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Screening of high-yielding Swarna lines developed through MABB for resistance against bacterial blight and blast diseases

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Abstract

Rice cultivation has two significant challenges: BB and blast diseases. There are a number of Indian rice variety that are extremely sensitive to these diseases, one of which is the well-known Swarna mega-rice variety. The resistance genes *Xa21* and *Pi54*, which provide protection against bacterial blight (BB) and blast diseases, were incorporated into a Near Isogenic Line (NIL) of Swarna that already had the yield-boosting gene *OsSPL14* using marker-assisted backcross breeding. Ten best introgressed lines were selected at the ICF_6 stage and subjected to phenotypic screening in a standardized blast nursery to evaluate resistance to blast disease and under controlled conditions for bacterial leaf blight. The lines exhibited outstanding performance and showed good tolerance to biotic stresses such as bacterial blight and blast.

Keywords: Bacterial blight, blast, yield, MABB

Introduction

Rice is among the three most extensively cultivated crops globally, alongside wheat and maize. India's rice demand is projected to reach 130 million tonnes by 2030 and 160 million tonnes by 2050, based on an estimated daily consumption of 189 g per person and an average annual population growth rate of 1.8%. To address this need, it is essential to enhance rice productivity and production (Guru *et al*. 2018) [6]. Abiotic and biotic stresses are the primary causes of significant yield reductions in several crops. One of the most harmful types of biotic stress that rice is subjected to is bacterial leaf blight (BB), which is caused by *Xanthomonas oryzae* pv. *Oryzae (Xoo*). According to Kumar *et al*. (2012) [13], the severity of BB can result in a reduction of rice yield of up to the extent of 85 percent. Researchers have found a total of 48 resistance genes from various rice sources to date (Jiaxin Xing *et al*., 2021; Fiyaz *et al*., 2022; Chen *et al*., 2020) [10, 5, 4]. Sundaram *et al*. (2008) [28] and Hue ThiNguyen *et al*. (2018) [9] have demonstrated that the *Xa21* gene, which is both dominant and resistant, derives from the wild species *Oryza longistaminata* that is native to Africa. This gene has been found to offer resistance that is both widespread and extensive.

The fungus *Magnaporthe oryzae* produces rice blast disease, which can lead to significant yield losses of 70-80% in severe cases, posing a substantial threat to rice production. Sahu PK *et al*. 2022 [22] reported the existence of around 146 important rice blast resistance genes (R-genes). The *Pi54* gene, derived from the Tetep cultivar, has demonstrated strong effectiveness against Indian blast isolates (Sharma *et al*., 2002) [23]. In 1982, Maruteru, Andhra Pradesh, India, introduced Swarna, also known as MTU7029, a long-duration, medium-bold grain type mega-rice variety. Swarna experiences significant reductions in crop output because of its high vulnerability to bacterial blight and its low resistance to blast disease. With the use of the marker-assisted backcross breeding (MABB) technique, an effort was made to incorporate *Xa21* and *Pi54* genes into the high-yielding Swarna variety that already contains the yield improving gene *OsSPL14*.

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This action was taken in an attempt to enhance the yield of the line.

Materials and Methods

Plant materials

A NIL of Swarna (IR121047-2-2-1) susceptible to bacterial blight and blast but possessing *OsSPL14* for panicle branching was crossed with a NIL of ISM (ISM-12-305-106) containing resistance genes *Xa21* and *Pi54* against BB and blast, respectively (Rekha *et al*. 2018) [21]. The crossing programme began in the Kharif season of 2015 and concluded in the Kharif season of 2021. During the ICF_6 generation, 10 best lines were selected for assessing their resistance to BB and blast disease, along with resistant and susceptible checks.

Assessing the intercross developed lines of Swarna for resistance to bacterial blight.

The enhanced intercross derived Swarna lines were planted in the field and assessed for their resistance to BB, in comparison with their parents in the Rabi 2020-21 and Kharif 2021. The negative parent (Swarna) and positive parent (ISM) checks for bacterial leaf blight were also included in the evaluation. A highly infectious strain, IX0-20, sourced from Telangana, of *Xanthomonas oryzae* pv. *Oryzae* was used for the screening process. The bacterial culture was cultivated on Hayward's agar media and placed in an incubator at 28 °C for 96 hours. Following the completion of the incubation time, the bacterial culture was collected and diluted to a final concentration of 108 colony-forming units per milliliter (Preece *et al*., 1982) [18]. In 1973, Kauffman *et al*. developed a leaf clipping approach that involves cutting leaf tips with *Xoo*-infected scissors to expose crosscut veins to *Xoo* suspension. The IRRI SES score was used to assess the average percentage of diseased leaf area on the top three leaves of the plants 15 days after inoculation.

Assessing the intercross developed lines of Swarna for blast resistance

The Swarna intercross derived lines, together with their parent lines NIL of Swarna (IR121047-2-2-1) and NIL of ISM (ISM-12-305-106), were assessed for blast resistance in UBN beds during the Rabi 2020-21 and Kharif 2021 seasons, compared to the negative check (HR12) and positive check (Tetep). Pathogen cultures were prepared by breaking down 10-day-old mycelia slants in 5 milliliter of water and then placing them on Mathur's medium for sporulation. After incubating for 8 to 10 days at a temperature range of 25 to 28 degrees Celsius, the plates were rinsed with 10 milliliters of water to create a spore suspension. The concentration of the pathogen *Magnaporthe oryzae* spore suspension was adjusted to 1 × 105 spores/ml. 15-day-old seedlings were inoculated with around 40 cc of spore suspension (Local IIRR isolate-SPI 40) of the blast infectious agent using a low-volume plastic sprayer. Sprinklers were employed to moisten the environment with water 3-4 times a day to maintain elevated humidity levels. Disease resistance and susceptibility scores were recorded on a 0-9 scale when standard blast lesions were observed on each line.

Results and Discussion

The yield of rice is considerably reduced by various biotic stresses such as bacterial blight (BB) and blast infections (Tanweer *et al*., 2015) [28]. Because of the country's rapidly evolving environment, these two diseases have been identified in many regions, and widely grown varieties such as Swarna are especially susceptible to them. Resistance genes can be incorporated into popular cultivars like Swarna to provide improved varieties for a range of races/isolates of BB and blast diseases.

Swarna (MTU7029) is a mega rice variety developed and released by ANGRAU in 1982. It is known for its adaptability, brown hull color, dark green foliage, high tillering ability, semi-dwarf plant stature, long duration, and medium bold grains. Indian farmers utilize Swarna for farming in kharif and Rabi seasons. It is derived from the cross Vasistha and Mahsuri, as reported by Revathi *et al*. (2020) [15] and Anila *et al*. (2018) [1]. In 2019, there was a notable request for 397.25 quintals of breeder seed.

Phenotypic assessment for resistance to bacterial leaf blight

A robust resistance to the disease was demonstrated by each of the ten intercross-derived lines, with lesion lengths ranging from one centimeter to six centimeters and SES scores which ranged from one to three (Fig 1; Table 1). With a disease score of nine and a lesion length that was greater than twelve centimeters, the recurrent parent Swarna demonstrated that it was also susceptible to bacterial blight disease. It was determined that the donor parent NIL of ISM (ISM-12-305-106) had an immune level of resistance. The disease score was one, and the lesion length ranged from zero to less than one centimeter. The diseases known as bacterial blight and blast are extremely damaging and have the potential to reduce plant output by as much as fifty percent (Sharma *et al*., 2017). *Xa21* gene, which encodes a leucine-rich repeat receptor-like kinase (LRR-RLK) gene, was the first R gene to be cloned. This gene was originally derived from *Oryza Longistaminata*, according to Song *et al*. 1995 [25].

In spite of the fact that it is typically preferable to combine many genes into high-quality rice varieties, there have been examples in which a prominent BB resistance gene, such as *Xa21*, has demonstrated the ability to exhibit strong resistance to the disease. It is well known that *Xa21* is significantly resistant to a number of different strains of the bacterial blight pathogen that is prevalent in India. To provide resistance to the bacterial blight disease in the Swarna variety, we utilised the *Xa21* gene in our research work.

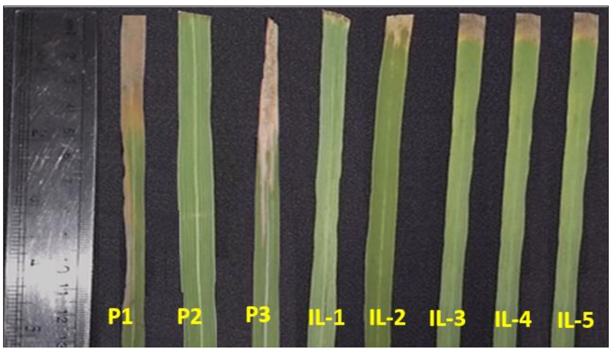

Fig 1: Phenotypic screening of improved Swarna lines at ICF_6 generation for resistance to BB disease

Fig 1. Phenotypic screening of the selected ICF_6 lines exhibited resistance to BB with a score of '1'. P1- Swarna (susceptible check), P2- ISM-12-305-106 (NIL of Improved Samba Mahsuri (*Xa21+Pi54*); resistance check), P3-

IR121047-2-2-1 (*OsSPL14*), IL-1 to IL-5- Intercross derived lines of Swarna.

Table 1: Screening Improved breeding lines of Swarna (at ICF_6) along with the parents, with DX020 isolate of *Xanthomonas oryzae* pv. *oryzae* to assess their bacterial blight resistance

S. No.	Parents and Checks	Reaction against bacterial leaf blight disease		
		Lesion length (cm)	Score	Immune/ Resistant/Moderately resistant/Susceptible/ Moderately susceptible/Highly Susceptible
1.	Swarna	12	9	Susceptible
2.	ISM-12-305-106	Less than 1	1	Resistant
3.	IR121047-2-2-1	Less than 12	9	Susceptible
S. No.	**Improved breeding lines (ICF_6)**	**Lesion length (cm)**	**Score**	**Resistant/Moderately resistant/Susceptible**
1.	Swarna-IL-1	Less than 3	1	Resistant
2.	Swarna-IL-2	Less than 2	1	Resistant
3.	Swarna-IL-3	Less than 1	1	Resistant
4.	Swarna-IL-4	Less than 1	1	Resistant
5.	Swarna-IL-5	Less than 2	1	Resistant
6.	Swarna-IL-6	Less than 2	1	Resistant
7.	Swarna-IL-7	Less than 2	1	Resistant
8.	Swarna-IL-8	Less than 1	1	Resistant
9.	Swarna-IL-9	Less than 1	1	Resistant
10.	Swarna-IL-10	Less than 1	1	Resistant

Phenotypic screening for blast resistance

There was a high level of resistance to blast disease across all ten intercross-derived lines, with scores ranging from 2-3. Both the negative check, HR12,TN1, and the recurrent parent, NIL of Swarna (IR121047-2-2-1), displayed a high susceptibility to blast disease, as indicated by a score of 9. The positive check, Tetep, and NIL of ISM (ISM-12-305-106) on the other hand, revealed a high level of resistance to blast disease, with scores of 1 and 3, respectively (Figure 2; Table 2). According to Khush and Jena (2009) [12], rice blast is a particularly severe disease that is brought on by the fungus *Magnaporthe grisea*. This disease can cause yield losses of between 70 and 80 percent. As demonstrated by Sharma *et al*. (2010) [24] and Ramkumar *et al*. (2011) [20], *Pi54* is a major gene that confers resistance to a large number of blast pathogen isolates in India. The *Pi54* gene, which codes for an NBS–LRR protein, was successfully isolated and duplicated from the indica cv. Tetep strain for the first time. According to Rai *et al*. (2011) [19], it offers resistance against Indian rice blast isolates that is extremely broad. The efficiency of the gene in giving a high level of resistance was demonstrated in a previous study that was conducted by Balachiranjeevi *et al* (2015) [3]. According to the findings of Prasad *et al*. (2011) [17], Samba Mahsuri breeding lines that included the gene showed a high level of resistance to blast diseases in trials conducted in many locations. When compared to the original parent, Swarna, the improved cultivars of Swarna had much better levels of resistance to bacterial leaf blight and blast.

Fig 2: Phenotypic screening of improved Swarna lines at ICF_6 generation for resistance to blast disease

Figure 2. Phenotypic screening of five selected intercross derived lines IL-1 to IL-5 showed a resistance against blast disease with the score of 2-3 as per IRRI-SES Scale, P1-ISM-12-305-106 (NIL of Improved Samba Mahsuri (*Xa21+Pi54*); resistance check) P2- Swarna (susceptible check), P3-Tetep (Resistance check), P4- IR121047-2-2-1 (*OsSPL14*), P5- TN1, P6- HR-12 (Susceptible check), IL-1 to IL-5- Intercross derived lines of Swarna.

Table 2: Screening Improved breeding lines of Swarna (at ICF_6) along with the parents, with SPI-40 isolate of *Magnaporthe oryzae* to assess their blast resistance a (scoring done as per IRRI-SES scale; IRRI 2013)

S. No.	Parents and Checks	Reaction against blast disease	
		SPI-40	
		Score	Resistant/Moderately Resistant/Susceptible
1.	Swarna	9	Susceptible
2.	ISM-12-305-106	3	Resistant
3.	Tetep (Resistance check)	1	Resistant
4.	HR-12 (Susceptible check)	9	Susceptible
5.	IR121047-2-2-1	9	Susceptible
S. No.	**Improved breeding lines (ICF_6)**	**Score**	**Resistant/Moderately Resistant/Susceptible**
1.	Swarna-IL-1	3	Resistant
2.	Swarna-IL-2	3	Resistant
3.	Swarna-IL-3	2	Resistant
4.	Swarna-IL-4	3	Resistant
5.	Swarna-IL-5	3	Resistant
6.	Swarna-IL-6	3	Resistant
7.	Swarna-IL-7	2	Resistant
8.	Swarna-IL-8	3	Resistant
9.	Swarna-IL-9	2	Resistant
10.	Swarna-IL-10	2	Resistant

Conclusion

The improved Swarna lines with resistance to bacterial leaf blight and blast, produced in this present study, could provide a significant benefit to farmers growing the Swarna rice variety in fields impacted by both diseases. The enhanced Swarna lines produced in this research can serve as donors to transmit resistance to BB and blast to other varieties.

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