**Introgression of major blast resistance gene *Pi-2* into Elite Rice Cultivar Yairipok Phou through Marker Assisted Selection**

**Abstract**

Rice blast disease caused by the fungus *Magnaporthe oryzae* is one of the most devastating diseases causing massive losses worldwide. Development of blast resistance of rice varieties cultivated in blast endemic areas is one of the most important objectives of rice breeding programs. The objective of the present investigation is to develop breeding lines utilizing YAIRIPOK PHOU× C101A51 through the pedigree method along with MAS. Therefore, marker-assisted selection, which has got the enormous potential to transfer gene(s) of interest from donor to diverse rice cultivars with high precision within the short breeding cycle is the best choice to deploy the selected blast-resistant *Pi2* gene into the genotype of Yairipok Phou. One hundred and twelve F2 plants produced through selfing were screened with gene-specific markers *Pi2(i)* to identify the plants carrying the *Pi2* gene. Out of the 112 F2 plants that were subjected to foreground analysis, 25 F2 plants (F2-8, F2-35, F2-39, F2-41, F2-44, F2-48, F2-53, F2-55, F2-56, F2-60, F2-61, F2-62, F2-64, F2-70, F2-73, F2-75, F2-80, F2-86, F2-88, F2-89, F2-90, F2-94, F2-101, F2-105 and F2-106 were found to be homozygous for the target genes (*Pi2Pi2*. Out of the 25 F2 lines with the target genes in homozygous condition, seven lines *viz*., F2-39, F2-60, F2-70, F2-73, F2-75, F2-86, and F2-90 exhibited yield per plant better or on par with RP Yairipok phou coupled with more or less difference concerning a number of productive tillers and 1000 seed weight over the recurrent parent RP Yairipok Phou. These lines with desirable agronomic traits and blast resistance were selected and need to be advanced and evaluated further for their yield parameters along with disease resistance.

**Keywords:** Blast resistance, Marker Assisted Selection, Rice,Gene transfer

**Introduction**

Rice blast (*Pyricularia oryzae* L.) is caused by the fungus *Magnaporthe oryzae* (Couch and Kohn 2002). It infects and produces lesions on the following parts of the rice plant: leaf, leaf collar, culm, panicle neck node and panicle. The pathogen is most common on leaves, causing leaf blast during the vegetative stage of growth, and on neck nodes and panicle branches during the reproductive stage, causing neck blast (Bonman 1992). Leaf blast lesions reduce the net photosynthetic rate of individual leaves to an extent far beyond the visible diseased leaf fraction. The pathogen can affect all the above-ground parts of a rice plant viz., leaf, node, collar, neck, parts of panicle, and sometimes leaf sheath at different growth stages (Pinnschmidt *et al*. 1994). Blast disease of rice was first reported in California rice in 1996. Blast disease can cause severe loss of yield up to 50-85 % (Teng and Revilla 1996). It is widely distributed and occurs in every region of the world where rice is grown (Bonman 1992). Among the several diseases, Rice blast caused by *Magnaporthe oryzae* is one of the most devastating and destructive diseases of rice worldwide (Zeigler *et al*. 1994). Rice blast is economically the major fungal disease because of its worldwide distribution. Rice blast disease, caused by the heterothallic ascomycete, is pervasive globally and hampers rice productivity to the extent of 50–90 percent (Ellur *et al.* 2015). The fungus has the potential to infect all the aerial parts of the plant except the root at any stage of plant growth through the production of lesions on the leaves, nodes, and panicles (Picco and Rodolfi 2002). About 50 species of the grass family are infected by *M. grisea*, including rice, wheat, and barley. The blast disease was first observed in India in 1919, with estimated yield losses of 4 percent (Padmanabhan 1965). Rice blast has been found in more than 85 rice-producing countries worldwide and the estimated annual loss of rice due to blast was enough to feed 60 million people for one year (Parker *et al*. 2008). Yairipok phou is a popular variety with an excellent eating quality of local preference with a milled rice recovery of about 70 percent. However, the variety is moderately susceptible to blast disease and rice sheath blight. The variety withstands rice gall midge and stem bore infestations to a considerable extent. Yairipok phou performed well as a main paddy crop under the rainfed wetland ecosystems of Manipur valley and similar situations in the NEH Region. C101A51 is a isogenic line containing broad spectrum blast resistance gene *Pi2* which is used as a donor parent for introgression program. The objectives of the present investigation is to develop blast-resistant genotypes through marker-assisted selection (MAS) in an F2 population derived from the cross of C101A51 (resistant cultivar) and a susceptible rice cultivar, Yairipok phou.

**Materials and methods**

***Plant material and DNA isolation.***

In the present study, three genotypes were used in the crossing programme *viz*., Yairipok phouas recurrent parent, C101A51 as donor parents, HR-12 (negative check) and Tetep (positive check). The seed materials used in the experiment were obtained from the Central Agriculture University, Imphal and ICAR Complex for NEH Region, ICAR Manipur Centre, Lamphelpat, Imphal West-795004**.** Genomic DNA was extracted from young leaf tissue by using CTAB (Cetyl- Tri Methyl Ammonium Bromide) method as described by Murray and Thompson (1980). Quantification of DNA was done by analyzing the purified DNA on 0.8 % agarose gel with diluted uncut lambda DNA as a standard. The banding pattern was observed and recorded using a gel documentation unit (Alpha Innotech, USA).

***Method of Gene Transfer***

The recurrent parent CAUR-1 was crossed to the donor parent C101A51 to transfer *Pi2* genes for resistance to the blast disease. The resulting F1 plants with genes (*Pi2)* were selfed to produce an F2 generation. The resulting F2 plants were confirmed for the presence of the target gene and were studied for disease resistance and yield parameters. Blast screening was done at Uniform Blast Nursery, Genetics and Plant Breeding, CAU, Imphal, during the *Kharif* season favor the blast disease development using a 0–9 scale following the standard evaluation system (SES; IRRI, 2002).

***Foreground selection.***

In the current study, gene-specific marker Pi2(i) was used for the foreground selection to identify the positive plants with target genes *Pi2.* Foreground selection was carried out in the F2 population to identify the plants possessing the target resistance gene and the details of the target blast-resistant gene along with the markers used for foreground selection are given in Tab. 1.

Table. 1 Functional marker used for foreground selection.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sl. No | Gene | Marker | Sequence | Chr.No. | ‘R’ allele (bp) | ‘S’ allele (bp) | Reference |
| **1** | *Pi2* | Pi2(i) | CAGCGATGGTATGAGCACA | 6 | 450 | 282 | Xu *et al*. 2013 |
| CGTTCCTATACTGCCACATG |

**Result**

***Validation of Parents for Blast Resistance***

Donor and recipient parents were differentiated using polymorphic SSR marker using gene-specific marker Pi2(i). *Pi2* gene showed polymorphism between the recurrent parent and donor parent. The Pi2(i) marker for the *Pi2* gene was amplified at 450bp in donors (C101A51) and at 282bp in recipient parent Yairipok phou (Fig. 1).



 **450bp**

 **282bp**

 **L A B**

Fig. 1 Marker validations in the parents for target gene with gene-specific marker Generation and Confirmation of F1s

In the current study, F1s obtained from the cross were raised at the Department of Genetics and Plant Breeding department, CAU, Imphal, during *Kharif*, 2022. These F1s were confirmed for their hybridity using the gene-specific markersPi2(i)which was polymorphic between the donor and recurrent parents. A total of 15 F1 plants were obtained from the cross Yairipok phou x C101A51. Ten out of 15 F1 (Yairipok phou x C101A51) plants were identified to possess the *Pi2* gene in the heterozygous condition (*Pi2pi2)* (Fig. 2). These confirmed heterozygous F1 plants were tagged before flowering for further use in MAS programme.



 **L P1  P2 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15**

Fig. 2 Hybridity confirmation of the F1s for *Pi2* gene using Pi2(i) marker (L: 100bp ladder; P1: RP YAIRIPOK PHOU; P2: IR-64; 1-15)

***Phenotyping of F2 population lines for blast disease***

F2 plants which were developed by selfing of F1 plants were screened for blast resistance during *Kharif* 2022 as described in materials and methods. Blast screening was done at Uniform Blast Nursery, Genetics and Plant Breeding, CAU, Imphal. The susceptible check HR-12 displayed high susceptible reaction with a score of ‘9’. The donor parents C101A51 (positive checks) which possesses the *Pi2* gene, showed a high level of resistance to rice blast with a ‘3’ disease score, and recurrent parent Yairipok phou which is moderately susceptible to the blast disease showed the presence of disease lesions not more than 50 percent leaf area with disease scoring scale ‘5’ exhibiting a moderately susceptible reaction to the native of the blast pathogen. The introgressed lines (*i.e.,* heterozygous F1 plants) developed through marker-assisted breeding, which was sown in two rows displayed a high level of resistance to rice blast disease without any lesions on their leaves with a score of ‘3’ (Table 2). Similarly, most of the F2 plants which were sown in three rows in the UBN bed displayed a high level of resistance for rice blast with no lesions on the leaves. All the plants in F2 lines showed small brown specks of pin head size without a sporulation center on the leaves mostly found on the lower leaves with a disease score of ‘3’. In this study, it is observed that even the recurrent parent was not highly susceptible to the blast disease (exhibiting scale a ‘5’, moderately susceptible). However, F2 lines derived from the recurrent parent showed complete resistance to the blast disease, indicating that the target genes are highly resistant to the tested isolates.

Table 2. Blast disease scoring of the gene pyramided lines.

|  |  |  |  |
| --- | --- | --- | --- |
|  **Sl. No.** | **Plant Identity** | **Disease Score** | **Disease Reaction** |
| 1 | Yairipok phou | 5 | Moderately Susceptible |
| 2 | C101A51 | 3 |  Medium Resistant |
| 3 | HR-12 | 9 | Highly susceptible |
| 4 | F1 | 3 | Medium Resistant |

***Genotyping F2 generation for blast-resistant genes utilizing Pi2(i) gene-specific maker.***

All the F2 plants that were uprooted from UBN beds along with the recurrent parent (Yairipok phou) were transplanted in the main field during *Kharif*, 2022. A total of 112 F2 plants were grown at Genetics and Plant Breeding, CAU, Imphal during *Kharif*, 2022. All the F2 plants were checked for the presence of the gene *(Pi2)* using the molecular markers Pi2(i). Fig. 3 shows the result of foreground selection in F2 plants (1-16) along with parents for the *Pi2* gene. Out of 112 F2 plants screened, fifty-five plants were found the *Pi2* gene in heterozygous condition whereas twenty-five plants were identified in homozygous condition (Table 3). But for the current work which was framed to attain durable resistance, the plants introgressed with the gene are desirable. Therefore, the twenty-five F2 plants that were identified to be a desirable gene for the genes based on foreground selection were tagged in the field for further evaluation of yield and agronomic performance.

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 **P1 P2 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16**

Fig. 3 Foreground selection in F2 plants along with parents for *Pi2* gene using Pi2-(i) marker (L: 100bp ladder; P1: RP Yairipok Phou; P2: IR-64; 1-16)

Table 3. List of F2 lines produced for *Pi2 gene* for both heterozygous and homozygous conditions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  **Sl. No** | **Total Plants produced** |  **No. of plants with*****Pi2* gene****(heterozygous)** |  **No. of plants with*****Pi2* gene****(homozygous)** | **No. of plants with no*****Pi-2* gene** |
| 1 | 112 | 54 | 25 | 33 |

***Evaluation of the F2 Lines for Yield and Agro- Morphological characters***

The agro-morphological evaluation was carried out in twenty-five F2 lines during *Kharif*, 2021-22 at COA, CAU, Imphal. Data was recorded for fourteen characters *viz*., days to flowering, plant height (cm), days to maturity (day), the total number of tillers per plant, total number of effective tillers per plant, panicle length (cm), number of filled grains per panicle, grain yield per plant (g), grain length (mm), grain width (mm), grain length/width (mm), flag leaf length (cm), flag leaf width (cm), and 1000 seed weight (g) along with values of range are presented. The technique of marker-assisted foreground selection using a co-dominant marker has been efficient in determining the zygosity of the resistance gene accurately (Hospital, 2009). As the marker used for foreground selection in the current research i.e., Pi2(i) was based on the gene itself, there was hardly any chance of recombination between genes and markers. Therefore, the efficacy of foreground selection was very high. Out of the 25 F2 lines with the target genes in homozygous condition, seven lines *viz*., F2-39, F2-60, F2-70, F2-73, F2-75, F2-86, and F2-90 exhibited yield per plant better or on par with RP Yairipok phou coupled with more or less difference concerning a number of productive tillers and 1000 seed weight over the recurrent parent RP Yairipok phou. These lines with desirable agronomic traits and blast resistance were selected and need to be advanced and evaluated further for their yield parameters along with disease resistance.

**Discussion**

Use of molecular markers in breeding has opened easier way to select the desired allele in a short duration. Marker assisted selection for target trait(s)/gene(s) also known as foreground selection, facilitates identification of plants possessing gene of interest at early seedling stage and thus reduces the population size by half in a backcross breeding programme and number of generations for screening thus facilitating elimination of undesirable lines at early generations (Ribaut and Hoisington 1998; Joshi *et al*. 1999) in addition to several other advantages. One hundred and twelve F2 plants produced through selfing were screened with gene-specific markers Pi2(i) to identify the plants carrying the *Pi2* gene. Out of the 112 F2 plants that were subjected to foreground analysis, 25 F2 plants (F2-8, F2-35, F2-39, F2-41, F2-44, F2-48, F2-53, F2-55, F2-56, F2-60, F2-61, F2-62, F2-64, F2-70, F2-73, F2-75, F2-80, F2-86, F2-88, F2-89, F2-90, F2-94, F2-101, F2-105 and F2-106 were found to be homozygous for the target genes (*Pi2Pi2*). Among all the plants that were grown in UBN, some plants could be with the target gene (*Pi2*), while some of them might not have the target gene (*Pi2*). But irrespective of gene status all the plants showed high resistance to the isolate tested here. The possible explanations for this are given below. The wide spectrum resistance of the gene was clearly demonstrated in earlier studies by Inukai *et al*. 1994; Jiang *et al*. 2015; Chen *et al*. 1996 for *Pi2* gene in conjunction with other genes while Prashanthi *et al*. (2010); Liu *et al*. (2002a); Fjellstrom *et al*. (2006); Balakrishnan *et al.* (2014); Ellur *et al.* (2015) witnessed the high resistance of *Pi2* gene with the combination of other genes. Ellur *et al.* (2015) employed marker-assisted backcross breeding to pyramid the blast resistance genes *Pi2* and *Pi54* and bacterial blight resistance genes *Xa13* and *Xa21* into the genetic background of Pusa Basmati1121 (PB1121) and Pusa Basmati 6 were used as recurrent parents. The genotypes namely, Pusa 1602 with *Pi2*, Pusa 1603 carrying Pi54; SPS97, and Pusa 1460 possessing *Xa13 + Xa21*, were used as donor parents. The results obtained in this study was in accordance with the results of Liu *et al*. (2002a); Balakrishnan *et al.* (2014), who used marker-aided selection for both foreground as well as background genotypes. Many reports for the effectiveness of the gene *Pi2* either singly or in combination with other genes are available (Chen *et al*. 1996; Mackill and Bonman 1992; Prashanthi *et al*. 2010). The agro-morphological evaluation was carried out in twenty-five F2 lines during *kharif*, 2021-22 at COA, CAU, Imphal. Data was recorded for fourteen characters *viz*., days to flowering, plant height, days to maturity, number of productive tillers per plant, number of effective tillers, panicle length, number of filled grains per panicle, grain yield per plant, grain length, grain width, grain length/width ratio, flag leaf length, flag leaf width, and 1000 seed weight. Seven lines with *Pi2* gene *viz*., F2-39, F2-60, F2-70, F2-73, F2-75, F2-86, and F2 showed improved performance over the recurrent parent concerning grain yield per plant (g). Among these, three genotypes *viz*., F2-75 (35.63 g), F2-60 (32.26 g), and F2-50 (31.74 g) exhibited significant yield increase over the recurrent parent RP Yairipok phou (22.9 g).

**Conclusions**

The gene-specific markers used in this research Pi2(i) for the *Pi2* gene is recommended for screening germplasm and segregating lines in the development of blast-resistant rice varieties. The prominent F2 lines performing better and on par with the recurrent parent need to be further improved with the introgression of more major genes coupled with selfing to attain homozygosity, which could be used as breeding materials for developing blast resistance genotypes. The F2 lines carrying the target gene in homozygous condition (*Pi2Pi2*) need to be advanced and evaluated further in hop spots with different isolates for their stability and durability of the resistance. The F2 lines carrying the target genes (*Pi2)* need to be advanced and evaluated further in hop spots with different isolates for their stability and durability of the resistance.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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