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Shivashankaragouda Patil
Ph.D Student, Department of Genetics and Plant Breeding, College of Agriculture, UAS, Dharwad, Karnataka, India

JR Diwan
Professor, Department of Genetics and Plant Breeding, College of Agriculture, UAS, Dharwad, Karnataka, India

K Mahantashivayogayya
Ph.D Student, Department of Genetics and Plant Breeding, College of Agriculture, UAS, Dharwad, Karnataka, India

Mahadeva Swamy
Ph.D Student, Department of Genetics and Plant Breeding, College of Agriculture, UAS, Dharwad, Karnataka, India

D Pramesh
Ph.D Student, Department of Genetics and Plant Breeding, College of Agriculture, UAS, Dharwad, Karnataka, India

Vikas V Kulkarni
Ph.D Student, Department of Genetics and Plant Breeding, College of Agriculture, UAS, Dharwad, Karnataka, India

Abhishek V Karadagi
Ph.D Student, Department of Genetics and Plant Breeding, College of Agriculture, UAS, Dharwad, Karnataka, India

Corresponding Author:
Shivashankaragouda Patil
Ph.D Student, Department of Genetics and Plant Breeding, College of Agriculture, UAS, Dharwad, Karnataka, India

Response of rice genotypes for their interaction with *Bacillus subtilis* for blast disease resistance

Shivashankaragouda Patil, JR Diwan, K Mahantashivayogayya, Mahadeva Swamy, D Pramesh, Vikas V Kulkarni and Abhishek V Karadagi

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Abstract

Rice (*Oryza sativa* L.) is a staple food source for nearly 3.5 billion people and has occupied an enviable prime place among the food crops cultivated around the world. Major diseases causing yield losses in rice include bacterial blight, blast, sheath blight, and tungro virus which adversely affect rice yield. Among these diseases, rice blast caused by the *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*) is one of the most damaging diseases of rice in many regions of the world. The disease occurs on leaves, stems and seeds of the cultivated crop. In view of these, the present investigation was carried out at the Agricultural Research Station (ARS), Gangavathi, University of Agricultural Sciences (UAS), Raichur during *Kharif* 2021, to evaluate the response of twenty rice genotypes in Uniform Blast Nursery (UBN) for blast incidence when they were treated with *Bacillus subtilis* and untreated condition. The *B. subtilis* significantly reduced the blast incidence in treated IR-64, GNV 1905 and IET-25220 genotypes compared to untreated, whereas 17 genotypes showed moderately resistant to blast disease. The resistance mechanism offered in these genotypes may be due to the interaction of *B. subtilis* with plants, which might have activated the defense response in the plants by triggering the Induced Systemic Resistance (ISR) by potentially regulating the defense-related genes in salicylic acid (SA)- and Jasmonic acid (JA)-dependent signalling pathways in plants. These findings suggest that *B. subtilis* could be a potential bio inoculum for rice to control blast disease and enhance yield.

Keywords: Rice (*Oryza sativa* L.), *Magnaporthe oryzae*, blast disease, *Bacillus subtilis*, induced systemic resistance (ISR)

Introduction

Rice (*Oryza sativa* L.) is a chief nourishment basis for nearly 3.5 billion people and has occupied an enviable prime place among the food crops cultivated around the world. Among various biotic factors affecting rice, blast disease is the most disastrous, causing 70-80 per cent yield loss. Rice blast is instigated by *Magnaporthe grisea* (anamorph: *Pyricularia oryzae*) which is the most damaging and widespread disease of rice across the world. Blast epidemic will cause the complete downfall of seedling at the nursery and in field condition and causes up to 70 - 80 per cent of total yield reduction. The pathogen can survive in the seed and causes damage to the seedlings. Hence seed treatment is one effective method to stunned the losses**.** Utilizing treated seeds can prevent rice blast disease caused by infected rice seeds. However, reliance on chemical fungicides for seed treatment presents various drawbacks, including pathogen resistance development, environmental harm, and health risks to farmers and consumers. These limitations underscore the necessity for alternative non-chemical seed treatment approaches, such as the use of microbial antagonists. Treatment of rice seeds with B. subtilis has demonstrated efficacy in reducing both the occurrence and intensity of rice blast disease, with reductions ranging from 10 to 72.4 per cent. (Hashim *et al.*, 2019) [3].

Material and Methods

The experimental material for the assessment of rice genotypes for their interaction with *Bacillus subtilis* for blast disease resistance were collected from Agriculture Research Station, Gangavathi. The seeds were sown in the seed bed on 23rd March 2022 in Uniform

Blast Nursery (UBN) following the sandwich technique and boundary rows remained circulated with susceptible variety as infector *i.e.,* HR-12. The multiplied virulent inoculum of *Pyricularia oryzae* (Gangavathi isolate) was inoculated uniformly and covered with a polythene sheet to maintain humidity. *Bacillus subtilis* strain was obtained from the Centre for DNA Fingerprinting Diagnosis (CDFD) Hyderabad. *B. subtilis* was multiplied in Luria broth as per the method adopted by Xie *et al.,* (2015) [10]. Pure culture of *B. subtilis* was inoculated into the Lurea broth and kept for incubation after four days of incubation, broth culture was mixed with lignite powder as carrier material in the ratio of 1:2. Then paddy seeds were treated with carrier material as per the method adopted by Amruta *et al.* (2018) [1] then treated seeds were sown in the UBN (Uniform Blast Nursery) and untreated seeds were kept as control. The scoring for blast disease was done after 22 days after sowing (DAS) based on the (IRRI, 2006) 0-9 scale.0-3 is resistant, 4-6 is moderately resistant and 7-9 is susceptible. Based on disease scoring evaluation, 20 genotypes were regarded as into three distinct assemblies: highly resistant, moderately resistant and susceptible.

Results and Discussion

The 20 genotypes which were treated with *B. subtilis* and untreated (without *B. subtilis* treatment) were sown in UBN (Uniform Blast Nursery). Based on natural blast disease occurrence, the reactions of 20 genotypes (both treated and untreated) were assessed. All the measures were taken to ensure the occurrence of maximum disease pressure by planting the infector (HR 12) after every 5 test genotypes and all along the borders. The disease reactions were scored after 22 days after sowing (DAS) grounded on the (IRRI, 2006) [5] 0-9 scale for treated and untreated genotypes given in Table 1.

The findings of the scoring in untreated revealed that 1 line **(**IIRR-93R**)** with a score of 2 was found to be resistant, 13 were moderately resistant (score 5-6) and 6 lines were susceptible (score of 7 to 9) among those twenty lines. About 1(5%) of the 20 genotypes were resistant (scoring 2), 13 (65%) were moderately resistant and six (30%) were susceptible genotypes. Of the 20 genotypes, none of them showed immune to leaf blast disease with a score of 0. Hence, none of the genotypes evaluated under the present investigation were originate to be immune.

Among the 20 genotypes treated with *B. subtilis*, 14 lines were found to be resistant (1-4), 6 genotypes were found to be moderately resistant (5) and none of the genotypes were initiate to be susceptible. Of the 20 resistant genotypes, IIRR-93R shown the resistant phenotypic score 1.

From the above study *B. subtilis* treated rice plants have shown resistance to leaf blast disease in rice Plate 1 and 2. The rice genotypes (untreated) IR 64, GNV 1905 and IET-25220 which have shown susceptible reaction to blast disease were shown resistant to blast disease when they were treated with *B. subtilis*. The rice genotypes (untreated) MTU 1010 and IET-28695 which are susceptible have shown moderately resistance to blast disease when they were treated with *B. subtilis*. The rice genotypes (untreated) GNV 1108, IET-27870, RNR 15048, GNV 1109, IIRR-93R, GNV 10-89, Gangavathi Sona, BPT-5204, Asaliya, Ralhu galli, Gowri Sanna, Madrasa sanna and Jasmin black have shown moderately resistance to blast disease have shown resistance to blast disease when they were treated with *B. subtilis.*

These findings are similar to He *et al.* (2019) [4], Amruta *et al.* (2018) [1], Prasanna Kumar *et al.* (2017) [8] and Christopher and Newton (2017) [2].

B. subtilis is gram-positive bacteria that produces many antibiotics and enhance the development of the plant and also it activates a defense response in the plants by triggering the Induced Systemic Resistance (ISR) by potentially regulating the defense-related genes in salicylic acid (SA)- and Jasmonic acid (JA)-dependent signalling pathways in plants. Ongena *et al.* (2007) [7], Kloepper *et al.* (2004) [6] and Van *et al.* (2008) [9]. Hence, in the present study, it was noticed that treated genotypes have shown resistance to moderate resistance reaction against the blast disease and none have shown susceptible reaction compared to untreated. The resistance mechanism offered in these genotypes might remain due to the interaction of *B. subtilis* with plants, which might have triggered the Induced Systemic Resistance (ISR), which offered a natural resistance to plants in contradiction of the leaf blast disease.

Conclusion

Rice is an significant Asian cereal crop, is considered as an essential nourishment produce for developing countries and a staple meal for the entire global human population. Improvement of a crop mainly demands the occurrence of wide genetic variability. The level to which natural variation among host genotypes in their associated microbes translates into fitness differences has to be determined. The information considering the nature and kind of association among various traits is a precondition for any upbringing programme which mainly focuses on developing high-yielding crop varieties. Keeping all these points under consideration, the present study concentrated on response of rice genotypes for their interaction with *B. subtilis* for blast disease resistance.

A set of twenty rice genotypes (both treated and untreated) were evaluated for blast disease resistance in a uniform blast nursery with *M. oryzae* isolate, results revealed that *B. subtilis* treated genotypes have shown resistance to *M. oryzae* than untreated genotypes. Untreated three genotypes *viz.,* GNV 1905, IET 25220 and IR-64 which were susceptible to blast disease have shown resistance to blast disease when they were treated with *B. subtilis,* whereas three genotypes *viz.,* IET 28695, Gangavathi Sona and MTU 1010 have shown susceptibility to blast disease in untreated conditions have shown moderate resistance to blast disease.

Table 1: Screening of rice genotypes for leaf blast disease in UBN

Sl. No.	Genotypes	Untreated		Treated	
		Disease scoring	Disease reaction type	Disease scoring	Disease reaction type
1	GNV -1108	6	MR	3	R
2	IET 27870	6	MR	3	R
3	IIRR-93R	2	R	1	R
4	GNV 1905	7	S	4	R
5	GNV 1109	6	MR	5	MR
6	IET 25220	8	S	4	R
7	IET 28695	7	S	5	MR

8	RP-BIO-226	6	MR	5	MR
9	IET-28706	6	MR	5	MR
10	Asaliya	5	MR	3	R
11	Ralhu galli	5	MR	3	R
12	Gowri Sanna	6	MR	4	R
13	Madrasa sanna	5	MR	3	R
14	Jasmin Black	6	MR	4	R
15	Gangavathi Sona	8	S	5	MR
16	BPT-5204	6	MR	3	R
17	IR-64	8	S	4	R
18	RNR 15048	5	MR	2	R
19	GNV 10-89	5	MR	3	R
20	MTU 1010	8	S	5	MR

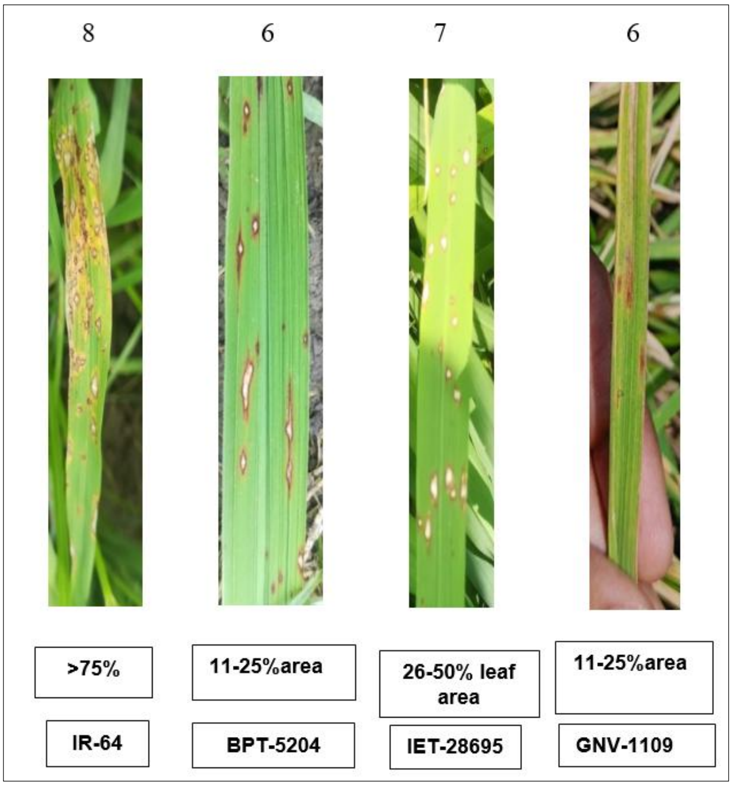


Plate 1: Scale and percentage of leaf area infected by lesions observed in untreated genotypes

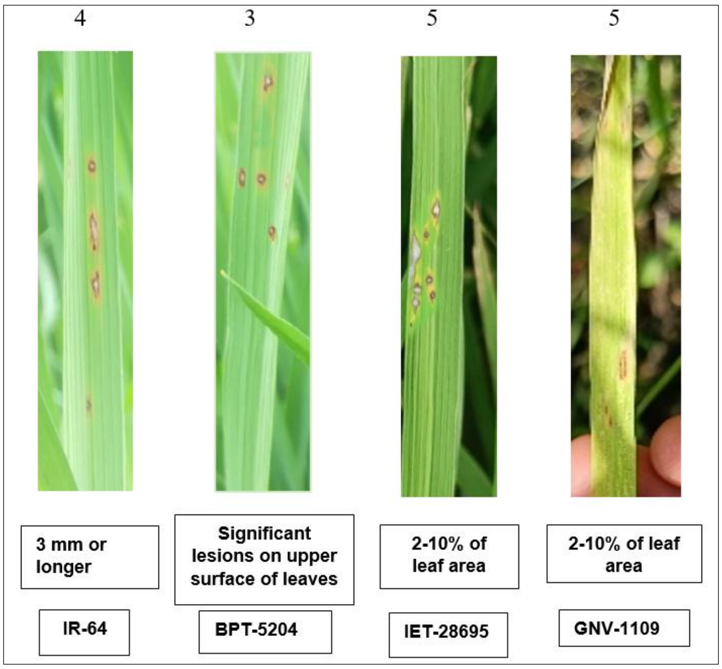
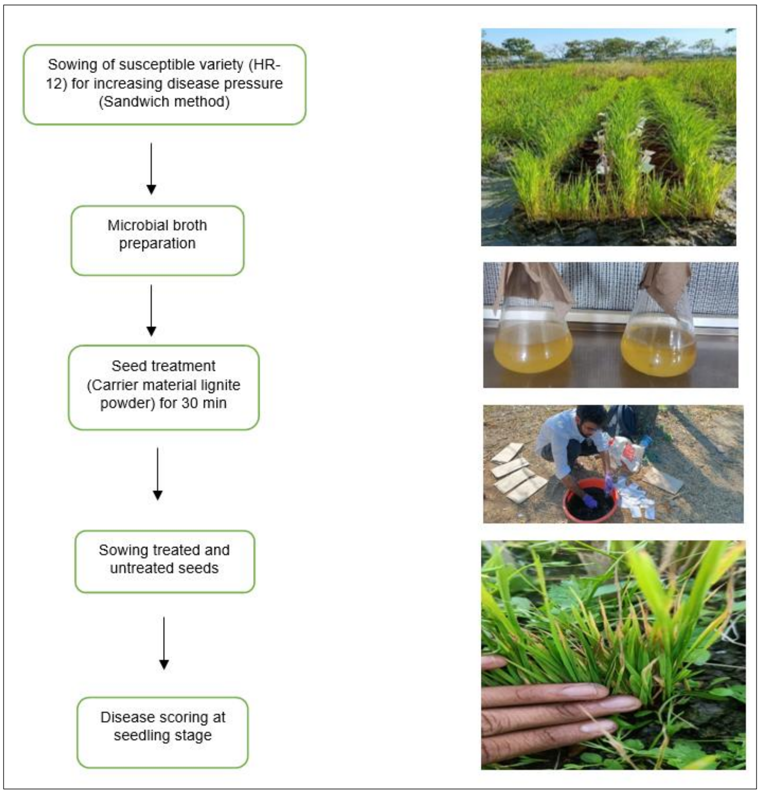


Plate 2: Scale and percentage of leaf area infected by lesions observed in *Bacillus subtilis* treated genotypes

Flow chart for milestone activities for investigations



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