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Identification of blast-resistant advanced rice breeding lines through uniform blast nursery screening

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Abstract

Rice blast caused by *Magnaporthe oryzae* is a major constraint in rice production, leading to severe yield losses under favourable conditions. Developing resistant varieties is considered the most effective and eco-friendly strategy to manage the disease. RNR 15048 (Telangana Sona), a widely cultivated variety known for its fine grain quality and low glycemic index, lacks durable resistance to blast. To enhance the resistance, a breeding programme was carried out at the Rice Research Unit, ARI, Rajendranagar, Hyderabad. The major blast resistance gene *Pi54* was introgressed into RNR 15048 through marker-assisted pedigree breeding using MTU1010 NILs as the donor source. Altogether 40 advanced F₆ breeding lines were evaluated during the *Rabi* season under Uniform Blast Nursery conditions. Standard inoculation methods were followed to ensure uniform disease pressure across all the test entries. Disease reactions were recorded using the IRRI Standard Evaluation System on 0-9 scale. The inclusion of TN1 (susceptible) and NLR 34449 (resistant) checks confirmed the reliability of the screening process. Among the lines evaluated, 10 were highly resistant, 20 were resistant, and 10 were moderately resistant. The susceptible check, TN1, recorded a disease score of 9, while NLR 34449 showed a score of 1, indicating effective screening conditions. Overall, the study serves as a platform to identify promising lines combining blast resistance with desirable agronomic traits for further breeding programmes.

Keywords: Blast, resistant, *Pi54*, screening, rice

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops worldwide and serves as the primary staple food for more than half of the global population. Rice plays a critical role in global food and nutritional security (Thapa & Bhusal, 2020) [1]. At present, global demand for rice continues to rise due to population growth and improved living standards. Projections indicate that rice production must increase by nearly 30% by 2030 to meet future food requirements (Devanna *et al.*, 2022) [3]. Both abiotic and biotic stresses significantly affect rice production. Among them, rice blast caused by *Magnaporthe oryzae* is the most destructive and economically significant disease, resulting in yield losses of 70% to 80% under severe conditions (Miah *et al.*, 2013) [2]. Although fungicides can reduce disease severity, their application is often uneconomical and environmentally unsafe, particularly in developing countries. Consequently, the development of blast-resistant rice cultivars remains the most effective, cost-efficient and environmentally sustainable strategy for managing rice blast and ensuring stable rice production. The pathogen shows high genomic variability, driven by transposable elements and the rapid evolution of effector genes. This dynamic nature allows the pathogen to adapt and overcome resistance genes introduced into cultivated varieties quickly. As a result, there is a constant need to discover and incorporate new resistance (R) genes to combat emerging pathogenic races effectively. In view of this challenge, the present study emphasized phenotypic screening of advanced breeding lines to identify genotypes with stable and effective resistance to blast disease, thereby contributing to the development of durable resistance in rice.

2. Materials and Methods

Near-isogenic lines derived from RNR 15048 × MTU1010 NIL that are available at Rice Research Unit, ARI, Hyderabad were used in the present investigation. These NILs possess a long slender grain type carrying blast resistance gene *Pi54*.

Two control genotypes, TN1 and NLR 34449, were used as susceptible and resistant checks, respectively, during phenotypic screening in the Uniform Blast Nursery. A total of 40 advanced breeding lines at F6 generation derived from the cross between RNR 15048 (Telangana Sona) and MTU1010 NIL (*Pi54* donor) was selected for phenotypic evaluation during *Rabi* 2025-26. RNR 15048, an elite, short-duration (125 days), high-yielding variety with a low glycemic index (~51.7) with desirable cooking quality, served as the female parent, while MTU1010 NIL was used as the male parent for introgression of blast resistance. The entire study, including screening and evaluation, was undertaken at the Rice Research Unit, Agricultural Research Institute, Rajendranagar, Hyderabad.

2.1. Isolation and maintenance of blast culture

Scraping Method

Rice leaves showing blast symptoms were collected and cut into small pieces. These pieces were surface-sterilized with 0.1% mercuric chloride and washed 3-4 times with sterile distilled water. The cleaned pieces were then placed on leaf extract agar in sterile Petri dishes under aseptic conditions and incubated at 27 °C for 3-4 days, until fungal growth was visible. After appearance of fungal growth, a small portion of the mycelium was carefully picked and transferred onto fresh agar plates under aseptic conditions. These plates were then incubated at 27 °C to promote further growth. Once the culture became well established, it was sub-cultured onto agar slants in test tubes for storage and routine maintenance.

Mass Multiplication on Oat Meal Agar (OMA)

A seven-day-old actively growing culture was aseptically transferred to sterile oatmeal agar plates. These plates are incubated at 27-28 °C for 8-10 days to allow sufficient fungal growth and sporulation. The pathogen produces simple, grey conidiophores bearing terminal, pear-shaped and 2-septate conidia.

Uniform Blast Nursery Method for Screening

Screening for blast resistance was conducted using the Uniform Blast Nursery (UBN) method. The nursery consists of raised beds measuring 10 m × 1 m, which were prepared with farm yard manure (FYM) with the recommended dose of fertilizers. The test entries were sown in rows about 50 cm long, with a spacing of 10 cm between rows. To maintain enough disease pressure, the highly susceptible variety, TN1, was planted as border rows around the nursery and also after every ten test entries. This arrangement will facilitate the uniform spread of the pathogen. The test lines were sown perpendicular to the border rows to enhance exposure to inoculum. Relative humidity was maintained using regular water sprinkling. To further enhance moisture levels and increase disease pressure on the test entries, the nursery beds were covered with polythene sheets during night.

Pathogen Inoculation and Disease Scoring

A spore suspension of *Magnaporthe oryzae* is prepared from

a 7-to 10-day-old culture grown on oatmeal agar at 25-28 °C. The culture plates were washed with sterile distilled water, and the fungal growth, including mycelium and conidia, was gently scraped. The suspension was shaken for a few minutes to release spores and then filtered through muslin/cheese cloth to remove debris. Spore concentration was adjusted to approximately 1×10^5 conidia ml⁻¹ using a haemocytometer, and a small amount of Tween-20 was added to the spore suspension to ensure uniform spreading. The prepared inoculum was evenly sprayed onto 15-21-day-old seedlings in the Uniform Blast Nursery using a hand sprayer in the evening, ensuring complete foliage wetting. High humidity, which essential for disease development, was maintained by regular sprinkler irrigation during the day, taking care not to wash off the inoculum immediately after spraying. Disease development was monitored, and scoring was carried out 15 days after inoculation using the Standard Evaluation System (SES, 2013). Based on lesion severity, the entries were grouped into highly resistant (0-1), resistant (1.1-3), moderately resistant (3.1-5), moderately susceptible (5.1-6), susceptible (6.1-8.9) and highly susceptible (9).

3. Results and Discussion

The study aims to identify rice genotypes with improved resistance to blast disease through screening under Uniform Blast Nursery (UBN) conditions. A total of 40 advanced breeding lines, along with parental lines RNR 15048, MTU1010 NILs, and the check varieties TN1 (susceptible) and NLR 34449 (resistant), were evaluated. Disease severity was assessed using the IRRI Standard Evaluation System (SES, 2013) [12] on a 0-9 scale. Adequate disease pressure was confirmed by the high susceptibility of TN1, which scored around 9, indicating the reliability of the screening conditions. Based on SES scoring, the breeding lines are grouped into different resistance classes. Of the 40 advanced lines evaluated, 10 showed high resistance (score 0-1), 20 were classified as resistant (1.1-3.0) and 10 exhibited moderate resistance (3.1-5.0).

All 40 advanced breeding lines, along with the parental genotypes, exhibited highly resistant to moderately resistant reactions against blast disease, with scores ranging from 1 and 5 on the IRRI 0-9 SES scale. Among them, 10 lines CDR-7, CDR-9, CDR-10, CDR-13, CDR-14, CDR-16, CDR-18, CDR-22, CDR-32 and CDR-37 exhibited strong resistance with a score of 1 and were categorized as highly resistant. A total of 20 lines CDR-1, CDR-2, CDR-3, CDR-5, CDR-6, CDR-8, CDR-11, CDR-12, CDR-17, CDR-19, CDR-20, CDR-23, CDR-24, CDR-28, CDR-29, CDR-30, CDR-31, CDR-33, CDR-35 and CDR-40 recorded a score of 3 and were classified as resistant, while 10 lines CDR-4, CDR-15, CDR-21, CDR-25, CDR-26, CDR-27, CDR-34, CDR-36, CDR-38 and CDR-39 showed moderate resistance with a score of 5. The parental lines, RNR 15048 and MTU1010 NIL, showed moderate resistance and resistance with scores of 5 and 3, respectively.

Table 1: Blast disease reaction of rice breeding lines under UBN conditions.

S. No	Lines	Score (0-9)	Disease reaction
1	CDR-1	3	R
2	CDR-2	3	R
3	CDR-3	3	R
4	CDR-4	3	R
5	CDR-5	3	R
6	CDR-6	3	R
7	CDR-7	1	HR
8	CDR-8	3	R
9	CDR-9	1	HR
10	CDR-10	1	HR
11	CDR-11	1	HR
12	CDR-12	1	HR
13	CDR-13	3	R
14	CDR-14	1	HR
15	CDR-15	1	HR
16	CDR-16	3	R
17	CDR-17	3	R
18	CDR-18	5	MR
19	CDR-19	5	MR
20	CDR-20	5	MR
21	CDR-21	5	MR
22	CDR-22	5	MR
23	CDR-23	5	MR
24	CDR-24	5	MR
25	CDR-25	5	MR
26	CDR-26	3	R
27	CDR-27	5	MR
28	CDR-28	5	MR
29	CDR-29	3	R
30	CDR-30	3	R
31	CDR-31	3	R
32	CDR-32	3	R
33	CDR-33	3	R
34	CDR-34	1	HR
35	CDR-35	1	HR
36	CDR-36	1	HR
37	CDR-37	3	R
38	CDR-38	3	R
39	CDR-39	3	R
40	CDR-40	3	R
Parent 1	RNR 15048	5	MR
Parent 2	MTU1010 NIL	3	R
Resistant check	NLR 34449	1	HR
Susceptible check	TN-1	9	HS



Fig 1: Phenotypic screening for blast resistance using *Magnaporthe grisea* culture. NLR 34449-Resistant check; TN-1- Susceptible check; CDR-1 to CDR-5 Advanced breeding lines possessing the *Pi54* gene.

In contrast, the susceptible check, TN1, recorded a score of 9, indicating severe infection and confirming the presence of

sufficient disease pressure. The resistant check, NLR 34449, showed a score of 1, validating the effectiveness of the screening. Overall, the clear distinction between resistant, moderately resistant and susceptible reactions demonstrated that the Uniform Blast Nursery conditions were adequate for reliable evaluation of blast resistance in the breeding material. Similar field screening experiments aimed at identifying location-specific blast-resistant lines have also been carried out by Sadhana *et al.* (2023) ^[4], Umamahesh *et al.* (2024) ^[5] and Sai Akhila *et al.* (2025) ^[8], who reported comparable findings in their respective studies.

4. Conclusion

In the present study, the major dominant blast resistance gene *Pi54* gene was successfully introgressed into RNR 15048 (Telangana Sona) through marker-assisted pedigree breeding. The developed breeding lines carrying *Pi54* gene demonstrated a high level of resistance to blast under station conditions. Due to broad-spectrum resistance of *Pi54* gene against prevalent races of *Magnaporthe oryzae* in India, these lines are also expected to perform consistently across different regions of Telangana and other parts of South India. The improved lines retain the key characteristics of the recurrent parent RNR 15048, including its short duration, high yield potential and preferred short slender grain type with good cooking quality, while incorporating durable resistance to blast disease.

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