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Assessment of Leaf Blast Isolates Virulence on Rice Genotype under Shade House at NARC, Khumaltar, Nepal

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Abstract

Blast disease caused by *Magnaporthe grisea* (Hebert) Barr. anamorph *Pyricularia oryzae*, is an important fungal disease of rice known to occur in most rice producing areas of the world. In Nepal, the disease causes 10%-20% yield reduction in susceptible varieties, but in severe case, it goes up to 80% to measure virulence of 5 isolates of *P. grisea*, obtained from various location, against 10 genotypes of rice, a shade house experiment was carried out under inoculated condition at NARC (Nepal Agricultural Research Council), Khumaltar, during August, 2015. Isolates from Gokarna and Madhyapur Thimi had highest aggressiveness, and it was lowest in Kirtipur and Lalitpur isolates. Considerable pathogenic variations were observed in the five isolates, however no distinct differential reactions were found on the genotypes. Highest disease severity was found in Taichung-176 and least in Manjushree, IR 87760-15-2-2-4, IR 70210-39-CPA-7-1 and NR 11105-B-B-20-2-1 under shade house.

Keywords: Rice blast; Genotypes; *Magnaporthe grisea*; Isolate

Introductions

Blast disease is one of the most devastating diseases of both the seasons that occur in all rice growing areas. The disease, caused by *Magnaporthe oryzae* (Hebert) Barr., anamorph *Pyricularia oryzae* Rossman et al. [1] is an important fungal disease of rice known to occur in most rice producing areas of the world [2]. Rice blast is the most common and destructive disease in irrigated rice of both temperate and subtropical areas of East Asia Bonman et al. [3]. The pathogen is highly adaptable to various environmental conditions and can be found in irrigated lowland, rain-fed upland, or deep-water rice fields [4]. The disease results in yield loss as high as 70%-80% [2] when predisposition factors (high mean temperature, relative humidity higher than 85%-89%, presence of dew, drought stress and excessive nitrogen fertilization) prevail [5] In Nepal, the disease causes 10%-20% yield reduction in susceptible varieties, but in severe case, it goes up to 80% Manandhar et al. [6]. Blast epidemic causes complete loss of seedlings in nursery and epidemics in the field [7,8].

Host resistance, even being a preferred tactic for blast management, sometimes fails due to evolutionary changes in the pathogen population Correa-victoria et al. [9]. In blast prone areas, resistant cultivars have an expected field life of only 2-3 growing seasons due to the generation of newly virulent forms of the fungus Leung and Shi [10]. The breakdown of the more complex resistance that is inherited through several genes also occurs, but more gradually taking longer time Barman et al. [11].

Hence, the successful breeding program for resistance to blast should be concentrated on effective and efficient screening techniques [7] study of variability and population biology of the blast fungus [12,13] behavior of resistant genes Mc Couch et al. [14] and host parasite interaction in rice-blast pathosystem Notteghem et al. [15] are essential. Therefore, identification of the durable new sources of resistance and their development are necessary for blast management. So this study was conducted to determine virulence of *Magnaporthe grisea* isolates obtained from different location of Kathmandu valley on rice genotypes in shade house.

Materials and Methods

The experiment was conducted in a shade house of division of plant pathology, NARC, Khumaltar, Lalitpur, during August, 2015. In this study, five different isolates of leaf blast originating from Kathmandu valley were tested against 10 rice genotypes/varieties, with various reaction levels to leaf blast, to find out virulence of the isolates. Ten genotypes/varieties of rice, appearing Highly Susceptible (HS) to Resistant (R) in field were selected for shade house experiment .Highly susceptible variety "Taichung-176" was taken as a check (Table 1).

The rice genotypes were grown inside a screen house in plastic trays (50×25×10 cm^3) at NARC, Khumaltar and Lalitpur. Fifteen plastic trays were filled each with 5 kg dry soil taken from the farm of plant pathology division, NARC. There were 5 cages, and 3 trays were put per cage. The soil was fertilized with NPK @ 0.4:0.15:0.1 g/kg soil @ 150:50:50 kg/ha and FYM @ 10 ton/ha before filling the trays. In a tray, 10-15 seeds of a genotype were sown in 3 lines (parallel to length of tray) 2 cm apart and 1-1.5 cm seed to seed and covered with a thin layer of soil on 3rd August, 2015. Three lines represented three replications. Three trays were kept in one iron cage.

First tray contained first-4 genotypes, second tray contained next four genotypes and third tray contained last two genotypes, thus 10 genotypes in each cage. There were five cages for five isolates and each

cage was separated by a plastic sheet, so as to protect the cross inoculation of fungal spores of one isolate from one cage to another due to air drift Seedlings were irrigated daily after germination (Tables 2-5).

Code	Genotypes	Reaction
V_1	IR 87760-15-2-2-4	R
V_2	IR 70210-39-CPA-7-1	R
V_3	NR 11130-B-B-B-3	R
V_4	NR 1105-B-B-16-2	R
V_5	NR 11142-B-B-B-9	R
V_6	Khumal-10	R
V_7	Manjushree	R
V_8	NR 11011-B-B-B-B-33	R
V_9	NR 11105-B-B-20-2-1	MR
V_{10}	Taichung-176	HS

Table 1: List of genotypes selected for shade house experiment.

Cage 1		Cage 2		Cage 3		Cage 4		Cage 5	
Tray 1	V_1I_1	Tray 1	V_1I_2	Tray 1	V_1I_3	Tray 1	V_1I_4	Tray 1	V_1I_5
	V_2I_1		V_2I_2		V_2I_3		V_2I_4		V_2I_5
	V_3I_1		V_3I_2		V_3I_3		V_3I_4		V_3I_5
	V_4I_1		V_4I_2		V_4I_3		V_4I_4		V_4I_5
Tray 2	V_5I_1	Tray 2	V_5I_2	Tray 2	V_5I_3	Tray 2	V_5I_4	Tray 2	V_5I_5
	V_6I_1		V_6I_2		V_6I_3		V_6I_4		V_6I_5
	V_7I_1		V_7I_2		V_7I_3		V_7I_4		V_7I_5
	V_8I_1		V_8I_2		V_8I_3		V_8I_4		V_8I_5
Tray 3	V_9I_1	Tray 3	V_9I_2	Tray 3	V_9I_3	Tray 3	V_9I_4	Tray 3	V_9I_5
	$V_{10}I_1$		$V_{10}I_2$		$V_{10}I_3$		$V_{10}I_4$		$V_{10}I_5$

Table 2: Layout of experiment in a shade house at Khumaltar, Lalitpur, during August, 2015 (V=Genotype, I=Isolate).

S. No	Location	Symbol	District
1	Kirtipur	I_1	Kathmandu
2	Gokarna	I_2	Kathmandu
3	Mulpani	I_3	Kathmandu
4	Khumaltar	I_4	Lalitpur
5	Madhyapur Thimi	I_5	Bhaktapur

Table 3: Blast infected leaf samples of Taichung-176, variety rice were collected from five different location of Kathmandu valley to isolate the pathogen as illustrated.

The pathogen (*Pyricularia grisea*) was isolated in the laboratory of plant pathology division, NARC, Khumaltar, Lalitpur, during August, 2015. For inoculation of rice plants by *P. grisea*, mass production of the fungal spores was done. The spore suspension was diluted to a concentration of 105 spores per mL adding sterile distilled water. A final volume of 200 mL was prepared of each isolate. Eighteen days old seedlings grown in plastic trays inside cages, were inoculated with one isolate/cage with the spore suspension of 105 spores mL, with a hand sprayer at late afternoon (4 pm).

Five days after inoculation, disease scoring for leaf blast was done in a 0-9 scale as developed by IRRI (International Rice Research Institute) [16] by randomly selecting five plants from each replication of each genotype. Disease intensity (severity) of leaf blast was calculated by using the following formula and mean values were computed for each genotype.

Analysis of variance for all parameters was carried out as per the procedures given in Genstat Discovery edition and R software (3.2.2). DMRT for mean separation was done from the references of Gomez and Gomez [17].

$$Disease\ Intensity\ (\%) = \frac{Sum\ of\ all\ numerical\ ratings}{Total\ no.\ of\ plants\ observed \times Maximum\ rating} \times 100$$

Results and Discussion

Reaction of rice genotypes against leaf blast isolates

Among the ten genotypes, IR 87760-15-2-2-4 (V_1) and IR 70210-39-CPA-7-1 (V_2) showed resistant reaction with lowest mean value (1.6) each, followed by NR 11130-B-B-B-3 (V_3) (1.8). NR 11011-B-B-B-B-33 (V_8) and Taichung-176 (V_{10}) showed susceptible reaction (5.8 and 6.4, respectively) (Table 1). Isolates I_5 (4.3) and I_2 (3.7) were more aggressive with highest mean values. The isolate from Bhaktapur (I_5) developed highly susceptible reaction (9) with Taichung-176 (V_{10}), susceptible with NR 11011-B-B-B-B-33 (V_8) (7) and Manjushree (V_7) (6), and moderately resistant reaction with NR 11105-B-B-20-2-1 (V_9) (4), IR 87760-15-2-2-4 (V_1) (3), NR 11142-B-B-B-9 (V_5) (3), NR 11130-B-B-B-3 (V_3) (3) and Khumal-10 (V_6) (3) genotypes, and resistant reaction with IR 70210-39-CPA-7-1 (V_2) (2). Similarly, isolate from Gokarna (I_2) developed susceptible reaction with Manjushree, NR 11011-B-B-B-B-3 and Taichung, moderately resistant reaction with

NR 11142-B-B-B-9, Khumal-10 and NR 11105-B-B-20-2-1 and resistant reaction with other 4 genotypes (Table 1). Other isolates had mean values 2.5 or below. In screen house, differential symptoms of leaf blast were caused by five isolates of *P. grisea* on rice leaves (Figure 1).

Isolates from Kirtipur (I_1), Mulpani (I_3) and Lalitpur (I_4) could not develop susceptible reaction in any of the genotypes except Taichung-176 (V_{10}) and NR 11011-B-B-B-B-33 (V_8) while they developed resistant reaction in remaining eight genotypes.

Figure 1: Symptoms of leaf blast on rice caused by five isolates of *P. grisea* in a shade house at NARC, Khumaltar, during 2015.

Reaction of rice genotype

Isolates	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	Mean
I_1	1 (R)	1 (R)	2 (R)	2 (R)	1 (R)	2 (R)	2 (R)	1 (R)	5 (MS)	5 (MS)	2.2
I_2	1 (R)	2 (R)	2 (R)	2 (R)	1 (R)	2 (R)	2 (R)	1 (R)	5 (MS)	5 (MS)	2.3
I_3	2 (R)	1 (R)	1 (R)	1 (R)	2 (R)	1 (R)	2 (R)	3 (MR)	5 (MS)	7 (S)	2.5
I_4	1 (R)	2 (R)	1 (R)	2 (R)	4 (MR)	4 (MR)	4 (MR)	6 (S)	5 (MS)	6 (S)	3.7
I_5	3 (MR)	2 (R)	3 (MR)	3 (MR)	3 (MR)	3 (MR)	4 (MR)	6 (S)	5 (MS)	9 (HS)	4.3
Mean	1.6	1.6	1.8	2	2.2	2.4	2.8	3.4	5.8	6.4	

Table 4: Reaction of 10 rice genotypes against 5 isolates of *P. grisea* in shade house, at Khumaltar, Lalitpur, during 2015. I1: Kirtipur; I2: Gokarna; I3: Mulpani; I4: Khumaltar, I5: Madhyapur Thimi; V: Genotypes; R: Resistant; MR: Moderately Resistant; MS: Moderately Susceptible; S: susceptible; HS: Highly Susceptible.

As the reaction of 10 genotypes was varied with 5 isolates, the isolates from different location may also be different races of the pathogen. The isolates from Kirtipur (I_1) and Khumaltar (I_4) may be one race, from Gokarna (I_2) and Madhyapur Thimi (I_5) an another race and from Mulpani (I_3) an another race. The isolate of the same race are also close to each other geographically. Further studies under various conditions and locations should be done to verify the results.

Virulence of leaf blast isolates on rice genotypes

The five leaf blast isolates varied considerably in developing leaf blast disease on ten rice genotypes in shade house. The isolate from Madhyapur Thimi (I_5) appeared most virulent and also significantly different from all the others, with a disease index of 55.56%, followed by the isolate from Gokarna (I_2), (46.67%). The isolate from Kirtipur (I_1), (17.78%) and Khumaltar (I_4), (27.67%) were least virulent (Figure 2).

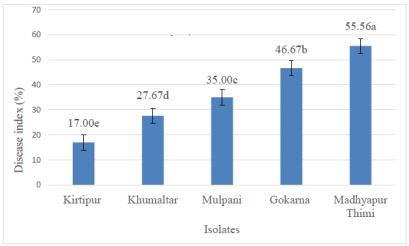

Figure 2: Graph showing the disease severity with error bars and statistical analysis of leaf blast on rice caused by five isolates of *Pyricularia grisea* in a shade house at Khumaltar, Lalitpur, during August, 2015.

Source	Degree of Freedom	Sum of Square	Mean Square	Mean sum of Error	F-value	Prob
Treatment	1	2745.6	2745.6	3	4.8	
Residual	13	39.7	3.1	36.33	899.1	2.19e-13 ***

Table 5: Mean disease index and mean sum of error isolates, *** highly significantly different by DMRT at 5 % level.

Highest aggressiveness in isolates from Bhaktapur and Gokarna on the tested lines showed higher diversity of the blast population in Bhaktapur and Gokarna. In 2013, in Bhaktapur, there has been observed the severe outbreak of neck blast in Chinese variety, i.e. DY-69 NARC [19] which was considered to be the factor of aggressiveness of blast isolates. Among all study sites, the favorable condition for the blast disease appeared in Gokarna. The variations in the mean neck and leaf blast severity at different locations suggested that the pathogen population was highly variable and genotype-specific and also weather conditions would be more conducive in some locations over others. The relative effect of weather conditions at Patancheru, India, was minimized by artificial inoculation of *P. grisea* at appropriate stage of the crop and use of sprinkler irrigation for maintenance of blast-conducive weather conditions Thakur et al. [20]. This facilitated fair assessment of genotypic response to blast infection and development at Patancheru location. In addition to genetic differences in mini-core, there were several weather factors that influenced blast infection and symptom expression under field conditions [2,21-22]. The genetic makeup and environmental conditions would be the prominent factors for the differential interactions. In general, the result indicated that the same strains were capable of causing different types of blast symptoms under suitable agro-ecological conditions [23,24].

Conclusion

Disease severity was significantly higher in rice genotypes with Bhaktapur isolate, followed by Gokarna, Mulpani and Lalitpur, and least with Kirtipur isolate. Disease development was slowest in IR 70210-39-CPA-7-1 and fastest in Taichung-176, with considerable pathogenic variations among the five isolates. So it is concluded that the genotypes i.e. IR 87760-15-2-2-4, IR 70210-39-CPA-7-1, NR 11142-B-B-B-9, NR 11130-B-B-B-3, Khumal-10, NR 1105-B-B-16-2 and NR 11105-B-B-20-2-1 were found resistant to different isolates of *P. grisea* in shade house. Therefore, these genotypes should be tested in field at different location to verify their performance. Madhyapur Thimi and Gokarna sites could be selected as hot spots for screening rice genotypes against leaf blast, because the isolates from these areas were very aggressive. As the reaction of some rice genotypes to leaf blast isolates varied abruptly, there might be presence of various rice blast races in Kathmandu valley.

Author Contributions

As a main authors, I (Prakash Ghimire), had contributed a diligence and novel idea initially from initiation of Research to final of manuscript Preparation. Prof. Dr. Gopal Bahadur KC, Ph.D., Asst. Dean (Academic), Institute of Agriculture and Animal Science, Kirtipur, had contributed his persistent encouragement, constant supervision, excellent guidance and invaluable suggestions throughout the course of this investigation and during preparation of the manuscript. Similarly, Mr. Gopal Prasad Parajuli, senior scientist, Nepal Agriculture Research Council (NARC), Khumaltar had inspired in selecting the research area; promotive stimulation and suggestions in the entire course of study as well in manuscript preparation. I equally owe and express my deep sense of gratitude and heartfelt thanks to Prof. Dr. Sundar Man Shrestha, for his invaluable suggestions, prudent advice and constructive comments provided during the course of study to this final manuscript preparation.

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References

1. Rossman AY, Howard RJ, Valent B (1990) Pyricularia grisea, The correct name for the rice blast disease fungus. Mycologia 82: 509-512.
2. Ou SH (1985) Rice disease. Second edition. Commonwealth Agricultural Bureau International Mycological Institute. Farham House pp: 380.
3. Roumen EC (1992) Leaf age related partial resistance to Pyricularia oryzae in tropical lowland rice cultivars as measured by the number of sporulating lesions. Phytopathology 82: 1414-1417.
4. Rao KM (1994) Rice Blast Disease Daya Publishing House pp: 1-9.
5. Piotti E, Rigano MM, Rodino D, Rodolfi M, Castiglione S, et al. (2005) Genetic Structure of Pyricularia grisea (Cooke) Sacc. Isolates from Italian Paddy Fields. J Phytopathology 153: 80-86.
6. Manandhar HK, Shrestha K, Amatya P, Mathur SB (1992) Seed-borne diseases. In: Plant Diseases, Seed Production and Seed Health Testing in Nepal. Danish Government, Institute of Seed Pathology for Developing Countries pp: 59-74.
7. Chaudhary RN (1996) Techniques for screening rice genotypes for resistance to the blast fungus and its crude extract toxin. Thesis Kasetsart Universityp pp: 97.
8. Teng PS, Klein-Gebbink HW, Pinnschmidt H (1991) An analysis of the blast pathosystem to guide modeling and forecasting. In: Rice blast modeling and forecasting. International Rice Res Institute pp: 1-30.
9. Correa-Victoria FJ, Zeigler RS, Levy M (1994) Virulence characteristic of genetic families of Pyricularia grisea in Colombia. In: Leong SA, PS Teng (Eds), Rice Blast Disease. CAB International, IRRI, Wallimgford, Oxon OX10 8DE, UK.
10. Shi ZX (1994) Genetic analysis and rapid mapping of a sporulation mutation in Magnapothe grisea. Mol Pl- Microbe Interaction 7: 113.
11. Barman JM (1990) Host plant resistance for rice plant management in the tropics third international conference on plant protection on tropics: Magnaporthe grisea from India that are pathogenic to finger millet and to rice. Myco Res 104: 161-167.
12. Correa-Victoria FJ, Zeigler RS (1993) Pathogenic variability in Pyricularia grisea at a rice blast hot spot site. Plant Dis pp: 1029-1034.

13. Zeiglar RS, Tohme J, Nelson R, Levy M, Correa-Victoria FJ, et al. (1994) Lineage exclusion: A proposal linking blast population analysis to resistance breeding, Zeigler RS, Leong SA, Teng PS (Eds.), Rice Blast Disease. CAB International IRRI UK.
14. McCouch S, Nelson R, Tohme J, Ziegler RS (1994) Mapping blast resistance genes in Rice. CAB International, Wallingford, UK, in association with IRRI, Philipines.
15. Nottéghem JL, Tharreau D, Silué D, Roumen E (1994) Present knowledge of rice resistance and strategies for Magnaporthe grisea pathogenicity and a virulence gene analysis. Rice Blast Disease. CAB International, Wallingford, Oxfordshire UK.
16. IRRI (1996) Standard Evaluation System (SES) for Rice. (4th edn) Rice Res. Institute, Los Baños, Philippines.
17. Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research (2 Edition). John wiley and sons, NewYork.
18. Mekwatanakarn P, Kositrantana W, Levy M, Zeigler RS (2000) Pathotype and avirulence gene diversity of Pyricularia grisea in Thail and as determined by rice lines near- isogenic for major resistance genes. Plant Disease 84: 60-70.
19. NARC (2013) Annual report of Nepal Agriculture Research Council.
20. Thakur RP, Sharma R, Rai KN, Gupta SK, Rao VP (2009) Screening techniques and resistance sources for foliar blast in pearl millet. J Agri Res 7: 1-5.
21. Patel RP, Tripathi SK (1998) Epidemiology of blast of finger millet caused by Pyricularia grisea (Cke) Sacc. Adv in Plant Sci 11: 73-75.
22. Kumar VBS, Bhatt A, Nagaraju S (2007) Virulence analysis of Pyricularia grisea (Cke) Sacc on different finger millet genotypes and cultivars to determine race differential lines. Environment and Ecology 25: 190-192.
23. Koutroubas DS, Katsantonis D, Ntanos DA, Lupatto E (2009) Blast fungus inoculation reduces accumulation and remobilization of pre-anthesis assimilates to rice grains. Phytopathol Mediterr 48: 240-252.
24. Sreenisaprasad S, Chipili J, Muthumeenakshi S (2001) Diversity and dynamics of phytopathogenic fungi: Application of molecular tools. In: Proceedings of the 11th Mediterranean Phytopathological Congress, University of Evora Portugal. pp: 21-22.