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Research Article

Screening of rice genotypes for leaf blast resistance under field condition

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ABSTRACT

Blast, caused by *Pyricularia grisea* (Sacc.) is the most destructive disease of rice in Nepal. To identify the sources of leaf blast resistance in rice genotypes, a field experiment was conducted under natural epiphytotic condition at National Plant Pathology Research Centre (NPPRC), Khumaltar, Lalitpur, Nepal during summer season in 2018 and 2019.A total of 128 rice genotypes in 2018 and 291during 2019 including resistant check (Sabitri) and susceptible check (Shankharika/Mansuli) were tested. Field experiment was conducted in single rod row design. Leaf blast disease assessment was done according to 0-9 scale. During 2018, 59 entries were highly resistant (Score 0),34 resistant (Score 1),26 moderately resistant (Score 2-3),5 were moderately susceptible (Score 4-5),4 susceptible (Score 6-7) and none of them were highly susceptible (Score 8-9) to leaf blast. Similarly, in 2019, 6 lines were highly resistant, 70 resistant,196 moderately resistant, 15 lines were moderately susceptible, 4susceptible and none of them were highly susceptible to the disease. Only, one genotype NR2179-82-2-4-1-1-1-1 (Score 1) was found resistant in both years. Similarly, genotype NR2182-22-1-3-1-1-1 (Score 2-3) was found moderately resistant. Some of the genotypes were found resistant in 2018 which become moderately resistant in 2019, they were NR2180-20-2-5-1-1-1-1,IR97135-8-3-1-3, IR98786-13-1-2-1,NR2181-139-1-3-1-1-1-1, andIR13F402.So, findings of these resistant and moderately resistant genotypes could be used in resistant source for the development of leaf blast resistant rice varieties through hybridization in future.

Keywords: Blast, Disease assessment, Genotypes, Rice, Resistant

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INTRODUCTION

Rice blast caused by *Pyricularia grisea*, has been a continuous threat to rice production in Nepal (Manandhar, 1987). Rice is the staple food for more than half of the world's population. Worldwide, rice was grown in an area of 165.2 million ha with a total production of 741.0 million tons in the year 2016 (FAOSTAT, 2017). In Nepal, rice ranks the first position in terms of area and production, covering 1.49 million ha with total production of 5.6 million ton in the country with the productivity of 3.76 t/ha (MoALD, 2019). Government of Nepal (GoN), Nepal Agricultural Research Council (NARC) has been playing a significant role to improve the rice productivity in the country. The current production is not sufficient to meet the demand of growing population and ensure food security in the country (Shrestha et al., 2020a; Shrestha et al., 2020b).The rice productivity is greatly affected by diseases. Rice blast is locally known

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as"Maruwa Rog" in Nepali language. The disease causes 10-20% yield reduction in Nepal in susceptible varieties, but in severe case it went up to 80% (Manandhar et al., 1992). The disease is more devastating in valleys, river basins, foot-hills and hills of Nepal, although it is prevalent throughout the rice growing areas in the country. The disease has been a serious problem in rice cultivation almost every year, especially in the area where blast susceptible cultivars are grown. Because of its pressure, both the quality and quantity of rice grain and straw yield have reduced considerably. Rice blast with high pathogen plasticity and mutation rate considered as most damaging disease in rice. Blast resistance, tended to be unreliable, with resistance often failing, or broken down, under field conditions, therefore there is always continuous search for resistant donors/lines.

Although fungicides can be used to control rice blast, they generate additional costs in rice production and chemical contamination of environment and foods. Therefore, the use of resistant varieties (host plant resistance) is thought to be one of the most economically and environmentally efficient ways of crop protection. Continuous efforts have been made to combat rice disease for many years (Manandhar, 1987). The efforts mainly include development of rice varieties resistant to blast (Manandhar et al., 1985). Though many resistant varieties to *Magnaporthegrisea* have been developed, the resistance is not long lasting, because the high pathogen plasticity in the fields makes single resistance gene break down after three to five years of the cultivar release (Lang et al., 2009). So the present study was carried out to screen different rice genotypes along with resistant (Sabitri) and susceptible (Sankharika) check to find the sources of leaf blast resistance.

MATERIALS AND METHODS

Experimental site

The field experiment was conducted at field of National Plant Pathology Research Centre (NPPRC) Khumaltar, Lalitpur, Nepal during summer season in 2018 and 2019 under rain-fed conditions. The latitude, longitude and altitude of the experimental site are $27^0$39’13’’N, $85^0$19’36’’ E, and 1340 masl respectively. The rice genotypes were obtained from National Rice Research Program, Hardinath, Dhanusha which consist of improved and pipeline entries.

Experimental materials, design and setup

A total of 128and 291rice genotypes were evaluated against rice leaf blast during 2018 and 2019 respectively. Trial was conducted during summer season under natural epiphytotic condition in a rodrow design with single replication. Sabitri and Sankharika/Masuli were used as resistant and susceptible checks which were repeatedly planted in $19^{th}$and 20^{th}rows respectively. Conducive environment for blast development was created by planting four rows of maize around the experimental plot before 35 days of seeding of tested genotypes. Maize served as wind break and created humid condition inside the experimental field which allowed landing and germination of conidia of the pathogen available in the air. Similarly, two rows of Chainung-242 (susceptible local rice) variety were seeded inside the plot as spreader rows to enhance the level of inoculums. After one month, the test entries were sown in 15 cm raised dry seed bed and seeds of each genotypes were seeded separately and continuously at one meter row length with 10 cm apart. Chemical fertilizer was applied @ 100:50:0 NPK kg/ha as basal dose.

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Disease Assessment

Leaf blast disease scoring was done 25 days after sowing at seedling stage using 0-9 scale (Table 1) as described by IRRI (1996). In total, three times disease scoring was done at an interval of 10 days.

Table 1. Scale for leaf blast disease assessment

Scale	Infection	Host Response
0	No lesions observed	Highly resistant (HR)
1	Minute brownish non-sporulating spots of pin point size under lower leaves.	Resistant (R)
2	Round,slightly prolonged necrotic gray spots, of 1-2mm in diameter,with a well-defined brownish margin, little sporulating lesions mostly found on the lower leaves.	Moderately Resistant (MR)
3	Spot same as in 2, but with a notable number of spots on the upper leaves.	
4	Typically, heavy sporulating blast spots with 3 mm or more in length causing less than 2 % infection on leaf.	Moderately Susceptible (MS)
5	Typical blast lesions of 3 mm or longer infecting 2-10 % of the leaf area	
6	Typical blast lesions of 3 mm or longer infecting 11-25 % of the leaf area	Susceptible (S)
7	Typical blast lesions of 3 mm or longer infecting 26-50 % of the leaf area	
8	Typical blast lesions of 3 mm or longer infecting 51-75 % of the leaf area	Highly Susceptible (HS)
9	Typical blast lesions of 3 mm or more longer infecting more than 75 % leaf area	

(*Source: IRRI,1996)*

Statistical Analysis

Data were analyzed statistically using Microsoft Excel package. Clustering of rice genotypes was done in R-studio using r-package ape ver. 5.4-1 (Paradis and Schliep, 2019).

RESULTS

Meteorological information

The weather parameters like temperature, relative humidity (RH) and rainfall varied during the experimental period. In 2018, the maximum, minimum temperature and RH were recorded as 29.23ºC, 19.17ºC and 86.25% respectively. The rainfall ranged from 2.9 mm to 165.4 mm. The rainfall was recorded highest during the last week of July and decreased thereafter and was least at third week of September (Figure 1). Similarly in 2019, the maximum temperature recorded was 29.9ºC, minimum temperature 20ºC and RH 86.3%. The rainfall was recorded from 7 mm to 242 mm. Maximum rainfall was in the second week of July and minimum in first week of August (Figure 2).

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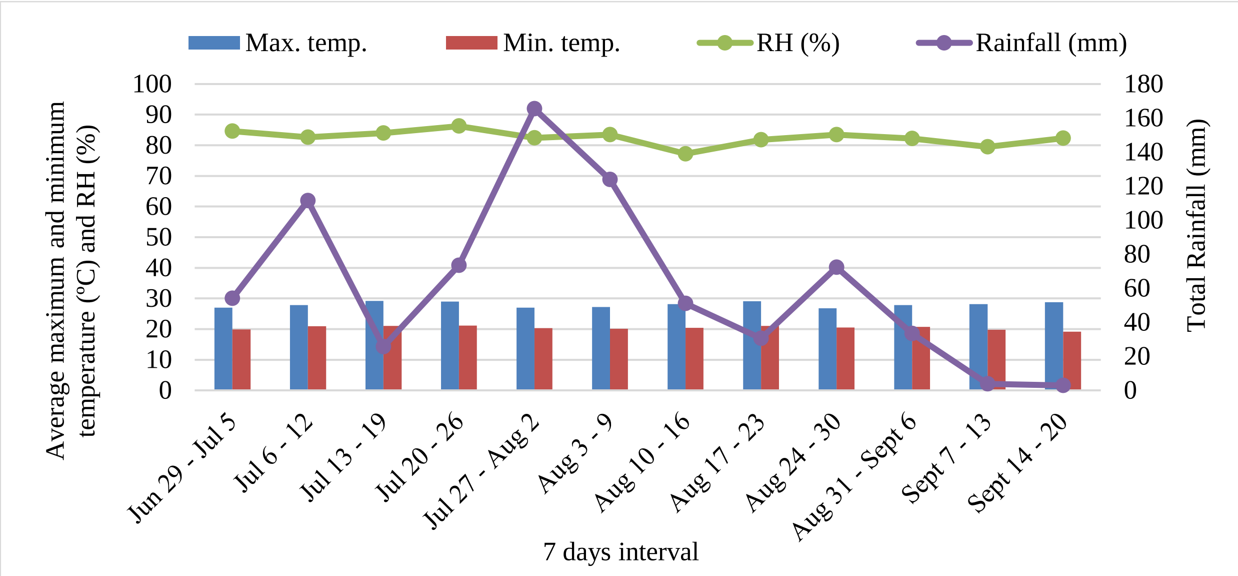

Figure 1: Weather data during experimental period (June 29 to September 20, 2018) at Khumaltar, Lalitpur

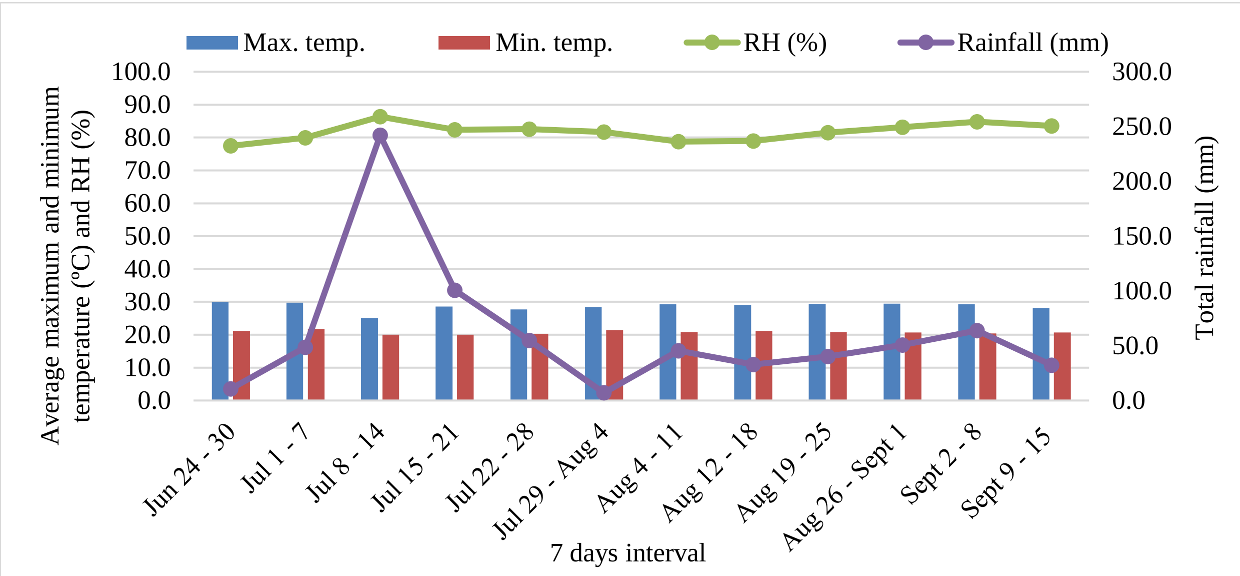

Figure 2: Weather data during experimental period (June 24 to September 15, 2019) at Khumaltar, Lalitpur

Rice genotypes categorization on the basis of disease score

During 2018,128 lines were evaluated against leaf blast disease. Among the genotypes, 59 were highly resistant, 34 lines were resistant, 26 lines were moderately resistant, 5 were moderately susceptible and 4 lines were susceptible. None of them were highly susceptible to leaf blast (Figure 3, Table 2, Table 3).Lowest disease severity was observed in Sabitri (Score 0)followed by other 58 entries whereas Sankharika (Score 6),IR108541:6-36-1-20-B-B(Score 6), Masuli(Score 7) and IR108541:12-27-1-11-B-B (Score 7) were susceptible.

Similarly, in 2019, out of 291 genotypes, 6 lines were highly resistant, 70 were resistant, 196 moderately resistant, 15were moderately susceptible,4 lines PANT DHAN-2 (Score 6),

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NR2192-16-1-1-1-1 (Score 6), NR2199-54-2-1-2-1 (Score 6) and Masuli/Sankharika (Score 7) were susceptible and none of them were highly susceptible to disease(Figure 3, Table 2, Table 3).

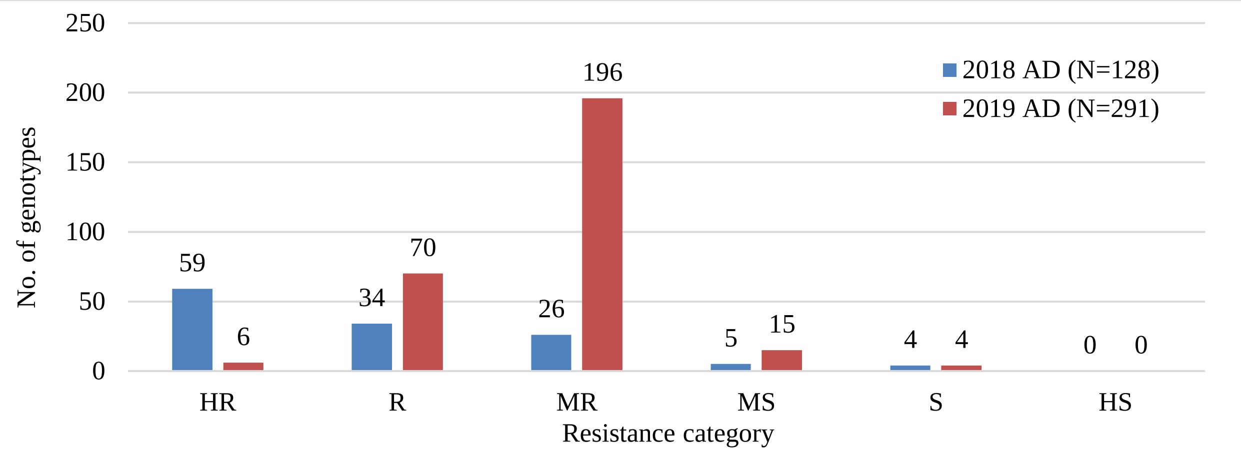

Figure 3: Rice genotypes showing different level of resistance to leaf blast during 2018 and 2019 at NPPRC, Khumaltar, Lalitpur

Table 2: Rice genotypes showing resistant response for leaf blast disease at NPPRC, Khumaltar, Lalitpur in 2018 and 2019

Experimental year	Resistant (R)Genotype (Score 1)
2018	NR2180-20-2-5-1-1-1-1,IR97135-8-3-1-1,NR2182-4-4-3-2-1-1,NR2188-3-2-4-1-1, NR2170-150-5-3-2-1-1-1,NR2175-66-2-3-1-1, NR2157-144-13-1-2-4-5, NR 2179-82-2-4-1-1-1-1, NR 2168-65-1-1-1-1-1-1-1, NR2182-58-1-3-1-1-1, NR2182-33-3-2-1-1-1, IR12F578, IR102885-2-74-17-2-3, NR2157-144-1-3-1-1, NR2157-122-1-2-1-1-1, 2015SA-22, B11598C-TB-2-1-B-7, IR14L537, IR14L546, IR97135-8-3-1-3, IR98786-13-1-2-1, IR14D198, IR96279-39-3-1-2, IR95809-25-1-1-1, IR98846-2-1-4-3, Radha-4, Hardinath-3, IR86635-B-B-25-4, NR2181-139-1-3-1-1-1-1, IR99739-2-1-1-2-1, IR98785-10-1-1-3, IR13F228, IR13F402 and IR108541:6-36-3-9-B-B.
2019	SABITRI (RC),IR16L1421,IR103587-22-2-3-B,GSR310,TP536,IR14L363,IR106529-2-40-3-2-B,NR2179-82-2-4-1-1-1-1,TP30583,TP30588,TP30549, SVIN188, TP29784, IR99742:2-2-22-4-1-9-B, TP30251, IR97073-32-2-1-3, TP30582, SVIN255, IR98853-6-1-3-2, NR2157-122-1-2-1-1-1, IR14L145,IR15L1065, IR100638-12-AJY3-CMU2, IR106523-25-34-3-2-B-23-1, NR2181-465-1-1-1-1-1, NR2182-58-1-3-1-1, NR2192-66-3-1-3-1, IR106523-25-34-3-2-B-5-3, TP26777,NR2187-25-2-3-3-1, NR2175-66-2-3-1-1,NR2188-13-5-2-5-1, NR2187-32-4-6-1-1,NR2187-6-2-2-1-1,HHZ23-SAL13-4-SAL,SARBATI, IR16L-1753, IR98849-2-1-4-3,IRKASTURI BASMATI,PUSA-1509, IR11L-412,NR2189-42-1-1-1-1-1,IR16L-1844,IR16L-1591,IR17L-1420, IR16L-1421,IR 12A-173, IR14L-261,IR 93346-1-B-B-7-1RGA-2-RGA,IR -11-159, IR101-152,HHZ7-DT3-Y1-Y1, NR2184-17-1-1-1-1, NR2187-33-1-3-5-1, SVIN056, SVIN083, SVIN066, NR2199-19-1-1-1-1,NR 2184-34-1-1-2-1,IR106523-25-34-3-3-B-45-1, IR16L1591,IR16L1742, NR219187-1-1-3-1, NR2210-11-1-2-1-1,SVIN074,SVIN156,SVIN244,SVIN 224, SVIN279 and TP 30531.

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Table 3: Rice genotypes showing moderately resistant response for leaf blast disease at NPPRC, Khumaltar, Lalitpur in 2018 and 2019

Experimental year	Moderately Resistant (MR) Genotype (Score 2-3)
2018	IR15D110,Plant-1,Plant-2, IR95786-9-2-1-2, IR108196-1-B-B-3-2-5,NR82-31-1-1-2-1-1,NR2179-6-1-1-4-1-1,Lalka Basmati,IR 91326-7-13-1-1, IR103575-76-1-1-B, IR08L181, Radha-11, Anmol masuli, IR05F102, IR108541:1-23-1-14-B-B, IR108541:1-70-1-21=B-B,IR108541:6-29-1-9-B-B,IR108541:6-29-3-3-B-B,IR108541:6-36-1-19-B-B, IR108541:6-36-3-8-B-B, IR108541:8-66-1-4-B-B, IR108541:8-66-2-12-B-B,NR2175-34-1-3-1-1-1-1-1,Kalanuniya, NR 2182-22-1-3-1-1-1 and IR 103588-77-1-2-3.
2019	IR15T1133, IR99993-B-B-RGA-1RGA-2RGA, IR14L245,TP30566,IR101465-5-25, TP30539, TP24172, NR2193-6-3-1-1-1, NR2169-10-1-1-6-2-1-3-1, NR2187-33-1-2-1-1-1, IR15D1031, NR2187-33-2-3-4-1,TP30528,TP29766,IR15L1018,IR14D134, SVIN123, SVIN207,NR2184-23-3-1-2-1,NR2182-33-3-2-1-1-1,TP30257,IR99742:2-11-17-1-9-B,IR103575-76-1-1-B, IR12L353, IR14L560,IR14L537,IR98786-13-1-2-1, SVIN238,HHZ26 DT1-L11-L11,HHZ25-DT9-Y1-Y1,IR13F402, NR2181-139-1-3-1-1-1-1-1, NR2170-5-5-1-6-1-1-3-1,IR95784-21-1-1-2,IR12L355,IR97135-8-3-1-3,IR1065232534-3-2-B-44-1, IR106523-25-34-3-2-B-1-2,IR106523-25-34-3-2-B-44-3,IR106523-25-34-3-2-B-2-2, IR106523-25-34-3-2-B-1-1,NR 2181-60-4-1-2-1-1-1-1, NR2158-13-1-2-4-5,NR2187-2-2-2-3-1, TP30617, NR2182-31-1-1-2-1-1, NR2188-13-3-4-3-1, NR2168-44-2-1-1-1-2-1-1, NR2157-144-1-3-1-1,SVIN096,NR2184-187-1-1-1-1,NR2191-172-2-1-1-1, NR2191-1-2-3-2-1, NR2184-56-1-1-1-1,NR2181-15-1-1-6-1-1-1,NR2187-6-2-2-1-1,NR2187-25-2-4-3-1, NR2187-32-4-2-2-1,SUGANDHIT DHAN 1, NR2175-34-1-3-1-1-1-1,DEGORA, IR16L-1636, IR16L-1792, IR16L-1657, IR17L-1365, IR17L-13837, IR17L-1481, IR16L-1801, IR83373-13-2-3-3, IR-11N313,NR2184-149-1-14-1-1,IR16L-1795,IR17L-1314, IR17L-1571, IR17L-1317,HHZ2-DT7-DY1, IR14F-717, IR14L-245,IR16L-1411, IR11A-106, IR-11A-151,IR-06-151, IR09L-270,HHZ10-DT7-Y1-Y1,NR2158-13-1-2-4-5,NR2187-25-1-2-4-1,NR2187-33-2-3-4-1,NR2189-7-1-1-1-1,NR2189-11-4-1-2-1, NR2191-221-4-1-1, NR2199-54-2-1-6-1, NR2212-3-2-4-1-1,SVIN093,SVIN115,SVIN084,SVIN096,SVIN102,NR 2189-1-1-1-2-1,NR 2191-6-2-1-2-1,NR 2191-6-2-4-5-1,NR 2191-6-2-6-2-1,NR2191-18-1-3-4-1, NR2192-21-1-1-1-1,NR2200-8-1-1-2-1,IR106523-25-34-3-2-B-1-2, IR16L1755, IR16L1844, IR16L1836,IR16L1795, IR16L1661, IR16L1637, IR16L1678, NR2187-6-5-1-1-1,NR2188-1337-1-5-1,NR2192—178-1-1-1-1, NR2192-32-1-1-2-1,SVIN120,SVIN141,SVIN204,SVIN181,SVIN055, SVIN082, SVIN053,SVIN051,NR 2287-2-1-4-1-1,NR 2192-62-1-1-2-1, NR2193-22-2-1-2-1,NR2193-32-1-1-3-1,SVIN056,SVIN109,SVIN072,SVIN268,SVIN149,SVIN188, SVIN123, SVIN121,SVIN209, IR99761-196-52-2-12-B, IR16L1713, IR16L1708,SVIN277, SVIN241, SVIN224,SVIN234,SVIN238,SVIN231, IR16L1637, TP30529, TP30523, IR16L1829, NR2169-10-4-1-1-1-1-1,IR 15L1717, NR2184-6-1-1-4-1,NR2189-1-1-1-2-1, IR90020:22-283-B-4, NR2188-13-3-4-5-1, IR106523-25-34-3-1-B-45-1,NR2191-1-6-2-1-2-1,NR 2191-1-6-2-4-5-1,NR2180-20-2-5-1-1-1-1,IR16L-1831, IR17L-1323, IR15L-1008, IR15T-1133,BH5-86FNR-11R-2-11,NR-2181-465-1-1-1-1-1, NR2182-22-1-3-1-1-1, NR2286-9-1-3-1-1,NR2187-33-1-2-1-1,NR2188-9-1-1-1-1,SVIN 071, NR2184-50-1-1-1-2, NR2191-80-1-2-1-1, NR2192-7-1-1-1-1, NR2210-15-1-1-5-1,IR106523-25-34-3-2-B-1-3, IR16L1855,NR2187-4-1-2-1-1,NR2191-236-3-1-3-1,SVIN195,SVIN172, NR2184-9-2-1-3-1,NR2190-41-2-1-2-1,NR2210-9-1-1-1-1,SVIN079,SVIN108,SVIN179,SVIN168,IR 16L1743, IR16L1815, IR16L1769,SVIN 221, SVIN248 and SVIN253.

Cluster analysis

Rice genotypes were classified into five clusters viz. cluster I (highly resistant), cluster II (resistant), cluster III (moderately resistant), cluster IV (moderately susceptible) and cluster V (susceptible) based on similarity in disease reactions among 128 rice genotypes in 2018 and 291 in 2019. In cluster I, 59 rice genotypes which comprises 46% of total were highly resistant, 34 were resistant belonging to cluster II representing 27% in total during 2018. Similarly, in cluster III, 26 entries were moderately resistant which represents 20% and 5 entries were in

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cluster IV (4% of total rice genotypes) which represents moderately susceptible genotypes, whereas only 4 entries were in cluster V representing 3% of total were susceptible to leaf blast disease (Figure 4).

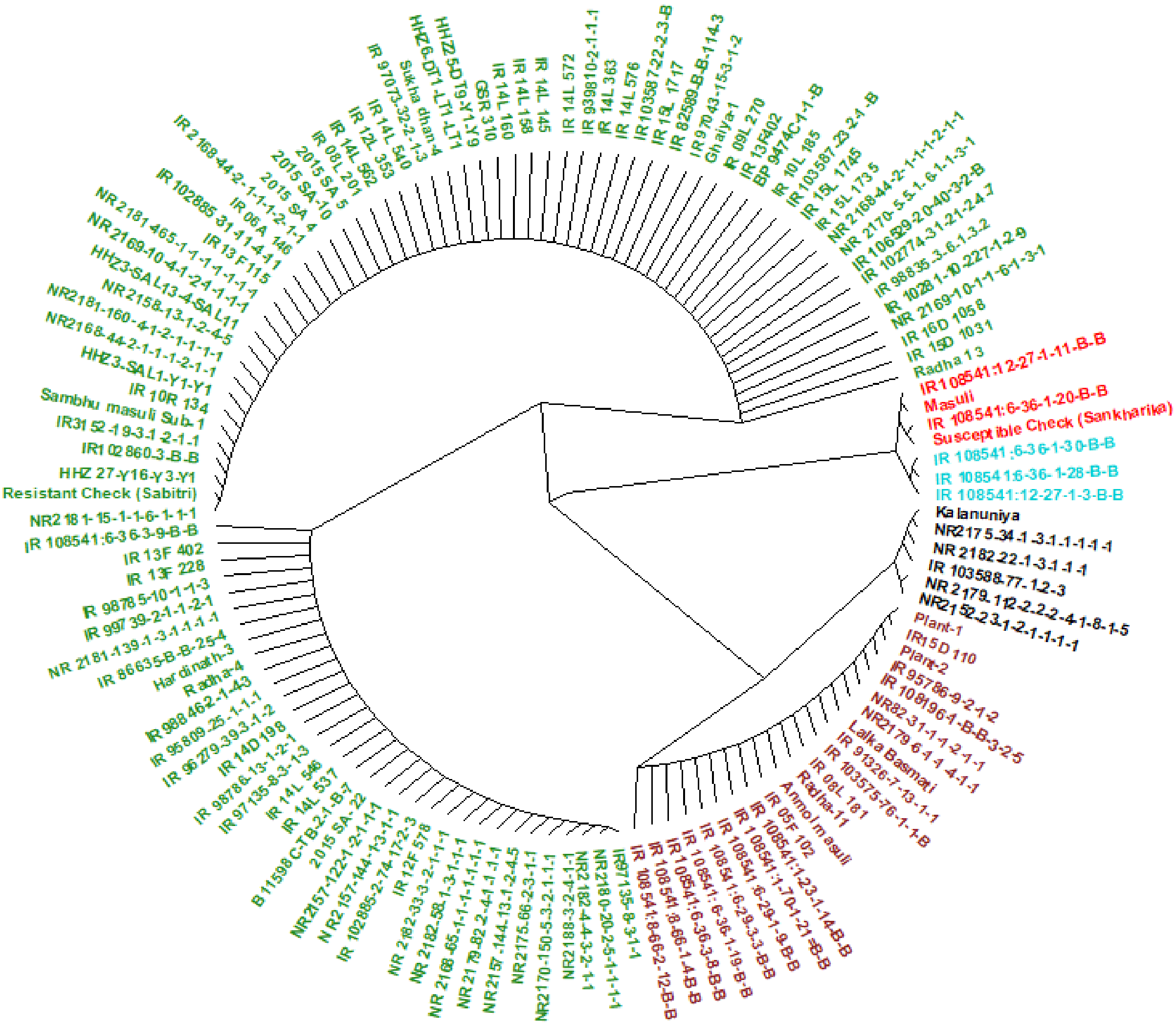

Figure 4:UPGMA clustering of 128 rice genotypes based on final scoring for leaf blast disease in 2018

In 2019, cluster I has only 6entries which represents 2% of total rice genotypes were highly resistant, 70 were resistant belonging to cluster II representing 24% in total. Similarly, in cluster III, a large no. of entries, 196 were moderately resistant which represents 67% and 15 entries were in cluster IV which represents moderately susceptible genotypes comprising 5% of total rice genotypes, whereas only 4 entries were in cluster V representing 1% were susceptible to leaf blast disease (Figure 5).

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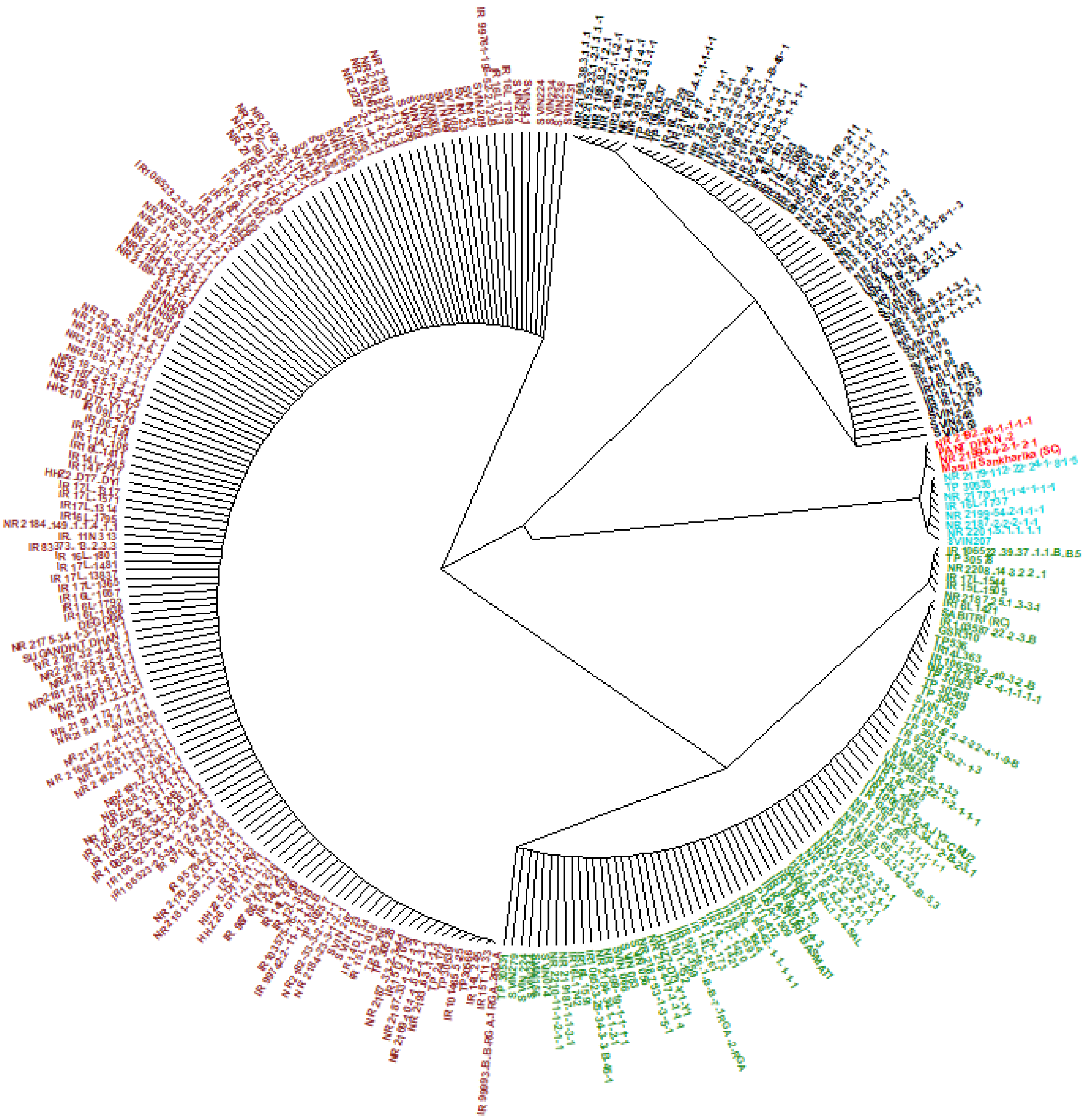

Figure 5: UPGMA clustering of 291 rice genotypes based on final scoring for leaf blast disease in 2019

DISCUSSION

Use of resistant cultivars is the most effective way to manage rice blast disease. The rice genotypes showed different level of resistance ranging from highly resistant to susceptible against leaf blast disease. None of them were highly susceptible in both years. Resistance and susceptible interaction on rice conferred by single amino acid substitution in *Pi-ta* leucine rich domain (LRD) or in the AVR-*Pi-ta*$_{176}$ protease motif that result in loss of resistant in plant and also disturb the physical interaction among them was reported by Jia et al. (2000).Thus, the rice genotypes used in this study having different genetic background showed different interaction to leaf blast. Such result was also supported by the work of Koh et al. (1987), Chaudhary (2001) and Puri et al. (2006). Blast is damaging under tropical low land condition (Bonman et al., 1989). Thruston (1998) reported upland dry seedbed was more favorable in

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blast development at seedling stage. Bonman and Mackill (1988), and Gill and Bonman (1988) also mentioned water stress enhance lesion size and disease severity in case of blast.

Screening of rice genotypes showed various level of leaf blast resistance in the tested genotypes during 2018 and 2019.Most of them were highly resistant, resistant and moderately resistant to blast in both years indicating good sources of resistance in the available genotypes. During 2018 most of the entries showed highly resistant than in 2019 which might be host specificity of pathogen, climatic factor or genetic character of genotypes. Environment influences the expressions of varieties develop from horizontal resistance and thus result in durable resistance (Suh et al., 2009). Besides, these other factor like moisture stress and excessive levels of nitrogenous fertilizer increases rice blast disease severity (Prabhu et al., 1996).Varieties having resistance to both leaf and neck blast have been most widely used for rice blast management (Bonman et al., 1992). Several researchers have reported Sabitri having higher degree of blast resistance (Chaudhary et al., 2005and Joshi et al., 2017).The most important challenge in front of the rice scientists is to do accumulation of resistance genes which could be used against continuously evolving and geographically diverse races of *P. oryzae* (Sharma et al., 2012). Thus, such studies need to be continued to monitor virulence of the blast pathogen and to identify new sources of resistance which will help in national breeding program for the development of blast resistant rice varieties in future.

CONCLUSION

Rice blast caused by *Pyricularia grisea* is the most destructive disease of rice in Nepal. Among rice genotypes, 46.09% were highly resistant, 26.56% resistant, 20.31% moderately resistant, 3.91%moderately susceptible and 3.13%were susceptible in 2018 whereas only 2.06% were highly resistant, 24.06 % resistant 67.35% moderately resistant, 5.15 %moderately susceptible and 1.38%were susceptible to leaf blast disease in 2019. Therefore, these resistant and moderately resistant genotypes with desirable agronomical characters can be used as donor parent in resistance breeding program for the development of leaf blast resistant varieties.

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Authors' Contributions

S. Baidyaand R.B. Yadaw guided research and revised the article for publication. P. Sharma conducted the trial and recorded data.P. Sharma and P.B. Magar wrote the final manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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