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## SCREENING OF RICE GENOTYPES AGAINST BLAST DISEASE (Pyricularia oryzae) UNDER DIRECT SEEDED RICE CONDITION AT GOKULESHWOR, BAITADI.

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#### **ABSTRACT**

Rice is the principal food crop of Nepal grown extensively under a wide range of agro-ecological conditions. However, rice production is not satisfactory due to a variety of biotic and abiotic factors, of which Rice blast (*Pyricularia oryzae*) is among the most important biotic factors responsible for yield losses in rice growing areas of Nepal. The experiment was conducted at Gokuleshwor, Baitadi, Nepal during July to November 2019 to screen rice genotypes against leaf blast disease under disease conducive upland nursery conditions. A total of 10 rice genotypes were tested in one factorial randomized complete block design with three replications. Diseases were scored beginning on the 39th day of sowing using disease rating scale of 0-9. In the test of 10 rice genotypes, 6 genotypes viz. Sabitri, Saga 4, INH12023, DR-11, Hardinath 3 and INH13140 were found resistant. Similarly, Chaite-5, INH14120 and INH14172 were moderately resistant. Similarly, Shankharika was highly susceptible to rice blast. This study can provide valuable information for rice leaf blast disease management, as well as how to utilize these resistant and moderately resistant genotypes in further resistance breeding.

Keywords: Rice, Rice Blast, Pyricularia oryzae, Screening, Resistant, Susceptible

### **INTRODUCTION**

Rice (Oryza sativa L.) is the second most important crop in the world after wheat (FAO,2020). It is the primary staple crop for more than 60% of the world's population and is cultivated over 167 million hectares with an annual production of 744.4 million tonnes of grains globally in the fiscal year 2020/21 (Bhatta et al. 2022, FAOSTAT, 2020, Katoch et al. 2019). In Asia, it is grown on more than 148 million hectares under a variety of ecosystems and water regimes with over 90% of global production and consumption (FAOSTAT, 2020). It also provides 30 - 80% of the daily calories consumed in Asia (Narciso and Hossain, 2002). Rice is the principal food crop of Nepal grown extensively under a wide range of agroecological conditions from low-land terai (60 m) to high mountain valley (3050 m) (Devkota, 2018). In Nepal, it is cultivated over 1,458,915hectare area with an annual production of 5,550,878 metric tons in the fiscal year 2019/20 (MoAD,2019/20). It is estimated that global paddy production will need to increase by 40% by 2030 in order to meet food demands (Khush, 2005). However, this demand will have to be met with less land, less water, less labor, and fewer chemicals (Katoch et al.2019). In Nepal, rice production is not satisfactory due to

a variety of biotic and abiotic factors, of which Rice blast (Pyricularia oryzae) is among the most important biotic factors responsible for yield losses in rice growing areas of Nepal (Dahal, Amatya, & Manandhar, 1992, Hobbs et al. 1996, Adhikari, 2004, Neupane and Bhusal, 2021). Rice blast, also known as "Maruwa Rog" in Nepali, was first reported in a Chinese manuscript from the Ming Dynasty as early as 1637 and interpreted as a "Rice Fever" (Ou,1972). It has caused significant yield losses in rice growing countries with an annual yield loss of 10-30% (Wilson and Talbot, 2009). The first record of Blast of rice in Nepal was in 1966, since then the pathogen has been connected to average yield losses of 10-20% in susceptible varieties. However, yield losses of up to 80% have also been reported in critical situations (Manandhar, 1987; Manadhar et al. 1992, Chaudhary, 1999, Magar et al., 2015).

The pathogen is primarily found on leaves, causing leaf blast during vegetative stage, and on neck or panicle branches during reproductive stage, causing neck blast (Bonman and Mackill,1988, Bonman and Khush,1992). The symptoms of leaf blast include elongated diamond-shaped lesions with gray or white centers and brown or reddish-brown margins. The infection of the stem nodes leads to barren

panicles causing "broken necks". The "neck blast" is considered to be the most destructive phase of the disease, and occurs when the fungus provides a brown or black ring of lesions around the node just below the panicle (Greer et al. 1997, Sesma, 2004, Shahriar et al, 2020). In managing the disease, seed treatments (Manandhar 1984, Upadhyaya, 1996), fungicide applications, resistant cultivars, agronomic practices, and biotechnological methods have proven useful. However, Host plant resistance is the best way to manage the disease as it is convenient, preferable cost effective, safe and practical means of plant protection for resource-poor farmers (Sharma,

1995, Ou, 1985; Bonman et al., 1992) Although with the appearance of new virulent races of the pathogen, resistance is weakened (Castano et al.1990; Haq et. Al., 2002; Chandrashekara et al., 2008). The blast pathogen has been found to be highly variable in terms of its ability to cause disease on newly released popular varieties (Chaudhary,1999). Thus, it is crucial to develop durable resistant rice varieties by using effective and efficient screening techniques (Chaudhary 1996). In this study, all attempts have been made to assess the level of resistance on different rice genotypes against blast disease in field condition at Gokuleshwor, Baitadi, Nepal.

#### MATERIALS AND METHODS

### Plant materials

A total of 10 rice genotypes improved and hybrid originating from the diverse sources were used in

the study. They were collected from National Rice Research Program, Hardinath, Dhanusha. Sankharika was used as susceptible check and Sabitri as resistant check.

Treatment	Genotype	
T1	Hardinath-3	
T2	Chaite-5	
T3	DR-11	
T4	Saga-4	
T5	Sabitri	
Т6	Sankharika	

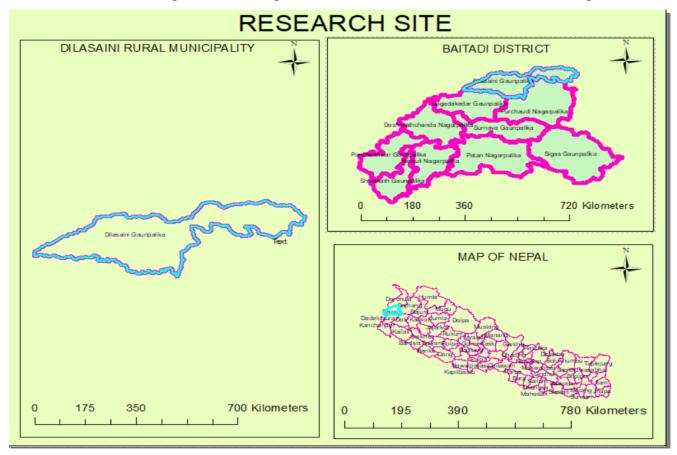
T7	INH 14120
T8	INH 14172
Т9	INH 12023
T10	INH 13140

Table 1 List of rice genotypes included in study at Gokuleshwor, Baitadi

### **Experimental design**

The experiment layout was laid out in one factorial randomized complete block design with

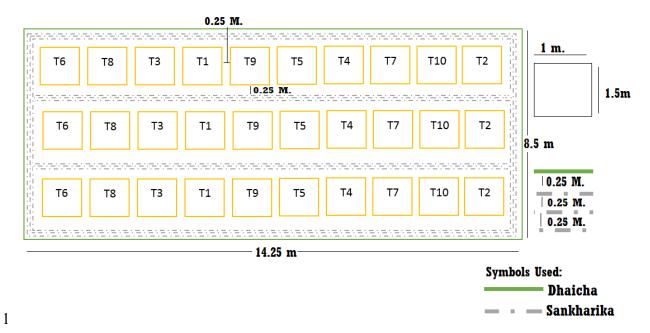
three replications having ten treatments during 2019 at the Gokuleshwor, Baitadi, Nepal.



A total of 10 genotype including resistant and susceptible were evaluated in the direct seeded upland nursery. The total size of the research plot was 147.88-meter square. The size of individual

plot was 1.5m x 1m. There was a gap of 0.5m between two experimental plots and gap of 1m between two replications. The pathogen was allowed to spread naturally in the test lines by

planting spreader rows around the nursery. Screening nurseries are designed according to the international specifications outlined by Jennings et al. (1979) to ensure a blast-friendly environment.







### **CULTURAL OPERATION**

Five grams of seeds of each test rice genotype were sown in the dry seed bed using the line sowing method. Rice seeds were then covered with pulverized soil. Farm yard manure @ 10 t/ha, was mixed into soil two weeks before

dhaincha sowing, and chemical fertilizers were diammonium applied through urea and phosphate, respectively @ 100: 50: 0 N: K2O: P2O5 kg/ha. Heavy dose of nitrogen and no potash was used to insure adequate infection. The half dose of nitrogen and full dose of phosphorus were applied as a basal dose at the time of final land preparation, and the remaining half dose of nitrogen was applied in two split doses: one fourth at 15 days after sowing (DAS) and the remaining one fourth at 25 DAS. Since rice seedlings need a significant amount of water, weekly irrigation was done. The manual weeding was done at the time of 25 DAS and 35DAS,

respectively. Irrigation was done as neded to ensure vigorous crop growth. Other intercultural operations were done as required.

### **DISEASE ASSESMENT**

The observations on disease appearance were recorded from each screened genotype along with the resistant and susceptible check planted after every ten varieties. A check variety for resistance and a check variety for susceptible were planted to ensure uniformity in the distribution of inoculum. The disease rating scale 0-9 was used to score diseases from the 39th day of sowing (IRRI, 2002).

Scale	Description	Host Behaviour
0	No lesion observed	Highly Resistant
1	Small brown specks of pinpoint size	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about	Moderately
	1-2 mm in diameter, with a distinct brown margin. Lesions are	Resistant
	mostly found on the lower leaves	
3	Lesion type same as in 2, but significant number of lesions on	Moderately
	the upper leaves	Resistant
1	Typical susceptible blast lesions, 3 mm or longer infecting less	Moderately
	than 4% of leaf area	Susceptible

- Typical susceptible blast lesions of 3mm or longer infecting 4- Moderately 10% of the leaf area Susceptible
- Typical susceptible blast lesions of 3 mm or longer infecting Susceptible 11-25% of the leaf area
- 7 Typical susceptible blast lesions of 3 mm or longer infecting Susceptible 26-50% of the leaf area
- Typical susceptible blast lesions of 3 mm or longer infecting Highly Susceptible 51-75% of the leaf area many leaves are dead
- 9 Typical susceptible blast lesions of 3 mm or longer infecting Highly Susceptible more than 75% leaf area affected

Table 2. Disease rating scale (0-9) used to score leaf blast in field at Gokuleshwor, Baitadi, 2019

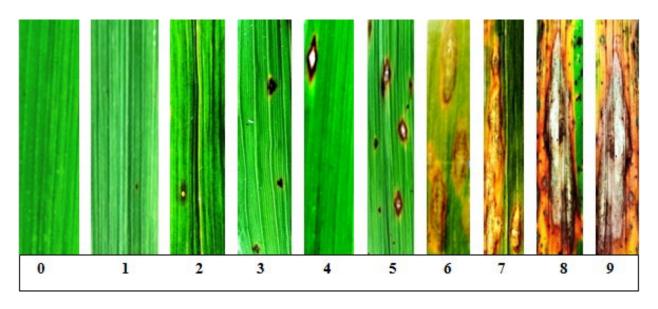


Figure 1 Leaf Blast disease scoring scale (0-9)

In order to assess the resistance and obtained from the experiments were divided into susceptibility of rice genotypes, the data five categories as resistant (R), moderately

resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS). Score 0 was considered as highly resistant

reaction, while 1 was considered moderately resistant, 2-5 moderately susceptible, 6-7 as susceptible, and 8-9 as highly susceptible.

Based on the scored value from estimation of the leaf area infection the severity % was calculated per plot by using the following formula:

### Estimation of area under disease progress curve (AUDPC)

The effect of disease severity on rice variety was integrated into area under disease progress curve (AUDPC) for the quantitative measure of epidemic development, disease severity and rate of progress which has no unit. AUDPC were computed, from the disease severities values from the formula given by Shaner and Finney (1977), Das, Rajaram, Mundt, and Kronstad (1992).

AUDPC= 
$$\sum_{i=1}^{n-1} \left[ \frac{X_{(i+1)} + X_i}{2} \right] (T_{i+1} - T_i)$$

Where

Xi = disease severity on first date

Ti= date on which the disease was scored

n= number of dates on which disease was scored

### STATISTICAL ANALYSIS

The recorded data were tabulated in excel data sheet and subjected to analysis by using the reference of Gomez and Gomez (1984). The data were processed to fit into R-studio and analysis was conducted using R 3.4.1 (R Core Team, 2017) and the agricolae version 1.1-8 package (Mendiburu, 2014). Based on ANOVA result, Duncan's multiple range test (DMRT) was performed to compare the genotypes. The treatment means were compared by the Least Significant Difference (LSD) test at 5% level (Gomez & Gomez, 1984; Kandel & Shrestha, 2019; Baral et al., 2016; Shrestha, 2019).

### RESISTANCE AND SUSCEPTIBILTY OF GENOTYPES

The genotypes were categorized into five categories based on the following AUDPC values:

Mean AUDPC	Category		
>420	Highly Susceptible	HS	
271-420	Susceptible	S	
181-270	Moderately Susceptible	MS	
91-180	Moderately Resistant	MR	
<90	Resistant	R	

Table 3: Categories of rice based on AUDPC value

### RESULT AND DISCUSION

### Disease severity on different date of scoring

**ANOVA** revealed a highly significant correlation between disease severity at 39 DAS and genotypes. The mean severity was 10.24 on the same day. Sabitri had the lowest severity on 39 DAS (2.96); Shankharika had the highest (41.11) followed by Chaite-5 (12.59). Genotypes and disease severity were also highly significant when analysed using ANOVA at 47th DAS. A mean value of 10.96 was seen for disease severity on the same day with Sabitri had the least severity (2.96) and Sankharika had the most severity (45.18) followed by chaite-5(13.70). Furthermore, based on analysis of variance (ANOVA), the relationship between disease severity and genotypes at 54 DAS was highly significant with a mean disease severity of 11.22.

In treatment Sabitri, the severity of disease was lowest (2.96) while Shankharika (46.29) had the highest disease severity, followed by Chaite-5 (14.44). In addition to this, an analysis of variance (ANOVA) showed highly significant correlation between disease severity at 62th ,69nd and 76th DAS and genotype with mean disease severity of 15.85, 17.53 and 25.036. The lowest disease severity was observed on Sabitri (5.18, 6.66 and 13.70) whereas highest disease severity was seen on Shankharika (50.00, 53.70, 58.14) on these subsequent days followed by Chaite-5 and INH 14172 respectively. These findings are in line with the finding of Chaudhary et al. (2004), Manadhar et al. (1985) and Acharya et al. (2019) with Sabitri being most resistant

genotype whereas Shankharika being most susceptible genotypes.

Ganatyna	Disease severity on							
Genotype	39 DAS	47 DAS	54 DAS	62 DAS	69 DAS	76 DAS		
Sabitri	2.96 <sup>d</sup>	2.96 <sup>d</sup>	2.96 <sup>d</sup>	5.18e	6.66 <sup>d</sup>	13.70 <sup>d</sup>		
INH 12023	$3.70^{\rm cd}$	3.70 <sup>cd</sup>	3.70 <sup>cd</sup>	7.03 <sup>de</sup>	9.25 <sup>cd</sup>	15.55 <sup>cd</sup>		
Saga 4	4.07 <sup>cd</sup>	4.81 <sup>cd</sup>	5.18 <sup>cd</sup>	8.14 <sup>cde</sup>	9.62 <sup>cd</sup>	19.62 <sup>bcd</sup>		
Hardinath 3 (DR-44)	5.92 <sup>cd</sup>	6.66 <sup>cd</sup>	6.66 <sup>cd</sup>	10.55 <sup>cde</sup>	11.66 <sup>cd</sup>	21.10 <sup>bcd</sup>		
INH 14120	6.66 <sup>bcd</sup>	7.21 <sup>bcd</sup>	7.21 <sup>cd</sup>	12.96 <sup>bcde</sup>	14.44 <sup>bcd</sup>	22.22 <sup>bcd</sup>		
INH 13140	8.14 <sup>bcd</sup>	8.14 <sup>bcd</sup>	8.14 <sup>bcd</sup>	14.07 <sup>bcd</sup>	15.55 <sup>bcd</sup>	22.96 <sup>bc</sup>		
DR-11	7.22 <sup>bcd</sup>	7.22 <sup>bcd</sup>	7.22 <sup>cd</sup>	14.07 <sup>bcd</sup>	15.18 <sup>bcd</sup>	22.96bc		
INH 14172	10.00 <sup>bc</sup>	10.00 <sup>bc</sup>	10.37 <sup>bc</sup>	16.10 <sup>bc</sup>	17.77 <sup>bc</sup>	26.66 <sup>b</sup>		
Chaite-5 (pk33319)	12.59 <sup>b</sup>	13.70 <sup>b</sup>	14.44 <sup>b</sup>	20.37 <sup>b</sup>	21.48 <sup>b</sup>	27.40 <sup>b</sup>		
Shankharika	41.11ª	45.18 <sup>a</sup>	46.29a	50.00 <sup>a</sup>	53.70 <sup>a</sup>	58.14ª		
Mean	10.24	10.96	11.22	15.85	17.53	25.03		
CV	37.78	36.52	36.64	30.29	30.21	19.86		
LSD	6.63	6.86	7.05	8.23	9.09	8.53		
Sem (±)	3.15	3.26	3.36	3.92	4.33	4.06		

Table 4: Severity of disease at different scoring date (CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 1% level of significance, Sem (±) indicates standard error)

### Categories of rice genotypes on the basis of mean AUDPC value

The mean AUDPC value ranged from 37.96 to 361.00 among the genotypes. Of the total 10 rice genotypes screened in the nursery, based on AUDPC value, none of the genotypes was highly resistant to the disease. However, 6 genotypes viz. Sabitri, Saga 4, INH12023, DR-11, Hardinath 3 and INH13140 were found resistant. Similarly, Chaite-5, INH14120 and INH14172 were moderately resistant. Similarly,

Shankharika was highly susceptible to rice blast. Significantly lowest AUDPC value was obtained in resistant check Sabitri (37.96) followed by Saga 4(52.66), which was at par with INH 12023(53.51). However, the highest AUDPC value was found in susceptible check variety Shankharika (361.00) followed by Chaite-5 (132.11). The treatments were compared using Duncan's multiple range test (DMRT)

	AUDPC	AUDPC	AUDPC	AUDPC	AUDPC	MEAN	Categor
Genotype	1	2	3	4	5	AUDPC	V
Chaite-5							
(pk33319)	105.18 <sup>b</sup>	98.51 <sup>b</sup>	139.25 <sup>b</sup>	146.48 <sup>b</sup>	171.11 <sup>b</sup>	132.11 <sup>b</sup>	MR
DD 11					114.70 <sup>bc</sup>		R
DR-11	57.77 <sup>bcd</sup>	50.55 <sup>bcd</sup>	$71.10^{cd}$	77.76 <sup>cde</sup>	de	74.37 <sup>cd</sup>	
Hardinath 3 (DR-					130.92bc		
44)	50.37 <sup>cd</sup>	46.66 <sup>cd</sup>	78.51 <sup>cd</sup>	95.92 <sup>bcde</sup>	de	$80.48^{\text{bcd}}$	R
INH 12023	29.62 <sup>cd</sup>	25.92 <sup>cd</sup>	47.40 <sup>cd</sup>	62.22 <sup>cde</sup>	102.40 <sup>cde</sup>	53.51 <sup>cd</sup>	R
INII 12140				102.40 <sup>bc</sup>	130.92bc		R
INH 13140	65.18 <sup>bcd</sup>	57.03 <sup>bcd</sup>	88.88 <sup>bcd</sup>	d	de	88.88 <sup>bcd</sup>	
INH 14120	55.53 <sup>bcd</sup>	50.52 <sup>bcd</sup>	93.30 <sup>bc</sup>	118.59 <sup>bc</sup>	155.55 <sup>bc</sup>	94.70 <sup>bc</sup>	MR
DHI 14170				103.70 <sup>bc</sup>	134.81 <sup>bc</sup>		MR
INH 14172	$80.00^{bc}$	$71.29^{bc}$	$97.77^{bc}$	d	d	97.51 <sup>bc</sup>	
Sabitri	23.70 <sup>d</sup>	20.74 <sup>d</sup>	32.59 <sup>d</sup>	41.48e	71.29e	37.96 <sup>d</sup>	R
Saga 4	35.55 <sup>cd</sup>	35.00 <sup>cd</sup>	48.88 <sup>cd</sup>	57.03 <sup>de</sup>	86.85 <sup>de</sup>	52.66 <sup>cd</sup>	R
Shankharika	345.18 <sup>a</sup>	320.18 <sup>a</sup>	385.18 <sup>a</sup>	362.96a	391.48a	361.00 <sup>a</sup>	S
Mean	84.81	77.64	108.29	116.858	149.01	107.32	
CV	37.01	36.52	31.19	30.12	23.63	28.65	
LSD	53.85	48.64	57.94	60.3792	60.42	52.75	
Sem (±)	25.63	23.15	27.58	28.73	28.76	25.11	

Table 5: Categories of rice based on AUDPC value (AUDPC: Area under disease progress curve, CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 1% level of significance, Sem (±) indicates standard error)

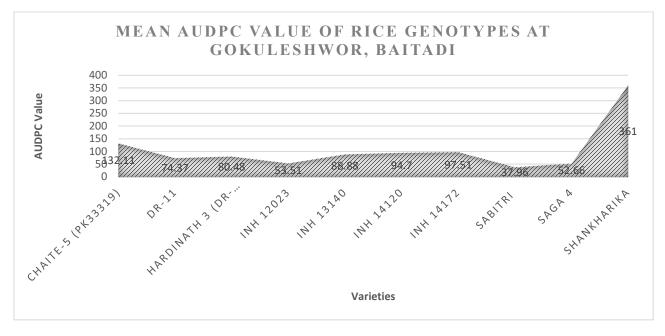


Figure 2 Mean AUDPC value of rice genotypes at Gokuleshwor, Baitadi

### **CLUSTER ANALYSIS**

Based on AUDPC values in field experiment, rice genotypes were divided into five cluster groups: cluster I (resistant genotypes), cluster II (moderately resistant), cluster III (moderately susceptible), cluster IV (susceptible), cluster V (highly susceptible). In cluster I, 6 genotypes were grouped as resistant, which represents 60% of the total

genotypes. In cluster II, 03 genotypes were grouped, as moderately resistant which represents 30% of the total genotypes. In cluster III no genotypes were grouped for moderately susceptible. In cluster IV, 1 genotype was grouped as resistant, which represent 10% of the total genotypes whereas in Cluster V, no genotypes were grouped for highly susceptible.

	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5
Cluster1	0.000	54.706	84.281	38.737	229.067
Cluster2	54.706	0.000	29.724	16.644	283.674
Cluster3	84.281	29.724	0.000	45.664	312.971
Cluster4	38.737	16.644	45.664	0.000	267.333
Cluster5	229.067	283.674	312.971	267.333	0.000

Table 6 Cluster analysis of rice genotype based on mean AUDPC value at Gokuleshwor, Baitadi

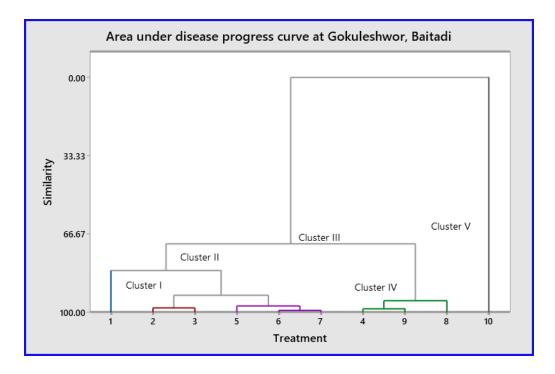


Figure 3 UPGMA dendrogram based on AUDPC of 10 rice genotypes at Gokuleshwor, Baitadi

Experiment was conducted in the upland to create ideal conditions for disease development, because water stress is known to positively affect the disease as reported by Bonman and Mackill (1988), Gill and Bonman (1988), Acharya et al. (2019). There were significant differences in mean AUDPC values among the rice genotypes at 39,47, 54, 62, 69 and 76 days after sowing (DAS). Since each genotype has a unique genetic makeup, most of them showed variable responses towards the pathogen. AUDPC values were the lowest in the resistant check rice genotype Sabitri (6.48), whereas the highest AUDPC values were recorded in the susceptible check Shankharika. Genotype-specificity of the pathogen was confirmed by the differences in the severity and AUDPC of blasts between genotypes.

During the study period, the weather at the research site was almost favourable for the development of blast disease, which included temperatures (13-37°C) and high humidity (84.66%). Results present here are comparable with those reported earlier for various locations across the country.

### **SUMMARY AND CONCLUSION**

Most farmers in Nepal lack resources, so cultivars with high resistance to blast diseases are very important. Baitadi district's Hilly region is quite backward in its use of new technology.

In this investigation, exotic and indigenous rice genetic resources were assessed for their reaction to blast disease. The present study concluded that genotypes differed significantly in blast severity and AUDPC owing to their genetic backgrounds. The results of the screening revealed that among the 10 rice genotypes tested, none were resistant to P. oryzae. A difference in disease severity was observed among rice genotypes, possibly due to variation in genetic diversity. Breeders could use those genotypes (DR-11, Hardinath 3 (DR-44), INH 12023, INH 13140, Sabitri and Saga 4) showing resistant reactions and encourage them to participate in yield evaluation trials in the future. Since the virulence of Pyricularia strains changes, it is necessary to constantly characterize any resistance genes found in these genotypes. Therefore, genotypes need to be evaluated under various environmental conditions and with various isolates before recommending release.

### **CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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