

Original Research Article

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Screening of the Breeding Lines of MTU1010 for Their Resistance against Bacterial Blight and Blast

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ABSTRACT

In the present study, we have introgressed two major genes, viz., *Xa21* and *Pi54* conferring resistance against bacterial blight (BB) and blast, respectively into an Indian rice variety MTU1010. Breeding line of Akshyadhan (RP6132) possessing *Xa21* and *Pi54* was used to transfer the target traits into the susceptible parent MTU1010, which is highly susceptible to both BB and blast diseases, which limits its spread to the disease endemic areas. Hence, an attempt was made to incorporate BB (*Xa21*) and blast (*Pi54*) resistance genes from breeding line of Akshyadhan which is highly resistant to the rice bacterial blight and blast diseases caused by the pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and blast fungus *Magnaporthe grisea* respectively. The SES scale (IRRI, SES 2013) was used for visual scoring of bacterial blight and blast resistance in the segregating population and the resultant resistant progenies were advanced. This work demonstrates the successful application of Mendelein ratio for targeted introgression of a dominant BB and blast genes (*Xa21* + *Pi54*) into a most popular rice variety, MTU 1010, a short duration, high yielding; long slender rice variety occupied maximum area in India particularly during dry-season. Our study exemplifies the improvement of the targeted popular variety MTU1010 for the multiple target traits.

Keywords

Rice, Bacterial blight, Blast, Resistant genes, Marker-aided selection, RILs

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Introduction

Rice blast disease was first reported as 'rice fever disease' in China by Soong Ying-shin in 1637 in India it was first reported in the Tanjavur delta of Tamil Nadu in 1913 (Srijan *et al.*, 2015). MTU1010, a short duration rice variety released in 2000 derived from the cross Krishnaveni/IR64, is extremely popular with farmers and has been planted for many years in a minimum of one million hectares. This variety also possesses brown plant

hopper resistance with long slender grains. However, MTU1010 is highly susceptible to both BB and blast diseases, which limits its spread to areas where the two diseases are endemic. In the absence of effective chemicals or any other methods of control agents against BB pathogen (Devadath *et al.*, 1989), resistance breeding is considered as the most economical and ecofriendly strategy for management of the disease and achieving yield stability. As the availability of several resistance genes to BB and blast, pyramiding

multiple genes into MTU1010 is considered as an ideal strategy to improve its resistance to these major diseases. Breeding for host-plant resistance is considered as the most economical and eco-friendly strategy for management of pests and diseases of crop plants and achieving yield stability. Molecular markers can accelerate resistance breeding efforts, as segregating plants can be selected on the basis of molecular marker alleles instead of their phenotypes and introgression of multiple resistance genes or gene pyramiding can be tracked easily in a population (Sundaram *et al.*, 2014).

Due to apparent changes in the climate, these two diseases may cause heavy yield loss. The deployment of resistance genes in rice breeding programme and cultivation of resistant varieties is considered as most effective, economical and environment friendly strategy to manage the plant diseases. Pyramiding blast and BB resistance genes in a single cultivar will help in tackling both the disease problems.

Two most devastating diseases in rice, blast caused by the fungus *Magnaporthe grisea* and bacterial blight caused by *Xanthomonas oryzae pv. Oryzae* throughout Asia and particularly in India, have plagued rice farmers since the beginning of rice cultivation (Ou, 1985) and can cause yield loss as high as 50% or more. Sometimes, both the diseases may occur together or at different period of growth stages causing severe loss to rice crop (POS, 2008). Due to apparent changes in the climate, these two diseases may cause heavy yield loss. The deployment of resistance genes in rice breeding programme and cultivation of resistant varieties is considered as most effective, economical and environment friendly strategy to manage the plant diseases. Rice is the principal staple food crop of the world and rice production has so far kept pace with the growing population, principally due

to cultivation of high-yielding, high-input demanding, and semi-dwarf varieties (Gnanamanickam, 2009). However, the introduction of semi-dwarf rice varieties and the large-scale use of inputs like fertilizers and insecticides have changed the dynamics of pests and diseases of rice, increasing their incidence significantly in the recent years. Bacterial blight (BB) and rice blast are the two most important diseases causing significant yield loss in rice (Zhang *et al.*, 2015), and they are endemic to several rice growing states of India (Production Oriented Survey, DRR, 2008). In Andhra Pradesh of India, the yield loss is very severe due to BB and blast (Rajarajeswari and Muralidharan, 2006, Sundaram *et al.*, 2008). It also implies to the newly formed state of Telangana. To minimize these problems, development of durable, broad-spectrum resistant varieties has been advocated (Jena and Mackill, 2008, Kumar *et al.*, 2014, Sundaram *et al.*, 2014).

Till date, at least 40 BB resistance (both dominant and recessive) genes have been identified (Bhasin *et al.*, 2012; Natraj Kumar *et al.*, 2012) and designated these in a series from *Xa1* to *Xa40* (Yang *et al.*, 1998; Sun *et al.*, 2004; Gu *et al.*, 2004; Cheema *et al.*, 2008; Kim *et al.*, 2015). Of these, *Xa21*, a major resistance gene, originally introgressed from *Oryza longistaminata* was observed to confer resistance to most Indian isolates of the bacterial pathogen. The gene has also been reported to confer durable resistance to the pathogen across many parts of the world including India (Sundaram *et al.*, 2014). To date, 101 blast-resistant genes (Rajashekara *et al.*, 2014) and 350 quantitative trait loci (QTLs) have been identified (Sharma *et al.*, 2012), with many fine-mapped and a few cloned. Among these, *Pi54*, a major blast resistant gene from the Vietnamese cultivar, Tetep has been identified to be highly effective under Indian conditions (Sharma *et al.*, 2010).

Materials and Methods

Plant material

Breeding lines of Akshydhhan carrying BB and blast resistant genes *Xa21* and *Pi54* were used to transfer resistant genes into MTU1010, a susceptible variety to both the diseases. In addition to this Taichung Native 1(TN1), HR12 were used as susceptible checks, while ISM, Tetep were used as resistant checks for BB and blast resistance respectively. The seeds of true F₁ plants obtained from the above mentioned cross between the parents were selfed to obtain F₂s and a total of 274 F₂ plants were obtained. The plants which were observed to be highly resistant under BB stress were forwarded to F₃ generation and screened for both the target stresses.

Isolation and characterization of the blast and bacterial blight pathogen

M. grisea was isolated from the blast infected leaf samples of the rice cultivar HR-12 on oat meal agar medium (Rathour *et al.*, 2006). To obtain a single spore colony, spore suspension was prepared and plated onto 4% water agar in Petri plates. After 10-12 hours of incubation at 25 ± 1°C, single germinating conidia were marked under a microscope and transferred to fresh culture medium. The purified culture was maintained in oat meal agar slants at 4°C.

To prove pathogenicity of the *M. grisea*, the fungus was mass multiplied on Mathur's medium (Rathour *et al.*, 2006). After 8-10 days of incubation at 25 ± 1°C, the plates were gently washed with distilled water to harvest conidia. The suspension was then filtered through muslin cloth and the spore concentration was adjusted to 1x10⁹ conidia/ml. Fifteen days old seedlings of rice variety HR-12 was inoculated by spraying the spore suspension (containing 0.2% Tween 20). The inoculated plants were kept in a humid

chamber maintained at 25°C and sprayed with water three to four times a day to maintain high humidity. The bacterial blight pathogen, *Xanthomonas oryzae pv. oryzae* was isolated from the infected leaf samples on modified Wakimoto's medium and maintained as pure culture at 4°C. The pathogenicity of the bacterial pathogen was confirmed on susceptible rice variety TN1.

Screening of the F₂ and F₃ population

Blast: The F₃ population along with respective parents was evaluated for their reaction to blast disease under uniform blast nursery. The plants were sown in rows and were surrounded with the densely sown spreader rows of susceptible cultivar HR-12. To create severe blast incidence additional inoculum was sprayed. For this, diseased leaves were chopped into pieces of 3-6cm long and scattered them over the plot. This was carried during prolonged wet weather to facilitate infections and polycyclic development of the disease. The seedlings at 4-leaf stage were sprayed with spore suspension of a highly virulent isolate of *M. grisea* (IIRR SP - 28). High humidity was maintained for good disease development. The disease reaction was recorded 15 days after inoculation on each plant following 0-9 scale (IRRI, 1996).

Bacterial blight: The F₂ and F₃ plants along with the checks ISM (resistant check) and TN1 (susceptible check) were transplanted in plastic trays. The bacterial pathogen was multiplied on modified Wakimoto's medium at 28 ± 1°C. Plants at maximum tillering stage were clip inoculated with three day old bacterial suspension (0.1 O.D.) (Kauffman *et al.*, 1973). Observations were recorded 14 days after inoculation by measuring the lesion length. The Lines were categorized as resistant (lesion length d" 4 cm), moderately resistant (lesion length 4.1-8 cm) or susceptible (lesion length > 8 cm) (Shanti *et al.*, 2001).

Results and Discussion

Phenotypic analysis of F₂ plants for Bacterial blight resistance

274 F₂ plants were obtained from the cross between NILs of MTU1010 and breeding lines of Akshyadhan. These were phenotyped for bacterial blight reaction. Out of 247 F₂ plants (Table-1) evaluated against BB, 97 plants were found resistant (lesion length <3), 54 were found to be moderately resistant (lesion length 3.1-5.0), 84 were found to be moderately susceptible (lesion length 5.1-7.0) and 39 were found to be highly susceptible (lesion length >7.1) (Table 1).

Phenotyping for bacterial blight and blast resistance genes in F₃ population

Ninety seven resistant plants (Table-1) obtained from screening of the F₃ population from the cross MTU1010 x Akshyadhan was advanced to F₃ generation. These were phenotyped with bacterial blight isolate and all were observed to be resistant to BB (lesion length <3cm). The selected lines were advanced further to obtain promising lines possessing both blast and bacterial blight resistance genes.

Acharya N.G Ranga Agricultural University (ANGRAU), Andhra Pradesh and Professor Jayashankar Telangana State Agricultural University (PJ TSAU), Telangana state played an important role in Indian agriculture by releasing many popular varieties and hybrids in almost all the crops especially in rice. Among the 10 mega rice varieties, four rice varieties viz., Cottondora Sannalu (MTU1010), Samba Mahsuri (BPT5204), Swarna (MTU7029) and Vijetha (MTU1001) are released from ANGRAU and three upcoming varieties viz., Telangana sona (RNR 15048), Kunaram sannalu (KNM 118) and Bathukamma (JGL 18047) from PJ TSAU,

have occupied more than 25% of area in India and more than half of the area in Telangana. Among which, Cottondora Sannalu (MTU1010), is covering >12% (Vanisree *et al.*, 2012) of Indian rice acreage because of its wider adaptability to the BPH resistance and its high yielding, but susceptible to bacterial blight (BB) and blast diseases, which causes significant yield losses in many states of India including Andhra Pradesh. In order to sustain the yield levels of rice cultivars like MTU 1010, it is imperative to improve the variety for disease resistance. In the absence of effective chemicals or any other methods of control agents against BB pathogen (Devadath *et al.*, 1989), resistance breeding is considered as the most economical and ecofriendly strategy for management of the disease and achieving yield stability. Pyramiding resistance genes is difficult to accomplish using conventional breeding strategy due to epistatic effects of genes controlling resistance and due to non-availability of screening facilities for multiple biotic stresses in addition to screening restricted only to specific seasons. Molecular markers can accelerate resistance breeding efforts (Sundaram *et al.*, 2008), as segregating plants can be selected on the basis of molecular marker alleles instead of its phenotype and introgression of multiple resistance genes can be tracked easily in a population.

Among several biotic stresses that cause significant yield losses in rice, bacterial blight (BB) and rice blast are the two major biotic stresses, particularly in Punjab, Andhra Pradesh, Haryana and Uttar Pradesh (including parts of Uttaranchal) mainly in the irrigated and rainfed low land ecosystems (Production Oriented Survey, 2008). Bacterial blight is one of the most destructive diseases of rice worldwide caused by *Xanthomonas oryza* pv. *oryzae* (Xoo) and the yield losses can be as high as 50% and can be assumed to epidemic proportions.

Table.1 Segregation pattern of F₂ generation against bacterial blight resistance

Cross	Frequency distribution for BLB disease score (cm)				Total no of plants (F ₂)	Observed frequency		Expected frequency	
	< 3	3.1 to 5.0	5.1 to 7.0	≥7.1		R	S	R	S
NILs of Akshyadhan	54	0	0	0	54	54	0	54	0
MTU1010	0	0	44	12	56	0	56	0	56
F ₂ population of Akshyadhan X MTU1010	97	54	84	39	274	151	123	154	120

Table.2 Screening details of F₃ generation against blast resistance

Cross	Frequency distribution for blast disease score					Total no of lines (F ₃)
	0	1-3	4-5	6-7	8-9	
NILs of Akshyadhan	0	51	0	0	0	51
MTU1010	0	0	0	33	21	54
NILs of Akshyadhan X MTU1010	0	67	30	0	0	97

Management of BB through application of chemicals/antibiotics is not commercially available (Devadath, 1989 and Gnanamanickam *et al.*, 1999). Hence, deployment of varieties with resistant genes is the only approach. Large scale and long term cultivation of varieties with single gene may enable the pathogen to overcome BB resistance. The most effective approach to combat bacterial blight is the use of resistant varieties with combination of different genes (Khush *et al.*, 1989). Till date, at least 40 BB resistance (R) genes conferring host resistance against various strains of *Xoo* have been identified. Suh *et al.*, (2013) reported that among the 40 R genes, six are physically mapped (*Xa2*, *Xa4*, *Xa7*, *Xa30*, *Xa33* and *Xa38*) and six are cloned (*Xa1*, *xa5*, *xa13*, *Xa21*, *Xa26=Xa3* and *Xa27*). Of these, *Xa21*, a major resistance gene, originally introgressed from *Oryza longistaminata* (Ronald *et al.*, 1992; Song *et al.*, 1995) was

observed to confer resistance to most Indian isolates of the bacterial pathogen.

The fungus *Magnaporthe oryzae* is the causal agent of rice blast disease and belongs to phylum, Ascomycota and family Magnaporthaceae. It is one of the most devastating diseases in at least 85 countries worldwide. The disease often results in a significant yield loss, as high as 70-80% during an epidemic (Ou, 1985). Hence there is an urgent need to improve this restorer line by incorporating resistance genes of bacterial blight and blast. As on date, 100 rice blast major resistance genes (R-genes) have been identified (Sharma *et al.*, 2012) and among the major blast resistance genes, *Pi-k^h*, which has been recently renamed as *Pi54* (Sharma *et al.*, 2010), exhibited resistance to predominant races of the pathogen in India (Sharma *et al.*, 2002). *Pi54* gene was originally identified from Tetep, a Vietnam

indica source and mapped on chromosome 11L with two tightly linked simple sequence repeat (SSR) markers TRS26 and TRS33 has been cloned (Sharma *et al.*, 2005). Ramkumar *et al.*, (2011) developed PCR based functional marker Pi54MAS and it was observed to perfectly co-segregate with no recombinants. The rice cultivar 'Tetep' has been found to be resistant to most of the pathogenic races occurring in India (Padmanabhan *et al.*, 1979). A recently developed Near Isogenic Line (NIL) line of Akshyadhan (Bhaskar *et al.*, 2016) with long slender grain type, *indica* rice variety derived from the cross Akshyadhan/RPBio Patho-2 possessing *Xa21* for BB and *Pi54* for blast along with good grain yield.

We selected only a single dominant gene each conferring resistance against BB (i.e. *Xa21*) and blast (i.e. *Pi54*) in the present study. Even though there are a few previous reports about breakdown of resistance conferred by a single BB resistance gene (Mew *et al.*, 1992, Khush *et al.*, 1989) in rice, till date there is no report about large-scale breakdown of resistance conferred by either *Xa21* or *Pi54* from India or abroad. Further, as per a recent report (DRR annual report, 2011-12), NILs of Samba Mahsuri and Swarna possessing only *Pi54* displayed resistance across multiple locations in India (DRR Progress report, Vol. 2, 2008-2013).

In conclusion, through the present study, we have developed F₂ and F₃ population of the popular variety MTU1010 possessing resistance against BB and blast. When we screened the 274 F₂ plants of MTU1010 against the predominant isolates of BB (DXO-020) and blast (SPI-40) (Table-1), the RILs were segregated by Mendelian ratio (9:3:3:1) and observed to be highly susceptible (120) and resistant (154) against both the diseases. Further, the highly resistant 97 plants were forwarded to F₃ generation

thus indicating that the RILs have tremendous potential for development of elite LS grain type, BB and blast resistance.

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