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Phenotyping of rice germplasm for blast resistance

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Abstract
Rice is a major cereal crop that contributes significantly to global food security and highly vulnerable to rice blast disease. Rice blast disease caused by *Magnaporthe oryzae* is one of the most destructive disease causing huge losses to rice yield in different parts of the world. The rapid genetic evolution of the fungus often overcomes the resistance after a few years of intensive agricultural use. Development of resistant cultivars is the most economic and effective strategy to control the disease. Therefore, an attempt has been made to identify resistant genotypes by screening a set of 355 rice germplasm accessions during *kharif* 2021 under Uniform Blast Nursery (UBN) using 0-9 scale SES, IRRI, Philippines. It was observed that the rice germplasm accessions showed variable responses against the rice blast pathogen and among the tested genotypes, out of 355 rice genotypes, thirty-two (32) genotypes were highly resistant with a score of 0 and 1, Twenty-one (21) genotypes were resistant with a score of 2, fifty-five (55) genotypes were moderately resistant with a score of 3, Three (3) genotypes were moderately susceptible with a score of 4, one hundred and one (101) genotypes were intermediate with a score of 5 and 6, thirty-nine (39) genotypes were susceptible and one hundred and twenty-nine (129) genotypes were highly susceptible. The information revealed from this study could be helpful for rice leaf blast disease management and the identified resistant rice genotypes could be used as prospective donors for the production of resistant varieties in various resistance breeding programs.

Keywords: Rice blast, uniform blast nursery, disease severity, disease resistance

Introduction

Rice plays a crucial role as a primary food source for over half of the global population, contributing 27% of the calories consumed in low and middle-income countries (Patil and Sharanagouda, 2017; Susanto *et al*., 2017; Estiati, 2019; Weerakoon and Somaratne, 2020) [38, 49, 13, 53]. Consequently, any decrease in rice production poses a significant risk to food security. Additionally, it has been emphasized that by 2030, rice production must increase by 40% to meet the growing demand (Khush *et al*., 2001) [26]. With the world's population rapidly expanding, this places food security as a major concern for the future.

Diseases and pests stand as significant factors that can severely impact rice production. Rice is susceptible to over 70 diseases caused by fungi, bacteria, viruses, or nematodes, and in severe instances, these diseases can lead to losses as high as 70-80% in specific rice ecosystems (Deepak and Prasanta, 2017) [12]. Among these diseases, blast disease, which is instigated by the fungus *Magnaporthe oryzae* (Anamorph: *Pyricularia oryzae*), stands out as one of the most devastating worldwide, primarily due to its extensive prevalence and its capacity for causing significant damage when conditions favor its development (Fahad *et al*., 2019) [14]. It is commonly referred to as rice fever disease and has been documented in approximately 85 countries where rice cultivation takes place across the globe (Thulasinathan *et al*., 2020)) [51]. Estimates indicate that this disease results in a yield reduction ranging from 10% to 30% (Sakulkoo *et al*., 2018) [44]. In the context of a disease outbreak, blast disease can lead to an astonishing 70-80% reduction in crop yields (Khush *et al*., 2009) [25].

While pesticides can offer a means of controlling blast disease, their frequent use may inadvertently promote the development of tolerance and evolution in pathogens, thereby posing a greater threat to the safety of rice production. Alternatively, a more cost-effective and environmentally friendly approach is to explore the resistance (R) genes within the host plant, which can limit the occurrence of blast disease (Khanna *et al*., 2015) [24].

Effectively managing blast disease necessitates ongoing breeding efforts to create cultivars that are resistant to it. The genome of *M. oryzae* contains abundant repetitive sequences and retro-transposons, allowing the fungus to frequently alter its pathogenicity or evade detection by its host through changes in effector molecules. This can break down the resistance provided by R genes, leading to disease epidemics (Dean *et al*., 2005)) [11]. Several factors, including weather conditions, disease prevalence, and the genetic stability of the pathogen, can influence these dynamics. To combat the ever-changing and geographically diverse pathogen strains, it is crucial to continually identify new sources of host plant resistance against the disease. Host plant resistance has proven to be the most effective strategy for managing blast disease. Therefore, the development of rice lines that are resistant to blast disease has become increasingly important. The objective of the current experiment is to screen rice germplasm and to select resistant genotypes against blast disease.

Materials and Methods

Description of study area and germplasm used

The experimental trial was laid out at Mountain Research Centre for Field Crops (MRCFC) Khudwani, Kashmir, India during Kharif 2021. Khudwani is located between 33°70'N latitude and 75°10'E longitude at an altitude of 1590 metres above mean sea level. The temperature during *Kharif* season ranges from 25°C-35°C with an annual precipitation of 80-120 cm. For screening rice germplasm against leaf blast disease, a total of 355 rice germplasm accessions (Table 1) both from indigenous and exotic sources, and some local landraces uncharacterized for blast resistance and being maintained at MRCFC, Khudwani were used in the present study. The rice genotypes were sown in raised beds with one row of each genotype having width 1m and row to row spacing 10 cm under uniform blast nursery (UBN) (Figure 1). The highly susceptible variety Mushkbudgi was used as spreader row around each bed to enhance natural infection and to minimize the chance of escape from infection (IRRI, 2015; Vasudevan *et al*., 2014) [20, 52].

Table 1: List of germplasm accessions used in the present study

Genotype	Genotype	Genotype	Genotype	Genotype	Genotype	Genotype	Genotype	Genotype
GS-2	GS-108	GS-174	GS-246	GS-312	GS-367	GS-473	GS-589	GS-649
GS-6	GS-109	GS-175	GS-247	GS-315	GS-368	GS-474	GS-590	GS-650
GS-7	GS-111	GS-176	GS-248	GS-316	GS-370	GS-476	GS-593	GS-651
GS-17	GS-112	GS-177	GS-249	GS-317	GS-378	GS-477	GS-594	GS-652
GS-21	GS-113	GS-178	GS-252	GS-318	GS-379	GS-478	GS-595	GS-653
GS-22	GS-114	GS-179	GS-253	GS-319	GS-380	GS-480	GS-596	GS-654
GS-23	GS-115	GS-180	GS-255	GS-320	GS-381	GS-484	GS-601	GS-655
GS-27	GS-116	GS-181	GS-256	GS-321	GS-382	GS-487	GS-602	GS-656
GS-29	GS-118	GS-182	GS-258	GS-322	GS-384	GS-491	GS-605	GS-657
GS-30	GS-120	GS-183	GS-259	GS-324	GS-385	GS-492	GS-608	GS-658
GS-31	GS-124	GS-184	GS-260	GS-325	GS-386	GS-496	GS-609	GS-659
GS-32	GS-125	GS-185	GS-261	GS-328	GS-387	GS-497	GS-610	
GS-33	GS-126	GS-188	GS-262	GS-329	GS-390	GS-499	GS-611	
GS-34	GS-128	GS-189	GS-263	GS-331	GS-391	GS-504	GS-612	
GS-35	GS-129	GS-190	GS-264	GS-332	GS-392	GS-520	GS-613	
GS-36	GS-130	GS-193	GS-266	GS-333	GS-394	GS-522	GS-614	
GS-37	GS-133	GS-194	GS-267	GS-334	GS-395	GS-523	GS-615	
GS-45	GS-134	GS-195	GS-269	GS-335	GS-396	GS-525	GS-616	
GS-47	GS-135	GS-197	GS-271	GS-336	GS-397	GS-527	GS-617	
GS-49	GS-139	GS-198	GS-273	GS-337	GS-398	GS-529	GS-618	
GS-50	GS-140	GS-199	GS-274	GS-338	GS-401	GS-535	GS-619	
GS-52	GS-142	GS-201	GS-275	GS-339	GS-403	GS-537	GS-620	
GS-57	GS-144	GS-202	GS-276	GS-340	GS-410	GS-539	GS-621	
GS-58	GS-148	GS-204	GS-277	GS-341	GS-416	GS-540	GS-622	
GS-59	GS-149	GS-205	GS-282	GS-342	GS-421	GS-541	GS-624	
GS-61	GS-150	GS-206	GS-284	GS-344	GS-436	GS-542	GS-625	
GS-62	GS-151	GS-207	GS-286	GS-345	GS-442	GS-546	GS-626	
GS-63	GS-152	GS-208	GS-288	GS-346	GS-444	GS-548	GS-627	
GS-66	GS-154	GS-209	GS-289	GS-347	GS-446	GS-554	GS-628	
GS-67	GS-155	GS-214	GS-290	GS-349	GS-447	GS-560	GS-630	
GS-69	GS-157	GS-216	GS-291	GS-350	GS-448	GS-569	GS-631	
GS-70	GS-158	GS-217	GS-292	GS-351	GS-450	GS-571	GS-632	
GS-72	GS-159	GS-218	GS-293	GS-355	GS-452	GS-575	GS-633	
GS-74	GS-161	GS-223	GS-294	GS-356	GS-453	GS-576	GS-634	
GS-75	GS-162	GS-224	GS-296	GS-357	GS-454	GS-579	GS-635	
GS-77	GS-166	GS-231	GS-303	GS-358	GS-455	GS-580	GS-637	
GS-79	GS-167	GS-234	GS-304	GS-360	GS-456	GS-581	GS-638	
GS-80	GS-168	GS-236	GS-305	GS-361	GS-459	GS-582	GS-640	
GS-81	GS-169	GS-237	GS-306	GS-362	GS-460	GS-583	GS-642	
GS-82	GS-170	GS-238	GS-307	GS-363	GS-462	GS-584	GS-643	
GS-88	GS-171	GS-242	GS-308	GS-364	GS-464	GS-585	GS-644	
GS-101	GS-172	GS-243	GS-309	GS-365	GS-467	GS-587	GS-647	
GS-103	GS-173	GS-245	GS-310	GS-366	GS-471	GS-588	GS-648	

Fig 1: Uniform Blast Nursery

Culture preparation, inoculation and disease scoring

Stock isolates will be revived from storage on pure agar slants with streptomycin at 10 mg/250ml of medium. To create a spore suspension, a 7-day-old blast culture that had been cultivated on oatmeal agar at a temperature between 25 °C and 28°C was utilized. This spore suspension, which contained 0.02% Tween-20, was evenly sprayed onto 15-day-old seedlings using a handheld, low-volume plastic sprayer, covering all the plants in UBN beds. The spraying of the plants was carried out in the evening and the humidity was maintained by periodically spraying water 3-4 times a day using sprinklers. It's worth noting that the inoculum was sprayed at least twelve hours before the water spraying, and care was taken not to apply water immediately after inoculation. The inoculated seedlings were observed for the development of blast lesions, and fifteen days after inoculation, the test entries were evaluated for leaf blast severity using the Standard Evaluation Scale (SES) for Rice (2015) by IRRI, Philippines (Table 2).

Table 2: Scale for blast disease assessment under field conditions

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

Statistical analysis

The data was 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.

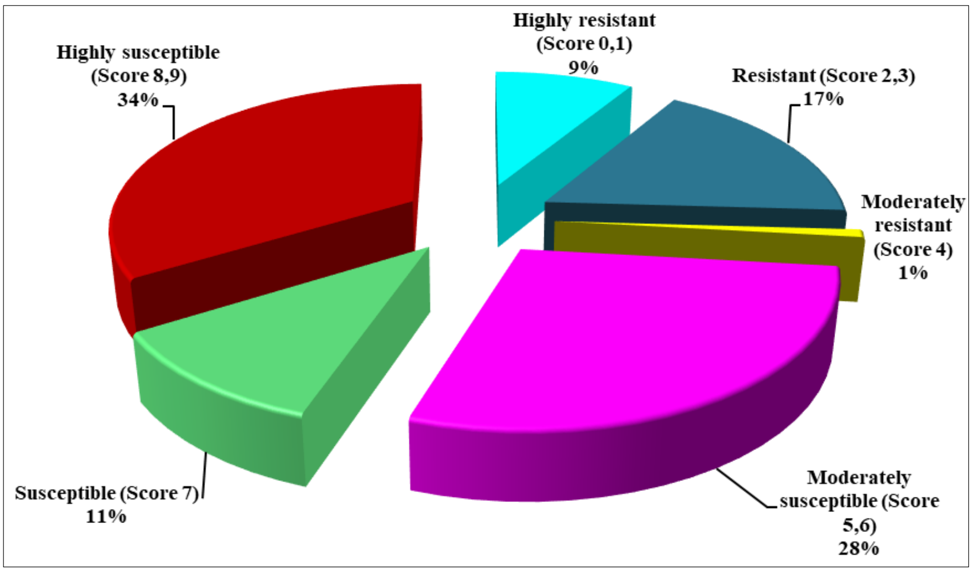

Fig 2: Frequency distribution of genotypes

Results

Based on the field experiment results (disease score), genotypes were classified into seven groups. Among the tested genotypes, out of 355 rice genotypes, thirty one (9%) were highly resistant (HR) with a score of 0-1, sixty one (17%) were resistant (R) with a score of 2-3, three (1%) were moderately resistant (MR) with a score of 4, one hundred one (28%) were moderately susceptible with a score of 5-6, 39 (11%) were susceptible with a score of 7 and one hundred twenty (34%) were highly susceptible with a score of 8-9 (Table 3, Figure 2).

Table 3: Disease reaction of germplasm accessions under uniform blast nursery (UBN)

Genotype	Score	Disease Reaction	Genotype	Score	Disease Reaction	Genotype	Score	Disease Reaction
GS-2	9	HS	GS-108	9	HS	GS-174	3	R
GS-6	9	HS	GS-109	1	HR	GS-175	3	R
GS-7	9	HS	GS-111	3	R	GS-176	1	HR
GS-17	9	HS	GS-112	7	S	GS-177	1	HR
GS-21	9	HS	GS-113	5	MS	GS-178	3	R
GS-22	9	HS	GS-114	8	HS	GS-179	8	HS
GS-23	9	HS	GS-115	8	HS	GS-180	7	S
GS-27	9	HS	GS-116	8	HS	GS-181	3	R
GS-29	9	HS	GS-118	8	HS	GS-182	3	R
GS-30	9	HS	GS-120	8	HS	GS-183	3	R
GS-31	9	HS	GS-124	9	HS	GS-184	7	S
GS-32	9	HS	GS-125	9	HS	GS-185	2	R
GS-33	7	S	GS-126	9	HS	GS-188	1	HR
GS-34	9	HS	GS-128	9	HS	GS-189	0	HR
GS-35	9	HS	GS-129	9	HS	GS-190	6	MS
GS-36	9	HS	GS-130	9	HS	GS-193	6	MS
GS-37	9	HS	GS-133	1	HR	GS-194	2	R
GS-45	9	HS	GS-134	1	HR	GS-195	2	R
GS-47	9	HS	GS-135	9	HS	GS-197	2	R
GS-49	9	HS	GS-139	9	HS	GS-198	6	MS
GS-50	9	HS	GS-140	9	HS	GS-199	7	S
GS-52	9	HS	GS-142	8	HS	GS-201	3	R
GS-57	9	HS	GS-144	8	HS	GS-202	3	R
GS-58	9	HS	GS-148	8	HS	GS-204	6	MS
GS-59	9	HS	GS-149	1	HR	GS-205	6	MS
GS-61	9	HS	GS-150	1	HR	GS-206	3	R
GS-62	9	HS	GS-151	1	HR	GS-207	3	R
GS-63	9	HS	GS-152	1	HR	GS-208	3	R
GS-66	9	HS	GS-154	7	S	GS-209	2	R
GS-67	9	HS	GS-155	7	S	GS-214	3	R
GS-69	9	HS	GS-157	1	HR	GS-216	6	MS
GS-70	9	HS	GS-158	8	HS	GS-217	3	R
GS-72	9	HS	GS-159	8	HS	GS-218	6	MS
GS-74	9	HS	GS-161	8	HS	GS-223	6	MS
GS-75	9	HS	GS-162	3	R	GS-224	6	MS
GS-77	9	HS	GS-166	3	R	GS-231	6	MS
GS-79	9	HS	GS-167	3	R	GS-234	6	MS
GS-80	9	HS	GS-168	5	MS	GS-236	6	MS
GS-81	9	HS	GS-169	3	R	GS-237	6	MS
GS-82	9	HS	GS-170	1	HR	GS-238	6	MS
GS-88	9	HS	GS-171	1	HR	GS-242	6	MS
GS-101	9	HS	GS-172	2	R	GS-243	6	MS
GS-103	9	HS	GS-173	0	HR	GS-245	6	MS

Genotype	Score	Disease Reaction	Genotype	Score	Disease Reaction	Genotype	Score	Disease Reaction
GS-246	6	MS	GS-307	7	S	GS-358	1	HR
GS-247	3	R	GS-308	5	MS	GS-360	3	R
GS-248	3	R	GS-309	5	MS	GS-361	5	MS
GS-249	3	R	GS-310	5	MS	GS-362	5	MS
GS-252	7	S	GS-312	5	MS	GS-363	4	MR
GS-253	7	S	GS-315	5	MS	GS-364	3	R
GS-255	6	MS	GS-316	7	S	GS-365	3	R
GS-256	3	R	GS-317	7	S	GS-366	5	MS
GS-258	3	R	GS-318	7	S	GS-367	5	MS
GS-259	3	R	GS-319	7	S	GS-368	5	MS
GS-260	3	R	GS-320	9	HS	GS-370	5	MS
GS-261	3	R	GS-321	3	R	GS-378	5	MS
GS-262	5	MS	GS-322	5	MS	GS-379	5	MS
GS-263	5	MS	GS-324	3	R	GS-380	5	MS

GS-264	5	MS	GS-325	3	R	GS-381	7	S
GS-266	6	MS	GS-328	3	R	GS-382	1	HR
GS-267	3	R	GS-329	2	R	GS-384	5	MS
GS-269	6	MS	GS-331	7	S	GS-385	5	MS
GS-271	1	HR	GS-332	1	HR	GS-386	5	MS
GS-273	3	R	GS-333	1	HR	GS-387	7	S
GS-274	3	R	GS-334	1	HR	GS-390	3	R
GS-275	3	R	GS-335	1	HR	GS-391	7	S
GS-276	3	R	GS-336	5	MS	GS-392	5	MS
GS-277	2	R	GS-337	1	HR	GS-394	5	MS
GS-282	3	R	GS-338	5	MS	GS-395	5	MS
GS-284	3	R	GS-339	7	S	GS-396	5	MS
GS-286	7	S	GS-340	5	MS	GS-397	5	MS
GS-288	7	S	GS-341	5	MS	GS-398	5	MS
GS-289	7	S	GS-342	3	R	GS-401	5	MS
GS-290	1	HR	GS-344	5	MS	GS-403	2	R
GS-291	1	HR	GS-345	1	HR	GS-410	2	R
GS-292	7	S	GS-346	5	MS	GS-416	1	HR
GS-293	3	R	GS-347	3	R	GS-421	1	HR
GS-294	3	R	GS-349	5	MS	GS-436	2	R
GS-296	3	R	GS-350	5	MS	GS-442	5	MS
GS-303	5	MS	GS-351	3	R	GS-444	5	MS
GS-304	7	S	GS-355	5	MS	GS-446	5	MS
GS-305	7	S	GS-356	4	MR	GS-447	5	MS
GS-306	7	S	GS-357	7	S	GS-448	5	MS

Genotype	**Score**	**Disease Reaction**	**Genotype**	**Score**	**Disease Reaction**	**Genotype**	**Score**	**Disease Reaction**
GS-450	5	MS	GS-548	9	HS	GS-622	7	S
GS-452	5	MS	GS-554	9	HS	GS-624	7	S
GS-453	5	MS	GS-560	9	HS	GS-625	5	MS
GS-454	5	MS	GS-569	9	HS	GS-626	9	HS
GS-455	9	HS	GS-571	9	HS	GS-627	5	MS
GS-456	5	MS	GS-575	9	HS	GS-628	5	MS
GS-459	5	MS	GS-576	9	HS	GS-630	9	HS
GS-460	5	MS	GS-579	9	HS	GS-631	7	S
GS-462	5	MS	GS-580	9	HS	GS-632	7	S
GS-464	5	MS	GS-581	9	HS	GS-633	7	S
GS-467	5	MS	GS-582	9	HS	GS-634	7	S
GS-471	5	MS	GS-583	9	HS	GS-635	5	MS
GS-473	5	MS	GS-584	9	HS	GS-637	9	HS
GS-474	5	MS	GS-585	9	HS	GS-638	4	MR
GS-476	5	MS	GS-587	1	HR	GS-640	7	S
GS-477	5	MS	GS-588	9	HS	GS-642	9	HS
GS-478	5	MS	GS-589	9	HS	GS-643	9	HS
GS-480	9	HS	GS-590	9	HS	GS-644	5	MS
GS-484	5	MS	GS-593	9	HS	GS-647	9	HS
GS-487	5	MS	GS-594	9	HS	GS-648	9	HS
GS-491	5	MS	GS-595	9	HS	GS-649	8	HS
GS-492	9	HS	GS-596	9	HS	GS-650	9	HS
GS-496	9	HS	GS-601	9	HS	GS-651	7	S
GS-497	9	HS	GS-602	9	HS	GS-652	7	S
GS-499	9	HS	GS-605	9	HS	GS-653	5	MS
GS-504	2	R	GS-608	9	HS	GS-654	6	MS
GS-520	9	HS	GS-609	9	HS	GS-655	7	S
GS-522	9	HS	GS-610	5	MS	GS-656	5	MS
GS-523	9	HS	GS-611	5	MS	GS-657	5	MS
GS-525	9	HS	GS-612	5	MS	GS-658	2	R
GS-527	9	HS	GS-613	1	HR	GS-659	3	R
GS-529	9	HS	GS-614	5	MS			
GS-535	9	HS	GS-615	5	MS			
GS-537	9	HS	GS-616	6	MS			
GS-539	9	HS	GS-617	1	HR			
GS-540	9	HS	GS-618	5	MS			
GS-541	9	HS	GS-619	7	S			
GS-542	9	HS	GS-620	8	HS			
GS-546	9	HS	GS-621	7	S			

HR= Highly resistant, R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, HS=Highly susceptible, S=Susceptible

Discussion

Rice blast is the most destructive among diseases affecting rice production due to its widespread occurrence and high prevalence in favorable conditions. While chemical fungicides have been employed to manage this disease, they come with drawbacks such as costliness (Panda *et al*., 2017; Sahu *et al*., 2018) [36, 43], reduced effectiveness under high disease pressure (Jeevan *et al*., 2020) [21], and the potential to promote resistance in the pathogens (Yamaguchi, 2004) [54]. Consequently, the most economical and environmentally friendly approach for combating rice blast disease is to utilize host resistance.

Utilizing host resistance is the most convenient, preferred, cost-effective, sustainable, safe, and practical method of protecting plants, particularly for farmers with limited resources (Sharma, 1995; Ou, 1985; Bonman *et al*., 1992) [45, 34, 8]. Although numerous resistant varieties have been developed, the continuous adaptability of the pathogen genome poses an ongoing threat to the efficacy of these cultivars (Patil *et al*., 2013) [38]. Therefore, it is crucial to identify new sources of host disease resistance to facilitate the development of resistant cultivars. To address this objective, we conducted an experiment involving the screening of 355 rice genotypes against blast disease in a uniform blast nursery (UBN). From the results it was clear that among the tested genotypes, thirty one (9%) were highly resistant (HR), sixty one (17%) were resistant (R), three (1%) were moderately resistant (MR), one hundred one (28%) were moderately susceptible, 39 (11%) were susceptible and one hundred twenty (34%) were highly susceptible indicating that the genotypes were diverse. The variation in the blast disease severity was observed in between the genotypes suggesting that the pathogen was host genotype-specific. The observed variation in disease severity among the rice genotypes can be attributed to both environmental factors that favored disease development and the genetic differences among the genotypes. Understanding these differences in how rice genotypes respond to the pathogen is important for breeding programs aimed at developing disease-resistant rice varieties. Similar field screening experiments were conducted for identification of blast resistant lines by Pasha *et al*., 2013, Chuwa *et al*., 2015, Kumar *et al*., 2015, Lee *et al*., 2015, Zewdu *et al*., 2017, Mustafa *et al*., 2018, Acharya *et al*., 2019, Arun *et al*., 2022. Sadhana *et al*., 2023 [37, 9, 27-28, 30, 56, 32, 1, 4, 35] screened 18 F3 breeding lines against rice blast under uniform blast nursery and scrutinized that among the breeding lines, 12 lines were found resistant with a score of 3 and 6 lines were found moderately resistant with a score of 5.

Conclusion

Our study on leaf blast screening has generated valuable germplasm options that breeders can use as parental material for transferring blast resistance traits in developing resistant breeding lines. Among the genotypes, GS-173, GS-189, GS-133, GS-134, GS-170, GS-171, GS-188, GS-271, GS-345, GS-382 and so on, showed highly resistant response against rice blast disease and thus could serve as better donors in various breeding programs. Before these identified resistant genotypes are considered for release, it is essential to thoroughly characterize their resistance genes under variable environmental conditions by exposing them to different isolates of *M. oryzae* and then evaluating their performance in different yield trials for desirable agronomic traits, with the ultimate goal of recommending them for cultivation by farmers.

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