Screening of rice genotypes for leaf blast resistance under field condition

Pratiksha Sharma1*, Prem Bahadur Magar1, Suraj Baidya1 and Ram Baran Yadaw2

1National Plant Pathology Research Centre (NPPRC), NARC, Khumaltar, Lalitpur, Nepal
2National Rice Research Program, NARC, Hardinath, Dhanusha, Nepal
*Correspondence: pratikshasharmakandel@gmail.com;
*ORCID: https://orcid.org/0000-0001-7010-0582
Received: July 05, 2020; Accepted: September 19, 2020; Published: October 30, 2020

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 epiphytic 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 291 during 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, 4 susceptible 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 NR2182-22-1-3-1-1-1-1 (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, and IR13F402. 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

Correct citation: Sharma, P., Magar, P.B., Baidya, S., & Yadaw, R.B. (2020). Screening of rice genotypes for leaf blast resistance under field condition. Journal of Agriculture and Natural Resources, 3(2), 276-286.DOI: https://doi.org/10.3126/janr.v3i2.32515

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 tons 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...
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 *Magnaporthe grisea* 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°39’13’’N, 85°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 128 and 291 rice 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 19th and 20th 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.
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 1-2 mm 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 29.23°C, 19.17°C 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 29.9°C, minimum temperature 20°C 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).
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, 15 were moderately susceptible, 4 lines PANT DHAN-2 (Score 6),
NR2192-16-1-1-1 (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](image)

**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,IR97135-8-3-1-1, NR2182-4-3-2-1-1, NR2188-3-2-4-1-1, NR2170-150-1-2-1-1, NR2175-5-2-5-1-1, NR2157-144-13-1-2-4-5, NR 2179-82-2-4-1-1-1, NR 2168-65-1-1-1-1-1-1, 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-1B-21-B-7, IR14L537, IR14L546, IR97135-8-3-1-3-1, 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-25-4, NR2181-139-1-3-1-1-1-1, IR99739-21-1-2-1, 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, 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-SAL-13-4-SAL, SABATRI, IR16L1753, IR98849-2-1-4-3, IRKASTURI BASMATI, PUSA-1509, IR11L412, NR2189-42-1-1-1-1-1, IR16L1844, IR16L1591, IR17L1420, IR16L1421, IR12A-173, IR14L261, IR93346-1-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, IR106523-25-34-3-3-3-B-45-1, IR16L1591, IR16L1742, NR219187-1-1-3-1, NR2210-11-1-2-1-1, SVIN074, SVIN156, SVIN244, SVIN224, SVIN279 and TP 30531. |
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
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](image)

In 2019, cluster I has only 6 entries 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).
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-ta176 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
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., 2005 and 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.

**ACKNOWLEDGMENTS**

Authors would like to acknowledge their gratitude towards NARC for financial support in this study. The research team of NPPRC, Khumaltar, Lalitpur is gratefully acknowledged for trail management and data recording. Sincere thanks to Coordinator of National Rice Research Program (NRRP), Hardinath for providing necessary genotypes. Many thanks to Mr. Mahesh Subedi, Scientist, National Plant Breeding and Genetics Research Centre, Khumaltar for doing cluster analysis.

**Authors’ Contributions**

S. Baidya and 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.
REFERENCES

Bonman, J. M., Estrada, B.A., & Bandong, J.M. (1989). Leaf and neck blast resistance in tropical lowland rice cultivars. *Plant Disease, 73*, 388-390.

Bonman, J.M., & Mackill, D.J. (1988). Durable resistance to rice blast. *Oryza, 25*, 103-110.

Bonman, J.M., Khush, G.S., & Nelson, R.J. (1992). Breeding rice for resistance to pests. *Annual Review of Phytopathology, 30*, 507-528. DOI: https://doi.org/10.1146/annurev.py.30.090192. 002451

Chaudhary, B. (2001). Rice blast: Pathogenic variability and host resistance. M.Sc. Thesis, Tribhuvan University, Institute of Agriculture and Animal Sciences, Rampur, Chitwan, Nepal.

Chaudhary, B., Shrestha, S.M., & Sharma, R.C. (2005). Resistance in Rice Breeding Lines to the Blast Fungus in Nepal. *Nepal Agriculture Research Journal, 6*, 49-56. DOI: https://doi.org/10.3126/narj.v6i0.3344

Gill, M., & Bonman, J.M. (1988). Effects of water deficit on rice blast. I. Influence of water deficit on components of resistance. *Journal of Plant Protection in the Tropics, 5*, 61-66.

IRRI. (2002). Standard evaluation system manual. International Rice Research Institute, Manila, Philippines. pp 35.

Jha, P., Sah, S.N. Yadaw, R.B., Mandal, A., Saphi, D.K., & Chaudhary, R.N. (2014). Evaluation of rice genotypes against bacterial leaf blight and blast. *Proceedings of the 27th National Summer Crops Workshop* (pp 281-290). Nepal Agricultural Research Council held at Kathmandu, Nepal.

Jia, Y., McAdams, S.A., Bryan, G.T., Hershey, H.P., & Valen, B. (2000). Direct interaction of resistant genes and avirulence gene products confers rice blast resistant. *The EMBO Journal, 19*, 4004-4014.DOI: https://doi.org/10.1093/emboj/19.15.4004

Joshi, P., Dangal, N.K., Chaudhary, B., Rai, S., & Sherpa, A.T. (2017). Evaluation of rice genotypes for resistance to leaf blast under field condition. In Y.P. Giri, B.N. Mahato, A.K. Gautam, S.P. Khatiwada, R. Shrestha, B.K. Joshi, B.B. Khatri, S.K. Rai, Y.N. Ghimire, B.P. Luitel, H.K. Upreti, K. Sah, P.N. Sharma, A.S.R. Bajracharya, S. Baidhya, J. Shrestha and S Manandhar (Eds). Proceedings of the 28th Summer Crops Workshop (pp 473-478), 17-18 March 2015. National Rice Research Program, Hardinath, Dhanusha, Nepal. Nepal Agricultural Research Council, Kathmandu, Nepal.

Koh, Y.J., Hwang, B.K., & Chung, H.S. (1987). Adult plant resistant to rice blast. *Phytopathology, 77*, 232-236.

Lang, N.T., Luy, T.T., Ha, P.T.T., & Buu, B.C. (2009). Monogenic lines resistance to blast disease in rice (*Oryza sativa* L.) in Vietnam. *International Journal of Genetics and Molecular Biology, 1*(7),127–136.

Manandhar, H.K. (1987). Rice diseases in Nepal .Plant Pathology Division, Department of Agriculture/HMG Nepal and Winrock International, USAID, pp 204.

Manandhar, H.K., Shrestha, K., & Amatya, P. (1992). Seed borne diseases.In S.B.Mathur, P.Amatya, K. Shrestha, & H.K. Manandhar(Eds.). Plant diseases, seed production and seed health testing in Nepal. Danish Government, Institute of Seed pathology for Developing Countries.

Manandhar, H.K., Thapa, B.J., & Amatya, P. (1985). Efficacy of various fungicides on the control of rice blast disease. *Journal of Institute of Agriculture and Animal Sciences, 6*, 59-74.

MoALD. (2019). Statistical information on Nepalese Agriculture 2075/76 (2018/19). Planning and Development Cooperation Coordination Division. Ministry of Agriculture and
Livestock Development. Government of Nepal. Singhadurbar, Kathmandu, Nepal. pp 9.

Paradis, E., & Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics, 35*, 526-528. DOI: https://doi.org/10.1093/bioinformatics/bty633

Prabhu, A.S., Filippi, M.C., & Zimmermann, F.J.P. (1996). Genetic control of blast in relation to nitrogen fertilization in upland rice. *PesquisaAgropecuariaBrasileria, 31*(5), 339-347.

Puri, K.D., Shrestha, S.M., Joshi, K.D., & K.C., G.B. (2006). Reaction of different rice lines against leaf and neck blast under field condition of Chitwan valley. *Journal of Institute of Agriculture and Animal Science, 27*, 37-44.

Sharma, T.R., Rai, A.K., Gupta, S.K., Vijayan, J., Devanna B.N., & Ray, S. (2012). Rice Blast Management Through Host-Plant Resistance: Retrospect and Prospects. *Agricultural Research,1*(1), 37–52. DOI: https://doi.org/10.1007/s40003-011-0003-5

Shrestha, J., Kushwaha, U. K. S., Maharjan, B., Kandel, M., Gurung, S. B., Poudel, A. P., Karna, M. K. L., & Acharya, R. (2020b). Grain Yield Stability of Rice Genotypes. *Indonesian Journal of Agricultural Research, 3*(2), 116 - 126. https://doi.org/10.32734/injar.v3i2.3868

Shrestha, J., Singh Kushwaha, U. K., Maharjan, B., Subedi, S. R., Kandel, M., Poudel, A. P., & Yadav, R. P. (2020a). Genotype × environment interaction and grain yield stability in Chinese hybrid rice. *Ruhuna Journal of Science, 11*(1), 47–58. DOI: http://doi.org/10.4038/rjs.v11i1.86

Suh, J.P., Roh, J.H., Cho, Y.C., Han, S.S., Kim, Y.G., & Jena, K.K. (2009). The Pi40 gene for durable resistance to rice blast and molecular analysis of Pi40 advanced backcross breeding lines. *Phytopathology, 99*(3), 243-250. DOI: https://doi.org/10.1094/PHYTO-99-3-0243

Thruston, H.D. (1998). Tropical plant diseases (2nd ed.). American Phytopathological Society. pp. 31-40. DOI: https://doi.org/10.1017/S0014479799223125