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Genetic improvement of RNR 15048 for blast resistance by using UBN phenotypic screening method

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Abstract
Rice blast, caused by *Magnaporthe oryzae*, remains one of the most severe biotic stresses, leading to major yield losses. Breeding for host resistance is the most sustainable and eco-friendly approach to mitigate these losses. RNR 15048 (Telangana Sona), a popular cultivar known for its desirable grain quality and low glycemic index, lacks durable resistance to blast. To enhance its resistance profile, a breeding program was undertaken at Institute of Rice Research, ARI, Rajendranagar, Hyderabad. Subsequently, *Pi54*, a major blast resistance gene, was introgressed into RNR 15048 through marker-assisted pedigree breeding using MTU1010 NILs as donors. Fifty F7 progenies were phenotypically screened for blast resistance during *Rabi* 2024-25 under UBN conditions for identification of improved lines. Advanced breeding lines were evaluated in a Uniform Blast Nursery (UBN) using SES (0-9 scale). Susceptible (TN1) and resistant (NLR 34449) checks validated the screening. Of these 50 advanced breeding lines, 21 showed resistant reaction with a disease reaction score of 3, while 6 lines were moderately resistant with a disease reaction score of 5 and other 23 lines showed Highly resistant reaction with a disease reaction score of 1. While Susceptible check TN1 showed highly susceptible reaction with a disease reaction score of 9 and resistant (NLR 34449) check is one.

Keywords: Rice blast, *Magnaporthe oryzae*, Pi54, marker-assisted breeding, RNR 15048

1. Introduction
Rice (*Oryza sativa* L.), a staple food for over half of the global population, plays a vital role in food and nutritional security. To meet the rising demand due to projected population growth, rice production must double by 2050. Among biotic stresses, *Magnaporthe oryzae*-induced blast disease remains the most destructive, accounting for up to 35% of total yield losses, and even higher (70-80%) during severe outbreaks. Despite the availability of chemical control, overuse of fungicides leads to resistance development in pathogen populations, posing challenges to sustainable production. Genetic resistance, governed by host resistance (R) genes, offers an eco-friendly and durable alternative. However, the high genomic plasticity of *M. oryzae*, facilitated by transposable elements and rapid effector gene evolution, enables it to overcome deployed resistance genes. This necessitates continuous identification and introgression of novel R genes to manage evolving pathogenic races. In this context, the present study focused on phenotypic screening of advanced breeding lines to identify genotypes carrying effective resistance against blast, thereby supporting durable resistance breeding programs.

2. Materials and Methods
In a collaborative effort between ICAR-IIRR and IRRI, near-isogenic lines (NILs) were developed in the MTU1010 background (IR121055-2-10-7) that carry the *Pi54* blast-resistance gene and exhibited a long, slender grain phenotype. Two control genotypes *viz*., TN1 and NLR34449 served as susceptible and resistant checks respectively, in the Uniform Blast Nursery during phenotypic evaluations. From the cross derived above, 50 advanced breeding progenies were selected and phenotypically screened for blast resistance at F7 generation. For further breeding, Telangana Sona (RNR15048) an elite, short-duration (125-day), high-yielding variety with a low glycemic index (~51.7) and favorable cooking traits served as the female parent. These were crossed with the MTU1010 NIL (Pi54 donor) to generate F7 breeding lines. All experimental and evaluation work was conducted at the Agricultural Research Institute, Rajendranagar, Hyderabad.

2.1 Isolation and Maintenance (Scraping Method)
Surface-sterilize blast-infected leaf fragments using 0.1 % $HgCl_2$, rinse 3-4 times with sterile water, then place on leaf-extract agar. Incubate at 27 °C for 3-4 days until fungal hyphae appear. Aseptically transfer emerging mycelium to fresh plates, incubate likewise, then subculture to slant tubes with sterile leaf-extract agar to establish pure cultures.

2.1 Mass Multiplication on Oat Meal Agar (OMA)
Using seven-day-old fungal plugs, inoculate oatmeal agar plates (optionally amended with streptomycin). Incubate at 28 °C for seven days to induce sporulation. The pathogen produces simple, grey conidiophores bearing terminal, pear-shaped and 2-septate conidia.

2.1 Uniform Blast Nursery (UBN) Setup
Prepare a 10 × 1 m bed enriched with FYM and recommended fertilizers. Sow the susceptible check TN1 around the borders and after every ten test entries. Plant breeding lines orthogonally. Maintain high humidity via sprinkler irrigation and night-time polythene covers to maximize disease pressure.

2.1.4 Pathogen Inoculation and Disease Scoring
Harvest spores from 7-day-old OMA cultures by washing plates with 10 ml sterile water, shaking for 5-10 min and filtering through cheesecloth. Adjust concentration to ~10^5 conidia/ml with 0.2 % Tween-20. Spray onto 15-day-old seedlings in the evening using a low-volume sprayer. Incubate overnight under high humidity, maintained by sprinklers 3-4 times daily. Monitor lesions; score disease severity 15 days post-inoculation using the SES 0-9 scale. Categorize lines: 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 primary objective of this work is to evaluate blast resistance in breeding lines along with parents RNR 15048, MTU 1010 NILs, ISM NILs and checks TN1 and NLR34449 using the Uniform Blast Nursery (UBN) protocol, with disease severity scored according to the IRRI Standard Evaluation System (SES, 2013). Based on SES thresholds, breeding lines were classified as resistant, moderately resistant or highly susceptible. Among the 50 advanced breeding lines, 23 exhibited highly resistant phenotypes (SES 0-1), 21 were categorized as resistant (SES 1.1-3.0) and 6 showed moderate resistance (SES 3.1-5.0).
All the 50 advanced breeding lines and parent genotypes exhibited resistance to moderate resistance against blast, with scores ranging from 1 to 5 on the IRRI 0-9 SES scale. Among them, 23 lines *viz*., JSA-1, JSA-2, JSA-5, JSA-6, JSA-11, JSA-12, JSA-14, JSA-17, JSA-19, JSA-21, JSA-22, JSA-22, JSA-24, JSA-26, JSA-27, JSA-32, JSA-33, JSA-38, JSA-39, JSA-40, JSA-42, JSA-44, JSA-45 and JSA-48 recorded a score of 1, indicating strong resistance. Twenty-one lines JSA-3, JSA-4, JSA-7, JSA-9, JSA-10, JSA-13, JSA-15, JSA-16, JSA-18, JSA-23, JSA-25, JSA-29, JSA-31, JSA-34, JSA-36, JSA-37, JSA-41, JSA-43, JSA-46, JSA-47and JSA-50 scored 3, also within the resistance category. 6 lines JSA-8, JSA-20, JSA-28, JSA-30, JSA-35 and JSA-49 scored 5, categorizing them as moderately resistant. Parent RNR 15048 and MTU1010 NIL also scored 3, indicating resistance. The susceptible check TN1 scored 9, confirming high disease pressure, while the resistant check NLR 34449 scored 1, validating screening effectiveness. The clear differentiation among resistant, moderately resistant and susceptible controls confirms that the Uniform Blast Nursery generated adequate pathogen pressure for reliable phenotypic assessment.

S. No	Lines	Score	Disease Reaction
1	JSA-1	1	HR
2	JSA-2	1	HR
3	JSA-3	3	R
4	JSA-4	3	R
5	JSA-5	1	HR
6	JSA-6	1	HR
7	JSA-7	3	R
8	JSA-8	5	MR
9	JSA-9	3	R
10	JSA-10	3	R
11	JSA-11	1	HR
12	JSA-12	1	HR
13	JSA-13	3	R
14	JSA-14	1	HR
15	JSA-15	3	R
16	JSA-16	3	R
17	JSA-17	1	HR
18	JSA-18	3	R
19	JSA-19	1	HR
20	JSA-20	5	MR
21	JSA-21	1	HR
22	JSA-22	1	HR
23	JSA-23	3	R
24	JSA-24	1	HR
25	JSA-25	3	R
26	JSA-26	1	HR
27	JSA-27	1	HR
28	JSA-28	5	MR
29	JSA-29	3	R
30	JSA-30	5	MR
31	JSA-31	3	R
32	JSA-32	1	HR
33	JSA-33	1	HR
34	JSA-34	3	R
35	JSA-35	5	MR
36	JSA-36	3	R
37	JSA-37	3	R
38	JSA-38	1	HR
39	JSA-39	1	HR
40	JSA-40	1	HR
41	JSA-41	3	R
42	JSA-42	1	HR
43	JSA-43	3	R
44	JSA-44	1	HR
45	JSA-45	1	HR
46	JSA-46	3	R
47	JSA-47	3	R
48	JSA-48	1	HR
49	JSA-49	5	MR
50	JSA-50	3	R
Parent 1	TN1	9	HS
Parent 2	NLR34449	1	HR

4. Conclusion
Here, We introgressed the dominant blast-resistance gene *Pi54* into RNR 15048 (Telangana Sona) via marker-assisted pedigree breeding. The resulting progeny carrying *Pi54* showed strong resistance to blast under station trials and are expected to exhibit similar performance across

Telangana and other South Indian states, as *Pi54* confers broad-spectrum resistance against prevalent Indian *Magnaporthe oryzae* races. These new lines are genetically akin to their female parent RNR 15048 preserving its short, slender grain and cooking characteristics while adding durable blast resistance and high yield.

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