Original Research Article

Identification of blast resistant breeding lines through Uniform Blast Nursery in rice (*Oryza sativa* L.)

Abstract:

Rice blast disease caused by the fungus *Magnaporthe oryzae* is the most devastating disease of rice causing considerable yield losses. Development of resistant varieties is the most effective and environment friendly strategy to combat losses. RNR 15048 (Telangana sona) (IET23746) is a popular local variety possessing a desirable, short-slender grain type with low glycemic index, good cooking quality and having *Pi2* gene for blast. However, it is moderately susceptible to bacterial blight disease and having no durable resistance to blast disease with weak culm. In this context, improvement of RNR 15048 for blast, bacterial leaf blight is important to reduce yield losses. Present study was carried out to screen and identify blast resistant breeding lines in Rice at Regional Agricultural Research station, Polasa, Jagtial, PJTSAU during *Rabi*, 2021-2022. 18 F_3 breeding lines were screened in uniform blast nursery along with parents RNR 15048, MTU 1010 NILs, ISM NILs. TN1 and NLR 34449 were used as the susceptible and resistant checks, respectively for screening blast resistance. Screening was done by following 0-9 Standard Evaluation Scale (SES) for leaf blast. Among the studied 18 F_3 breeding lines 12 lines were found resistant with score of 3 and 6 lines were found moderately resistant to screening reaction with score of 5. TN1 showed highly susceptible reaction with score of 9 and NLR 34449 showed highly resistant reaction with score of 1.

Key words: Rice, Blast, Uniform Blast Nursery, Resistant, Susceptible

1. Introduction:

Rice (*Oryza sativa* L.) is an important and most widely consumed cereal crop for more than half of the population, providing livelihood and nutritional security to the world's population. As the global population is expected to reach 9.77 billion by 2050, the rice production needs to be increased by two-fold from the current levels to ensure both global food and nutritional security. Around 52 per cent of the world's rice production is lost annually owing to the damage caused by biotic factors, of which 30 per cent is attributed to the attack of diseases [1]. Rice blast disease caused by the fungus *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*), is the most devastating disease of rice because of its wide distribution and its destructiveness under conducive conditions [2]. It is also referred as rice fever disease and has been reported in approximately 85 rice growing countries across the world [3]. During a disease pandemic, there is a 70-80% yield loss owing to blast [4]. 76 percent reduction in grain yield was observed when infection occurred immediately after flowering [5]. In India, blast disease was first recorded in 1913 and the first devastating epidemic was reported in 1919 in the Tanjore delta of erstwhile Madras state. Between 1980 and 1987, there were seven blast epidemics, which resulted in significant yield losses in the states of Himachal Pradesh, Andhra Pradesh, Tamil Nadu, and Haryana.

Even though blast disease can be controlled by using pesticides, but frequent usage could stimulate the tolerance and evolution of pathogens, which becomes a greater threat to the safety rice production. Alternatively, the most economical and an ecofriendly approach is to explore the host plant resistance (R) gene that limits the incidence of blast disease [6]. Effective management of blast disease require constant breeding efforts for development of resistant cultivars. *M. oryzae* genome is rich in repetitive segments and retro-transposons, which enable the fungus to frequently change its pathogenicity or avoid being recognised by its host by changing the effector molecules, breaking down the resistance provided by R genes and causing epidemics of disease [7]. These can be affected by several factors, including weather conditions, disease prevalence, as well as and the pathogen's genetic stability. In order to combat persistently changing and geographically diversified

pathogen races, it is necessary to continuously identify novel sources of host disease resistance. Host plant resistance proved to be the best strategy for blast disease management. Hence, development of blast resistant lines has gained importance. With the objective of identification of better breeding lines for blast resistance, blast screening was carried out in the present study.

2. Materials and Methods:

The material for the execution of current study was obtained by crossing RNR 15048 with MTU 1010 NILs (*SCM2*). F_1s were developed during *Rabi*, 2020-2021. F_1s were confirmed for heterozygosity using gene specific marker for *SCM2*. F_1s heterozygous for *SCM2* were crossed with the variety, Improved Samba Mahsuri NIL, possessing *Xa21+Pi54* during *Kharif*, 2021. Triple gene positive F_1s were selfed to develop F_2 seed during *Rabi*, 2021-2022 and F_2s were confirmed using gene specific markers and gene positive F_2s were selfed to develop F_3 seed during *Kharif*, 2022. F_3 seed developed was screened in uniform blast nursery during *Rabi*, 2021-2022. The pathogen strains were cultured and stored as described [8], was used for screening throughout the study as mentioned here under.

2.1 Isolation and maintenance of blast cultures

2.1.1 Scraping method:

Blast infected leaves were collected and made into bits these were surface sterilized using 0.1% mercuric chloride and washed in sterile distilled water for 3-4 times. Infected leaf bits were kept on leaf extract agar medium in a petri plate under aseptic condition and are incubated at 27°C for 3-4 days till mycelial growth is observed. The fungal mycelium is scraped from the infected leaf bit and transferred to a fresh petri plate containing sterile leaf extract agar. The petri plates are incubated at 27°C for further growth. After a significant amount of development, the fungus is then transferred to test tubes containing sterile leaf extract agar for culture establishment.

2.1.2 Mass multiplication on oat meal agar:

Oat meal agar is prepared by using ready-made OMA media. Seven days old pre inoculated fungal agar block is aseptically transferred to sterile Oat meal agar containing petri plates and these plates are incubated at 28°C for 7 days till sporulation is observed. Simple, grey conidiophores with terminal, pear-shaped, typically two septate conidia are produced by the fungus.

2.1.3 Uniform Blast Nursery (UBN) method of screening:

Uniform blast nursery is a 10 × 1 m bed and the soil is enriched with FYM and recommended dose of fertilizers. Local susceptible variety TN 1, is sown in border rows on all sides of the bed. The susceptible check variety is sown after every ten test entries (Fig.1). This helps to spread the inoculum. Breeding material to be tested is sown in rows perpendicular to the border rows. Relative humidity is maintained with water sprinklers. In order to maintain high humidity levels and to put more disease pressure on the entrances, the beds are covered with polythene covers at night.

2.1.4 Inoculation

A 7-day-old blast culture grown on oat meal agar at 25°C to 28°C is used to make the spore suspension, the plates were washed with 10ml of sterile distilled water to make a spore suspension. The collected water in a conical flask containing mycelia and spores was shaken well for 5-10 minutes, using a rotary shaker to detach conidia from the conidiophores of mycelia. To remove fungal debris, spore suspension was filtered through four layers of cheese cloth, and the spore concentration was then adjusted to roughly 10^5 spores per millilitre. The spore suspension containing Tween-20 (0.2%) was sprayed uniformly onto 15-day-old seedlings using a hand-held low volume capacity plastic sprayer on all the plants in UBN beds. The plants were sprayed in the evening hours and left overnight, undisturbed in a humidity chamber. Humidity was maintained by spraying water for 3-4 times a day using sprinklers. Inoculum was sprayed at least twelve hours

before spraying water. However, care was taken so that the water is not sprayed immediately after spraying. Inoculated seedlings were monitored for the development of blast lesions, fifteen days after inoculation the test entries were scored based on the leaf blast severity following Standard Evaluation Scale (SES) (2013) (Table1). Based on leaf blast scores recorded, breeding lines were categorized as highly resistant (0-1), resistant (1.1-3.0), moderately resistant (3.1-5.0), moderately susceptible (5.1-6.0) susceptible (6.1-8.9) and highly susceptible (9.0) (Table 2).

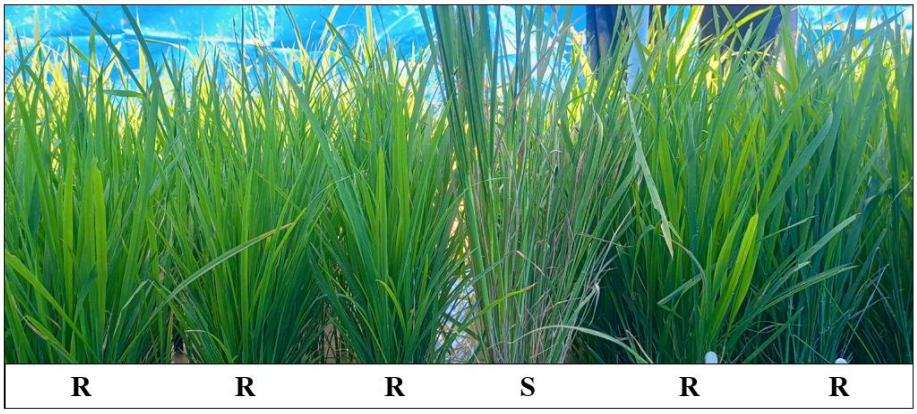

Fig 1. Screening of F_3 Breeding lines in Uniform Blast Nursery (UBN) at RARS, Jagtial.
R: Resistant line, S: Susceptible check-TN1

Table 1. Standard Evaluation System, IRRI (2013) scale for leaf blast in Rice.

Scale	Disease severity	Host response
0	Lesions are not present	Highly Resistant (HR)
1	Small brown specks of pin point size or large brown specks without sporulating centre	Resistant (R)
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves	Resistant (R)
3	Lesions type is same as in scale 02, but a significant	Resistant (R)

	number of lesions on upper leaf area	
4	Typical susceptible blast lesions, 3mm or longer infecting less then 4% of leaf area	Moderately Resistant (MR)
5	Typical susceptible blast lesions infecting 4-10% of leaf area	Moderately Resistant (MR)
6	Typical susceptible blast lesions infecting 11-25% of the leaf area	Moderately Susceptible (MS)
7	Typical susceptible blast lesions infecting 26-50% of the leaf area	Susceptible (S)
8	Typical susceptible blast lesions infecting 51-75% of the leaf area and many leaves are dead	Susceptible (S)
9	More than 75% of the leaf area affected	Highly Susceptible (HS)

3. Results and Discussion:

The main aim of the study is to screen and identify blast resistant breeding lines along with parents RNR 15048, MTU 1010 NILs, ISM NILs and checks TN 1 and NLR 34449 by following UBN method. Accordingly scoring was done using Standard Evaluation Scale (SES) (2013) system. Breeding lines were categorized as resistant, moderately resistant and highly susceptible according to the screening. Among the 18 F_3 breeding lines studied 12 lines were found to be resistant and 6 lines were found to be moderately resistant to screening reaction.

Table 2: Blast reaction of F_3 Breeding lines based on 0-9 scale (IRRI- SES, 2013) in rice.

S.No	Breeding Lines	Reaction to leaf blast	Disease reaction

		(Score 0-9 scale)	
1	SF-1	3	R
2	SF-2	3	R
3	SF-3	5	MR
4	SF-4	3	R
5	SF-5	3	R
6	SF-6	3	R
7	SF-7	3	R
8	SF-8	5	MR
9	SF-9	3	R
10	SF-10	5	MR
11	SF-11	5	MR
12	SF-12	5	MR
13	SF-13	3	R
14	SF-14	3	R
15	SF-15	3	R
16	SF-16	5	MR
17	SF-17	3	R
18	SF-18	3	R
	Parents and checks	**Reaction to leaf blast (Score 0-9 scale)**	**Disease reaction**
19	RNR 15048	3	R
20	MTU 1010 NIL	3	R
21	ISM NIL	3	R
22	NLR34449(resistant	1	R

	check)		
23	TN1(susceptible check)	9	HS

R: Resistant, MR: Moderately Resistant, S: Susceptible, MS: Moderately Susceptible, HS: Highly Susceptible

Among the lines screened for blast 12 breeding lines SF-1, SF-2, SF-4, SF-5, SF-6, SF-7, SF-9, SF-13, SF-14, SF-15, SF-17 and SF-18 have shown resistant reaction with a score of 3 and 6 breeding lines SF-3, SF-10, SF-11, SF-12 and SF-16 have shown moderately resistant reaction with a score of 5. Parents RNR 15048, MTU 1010 NIL and ISM NIL have shown resistant reaction with a score of 3. NLR 34449 and TN1 were reported resistant score of 1 and highly susceptible score of 9, respectively. Similar field screening experiments were conducted for identification of location specific blast resistant lines and results were reported [9,10,11].

Comment [mm1]: Add discussion. Please explain result this study, what the meaning of table 2. And comparaition with others study before

4. Conclusions:

These lines have to be forwarded to next generations, once attain the homozygosity to be tested in multi locations to identify best genotypes that show stable performance across Telangana and in AICRP trials. These lines could serve better in different locations across India where severe blast conditions prevails. These lines could also serve as donors to use in further breeding programmes.

References:

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