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# Identification of superior rice (*Oryza sativa* L.) genotypes for blast resistance through UBN Method (Uniform Blast Nursery)

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**Abstract**
Half of the world's population depends on rice as a staple food. Rice blast caused by *Magnaporthe oryzae* is one of the destructive diseases of rice causing enormous yield losses in several rice growing areas. Among the different management strategies host plant resistance is proved to be the best strategy to combat the blast disease. The current experiment was carried out to identify the superior rice (*Oryza sativa* L.) genotypes for blast resistance at regional agricultural research station (RARS) Polasa, Jagtial during *rabi* 2021-22. In this study, different rice genotypes comprising of 13 parents, 32 hybrids (including two commercial hybrids) along with suitable susceptible check variety (TN-1) were screened by adopting uniform blast nursery (UBN) technique and entries were scored using 0-9 scale of Standard Evaluation system (SES) developed by IRRI, 2002. In this experiment susceptible check TN -1 expressed highly susceptible reaction with score 8 on disease scale, it indicates that there is sufficient inoculum in the field to cause disease. Accordingly, on basis of disease scoring the genotypes were categorized as moderately resistant, moderately susceptible and susceptible genotypes. This experiment revealed that among the 45 tested genotypes none of the genotypes showed highly resistant to blast disease but 9 hybrids and 1 parental line was exhibited moderately resistant, 22 hybrids and 9 parents were moderately susceptible and one hybrid and 3 parents showed susceptible reaction to the blast disease.

**Keywords:** Parental lines, hybrids, rice blast, *M. oryzae* and uniform blast nursery

## Introduction
Rice (*Oryza sativa* L.) is the world's most important food crop and it feeds more than half of the world's population and provides 20% of daily calories (Carrijo *et al.*, 2017) [1]. The global area under rice cultivation is 165.2 million hectares with a production of 509.3 million tons per annum with productivity of 4600 kg $ha^{-1}$. In India, it is the staple food for more than 65% of population and the predominant food crop grown in area of about 45.8 million hectares with a production of 124 million tonnes and productivity of 2717 kg $ha^{-1}$. In Telangana State rice is grown in 3.2 million hectares with the production of 102.2 million tonnes and productivity of 3206 kg/ha (2020-2021) (www.indiastat.com).

Despite of its high productivity, yields of rice crop are low due to many biotic and abiotic constraints. Among the biotic constraints various diseases *viz*., 36 fungal, 21 viral and 6 bacterial diseases (Ou, 1985) [7] and insect pests causes major yield loss in rice crop. The rice blast caused by *Magnaporthe oryzae* (Hebert) Bar [formerly *Pyricularia oryzae* B. Couch] is one of the most important fungal disease effecting considerable loss in rice production. It is a widespread and damaging disease of cultivated rice and around 50 per cent of production may be lost in a field moderately affected by blast infection. Every year the fungus affects rice enough to feed an estimated 60 million people (Zeigler *et al*., 1994) [13]. In India, it was first registered in Thanjavur (Tanjore) delta of South India by Mc Rae in 1918. Between 1980 and 1987, seven explosion epidemics were occurred in Himachal Pradesh, Andhra Pradesh, Telangana State, Tamil Nadu and Haryana, leading in massive yield losses. Rice blast pathogen can infect the plant during nearly all growth stages of the crop. Recurrent epidemics and often breakdown of rice blast resistance causing yield losses of 20–100% have been registered over the last decades in India and Japan (Vasudevan *et al*., 2014; Sharma *et al*., 2012) [10]. Rice blast pathogen is dynamic in nature and can break down the resistance conferred by R genes, therefore resulting in disease epidemics. These can be influenced by several factors, including weather conditions, disease pressure and frequent variation in the *M. oryzae* population especially when the crop is grown in large areas.

With regard to plasticity of pathogen, development of broad spectrum and durable blast resistant cultivars is vital to combat this disease, which needed continuous efforts of breeders and plant pathologists. In Northern Telangana also it is causing much damage to the rice crop. Using host plant resistance has been proven to be the most effective and economical method to control rice blast disease (Fukuoka *et al*., 2009) [2] and it needs continuous breeding efforts for the development of resistant cultivars. Hence, identification and development of durable blast resistant rice hybrids has gained more importance. Keeping this in view, the present work was carried out with an objective of identification of superior rice genotypes for blast resistance by screening through uniform blast nursery method at Regional Agricultural Research Station, Polasa, Jagtial.

## Material and Methods

In this experiment a total of 46 genotypes were evaluated including 32 hybrids, 13 parental lines and one susceptible check. Among the 32 hybrid lines 30 hybrids were developed through crossing three testers with 10 cytoplasmic male sterile lines at RARS, Polasa, Jagtial and two commercial hybrids (hybrid checks) and the variety TN-1 used as the susceptible check. The details of the genotypes mentioned in Table No. 1. The screening experiment was conducted during *Rabi* 2021-2022 by adopting the Uniform Blast Nursery (UBN) technique. The genotypes were sown as a separate replication and screening data was not subjected to statistical analysis.

### Isolation and maintenance of blast cultures

Blast infected rice leaf samples showing brown spindle shaped lesions were collected from research fields of RARS, Polasa, Jagtial during *Kharif* 2021. To isolate the pathogen blast-infected leaf tissue showing typical blast symptoms are surface sterilized with 0.1% Sodium hypo-chloride and later washed in sterile distilled water for 3-4 times and then placed in a Petri plate containing oatmeal agar medium (rolled oats 20 g, agar 15 g and distilled water 1 liter). After incubating the cultures at 28 ± 1 °C for 7 days the culture was purified and maintained on OMA for further study.

### Mass multiplication on oat meal agar

The rice blast pathogen was mass multiplied on oat meal agar medium. Mycelial discs of 5 mm were cut from 7-day old culture of *M. oryzae* was transferred aseptically to sterile oat meal agar medium containing Petri plates (3 discs/plate) later these plates are incubated at 28±1 $^0$C for 8 to 10 days.

### UBN (Uniform Blast Nursery) method of screening for blast disease resistance

Uniform Blast Nursery (UBN) Method was followed in this screening method. UBN was a 10 x 1 m bed and the soil was pre-treated with FYM and recommended dose of fertilizers. Test entries were sown in 50 cm long rows by following the 10 cm row to row spacing. To establish a strong disease pressure for every 10 lines of test entries and around the nursery bed single row of susceptible check (TN-1) was sown. This aid in spreading the inoculum. Micro jet sprinklers were provided four times a day for 5-10 minutes to maintain high humidity and leaf wetness to promote infection and disease development. The beds were covered with polythene sheets during night to maintain high humidity and to increase the disease pressure on the test entries.

**Inoculation:** *M. oryzae* spore suspension was prepared from 10-day-old blast cultures grown on oat meal agar medium by scraping method. The plates were flooded with 10 ml of distilled water and the fungal growth containing mycelium and conidia was gently removed by scrapping with a sterile inoculation loop. Harvested spores were filtered through a double-layer muslin cloth, the resultant concentration was adjusted to $1\times10^5$ conidia $ml^{-1}$ using Heamocytometer and Tween 20 (0.02% vol/vol) (polyoxylethylene sorbitan monolaurate) was added to the suspension just before inoculation. Twenty-one-day old seedlings were artificially inoculated by spraying the inoculum on the foliage using a hand operated atomizer. The inoculum was sprayed in the evening hours till the entire plant surface became wet with conidial suspension and left overnight. To maintain optimum humidity, micro-Jet sprinklers were provided four times a day for 5 – 10 minutes during day time.

**Data recording:** Data on leaf blast severity was done after 10-15 days of post infection depending on the severity of the infection on each entry according to leaf blast disease severity scale developed by Standard Evaluation System of IRRI (SES, IRRI, 2002.) (Table. 2). Based on phenotypic scores, the genotypes were declared as highly resistant (0), resistant (1), moderately resistant (2-3), moderately susceptible (4-5), susceptible (6-7) and highly susceptible (8-9) (table 2.). Whenever differences observed in score in between the seedlings of the test entry, the higher value was considered for scoring. A graph was developed based on disease reaction of the genotypes considering the resistance reaction (score ≤ 3.0 on 0-9 scale) and susceptible reaction (score ≥ 4.0 on 0-9 scale) and represented in Figure.1. To complete Koch's postulates, re-isolations of the pathogen from the artificially inoculated leaves were made following the protocol previously described.

## Results and Discussion

With the objective of identifying the superior rice genotypes for blast resistance, blast screening experiment was carried out with 45 genotypes (13 parents, 30 hybrids, 2 hybrid checks) along with susceptible check variety (TN-1) for blast disease by adopting uniform blast nursery screening (UBN) technique and disease scoring was done using 0-9 standard evaluation system (SES) scale developed by IRRI-2002 (Ghimire *et al*., 2019) [3]. Blast disease severity was adequate (8 score on 1-9 scale) on the known susceptible line, TN -1 indicating a reliable disease screen conducted during *Rabi* 2021.

The experiment results revealed that, none of the evaluated genotypes recorded resistant to rice blast disease at RARS, Polasa, Jagtial. Among the evaluated 13 parental lines only one parental line RMS 24B exhibited moderately resistant reaction and three lines CMS 11B, CMS 14B and JMS 14B showed susceptible reaction against rice blast disease and remaining nine parental lines RMS 1B, RMS 2B, CMS 46B, CMS 59B, JMS 11B, JMS 20B, CGZR 2, CGZR 1 and ZINCORICE displayed moderately susceptible disease reaction towards blast disease.

Among the 30 cross combinations developed through crossing three testers with 10 cytoplasmic male sterile lines evaluated in uniform blast nursery revealed variable disease severity towards rice blast disease varying from score 3 to score 6 on 0-9 rice blast disease severity scale. Maximum number (21) of

hybrids showed moderately susceptible reaction to blast disease with disease severity score ranging from score 4 to score 5. Out of these 21 moderately susceptible hybrid lines only two lines *viz.*, CMS 11A x CGZR 2 and JMS 20A x CGZR 1 were recorded disease severity score 5. Eight lines exhibited moderately resistant reaction and remaining one hybrid JMS 20A x CGZR 2 found susceptible to blast disease with disease severity score 6. Among these 8 hybrids *viz*., RMS2A x CGZR 2, CMS 46A x CGZR 2, JMS 24A x CGZR 2, RMS 2A x CGZR 2, CMS 14A x CGZR 1, RMS 2A x ZINCORICE, CMS 11A x ZINCORICE and JMS 24A x ZINCORICE recorded lowest disease severity score - 3 compare to other hybrids evaluated in the present study. In this study two commercial hybrids (hybrid checks) KPH 473 and 27P 63 also evaluated to test the disease reaction against rice blast disease and reported moderately resistant and moderately susceptible respectively. The above detailed information was depicted in the table 1. Similar field investigations were done for identification of location specific blast resistant cultivars by Srijan *et al*. (2015) [11], Vinayak Turaider *et al*. (2017) [12], Saikiran *et al*. (2019) [9] and Krishna *et al*. (2020) [6].

**Table 1:** Screening of rice genotypes for leaf blast resistance during Rabi, 2021-2022

| S. No. | Entry | Disease Leaf score (0-9) | Disease Reaction |
|---|---|---|---|
| **Lines** | | | |
| 1. | RMS 1B | 5 | MS |
| 2. | RMS 2B | 5 | MS |
| 3. | CMS 11B | 6 | S |
| 4. | CMS 14B | 6 | S |
| 5. | CMS 46B | 5 | MS |
| 6. | CMS 59B | 4 | MS |
| 7. | JMS 11B | 4 | MS |
| 8. | JMS 14B | 6 | S |
| 9. | JMS 20B | 5 | MS |
| 10. | JMS 24B | 3 | MR |
| **Testers** | | | |
| 11. | CGZR 2 | 5 | MS |
| 12. | CGZR 1 | 4 | MS |
| 13. | ZINCORICE | 4 | MS |
| **Hybrids** | | | |
| 14. | RMS 1A x CGZR 2 | 4 | MS |
| 15. | RMS 2A x CGZR 2 | 3 | MR |
| 16. | CMS 11A x CGZR 2 | 5 | MS |
| 17. | CMS 14A x CGZR 2 | 4 | MS |
| 18. | CMS 46A x CGZR 2 | 3 | MR |
| 19. | CMS 59A x CGZR 2 | 4 | MS |
| 20. | JMS 11A x CGZR 2 | 4 | MS |
| 21. | JMS 14A x CGZR 2 | 4 | MS |
| 22. | JMS 20A x CGZR 2 | 6 | S |
| 23. | JMS 24A x CGZR 2 | 3 | MR |
| 24. | RMS 1A x CGZR 1 | 4 | MS |
| 25. | RMS 2A x CGZR 1 | 3 | MR |
| 26. | CMS 11A x CGZR 1 | 4 | MS |
| 27. | CMS 14A x CGZR 1 | 3 | MR |
| 28. | CMS 46A x CGZR 1 | 4 | MS |
| 29. | CMS 59A x CGZR 1 | 4 | MS |
| 30. | JMS 11A x CGZR 1 | 4 | MS |
| 31. | JMS 14A x CGZR 1 | 4 | MS |
| 32. | JMS 20A x CGZR 1 | 5 | MS |
| 33. | JMS 24A x CGZR 1 | 4 | MS |
| 34. | RMS 1A x ZINCORICE | 4 | MS |
| 35. | RMS 2A x ZINCORICE | 3 | MR |
| 36. | CMS11A x ZINCORICE | 3 | MR |
| 37. | CMS 14A x ZINCORICE | 4 | MS |
| 38. | CMS 46A x ZINCORICE | 4 | MS |
| 39. | CMS 59A x ZINCORICE | 4 | MS |
| 40. | JMS 11A x ZINCORICE | 4 | MS |
| 41. | JMS 14A x ZINCORICE | 4 | MS |
| 42. | JMS 20A x ZINCORICE | 4 | MS |
| 43. | JMS 24A x ZINCORICE | 3 | MR |
| **Hybrid checks** | | | |
| 44. | KPH 473 | 3 | MR |
| 45. | 27P 63 | 4 | MS |
| **Susceptible check** | | | |
| 46. | TN-1 | 8 | HS |

MR-Moderately Resistant, MS-Moderately Susceptible, S- Susceptible, HS-Highly susceptible

**Table 2:** Scale for blast disease assessment (IRRI, 2002)

| Scale | Infection | Host response |
|---|---|---|
| 0 | No lesions observed | Highly resistant (HR) |
| 1 | Minute brownish non-sporulating spots of pin point size under lower leaves. | Resistant (R) |
| 2 | Round, slightly prolonged necrotic grey spots, of 1-2 mm in diameter, with a well-defined brownish margin, little sporulating lesions mostly found on the lower leaves | Moderately resistant (MR) |
| 3 | Spot same as in 2, but with a notable number of spots on the upper leaves. | Moderately resistant (MR) |
| 4 | Typically, heavy sporulating blast spots with 3 mm or more in length causing less than 2%infection on leaf. | Moderately susceptible (MS) |
| 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 | Susceptible (S) |
| 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 blast lesions of 3 mm or more in longer infecting more than 75% leaf area | Highly susceptible (HS) |

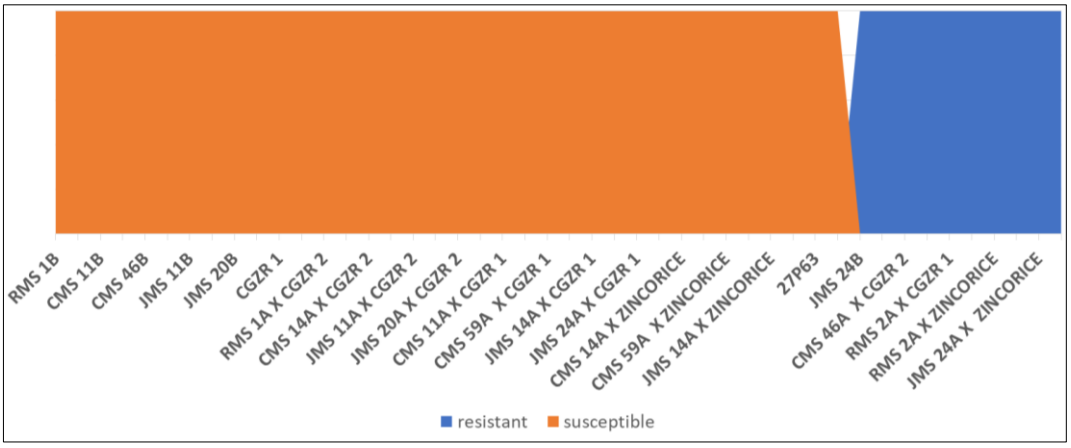


**Fig 1:** Showing the orange and blue area representing susceptible and resistant genotypes respectively.

## Conclusion

Based on the above findings, we identified 22.2% of genotypes resistant (Score 0-3) to leaf blast disease. These genotypes (9 hybrids and one parental line) can be used in breeding programme for developing leaf blast resistant lines. Further the study and time to time field evaluation of rice genotypes against leaf blast is entertained. In addition, there is a need for the multi-location evaluation of the rice lines that have been identified as blast resistant in the uniform blast nursery screens for the identification of stable sources of blast resistance.

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