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Validation of Linked Markers of Blast and Blight Resistance Genes among Black Rice Accession's of North East India

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ABSTRACT: North East India has rich diversity of rice's, among them three land races of black aromatic glutinous rice of Manipur i.e. Chakhao Poireiton, Black Chakhao and Chakhao Amubi have gained a lot of importance in domestic as well as International market. This black rice's are poor yielders and are susceptible to major biotic and abiotic stresses. In the present investigation, three black rice land races *i.e.*, Chakhao Poireiton, Black Chakhao and Chakhao Amubi were screened for blast and bacterial blight genes. To determine the presence of any blast and blight resistance genes, phenotyping on UBN using fungal isolates for blast, clip clipping with blb inoculum for blight in field was done and genotyping for two major blast (*Pi54* and *Pi2*) resistant genes and three major blight (*Xa21, xa13* and *xa5*) resistant genes were carried out by using functional markers (RM206, AP5659-5, pTA248, xa13 promoter and xa5FP, respectively). Both phenotyping and genotyping results have shown that black rice land races were highly susceptible to both blast and bacterial blight and have all the susceptible alleles for five genes. This investigation is helpful for designing improvement of blast and bacterial blight resistant versions of black rice by marker assisted pyramiding using molecular markers.

Keywords: Black rice, Chakhao Poireiton, Black Chakhao, Chakhao Amubi, blast resistance, bacterial leaf blight resistance, marker-assisted selection.

INTRODUCTION

Black rice is indigenous to North-East India and is extensively cultivated in Manipur, Odhisa, West Bengal and Jharkhand. Black rice known as Chakhao and its meaning is delicious rice in Manipuri language (*Chak* means rice; *ahoaba* means delicious). It is known as 'forbidden rice' or 'imperial rice'. It is also known as 'Emperor's rice' because in ancient days it was only used by kings for the consumption purpose. The two great qualities of black scented rice of Manipur are colour and scent. There are more than 200 types of black rice varieties around the world. Mostly black rice are grown in China, Sri Lanka, Indonesia, and India, and germplasm collection in above countries was 359, 50, 42, and 30 accessions, respectively (Kushwaha, 2016). A total of three landraces of black rice, *viz*., Chakhao Poireiton, Black Chakhao and Chakhao Amubi were mostly grown in Imphal East district of Manipur. Among them, Chakhao Poireiton has higher productivity and delicacy and so cultivated (43 %) by most of the farmers (Borah *et al.,* 2018).

In North Eastern hilly ecosystem of India, black rice occupies 3.51 M ha and produces 5.50 million tones which constitute 7.8 per cent of the total rice area and 5.9 per cent of the rice production. The productivity of NE region is around 1.57 t/ha, which is much below than the national average of 2.08 t/ha (Pattanayak *et al.,* 2006). Among many factors, which limits the Rice productivity in the NEH region of India, biotic stresses plays major role in reduction of productivity. Among the biotic stresses, blast (BL) and bacterial leaf blight (BLB) are the serious problems which do occur regularly in the NEH region, since the climate conditions are very much suitable for these deadly biotic stress agents.

The fungus *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*) is the causal agent of rice blast disease, belongs to Ascomycetaceae family causing high yield loss of 70-80% during an epidemic (Khush and Jena, 2009). In rice, more than 100 blast resistance genes and more than 350 QTL's have been identified out of which only 25 genes have been cloned (Devi *et al.,* 2020). Among them, two major blast resistance genes viz., *Pi2* and *Pi54* are known to be very effective when introgressed in different backgrounds (Jamaloddin *et al.,* 2020; Rekha *et al*., 2018; Kumar *et al.,* 2018). Bacterial blight (BB) is caused by *Xanthomonas oryzae pv. oryzae (Xoo)*, a major disease of rice. This disease in severe form can cause yield loss of about 74 to 81% (Srinivasan and Gnanamanickam, 2005). Till now 44 BLB genes have been identified (Kumar *et al.,* 2020). Out of 44 resistant genes only 11 genes viz. *Xa1, Xa3/Xa26, Xa4, xa5, Xa10, xa13, Xa21, Xa23, xa25, Xa27* and *xa41* were cloned. Among them, three major blight resistance genes (*Xa21, xa13* and *xa5*) were introgressed into many genetic backgrounds which include Samba Mahsuri, Tellahamsa and JGL1798 (Sundaram *et al*., 2008; Jamaloddin *et al.,* 2020 and Swathi *et al.,* 2019).

To develop resistant varieties, foreground selection is a pre-requisite process in marker assisted selection.

Unless the parents are polymorphic for the linked markers of the genes which are related to the trait of interest, the further selection of plants carrying the traits of interest is not possible in the progenies. The recent developments in plant biotechnology including marker-assisted selection (MAS) offer a choice of options for targeted pyramiding of major blast and BLB resistance genes in the genetic background of black rice varieties of North East India. Hence in the present study we have chosen to validate three blight and two blast resistance genes using gene specific markers in three land races of black rice of India.

MATERIALS AND METHODS

A. Black rice materials

Traditional varieties of black rice Chakhao Poireiton, Black Chakhao and Chakhao Amubi are used as parent collected from ICAR- NEH region, Manipur. Improved Sambha Mahsuri (possessing *Xa21*, *xa3* and *xa5*) was used as positive control as it has three BLB genes whereas Samba Mashuri (SM2545) having blast genes (*Pi54, Pi2)* developed at IIRR, Hyderabad was used as positive control for blast.

B. Screening for blast resistance

Chakhao Poireiton, Black Chakhao and Chakhao Amubi were sown on uniform blast nursery (UBN) at Indian Institute of Rice Research (IIRR), Hyderabad, India along with HR12 (tall low tillering variety with susceptibility to leaf blast) and SM2545 (possessing *Pi54* and *Pi2*) were used as susceptible and resistant checks, respectively. After 15 days of germination local *Magnaporthe oryzae* fungal virulent isolate (SPI-40) from Indian Institute of Rice Research (IIRR), Hyderabad, Telangana, India (Madhan Mohan, 2011), was used to screen these three black rice landraces. The plants were scored after one week of inoculation and evaluated on a 0–9 scale as per IRRI-SES scale (IRRI, 1996).

C. Screening for bacterial blight resistance

Four virulent isolates of the bacterial blight pathogen, *Xanthomonas oryzae pv. oryzae (Xoo)* collected from BB hot-spot locations in India, viz. IIRR *Xanthomonas* collection-020 (IX-020), IX-002, IX-066 and IX-049, were used to screen the parents for bacterial blight resistance under both glass house and field conditions. In the green house, disease severity is assessed based on lesion length measurement or estimation of diseased leaf area. These three black rice land races along with resistant (ISM) and susceptible (TN1) checks were clip-inoculated with a bacterial suspension of 10^9 cfu/ml at maximum tillering stage (45–55 days after transplanting) through the methodology of Kauffman *et al.* (1973) and were screened for bacterial blight after 14 days of inoculation. In addition to measurement of BB lesion length, the disease score was also calculated as per IRRI standard evaluation system (IRRI-SES) scale (IRRI 1996).

D. Marker assisted selection for blast and BB resistance

Chakhao Poireiton, Black Chakhao and Chakhao Amubi parents were screened for blast and BB resistance using functional markers. RM206, AP5659-5 markers were used to identify *Pi54, Pi2* blast genes, respectively. PCR-based STS marker pTA248 (Ronald *et al.,* 1992), xa13 prom (Basavaraj *et al.,* 2010), xa5FP (Iyer and McCouch *et al.,* 2007) were used to identify *Xa21, xa13* and *xa*5 genes, respectively (Table 1). PCR was performed using 1 U of Taq DNA polymerase (MBI Fermentas,Vilnius Lithuania, Bangalore Genei, Bangalore, India) and 1XPCR buffer (Genei, India) in 10µl reaction volume with a thermal profile of 94°C for 5 min (initial denaturation), followed by 35 cycles of denaturation at 94°C for 30s, annealing at 55°C for 1min, extension at 72°C for 1 min and a final extension of 10 min at 72°C. The amplified product of pTA248, xa13 prom, xa5FP and Pi54MAS were electrophoretically resolved on a 2% SeakemLE_ agarose gel (Lonza, Rockland, ME, USA), while AP5659-5 was electrophoretically resolved on a 4% Seakem LE_ agarose gels containing 0.5 mg/ml of ethidium bromide in 0.5XTBE buffer and visualized under UV.

Table 1: Details of primers used for the validation of blight and blast genes in black rice's.

Linked gene	Marker name	Primer sequence
xa5	Xa5FP	**F:**AGACGCGGGAAGGGTGGTTCCCGGA
		R:AGACGCGGGTAATCGAAAGATGAAA
		F:AGACGCGGGAAGGGTGGTTCCCGGA
		R:AGACGCGGGTAATCGAAAGATGAAA
xa13	xa13prom	**F:**TCCCAGAAAGCTACTACAGC
		R:GCAGACTCCAGTTTGACTTC
Xa21	pTA248	**F:**AGACGCGGGAAGGGTGGTTCCCGGA
		R:AGACGCGGGTAATCGAAAGATGAAA
Pi54	RM206	**F**:CCCATGCGTTTAACTATTCT
		R:CGTTCCATCGATCCGTATGG
Pi2	AP5659-5	**F:**CTCCTTCAGCTGCTCCTC
		R:TGATGACTTCCAAACGGTAG

E. Evaluation of agro-morphological characters

Thirty-day-old seedlings were transplanted in the main field at a spacing of 15 × 20 cm. Standard agronomic practices were followed to raise a healthy crop, which were evaluated during the wet season (June–November) in 2019. Data were recorded for the agronomic traits, viz. days to 50% flowering (DFF), mean plant height (cm), number of productive panicles per plant, panicle length (cm), 1000-seed weight, and grain type.

RESULTS

A. Disease reaction of black rice for blast and bacterial blight

Chakhao Poireiton, Black Chakhao and Chakhao Amubi parents were phenotypically screened for bacterial blight in main field and blast resistance in uniform blast nursery. All the parents showed high level of susceptibility to blast with a disease score of 8-9 and showed the presence of disease lesions in more than 70-80% leaf area with a disease score of 7-8 (Table 2).

B. Genotyping of black rice for blast resistance

Black rice varieties were screened for the presence of the target genes *Pi54, Pi2* using RM206 and AP5659-5 markers, respectively. Positive check having *Pi54* showed the amplicon size of 120bp and black rice varieties have showed the amplicon size of 190bp. For *Pi2* the resistant parent showed the amplicon size of 190bp and black rice varieties showed amplicon size of 220bp. All the three black rice varieties were observed to be susceptible for both the genes (Fig. 1).

Table 2: Disease reaction of black rice for blast and bacterial blight.

Genotypes	Blast score	Bacterial blight score
ISM	7	1
SM2545	0	8
Chakhao Poireiton	8	7
Black Chakhao	8	8
Chakhao Amubi	9	7

C. Genotyping of black rice for blight resistance

Black rice varieties were screened for *Xa21, xa13, xa5* genes using pTA248, xa13 prom and xa5FP, respectively, showed susceptibility for all the three blight genes. For *Xa21*gene resistant parent showed amplicon size of 900bp and black rice varieties showed amplicon size of 700bp. For *xa13* gene the resistant parent showed amplicon size of 490bp and black rice varieties are having amplicon size of 290bp. For *xa5* gene the resistant parent is having amplicon size of 134bp and black rice varieties are having amplicon size of 313bp and a common amplicon of 424bp (Fig. 1).

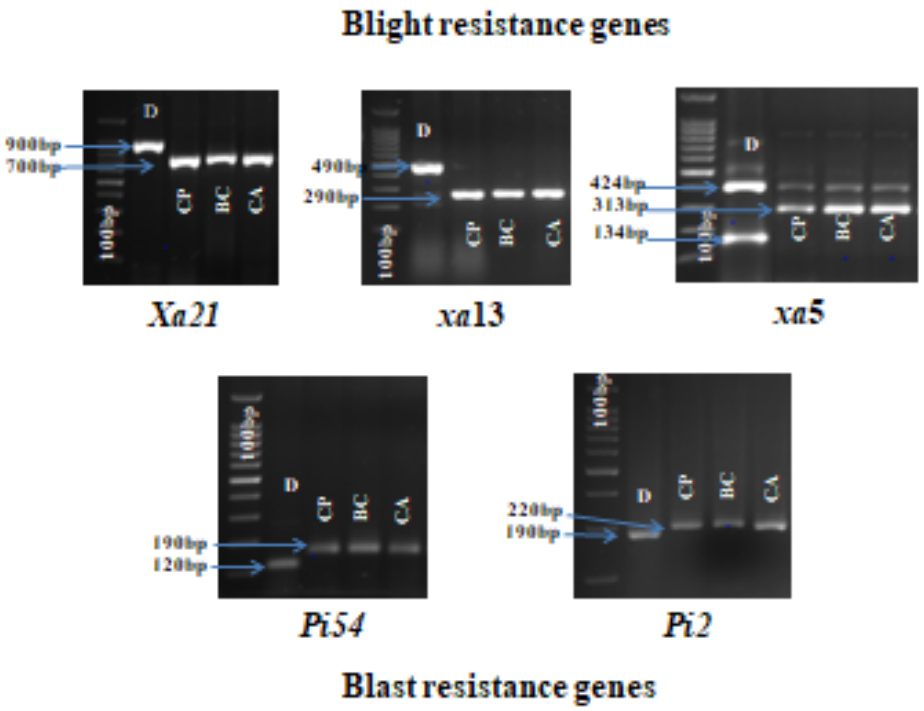

Fig. 1. Genotyping of black rice's for bacterial blight (*Xa21, xa13* and *xa5*) and blast (*Pi54* and *Pi2*) resistance genes. D: ISM donor for *Xa21, xa13* and *xa5*; D: SM2545 donor for *Pi54* and *Pi2;* CP: Chakhao Poireton; BC: Black Chakhao; CA: Chakhao Amubi.

D. Agro-morphological characters

Traditional black rice varieties are tall plants with 160-170 cm height, long bold grains having 22 to 28g test weight and 2.96 to 3.3 L/B ratio. They flower early but the numbers of productive tillers are significantly inferior compared to donors. They are long duration varieties having more than 150 days duration. Due to tall nature of plants they are highly susceptible to lodging. The agronomical morphological parameters recorded of Chakhao Poireiton, Black Chakhao and Chakhao Amubi along with gene donors are presented in Table 3.

Table 3: Agro-morphological performance of traditional black rice varieties.

Character	ISM	SM2545	Chakhao Poireiton	Black Chakhao	Chakhao Amubi	Critical difference
Days to 50% flowering	106	95	67	71	70	2.13
Plant Height (cm)	79.46	85.21	160	170	166	3.612
Productive tillers	12.67	16.00	11	11	13	2.943
Panicle length(cm)	19.68	18.45	21	22	23	3.289
Test weight	12.01	13.43	28	23.5	22.6	2.595
L/B ratio	2.78	2.95	3.16	2.96	3.3	NA

DISCUSSION

Chakhao Poireiton, Black Chakhao and Chakhao Amubi are traditional varieties of black rice grown in North-East parts of India particularly in Manipur. The two great qualities of black rice of Manipur are their colour and scent. Because of the health benefits of consuming black rice it is used as cuisine in five star hotels. But the black rice is susceptibility to biotic stress and is a poor yielder. Rice blast and Bacterial leaf blight are the major diseases affecting rice production and leads to significant yield loss (Khush and Jena, 2009). Therefore, development of host plant resistance is considered to be the best option for managing the diseases (Hulbert *et al*., 2001). Because of the highly variable nature of the pathogen, resistance conferred by single resistant genes has broken down and only those varieties possessing multiple resistance genes are effective over the years and at multiple locations. Realizing this, the present study aims at evaluation of north eastern Indian black rice viz., Black Chakhao, Chakhao Poreiton and Chakhao Amubi for resistance to rice blast and bacterial leaf blight disease through the application of molecular markers and stringent phenotypic selection. Marker assisted selection is a proven technology with high success rate. The molecular markers closely linked to the target traits were very useful for foreground selection. Two major genes i.e. *Pi54* (Sharma *et al*., 2005; Ramkumar *et. al*., 2011), *Pi2* (Zhou *et al.,* 2006) conferring resistance against blast disease and three major resistance genes (*Xa21, xa13*and *xa5)* conferring resistance for BLB were selected for evaluation of three land races of black rice.

In the present study both phenotyping and genotyping evaluation of black rice revealed that these landraces are highly susceptible to both bacterial leaf blight and blast and lack major blast (*Pi54* and *Pi2)* and blight (*Xa21, xa13*and*xa5*) genes. Several studies utilized these markers for improvement of popular varieties of rice. Swathi *et al*., (2019) utilized pTA248, xa13promoter and Pi54 MAS genes for the improvement of JGL 1798. Similarly, Jamaloodin *et al*., (2020) improved Tella hamsa for blast and blight resistance using similar markers.

CONCLUSION

As the domestic demand for the black glutinous aromatic rice of Manipur is increasing, efforts are needed to improve the existing landraces by introgressing major blast and BLB resistant genes through molecular breeding approach which increases their yield without losing the grain's quality, cooking quality and aroma and encouraging the farmers to cultivate black rice. To the best of our knowledge, there was no early work done on blast and bacterial blight in black rice varieties. The identified polymorphic foreground markers from the present study will be utilized for genotyping the backcross and intercross population possessing blast and blight genes.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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