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Screening of Backcross Derived High Yielding Lines of MTU1010 Developed through Marker Assisted Breeding for their Resistance against Bacterial Leaf Blight and Blast Diseases

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A B S T R A C T

Bacterial leaf blight and blast diseases are two major threats to rice production and many India rice varieties including the popular mega-rice variety, MTU1010 are highly susceptible to the above mentioned diseases. We used marker assisted backcross breeding to transfer two major genes, viz., *Xa21* and *Pi54* conferring resistance to bacterial leaf blight and blast diseases, respectively into the genetic background of a Near Isogenic line of MTU1010 possessing the yield enhancing gene, *Gn1a*. At BC_1F_6stage, ten best introgressed lines were selected and subjected for phenotypic screening against bacterial leaf blight under field conditions and for blast disease resistance under artificial condition in uniform blast nursery. These lines exhibited good performance with high level of resistance to two biotic stresses viz., bacterial blight and blast.

Introduction

Rice is one of the three major crops cultivated worldwide, in addition to wheat and corn. Based on the average growth rate of population (1.8% per annum) and estimated utilization of rice (about 189 g per day), the rice requirement by 2030is projected to be around 130 million tonnes and 160 million tonnes by 2050 in India. Enhancing the production and productivity of rice is the only alternative to meet this demand (Hossain, 1996; Mishra *et al.*, 2003). Biotic and abiotic stresses are the major factors causing massive yield losses in several crops. Among the biotic stresses, Bacterial leaf blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major destructive disease of rice, causing a yield

loss of up to 80% depending on the severity (Kumar *et al.,* 2012). So far, at least47 resistance genes have been identified from the different sources of rice (Chen *et al.,* 2020; Jiaxin Xing *et al.,* 2021; Fiyaz *et al.,* 2022).

Among these, *Xa21*, a major dominant resistant gene, originated from African wild species, *Oryza longistaminata*, was observed to confer broad spectrum resistance (Sundaram *et al.,* 2008; Hue ThiNguyen *et al.,* 2018). Similar to bacterial blight, rice blast disease caused by the fungus *Magnaporthe oryzae*, is also another major threat for rice production, and yield losses as high as 70-80% is noticed during severe incidence of the disease (Khush and Jena, 2009). As on date, more than 100 major rice blast resistance genes (R-genes) have been identified (Deepak *et al.,* 2021) and The gene *Pi54*, derived from the cultivar, Tetep was observed to be very effective against the Indian isolates of blast (Sharma *et al.,* 2002).

MTU1010 (IET15644; also known as Cottondora Sannalu) is a short duration, long slender grain type, mega-rice variety, released by the Andhra Pradesh Rice Research Station (APRRS), Acharya N. G. Ranga Agricultural University (ANGRAU), Maruteru, Andhra Pradesh, India, during the year 2000. MTU1010 is highly susceptible to bacterial blight and has only a moderate level of tolerance to blast disease, both of which cause significant yield losses in the variety. Through this study, we attempted to deploy the strategy of marker-assisted backcross breeding (MABB) to transfer *Xa21* and *Pi54* into the genetic background of a high yielding, near-isogenic line (NIL) of MTU1010 containing yield enhancing gene '*Gn1a'*.

Materials and Methods

Plant materials

A NIL of MTU1010 (ICF_5-16-59) possessing bacterial leaf blight and blast resistance genes of *Xa21* and *Pi54*, respectively was used to combine bacterial blight and blast resistance into a NIL of MTU1010 (IR121055-2-10-5), which is susceptible to both the diseases, but possessing *Gn1a* for increasing grain number. The crossing programme was initiated during *Kharif,* 2018. At BC_1F_6stage, ten best backcross derived lines were selected for screening against resistance to bacterial blight and blast diseases along with the respective resistant and susceptible checks, viz., Improved Samba Mahsuri (resistant check for bacterial blight), MTU1010 (susceptible check for bacterial blight), Tetep (resistant check for blast) and HR12 (susceptible check for blast).

Screening of the backcross derived lines of MTU1010 for bacterial blight resistance

The selected improved backcross derived lines of MTU1010 were transplanted in the field and screened for their resistance against bacterial blight along with their parents during Rabi 2020-21 (i.e. dry season 2020-21) and *Kharif* 2021 (i.e. wet season 2021) along with the susceptible (MTU1010) and resistant (ISM) checks for bacterial leaf blight. IX0-20, a virulent isolate (collected from Telangana) of *Xanthomonas oryzae* pv. *Oryzae* was used for the screening process. Hayward's agar media was used to grow the bacterial culture and it was incubated at 28°C for 96 hours. After incubation period, the bacterial culture was harvested and diluted to final concentration of 10^8cfu/ml (Preece *et al.,* 1982). Leaf clipping method developed by Kauffman *et al.,* (1973) was used, in which crosscut veins to be exposed to *Xoo* suspension by cutting off leaf tips with *Xoo* suspension infected scissor. The IRRI SES score (SES, IRRI 2013) developed for measuring diseased leaf area, mean percentage of diseased leaf area (% DLA) on the plants upper three leaves, were used to measure symptoms at 15 days after inoculation.

Screening of backcross derived lines of MTU1010 for blast resistance

The selected backcross derived lines of MTU1010 were screened along with their parents *Viz.,* NIL of MTU1010 (IR121055-2-10-5) and NIL of MTU1010 (ICF_5-16-59) for their blast resistance in UBN (Uniform blast nursery) beds during *Rabi*

2020-21 (i.e. dry season 2020-21) and *Kharif* 2021 (i.e. wet season 2021) along with the susceptible check (HR12) and resistant check (Tetep).

Cultures of blast pathogen was prepared from 10 day-old slants of mycelia were macerated in 5 ml of distilled water and plated on Mathur's medium (Mathur *et al.,* 1950) for sporulation. The plates were rinsed with 10 ml distilled water after 8 to 10 days of incubation at 25 to 28°C to create spore suspension.

Spore suspension of pathogen (*Magnaporthe oryzae)* was adjusted to concentration of 1×10^5 spores /ml. Seedlings of 15 days old were infected with about 30-40 ml of spore suspension (Local IIRR isolate-SPI 40) of the blast pathogen with the help of low volume plastic sprayer.

The water was sprayed 3-4 times per day to maintain high humidity using sprinklers. Scores were recorded for disease resistance and susceptibility, when the typical blast lesions developed on each lines using following standard 0-9 scale (IRRI - SES, 1996).

Results and Discussion

Rice production is constrained by many biotic stresses, among which, bacterial blight (BB) and blast diseases cause significant yield losses (Tanweer *et al.,* 2015). These two diseases are now endemic to several parts of the country due to rapidly changing climate and many popular cultivars like MTU1010 are highly susceptible to both diseases. Fortunately, resistance genes for a wide number of races/isolates of BB and blast are available; these genes can be introgressed to develop the improved version of popular cultivars like MTU1010 (Tabien *et al.,* 2002).

MTU1010 (CottondoraSannalu) is a high-yielding, short-duration, widely cultivated mega-variety of rice having long-slender grains developed and released by Acharya N G Ranga Agricultural University (ANGRAU) in 2000 and is extremely popular among farmers for cultivation in both wet (i.e. *Kharif*) and dry (i.e. *Rabi*) seasons in India due to its wider adaptability. It is derived from the cross Krishnaveni / IR64 (Arunakumari *et al.,* 2016) and had a very high demand of 397.25 quintals of breeder seed in the year 2019.

Phenotypic screening for bacterial leaf blight resistance

All the ten backcross derived lines showed highly resistance reaction to the disease with lesion length 1-6 cm, with a SES score of 1 to 3 (Figure 1; Table 1).

The susceptible check, TN1 and the recurrent parent, IR121055-2-10-5 showed susceptibility to bacterial blight disease with disease score of 9 and lesion length of > 12 cm, whereas the resistance check, ISM and donor parent, MTU1010 (ICF_5-16-59) showed a highly resistant reaction with a disease score of 1 and a lesion length of 0 to < 1cm.

Among the biotic stresses, bacterial blight and blast are the two most destructive diseases, reducing plant yield by up to 50% (Vasudevan *et al.,* 2002; Pradhan *et al.,* 2015). *Xa21* was the first cloned R gene in rice and encodes a leucine-rich repeat receptor-like kinase (LRR-RLK) gene, which originated from *O.longistaminata* (Song *et al.,* 1995).

Even though it is desirable to pyramid two or more genes in the genetic background of elite rice cultivars (Sundaram *et al.,* 2008; Sundaram *et al.,* 2009), there have been various reports, wherein a single major bacterial blight resistance gene like *Xa21* has showed the desired level of resistance against the disease (Hari *et al.,* 2011; Hari *et al.,* 2013; Balachiranjeevi *et al.,* 2018) and it is known that *Xa21* confers good level of resistance against multiple isolates of the bacterial blight pathogen in India. Therefore, in the present study, we deployed *Xa21* gene for imparting bacterial blight disease in MTU1010.

Table.1 Screening of backcross derived lines of MTU1010 (BC_1F_6) for bacterial leaf blight resistance and scoring details as per IRRI-SES scale (IRRI 2013)

Parents and Checks	Reaction against BB		
	DX020		
	Lesion length (cm)	Score	I/R/MR/MS/S/HS
MTU1010 (IR121055-2-10-5)	12	9	S
MTU1010 (ICF_5 -16-59)	< 1	1	R
MTU1010 (Susceptible Check)	< 12	9	S
ISM (Resistant check)	0	1	Immune
backcross derived lines (BC_1F_6)	Lesion length (cm)	Score	R/MR/S
IL-1	< 3	1	R
IL-2	< 3	1	R
IL-3	< 1	1	R
IL-4	< 1	1	R
IL-5	< 3	1	R
IL-6	< 1	1	R
IL-7	< 3	1	R
IL-8	< 1	1	R
IL-9	< 1	1	R
IL-10	<1	1	R

Table.2 Screening of backcross derived lines of MTU1010 (BC_1F_6) for blast resistance and scoring details as per IRRI-SES scale (IRRI, 2013).

Parents and Checks	Reaction against Blast	
	SPI-40	
	Score	R/MR/S
MTU1010 (IR121055-2-10-5)	9	S
MTU1010 (ICF_5 -16-59)	3	R
Tetep (Resistant check)	1	R
HR12 (Susceptible check)	9	S
backcross derived lines (BC_1F_6)	**Score**	**R/MR/S**
IL-1	3	R
IL-2	3	R
IL-3	3	R
IL-4	3	R
IL-5	4	R
IL-6	3	R
IL-7	3	R
IL-8	3	R
IL-9	3	R
IL-10	3	R

Fig.1 Phenotypic screening of backcross derived lines of MTU1010 against bacterial leaf blight disease. ISM (Improved samba mahsuri) (Resistant check), MTU1010 (Susceptible check); IL-1 to IL-8 - Backcross derived lines of MTU1010 with their parents.

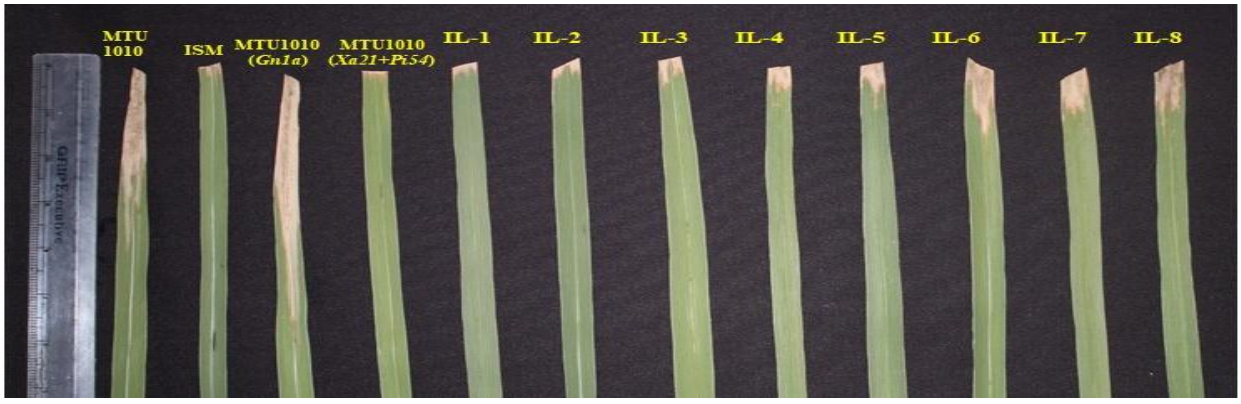
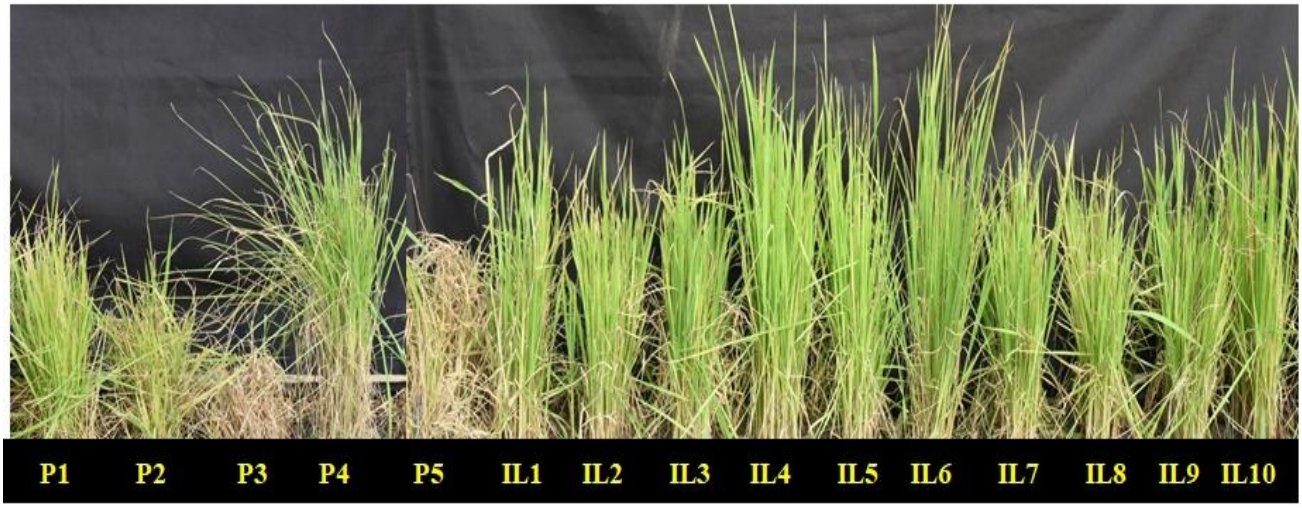


Fig.2 Phenotypic screening of the selected backcross derived lines of MTU 1010: IL1 to IL10 and P1-Tetep (Resistant check), P2-MTU1010, P3-MTU1010 (*Gn1a*), P4-MTU1010 (*Xa21 + Pi54*) and P5-HR12 (susceptible check) against blast disease following UBN Method.



Phenotypic screening for blast resistance

All the ten backcross derived lines were observed to be resistant to the blast diseases with a score of 3 - 4. The susceptible check, HR12 and the recurrent parent, NIL of MTU1010 (IR121055-2-10-5) were highly susceptible to blast disease with a score of 9, while the resistant check, Tetep and NIL of MTU1010 (ICF_5-16-59) were found to be highly resistant to the blast disease with a score of 1and 3(Figure 2; Table 2). Rice blast is a devastating disease, which is caused by the fungus *Magnaporthe grisea* (anamorph *Pyricularia oryzae*) and causes yield loss upto 70–80 % (Khush and Jena, 2009). *Pi54* is a major blast resistance gene that exhibits resistance against many blast pathogen isolates in India (Sharma *et al.*, 2010; Ramkumar *et al.*, 2011). The blast resistance gene *Pi54*, which encodes a NBS–LRR protein, was initially identified and cloned from the indica cv. Tetep and it confers broad-spectrum resistance against Indian rice blast isolates (Rai *et al.*, 2011). The gene was shown to be

effective in conferring good level of resistance in an earlier study (Balachiranjeevi *et al.,* 2015) and breeding lines of Swarna and Samba Mahsuri containing the gene have displayed excellent resistance against blast diseases in multi-location trials (Prasad *et al.,* 2011). The level of bacterial leaf blight and blast resistance in the improved versions of MTU1010 was observed to be significantly higher level than the original parent, MTU1010.

The improved versions of MTU1010 possessing bacterial leaf blight and blast resistance, developed in the present study may offer a distinct advantage to farmers MTU1010, whose fields are affected by both bacterial blight and blast. The improved lines of MTU1010 developed in this study can also be used as donors to transfer bacterial blight and blast resistance into other genetic backgrounds.

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