

Screening of Different Rice Genotypes against (*Pyricularia grisea*) Sacc. in Natural Epidemic Condition at Seedling Stage in Chitwan, Nepal

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Abstract

Numerous research has already established blast as the continuous and devastating threat to rice production in Nepal and on the contrary Nepalese farmers do not have efficient knowledge and understanding about the complexity of disease for the management of the blast epidemic development. The most effective physical tool seems to be provision of resistant genotypes obtained against screening of different rice genotypes: effective management practices against the complexity of blast pathogen. Experiments were conducted for screening 50 rice genotypes under natural epidemic condition against seedling blast (*Pyricularia grisea*) in Randomized complete block design at Chitwan. Rice grains were sown on July 6, 2015 at field and disease scoring was done on 21, 24, 27 and 30 DAS; Scoring was done based on the standard scale of 0-9 developed by IRRI. Based on the result Taichung-176 and Sankharika showed the highest percentage of incidence and severity of disease. Sabitri, however, was found to be most resistant among genotypes with the lowest percentage of incidence and severity during observation.

Keywords: Rice blast; *Pyricularia grisea*; Sabitri; Blast susceptible

Introduction

Blast is caused by *Pyricularia grisea*. It occurs in nearly all rice growing areas of the world. It is considered the most serious disease in both temperate and tropical rainfed environments. With increasing nitrogenous fertilizer and higher plant density, blast is known to be devastating [1]. Blast was first recorded in China in 1637. The causal organism was named *Pyricularia oryzae* by Cavara in Italy in 1891 and was renamed by Rossman 1990 to *Pyricularia grisea* [2].

Rice is truly a crop of global importance. Almost half the world's population, particularly in east and south east Asia, depends on rice as the major source of nutritional calories [3]. Every year it is estimated that rice blast destroys food more than enough to eat for 60 million people and 50% of the rice yield is lost in the field by the occurrence of blast [4]. Rice is the most prestigious food crop of Nepal. It is grown in a diverse environment ranging from tropical plains to foot of the mountain and higher elevation (3050 masl) in Chhumchure, Jumla. Nepal is considered as one of the origin centers of rice. It is one of the most important cereal crops in Nepal. Rice is grown in 1440 thousand ha and the productivity is 2.56 t/ha. It contributes nearly 20 per cent to the agricultural gross domestic product. Nepal has released fifty five rice varieties with full package of growing practices in the last 40 years. The coverage by improved varieties is 85 percent of the total rice cultivated land. Popularly cultivated improved varieties are Radha-4, Radha-12, Masuli, Sabitri, CH-45, Bindeswori in Terai, Khumal-4, Khumal-11, Taichung-176, Chaining in mid-hills and Chandanaath-3 in high hills (NARC 2014). Radha-12, Sabitri, Janaki possess higher level of resistance [5]. Seedlings of high yielding Masuli were affected in late June in Saradanagar, Rampur, Kiranganj, Mangalpur and Ratanagar area of the Chitwan district [6]. Radha-12 had 7 fold less neck blast than Masuli whereas other genotypes showed less neck blast than Masuli [5].

Rice blast genetic analysis confirmed gene for gene interaction that controls cultivar specificity in fungal plant interactions. Nuclear and mitochondrial genomes molecular analyses suggest that *M. grisea* pathogens remain in nature as different types of genetically distinct asexually reproducing populations [7]. An understanding of the molecular mechanism that governs host specificity should aid in the

development of new strategies for control of rice blast [8]. Mechanisms controlling host species specificity differ in basic compatibility factors that allow pathogens to infect particular species. PWL2 host species specificity genes have properties analogous to classical avirulence genes, which function to prevent infection of certain cultivars of particular host species. The PWL2 gene encodes a glycine-rich, hydrophilic protein with a putative secretion signal sequence [8]. Blast, caused by *Pyricularia grisea* Sacc has been a continuous threat to rice production in Nepal [9,10]. Blast epidemics result in a complete loss of seedlings in the seedbed [6,11-16].

Varying tools have been used as a blast management toolkit such as knowledge tools, communication, physical and policy tools. Each tool is rationalized in terms of having an effect either on initial inoculum or disease (x_0), the epidemic infection rate (R) or duration of epidemic (D). Certain tools like biological control agents and confirmatory serological tools are still unknown to blast control. Nepalese farmers do not have efficient knowledge and understanding about the use of fungicide and the effect of nutrient (nitrogen, phosphorus, potassium), and non-nutrient (silicon) amendments on blast epidemic development. Water management to reduce stress on plants at blast susceptible stages [17] are still in the dark to the Nepalese common farmers. Cultural practices seem ineffective due to no clear cut fallow period between any two rice seasons making blast pathosystem a continuous pathosystem and given the dispersal pattern of conidia initial inoculum will always be available for matching alloinfection. Hence, the most effective physical tool seems to be provision of resistant genotype. Seed possessing resistant genes to blast have been the basis for plant protection for centuries [13].

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Materials and Methods

Experimental setup

Field experiment was set up in Agronomy farm of IAAS, Rampur, Chitwan. The experiment was conducted in single factorial RCBD design with 3 replication. Each plot was 5 mX1 m, in each replication 50 rows was made to sow the 50 different genotypes. Seed was sown randomly in such a way that, genotypes was not repeated in line in the replications. Seeding was done in 2nd week of July.

Genotypes used as treatment:	
1) NR 10676-B-1-3-3-3	26) Madhya dhan -845
2) NR 10490-89-3-2-1	27) Sona Mansuli
3) NR 11105-B-B-27	28) Radha-22
4) NR 11052-B-B-B-B_6	29) Sankharika*
5) 08FAN10	30) Radha-11
6) NR10769-4-2-2	31) IR 87751-20-4-4-2
7) NR 10676-B-5-3	32) NR 11111-B-B-23
8) NR 11011-B-B-B-B-3	33) Manjushree-2
9) NR 11011-B-B-B-B-2	34) Taichung-176
10) Sugandha-2	35) Khumal-4
11) NR 11050-B-B-B-1	36) Kalo Masino
12) NR 11037-B-B-B-B-5	37) Radha-4
13) NR 11022-2-2-3-3-1	38) Sawa Mansuli
14) NR 11092-B-B-B-12	39) Ramdhan
15) NR 11042-B-B-B-1-1	40) Sabitri**
16) NR 11082-B-B-B-5-3	41) Bindheshwari
17) NR 11016-B-5-2-3-3-2	42) Sukkah-3
18) NR 11011-B-B-B-B-6	43) Savasab-1
19) NR 11139-B-B-B-21	44) Jethi Mansuli
20) NR 11050-B-B-B-B-2	45) Basmati Seto
21) NR 11115-B-B-31-3	46) Pusa Basmati
22) NR 11130-B-B-B-19	47) Sarju
23) NR 11105-B-B-16-2	48) Hardinath
24) NR 11111-B-B-23-2	49)Kanchi Mansuli
25) NR 11109-B-B-12-3-2	50) Makwanpure
Note: * = Susceptible Check	
** = Resistant Check	

Observation

Disease assessment

Disease incidence: Appearance of first symptoms of disease among all the plants germinated will be recorded. Here, total no. of plants in a row and Number of plants showing the symptoms will be recorded (Figures 1 and 2).

Percent disease incidence will be calculated by using the formula:

$$\% \text{ DI} = \frac{\text{Number of infected plants}}{\text{total number of plants germinated}} \times 100\%$$

Disease scoring: Disease scoring will be done according to standard scoring scale developed by International Rice Research Institute (IRRI) using a scale of 0-9.

- Small brown specks of pin point size.
- Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin, lesions are mostly

found on the lower leaves.

- Lesion type is the same as in 2, but significant number of lesion are on the upper leaves.
- Typical susceptible blast lesions, 3 mm or longer, infecting less than 4% of the leaf area.
- Typical susceptible blast lesions, 3 mm or longer, infecting less than 4-10% of the leaf area.
- Typical susceptible blast lesions, 3 mm or longer, infecting less than 11-25% of the leaf area.
- Typical susceptible blast lesions, 3 mm or longer, infecting less than 26-50% of the leaf area.
- Typical susceptible blast lesions, 3 mm or longer, infecting less than 51-75% of the leaf area, many leaves dead.
- Typical susceptible blast lesions, 3 mm or longer, infecting more than 75% of the leaf area

Disease intensity/index: Disease severity will be scored on the basis of standard scoring scale developed by International Rice Research institute (IRRI). 5 plants from each row showing the symptoms will be selected at random for observation and scored at a scale of 0-9 and average will be taken.

Disease severity will be calculated as:

$$\text{Disease severity}\% = \frac{\text{sum of all numerical rating}}{\text{no. of plants observed}} \times 100$$

The plants will be scored after 21 days of sowing and 3 days interval henceforth up to 30 days of sowing, giving 4 readings.

AUDPC:

$$\text{AUDPC} = \sum_i^{n-1} \left[\left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i) \right]$$

Where, y_i : initial infection percentage (disease score)

y_{i+1} : progressive infection percentage

$t_{i+1}-t_i$: time interval between the readings

Area under disease progressive curve

Total AUDPC value lied in the range of 17.78-210%. AUDPC 1&2 were calculated based on the disease severity percentage and calculated using formula as presented in the materials and methods above. Lowest total AUDPC was observed on Sabitri whereas highest was observed on Taichung-176 followed by pusa basmati, NR 11111-B-B-23-2, Sankharika and NR 10490-89-3-2-1. Based on the Total AUDPC value rice genotypes were listed on the five categories from resistant to highly susceptible which are shown in the Tables 1 and 2.

Discussion

Disease incidence was observed at 21, 24, 27 and 30 DAS. At 21 DAS, lowest disease incidence was observed in Kanchi Masuli followed by sabitri whereas highest disease incidence was observed in NR 10676-B-1-3-3-3-2 followed by sankharika and Pusa basmati. Similarly, during 24 DAS, 27 DAS and 30 DAS lowest disease incidence was observed in Sabitri whereas highest disease incidence at 24 DAS NR 11050-B-B-B-B-2 followed by NR 11050-B-B-B-B-1, NR 11016-B-5-2-3-3-2; at 27 DAS, NR 11050-B-B-B-B-2 followed by NR 11050-B-B-B-B-2 and NR 11016-B-5-2-3-3-2 were found to be highest and during 30 DAS NR 11050-B-B-B-B-2 followed by NR 11050-B-B-B-1 and NR 11105-

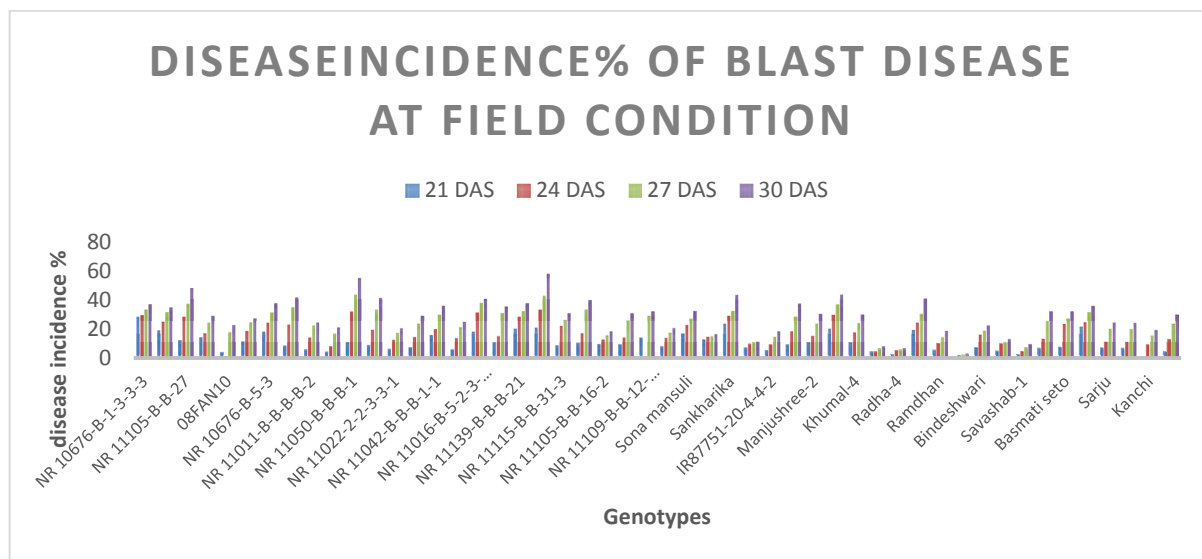


Figure 1: Disease incidence was observed at 21, 24, 27 and 30 DAS. At 21 DAS, lowest disease incidence was observed in Kanchi Masuli followed by sabitri whereas highest disease incidence was observed in NR 10676-B-1-3-3-3-2 followed by sankharika and Pusa basmati. Similarly, during 24 DAS, 27 DAS and 30 DAS lowest disease incidence was observed in Sabitri whereas highest disease incidence at 24 DAS NR 11050-B-B-B-B-2 followed by NR 11050-B-B-B-B-1, NR 11016-B-5-2-3-3-2; at 27 DAS, NR 11050-B-B-B-B-2 followed by NR 11050-B-B-B-B-2 and NR 11016-B-5-2-3-3-2 were found to be highest and during 30 DAS NR 11050-B-B-B-B-2 followed by NR 11050-B-B-B-B-1 and NR 11105-B-B-27 were found highest.

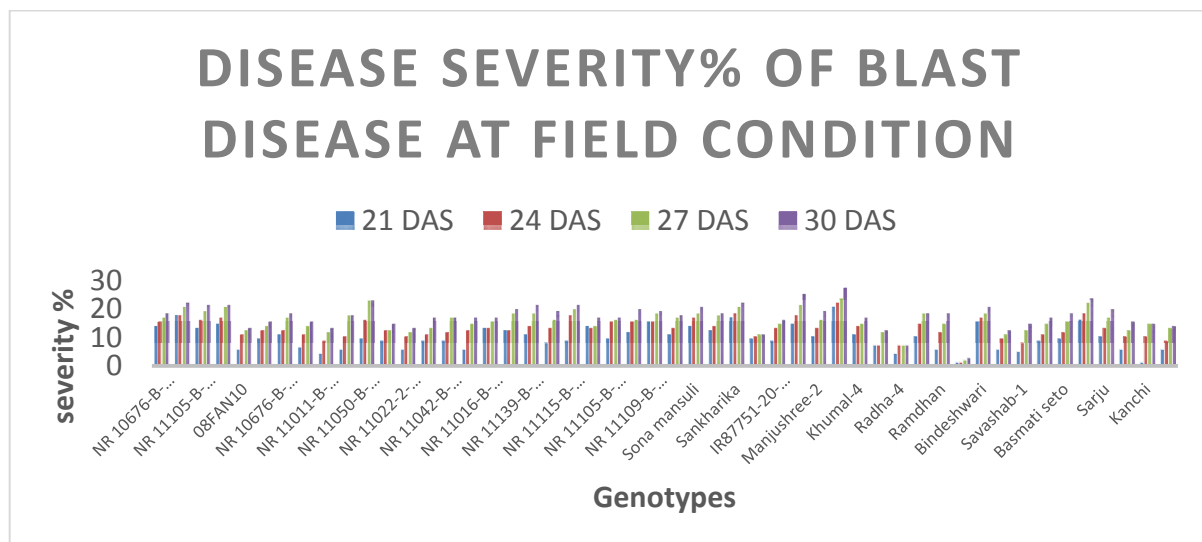


Figure 2: Based on the standard scoring scale of 0-9 developed by IRRI, disease were scored at 21, 24, 27 and 30 DAS and disease severity % was calculated as above mentioned formula(materials and methods). During 21 DAS lowest disease severity was observed on kanchi masuli followed by sabitri whereas on all other days of observation lowest disease severity % was observed on sabitri. However, during all days of observation Taichung-176 showed highest disease severity percentage along with varieties sankharika, pusa basmati, NR 11050-B-B-B-B-1 and NR 11111-B-B-23-2.

B-B-27 were found highest [18-20]. Sabitri was reported to be most resistant by Chaudary et al. [5]. Genotypes starting with NR initial were breeding lines developed by Nepal Agriculture Research Council (NARC) as they were developed for high hills and this experiment being conducted in the terai region might had induced blast incidence on these lines due to unsuitable temperature to the genotypes.

During 21 DAS lowest disease severity was observed on kanchi masuli followed by sabitri whereas on all other days of observation lowest disease severity % was observed on sabitri. However, during all days of

observation Taichung-176 showed highest disease severity percentage along with varieties sankharika, pusa basmati, NR 11050-B-B-B-B-1 and NR 11111-B-B-23-2. Experiment by Manandher et al. [11] presented sankharika to be most susceptible variety and established that it is adversely affected by blast pathogen whereas Taichung-176 were found to be highly susceptible variety by Manandhar et al. [9]. Kumar et al. [21] reported pusa basmati as most susceptible to blast disease.

Similarly sabitri showed lowest level of AUDPC value and was categorized as the resistant genotype along with Radha-4 which is

Genotypes	AUDPC1	AUDPC2	AUDPC 3	TOTAL AUDPC
NR 10676-B-1-3-3-3	44.44 ^{bcd}	48.49 ^{bcd}	53.33 ^{bcd}	146.67 ^{bcd}
NR 10490-89-3-2-1	53.33 ^{ab}	57.78 ^{abcd}	64.44 ^{abc}	175.56 ^{abcd}
NR 11105-B-B-27	44.44 ^{bcd}	53.33 ^{abc}	61.11 ^{abc}	158.89 ^{bcd}
NR 11052-B-B-B-B_08FAN10	47.78 ^{abcde}	56.67 ^{abcde}	63.33 ^{abc}	167.78 ^{abcde}
NR10769-4-2-2	25.56 ^{hijkl}	35.56 ^{ghijklmn}	38.89 ^{ghijkl}	100.00 ^{ijklm}
NR 10676-B-5-3	33.33 ^{defghijkl}	40.00 ^{efghijkl}	44.44 ^{cdefghij}	117.78 ^{efghijkl}
NR 11011-B-B-B-B-3	35.56 ^{cdefghijk}	44.44 ^{cdefghijkl}	53.33 ^{bcd}	133.33 ^{bcd}
NR 11011-B-B-B-B-2	26.67 ^{hijkl}	37.78 ^{ghijklm}	44.44 ^{cdefghij}	108.89 ^{ijklm}
Sugandha-2	20.00 ^{klm}	31.11 ^{klm}	37.78 ^{ghijk}	88.89 ^{lm}
NR 11050-B-B-B-1	24.44 ^{hijkl}	42.22 ^{defghijkl}	53.33 ^{bcd}	120.00 ^{efghijkl}
NR 11037-B-B-B-B-5	38.89 ^{bcd}	58.89 ^{abc}	68.89 ^{ab}	166.66 ^{abcde}
NR 11022-2-2-3-3-1	32.22 ^{defghijkl}	37.78 ^{ghijklmn}	41.11 ^{efghijk}	111.11 ^{hijklm}
NR 11092-B-B-B-12	24.44 ^{hijkl}	33.33 ^{klm}	37.78 ^{ghijk}	95.56 ^{klm}
NR 11042-B-B-B-1-1	30.00 ^{efghijkl}	36.67 ^{ghijklm}	45.56 ^{cdefghij}	112.22 ^{ghijkl}
NR 11082-B-B-B-5-3	31.11 ^{efghijkl}	43.33 ^{cdefghijkl}	51.11 ^{bcd}	125.56 ^{efghijkl}
NR 11016-B-5-2-3-3-2	27.78 ^{ghijkl}	41.11 ^{efghijkl}	47.78 ^{cdefghij}	116.67 ^{ghijkl}
NR 11011-B-B-B-B-6	40.00 ^{bcd}	43.33 ^{cdefghijkl}	47.78 ^{cdefghij}	131.11 ^{1 cdefghijkl}
NR 11139-B-B-B-21	37.78 ^{bcd}	46.67 ^{bcd}	57.78 ^{abc}	142.22 ^{bcd}
NR 11050-B-B-B-B-2	37.77 ^{bcd}	48.89 ^{bcd}	60.00 ^{abcde}	146.67 ^{bcd}
NR 11115-B-B-31-3	32.22 ^{defghijkl}	44.44 ^{cdefghijkl}	53.33 ^{bcd}	130.00 ^{cdefghijkl}
NR 11130-B-B-B-19	40.00 ^{bcd}	56.67 ^{abcde}	62.22 ^{abcd}	158.89 ^{bcd}
NR 11105-B-B-16-2	41.11 ^{bcd}	41.11 ^{efghijkl}	45.56 ^{cdefghij}	127.78 ^{defghijkl}
NR 11111-B-B-23-2	37.78 ^{bcd}	47.78 ^{bcd}	50.00 ^{bcd}	135.56 ^{bcd}
NR 11109-B-B-12-3-2	41.11 ^{bcd}	47.78 ^{bcd}	54.44 ^{bcd}	143.33 ^{bcd}
Madhya dhan -845	46.67 ^{bcd}	51.11 ^{bcd}	56.67 ^{abcde}	154.44 ^{bcd}
Sona mansuli	36.67 ^{bcd}	45.56 ^{bcd}	52.22 ^{bcd}	134.44 ^{bcd}
Radha-22	46.67 ^{bcd}	53.33 ^{abcde}	58.89 ^{bcde}	158.89 ^{bcd}
Sankharika	40.00 ^{bcd}	47.78 ^{bcd}	54.44 ^{bcd}	142.22 ^{bcd}
Radha-11	53.33 ^{ab}	58.89 ^{abc}	64.44 ^{abc}	176.67 ^{abcd}
IR87751-20-4-4-2	30.00 ^{efghijkl}	32.22 ^{klm}	33.33 ^k	95.56 ^{klm}
NR 11111-B-B-23	33.33 ^{defghijkl}	42.22 ^{defghijkl}	46.67 ^{cdefghij}	122.22 ^{efghijkl}
Manjushree-2	48.89 ^{abcde}	58.89 ^{abc}	70.00 ^{ab}	177.78 ^{abc}
Taichung-176	35.56 ^{cdefghijk}	44.44 ^{cdefghijkl}	53.33 ^{bcd}	133.33 ^{bcd}
Khumal-4	64.44 ^a	68.89 ^a	76.67 ^a	210.00 ^a
Kalo masino	37.78 ^{bcd}	43.33 ^{cdefghijkl}	47.78 ^{cdefghij}	128.89 ^{cdefghijkl}
Radha-4	22.22 ^{kl}	28.89 ^{lm}	36.67 ^{hijk}	87.78 ^{lm}
Sawa mansuli	17.78 ^m	22.22 ^m	22.22 ^{kl}	62.22 ^{mn}
Ramadhan	37.78 ^{bcd}	50.00 ^{bcd}	55.56 ^{bcd}	143.33 ^{bcd}
Sabitri	26.67 ^{hijkl}	40.00 ^{efghijkl}	50.00 ^{bcd}	116.67 ^{ghijkl}
Bindeshwari	4.44 ^m	5.56 ⁿ	7.77 ⁿ	17.78 ⁿ
Sukkah-3	48.89 ^{abcd}	53.33 ^{abcde}	58.88 ^{abcde}	161.11 ^{abcde}
Savashab-1	23.33 ^{ijkl}	31.11 ^{klm}	35.55 ^{jk}	90.00 ^{lm}
Jethi mansuli	20.00 ^{klm}	31.11 ^{klm}	41.11 ^{efghijk}	92.22 ^{lm}
Basmati seto	30.00 ^{efghijkl}	38.89 ^{efghijkl}	47.78 ^{cdefghij}	116.67 ^{ghijkl}
Pusa basmati	32.22 ^{defghijkl}	41.11 ^{efghijkl}	51.11 ^{bcd}	124.44 ^{efghijkl}
Sarju	52.22 ^{abc}	61.11 ^{ab}	68.89 ^{ab}	182.22 ^{ab}
Hardinath	35.56 ^{cdefghijk}	45.56 ^{bcd}	55.56 ^{bcd}	136.67 ^{bcd}
Kanchi	24.44 ^{hijkl}	34.44 ^{hijklm}	42.22 ^{defghijk}	101.11 ^{ijklm}
Makwanpure	17.78 ^m	37.78 ^{efghijklm}	44.44 ^{cdefghij}	100.00 ^{ijklm}
	22.22 ^{kl}	33.33 ^{ijklm}	41.11 ^{efghijk}	96.67 ^{klm}

Table 1: AUDPC values of rice genotypes.

Category	Range	Genotypes
Resistant	0-70	Sabitri Radha-4
Moderately resistant	71-120	Kalo masino Sukkah-3 NR 11011-B-B-B-B-2 Savashab-1 NR 11022-2-2-3-3-1 Kanchi Mansuli masuli Hardinath Radha-11 08FAN10 Makwanpure NR 11092-B-B-B-12 NR 11037-B-B-B-B-5 NR 11011-B-B-B-B-3 NR 11082-B-B-B-5-3 Ramadhan Jethi mansuli NR 10769-4-2-2 Sugandha-2

Moderately susceptible	121-140	<p>IR87751-20-4-4-2 Basmati seto NR 11042-B-B-B-1-1 NR 11130-B-B-B-19 Khumal-4 NR 11050-B-B-B-B-2 NR 11016-B-5-2-3-3-2 NR 10676-B-5-3 Manjushree-2 Madhya dhan-845 NR 11105-B-B-16-2 Sarju</p>
Susceptible	141-170	<p>Radha-22 NR 11111-B-B-23-2 NR 11011-B-B-B-6 NR 10676-B-1-3-3-3 NR 11139-B-B-B-21 Sawa Mansuli NR 11109-B-B-12-3-2 Bindeshwari NR 11105-B-B-27 NR 11115-B-B-31-3 NR 11050-B-B-B-1 Sona Mansuli NR 11052-B-B-B-B-6</p>
Highly susceptible	171-225	<p>NR 11111-B-B-23-2 Sankharika Pusa Basmati Taichung-176 NR 10490-89-3-2-1</p>

Table 2: Based on the AUDPC value rice genotypes are listed on the five categories from resistant to highly susceptible.

supported by Chaudary et al. [5] suggesting that Sabitri and Radha varieties to be resistant to blast pathogen; whereas Taichung-176 pusa basmati and Sankharika were categorized as the most susceptible varieties which coincides with the result presented by (Manandhar et al. and Manandhar et al.) and Kumar et al. [9,11,21]. Similarly, NR 11111-B-B-23-2 and NR 10490-89-3-2-1 were also categorized as the most susceptible and more conclusive result are yet to be drawn of these genotypes.

Conclusion

50 rice genotypes were sown in 6th July in Randomized complete block design at chitwan. The experiment was only limited to seedling stage and its purpose was to identify the resistant and susceptible variety among the different rice genotypes collected all over the country along with some of the breeding lines provided by the NARC khumaltar. Sankharika, Taichung-176, pusa basmati, NR 11111-B-B-23 and NR 10490-89-3-2-1 were found most susceptible and sabitri and Radha-4 were found to be resistant.

As Taichung-176, sankharika was found to be most susceptible to blast on both field and lab condition as NARC has described Taichung-176 as susceptible variety to mid-hills and sankharika to Terai region. Sabitri was found to be most resistant among all genotypes. Further research is recommended on the varieties mentioned above for further certainty; in addition, further research work such as comparison of plant yield with disease can be done and also molecular study of the plant varieties is further recommended.

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