considering the increasing incidence of BLS over large areas, which could be a significant barrier to rice production in Nepal, identification of resistant source through evaluation of diverse rice genotypes against BLS was realized imperative. Thus, the main objective of this study was to evaluate rice genotypes to explore bacterial leaf streak (BLS) resistance sources/genotypes.

## Materials and Methods

A total of 76 rice genotypes were collected from the National Rice Research Program (NRRP), Baniyina, Dhanusha, Nepal, along with a resistant check, Sabitri, and a susceptible check, TN-1 (these checks are normally used for BLB) (Table 1). The sources of rice genotypes were International Rice Research Institute (IRRI), Philippines, Yunnan Academy of Agricultural Sciences (YAAS), China, and NRRP, Nepal. Genotypes were chosen based on their genetic history, suggested geographical domains, maturity period, morphological and quality attributes. Furthermore, these genotypes were in various phases of selection, including NRON (National Rice Observation Nursery), IET (Initial Evaluation Trail), CVT (Coordinated Varietal Trail), CFFT (Coordinated Farmer’s Field Trial), and PVS (Participatory Variety Selection). Selected genotypes were screened against bacterial leaf streak of rice for two successive crop seasons (2018 and 2019).

### 2.1 Experimental procedure

The rice genotypes were tested in the experimental field of the National Wheat Research Program (NWRP), Bhairahawa, Nepal, which is located at 27°32’ N, 83°28’ E, and 105 masl. In both years, one gram seeds of each genotype were sowed in a seedbed of 1 m long row, 20 cm apart, in the last week of June in the year 2018 and 2019. The field was prepared and fertilized with N:P₂O₅:K₂O @ 120:40:30 kg ha⁻¹, with a half of the nitrogen dose and a full dose of phosphorus and potash administered as a basal dosage, 1/4th nitrogen applied at tillering, and the remaining nitrogen dose was applied at booting stage (Gupt et al., 2021). Twenty four days old seedlings were transplanted in a prepared field. Each genotype was transplanted as a single seedling at 15 cm apart in two rows of 1.5 m length and 20 cm inter-spaced. A pre-emergence weedicide i.e., Pendimethalin was...
Table 1. Average response of rice genotypes against bacterial leaf streak (BLS) across the year 2018 and 2019

| E. No | Genotypes | Source | Year 2018 | Year 2019 | Mean %DS Avg. HR |
|-------|------------|--------|-----------|-----------|-----------------|
|       |            |        | DRS | %DS | HR | DRS | %DS | HR |               |               |
| 1     | HHZ 27-Y16-Y3-Y1 | IRRI | 7   | 77.8 | S | 9 | 100 | HS | 88.9 | HS |
| 2     | IR 102860-3-B-B | IRRI | 7   | 77.8 | S | 9 | 100 | HS | 88.9 | HS |
| 3     | IR 95784-21-1-2  | IRRI | 3   | 33.3 | MR | 5 | 55.6 | MS | 44.4 | MS |
| 4     | 108196-1-B-B-3-2-5 | IRRI | 1   | 11.1 | R | 1 | 11.1 | R | 11.1 | R |
| 5     | NR 2182-4-3-2-1-1 | NRRP | 7   | 77.8 | S | 9 | 100 | HS | 88.9 | HS |
| 6     | NR 2182-31-1-2-1-1 | NRRP | 5   | 55.6 | MS | 5 | 55.6 | MS | 55.6 | MS |
| 7     | IR 14L 160   | IRRI | 3   | 33.3 | MR | 3 | 33.3 | MR | 33.3 | MR |
| 8     | IR 97096-15-1-1-3 | IRRI | 7   | 77.8 | S | 7 | 77.8 | S | 66.7 | S |
| 9     | IR 13F 228   | IRRI | 3   | 33.3 | MR | 1 | 11.1 | R | 22.2 | MR |
| 10    | CT 16658-5-2-35R-3-1 | IRRI | 1   | 11.1 | R | 3 | 33.3 | MR | 22.2 | MR |
| 11    | BP 9474C-1-1-B | IRRI | 1   | 11.1 | R | 1 | 11.1 | R | 11.1 | R |
| 12    | IR 3152-19-3-1-2-1-1 | IRRI | 3   | 33.3 | MR | 3 | 33.3 | MR | 33.3 | MR |
| 13    | IR 10A 134   | IRRI | 5   | 55.6 | MS | 5 | 55.6 | MS | 55.6 | MS |

DRS = Disease rating scale, DS = Disease severity, HR = Host response, IRRI = International Rice Research Institute, YAAS = Yunnan Academy of Agricultural Sciences, NRRP = National Rice Research Program
sprayed @ 2 ml L$^{-1}$ on the next day of transplanting to prevent germination of weeds. An insecticide Chlorpyriphos 50% EC + Cypermethrin 5% EC was sprayed @ 1 mL L$^{-1}$ at tillering, booting, and milking stage to reduce insect infestation (Gupt et al., 2021).

### 2.2 Isolation and inoculation

Rice leaves with a typical BLS symptom were collected from a local farmer’s field, chopped into 1 cm pieces, disinfected with 70% ethanol for 15 seconds, and then washed three times in distilled water. To leach out bacterial cells, leaf pieces were submerged in 300 µL sterilized water for 15 minutes in a microcentrifuge tube. A loop full bacterial suspension was streaked on (PSA) peptone sucrose agar (peptone 1.2%, sucrose 1.2%, and 2% agar) media plated in the Petri dish and incubated at 30 °C in a BOD (biological oxygen demand) incubator for 3-4 days to promote Xoc growth. Furthermore, a loop full of yellow-colored (Fig. 1), 3-4 days old colony was streaked on a new PSA media, incubated at 30 °C in a BOD incubator to obtain a pure culture of Xoc (Fig. 1).

Gram staining was done following the protocol of Gerhardt (1981), and further identification of Xoc was done based on characteristics described by Nino-Liu et al. (2006) (Fig. 2).

The pure culture of Xoc was dissolved in sterilized water to obtain a concentration of bacterial (Xoc) suspension @ $1 \times 10^8$ CFU mL$^{-1}$ and added a 100 µL L$^{-1}$ Tween 20 as a dispersing agent. Rice genotypes were inoculated 45 days after transplanting which coincides at stem elongation to booting stage (Chen et al., 2006) by following the technique developed by Kauffman (1973) with slight modifications (leaf tips were cut with a scissors and kept immersed in a bacterial suspension for 30 seconds. Leaves from five hills of each genotype were cut 2-3 cm from the tips with a sterilized scissor and then inoculated by dipping in the bacterial suspension for 30 seconds.

### 2.3 Disease assessment

Five inoculated hills were labeled with red wool for each genotype. The disease was scored 20 days after inoculation (heading to the milking stage). According to the Standard Evaluation System of Rice 5th edition (IRRI, 2013), BLS of rice was rated on a 0-9 scale depending on the percentage of leaf showing BLS symptoms (Table 2).

Disease severity was estimated by using the following formula (Waller et al., 2001) with slight modification as given below:

$$\% \text{ DS} = \frac{V}{N} \times 100$$  \hspace{1cm} (1)

where $DS$ = disease severity (%), $V$ = value of disease rating scale at final scoring of a disease, and $N$ = maximum value of disease rating scale.

### 2.4 Statistical analysis

Data entry, processing, and estimation of disease severity were performed in Microsoft Office Excel 2007. A cluster analysis of seventy eight rice genotypes based on mean disease severity over two year was performed using R (R Core Team, 2020).

### 3 Results

A pure bacterial culture streaked from a rice leaf displaying a typical BLS symptom resulted in the round, convex, mucoid, and yellow color colony on PSA media (Fig. 1). Moreover, Gram-staining of a bacterial colony revealed gram-negative, rod-shaped bacterial cells under oil immersion 100× binocular microscopes (Fig. 2). Furthermore, inoculation of rice leaves with an obtained pure culture of the bacteria developed water-soaked, yellow exudates in chain or streak on leaves (typical symptoms of BLS) (Fig. 3).

In 2018, out of 76 genotypes (excluding resistant and susceptible checks), fifteen genotypes were found resistant (R), fourteen genotypes were moderately resistant (MR), ten genotypes were moderately susceptible (MS), twenty-three genotypes were susceptible (S) and fourteen genotypes were highly susceptible (HS) based on disease severity (Table 1 & Fig. 4). Similarly, in the year 2019, among 76 rice genotypes, sixteen genotypes were resistance, twelve genotypes were moderately resistant, eight genotypes were moderately susceptible, seven were susceptible and thirty-three genotypes were found highly susceptible ((Table 1 & Fig. 4). Moreover, based on mean disease severity over two years, out of 76 rice genotypes, thirteen genotypes were categorized as resistant (mean DS <11.11%), fourteen as moderately resistant (mean DS range 11.12-33.33%), nine as moderately susceptible (mean DS-33.34-55.6%), six as susceptible (mean DS range 55.7-77.8%), and thirty-four as highly susceptible (mean DS range 77.9-100%) ((Table 1 & Fig. 5).

A cluster analysis of 78 genotypes (including both checks) based on the mean value of disease severity over two years revealed that the genotypes were clustered into five distinct clades. The cluster tree depicted thirteen resistant genotypes in clade I, fifteen moderately resistant genotypes in clade II, nine moderately susceptible genotypes in clade III, six susceptible genotypes in clade IV, and thirty-five genotypes in clade V (Fig. 6). The rice genotypes in clade I viz., IR 108196-1-B-B-3-2-5, IR 10A 134, NR 2168-44-2-1-1-1-2-1-1, B 11598C-TB-2-1-B-7, IR 14D 198, IR 96279-39-3-1-2, IR 103587-22-2-3-B, BP 9474C-1-1-B,
**Figure 1.** Three days old culture of *Xanthomonas oryzae pv. oryzicola* (Xoc) on Peptone Sucrose Agar (PSA) medium

**Figure 2.** *X. oryzae pv. oryzicola* (Xoc) cells after Gram staining under oil immersion 100X of binocular microscope

**Table 2.** Scale used for scoring bacterial leaf streak of rice in the field condition

| Scale | Diseased leaf area                                      | Host response          |
|-------|--------------------------------------------------------|------------------------|
| 0     | No lesions observed                                    | Immune                 |
| 1     | Small pin point size lesion <1 mm                     | Resistant              |
| 3     | Significant number of lesion <4% of leaf area)        | Moderately resistant   |
| 5     | Lesions infecting 4-10% of the leaf area              | Moderately susceptible |
| 7     | Lesions infecting 26-50% of the leaf area             | Susceptible            |
| 9     | Lesions infecting >75% of the leaf area               | Highly susceptible     |

Source: Standard Evaluation System of Rice (IRRI, 2013)
**Figure 3.** Response of rice genotypes: a- Resistant (R), b- Moderately resistant (MR), c- Moderately susceptible (MS), d- Susceptible (S) and e- Highly Susceptible (HS)

**Figure 4.** Frequency of rice genotypes at a scale 0-9 against bacterial leaf streak in the year 2018 and 2019
Figure 5. Frequency of rice genotypes based on % disease severity against bacterial leaf streak across two years (2018 and 2019)

Figure 6. Clustering of 78 rice genotypes based on an average disease severity across two years (2018 and 2019)
IR 10L 185, IR 15L 1735, IR 106529-20-40-3-2-B, IR 15D 1031 and IR 108541:12-27-1-3-B-B were found resistant.

4 Discussion

Rice production is limited by diseases, one of which, bacterial leaf streak (BLS), which is becoming more prevalent biotic constraint in Asia and Africa (Jiang et al., 2020). The most cost-efficient, environmentally friendly, and effective approach for reducing disease-related yield loss is to develop resistant cultivars by deploying genes conferring disease resistance. No single major gene conferring resistance to BLS has been identified to date, rather than a QTL, qBlsr5a, which is quite effective (Xie et al., 2014). However, the introgression of a non-host gene, Rox1, initially cloned from maize, into transgenic rice has been proved to confer a high level of resistance to BLS (Zhao et al., 2005).

Considering the increasing prevalence of BLS in Nepal in the last few years and increasing trends of growing hybrid varieties, the identification of resistance sources/genotypes against BLS is necessary. Screening of diverse rice genotypes under artificial epiphytotic conditions is one of the means for the identification of resistance sources (ai He et al., 2012). This study identified thirteen resistant, fourteen moderately resistant rice genotypes against BLS after evaluation of rice genotypes under artificial epiphytotic conditions. In a study, Xu et al. (1991) assessed 2017 rice accessions obtained from wild rice species generation and identified resistant thirty accessible. Similarly, Huang et al. (2008) also evaluated 1665 wild rice accessions and identified 57 resistant rice accessions. Moreover, Wonnii et al. (2015) evaluated four O. sativa and two O. glaberrima accessions against different Xoc strains that originated from Mali and the Philippines. They found two Oryza sativa accessions resistant to Xoc strains originated from Mali. Furthermore, Kanaabi et al. (2016) identified three resistant and eight moderately resistant to BLS, out of thirty-five rice genotypes evaluated in Uganda.

Identification, collection, and evaluation of wild rice species to identify major genes conferring resistance to race-specific (Jianlong et al., 1997; He et al., 1994) or QTLs that impart horizontal or field resistance to bacterial leaf streak (BLS) is necessary for the future (Sheng et al., 2005; ai He et al., 2012). Furthermore, this study confirmed the identity of the pathogen as Xanthomonas oryzae pv. oryzicola (Xoc) based on morphological aspects of the bacterial colony on PSA media, features of bacterial cells under a microscope after Gram staining, and symptoms on rice leaves following bacterial inoculation, as described by Nino-Liu et al. (2006).

5 Conclusion

In Nepal’s Terai/Plains region, bacterial leaf streak (BLS) is becoming more common, posing a new threat to rice production. Because no bactericides have been shown to be helpful against bacterial leaf streak, finding resistance genotypes has become as imperative strategy to manage BLS of rice. This study identified thirteen resistant rice genotypes, viz., IR 108196-1-B-3-2-5, IR 10A 134, NR 2168-44-2-1-1-1-2-1-1, B 11598CTB-2-1-B-7, IR 14D 198, IR 96279-39-3-1-2, IR 103587-22-2-3-B, BP 9474C-1-1-B, IR 10L 185, IR 15L 1735, IR 106529-20-40-3-2-B, IR 15D 1031 and IR 108541:12-27-1-3-B-B, whereas fourteen moderately resistant genotypes, viz., NR 2179-6-1-1-4-1-1, IR 3152-19-3-1-2-1-1, NR 2168-65-1-1-1-1-1-1, 2015 SA 10, IR 15L 1065, IR 14L 546, IR 15L 1717, IR 86515-19-1-2-1-1-1-1, IR 13F 228, CT 16658-5-2-35R-3-1, IR 103587-23-2-1-B, Anmol masulii, and NR 2179-82-2-4-1-1-1-1 against bacterial leaf streak of rice (BLS). These resistant genotypes could be used as resistance sources in breeding programs, whereas resistance and moderately resistant genotypes could be evaluated for yield potential in different geographical domains and, if found promising, could be released as bacterial leaf streak resistant varieties in Terai regions of Nepal.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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