

# **Inheritance and Segregation Pattern of genes *Xa21*, *xa5* and *xa13* causing Bacterial Leaf Blight (BLB) resistance in Rice (*Oryza sativa* L.)**

## **ABSTRACT**

The present study aims to investigate the genetic inheritance of resistance to bacterial leaf blight (BLB) in rice by analyzing the segregation of major resistance genes viz. *xa5*, *xa13*, and *Xa21* in two specific crosses. BLB, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a significant threat to rice cultivation worldwide. To manage this, resistant cultivars can be developed through pyramiding multiple resistance genes. This experiment involved 1392 BC<sub>2</sub>F<sub>2</sub> plants derived from crosses R1853-105-1-82-1×Improved Samba Mahsuri and BPT 2411×Improved Samba Mahsuri. Marker Assisted Selection for three BLB genes (*xa5*, *xa13*, and *Xa21*) was performed at earlier generations and segregation analysis was performed on true breeding plants. Field screening was conducted using clip inoculation method at maximum tillering stage. The result revealed that the population of cross R1853-105-1-82-1×Improved Samba Mahsuri was showed a 7:9 segregation ratio (Resistant: Susceptible) and resistant was governed by two recessive genes (*xa5* and *xa13*) while the population of cross BPT 2411×Improved Samba Mahsuri was found 55:9 (Resistant: Susceptible) segregation ratio and the resistant was governed by one dominant gene (*Xa21*) and two recessive genes (*xa5* and *xa13*). It verified the existence of all three resistance genes in the population. The produced lines will be used as breeding materials for the development of BLB-resistant cultivars.

**Keywords:** Rice, Bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae*, Resistant, Susceptible, Inheritance, Segregation.

## **Introduction**

Rice is the world's principal food source, feeds more than half of the world's population, mostly in tropical and subtropical Asia (Danaisilichaichon et al. 2023). It ranked second in global agriculture; it generates employment and revenue in rural areas while accounting for more than one-fifth of the calories consumed by 3 billion

people. Rice is farmed over an area of 165.04 million hectares with a global production of 776.46 million tons of paddy. About 90% of the rice worldwide is produced and consumed in Asia, while India generates 22% of the world's total rice production (FAOSTAT 2021). With 46.3 million hectares under cultivation and an annual yield of 130.29 million tons of paddy in 2022 (<https://www.indiastat.com>, 2022-23), India leads the world in both area and production (Dileep kumar *et al.* 2023).

India has the greatest area around 46.3 million hectares, producing 130.29 million tons at a productivity of 2809 kg/ha (Anonymous, 2023). Chhattisgarh, renowned as the "Rice Bowl of India," spans 43.48 lakh hectares, yielding 13.23 million tons with a productivity of 3045 kg/ha (Anonymous, 2023). Rice cultivation in Chhattisgarh state relies only on the monsoon, with an annual rainfall of 1200-1600 mm. During *Kharif*, paddy covers the most land and accounts for a significant portion of national paddy production.

In order to meet the demand brought forth by the world's population expansion and rising living standards, rice output must rise. To fulfill the demands of a growing population and rising incomes, the world's supply of staple cereal grains, such as wheat, maize, and rice, will have to double over the next three decades (Sao, *et. al.*, 2024). However, the impending threats of human-caused climate change, which increases biotic and abiotic stresses on agriculture, make this critical aim much more difficult to attain. Plant breeders and farmers are now dealing with the immediate consequences of climate uncertainty. Although rice production has greatly expanded since the green revolution, it still needs to keep up with the rising demand due to the world's fast growing population and depleting natural resources.

The sustainability of rice production and maintaining self-sufficiency is a massive task and presents great challenges for rice breeders and agricultural scientists due to the constraints imposed by static area, limited water, decrease in arable land, fast emerging new pathogens and pests, in addition to the adverse effects from climate change (Khush, 2005).

However, a large number of illnesses with bacterial, viral, and fungal origins limit the amount of rice that can be produced. One of the most destructive diseases

that affects entire rice acreages is Bacterial Leaf Blight (BLB) (Fiyaz *et al.*, 2016), which is caused by the gram-negative proteo-bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo) (Mew, 2003). Depending on the crop's stage, cultivar susceptibility, and environmental factors, BLB can result in severe yield losses of up to 80%. (Srinivasan and Gnanamanickam, 2005).

Rice disease, characterized by Kresak and leaf blight, affects rice growth stages and results in significant yield losses and grain quality issues. The leaf blight phase is the most distinctive and frequently seen, affecting photosynthetic area, output, and incomplete grain filling (Baliyanet *et al.*, 2016; Sombunjittet *et al.*, 2017).

Control of Bacterial Leaf Blight through Chemicals is ineffective thus; the best, most affordable, and ecologically safe way to manage BLB is through host-plant resistance (Khush *et al.*, 1989; Sombunjittet *et al.*, 2017). Researchers are working on evolving resistant cultivars and finding genes to protect against BLB. A total of 46 bacterial leaf blight resistance genes have been discovered and some of them have been introduced into popular high yielding rice varieties (Hsu *et al.*, 2020; and Chukwu *et al.*, 2019).

Marker-assisted backcrossing enables for the exact insertion of new resistance genes, which improves a genotype's overall resistance capabilities. Conventional breeding procedures, which might take eight to twelve years to generate a new variety, are inefficient and unreliable (Sundaram *et al.*, 2008). Marker-assisted selection (MAS) is a revolutionary approach to plant breeding that uses gene pyramiding to improve broad-spectrum resistance. Many prominent Indian rice cultivars are experiencing issues owing to biotic and abiotic stresses (Chen *et al.*, 2020). To achieve long-term resistance, many genes must be pyramided together.

Marker assisted backcross breeding (MABB) is a method used to develop durable resistance cultivars by pyramiding broad spectrum resistance genes. It involves studying the inheritance of these genes, which requires a thorough examination of the character's inheritance pattern. Breeding techniques change based on the character's inheritance pattern, and the number of populations required to capture the resistance gene is determined by its inheritance pattern. It is therefore

important to research the genetics of that attribute before continue with the breeding program.

Understanding the inheritance patterns of resistance genes can aid in developing resistant rice varieties. This study synthesizes current research on the major resistance genes, their inheritance patterns, and their application in rice breeding programs.

## Materials and methods

### Plant materials

In the present experiment, two genotypes viz., R1853-105-1-82-1 and BPT 2411 were used as recurrent parent with good agronomic characters along with good grain quality. Improved Samba Mahsuri is an improved variety having three genes for resistance to BLB (*xa5*, *xa13* and *Xa21*) is used as donor parent (Sundaram *et al.*, 2008). The experimental materials consist of the 1392 backcross (BC<sub>2</sub>F<sub>2</sub>) populations.

**Table 1. Characteristic features of the parents used in the present study**

SN	Genotypes	Features	BLB Resistance genes
1.	R1853-105-1-82-1	High yielding (6.5 t/ha) elite line with medium duration (130-135 days), possessing long bold grains.	-

2.	BPT 2411	High yielding (7.0-7.5 t/ha) with medium duration (140-145 days), medium slender grain, tolerant to BPH, Blast and stem borer, tolerant to water stress, Non-lodging and non-shattering	-
3.	Improved Samba Mahsuri (ISM)	High yielding with medium duration (135-140 days), fine grain variety possessing premium grain and cooking quality, resistant to bacterial leaf blight	<i>Xa21, xa13 and xa5</i>

### **Development of BC<sub>2</sub>F<sub>2</sub> population**

The genotype R1853-105-1-82-1 and BPT 2411 were crossed with Improved Samba Mahsuri. The resulting F<sub>1</sub> plants were backcrossed with recurrent parents to generate BC<sub>1</sub>F<sub>1</sub> plants (Fig 1&2), which were confirmed for all target genes using gene-specific markers. BC<sub>1</sub>F<sub>1</sub> plants were again backcrossed with recurrent parents to generate BC<sub>2</sub>F<sub>1</sub> plants, which were selfed to obtain BC<sub>2</sub>F<sub>2</sub> plants. These BC<sub>2</sub>F<sub>2</sub> plants were evaluated for disease resistance during *Kharif* (Wet season), 2023.

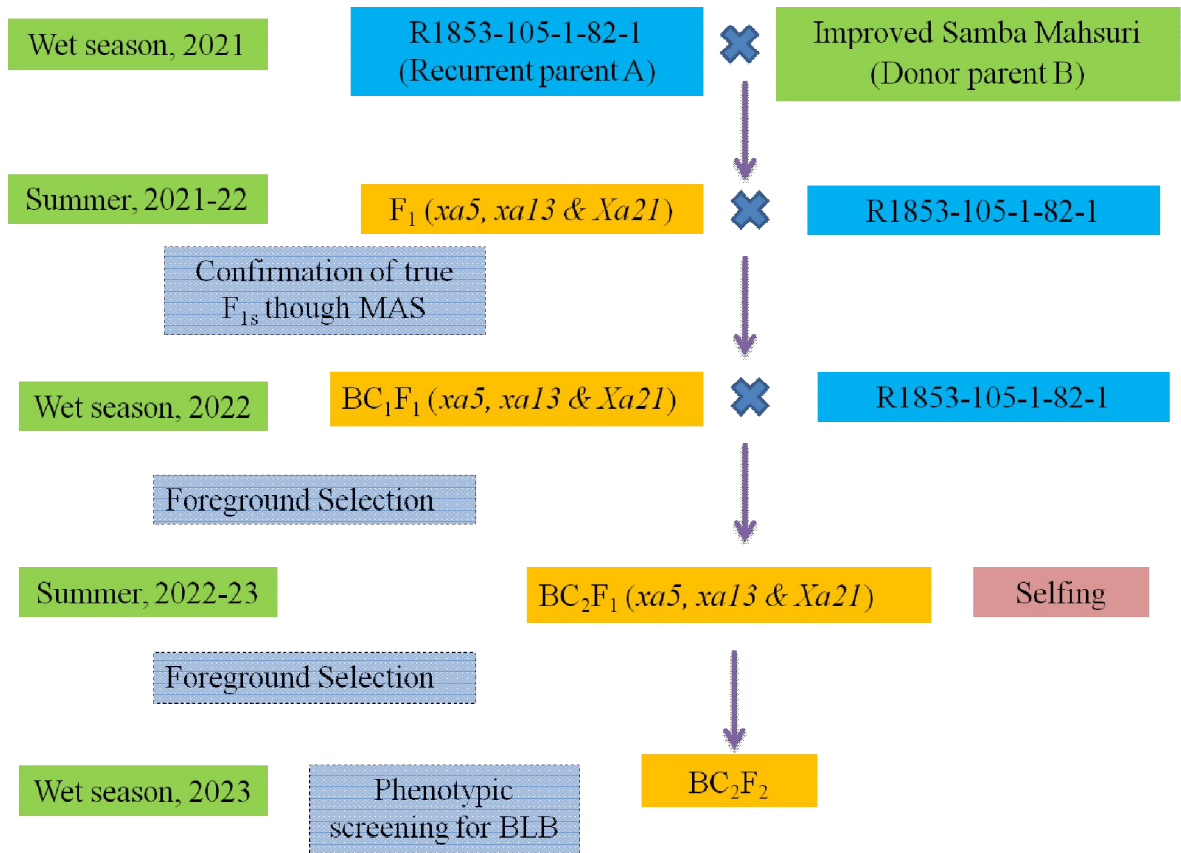


Fig1. Development of BC<sub>2</sub>F<sub>2</sub> population from cross R1853-105-1-82-1 × Improved Samba Mahsuri.

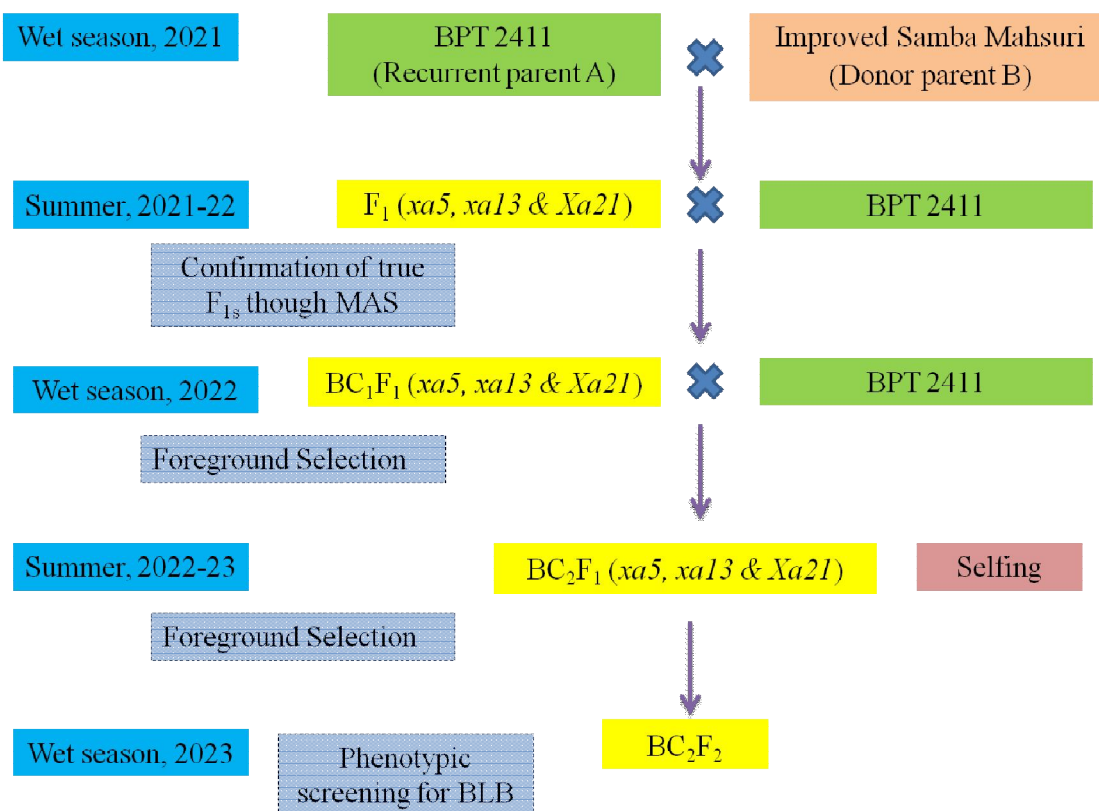


Fig 2. Development of BC<sub>2</sub>F<sub>2</sub> population from cross BPT 2411 × Improved Samba Mahsuri.

### Bacterial Culture and inoculation

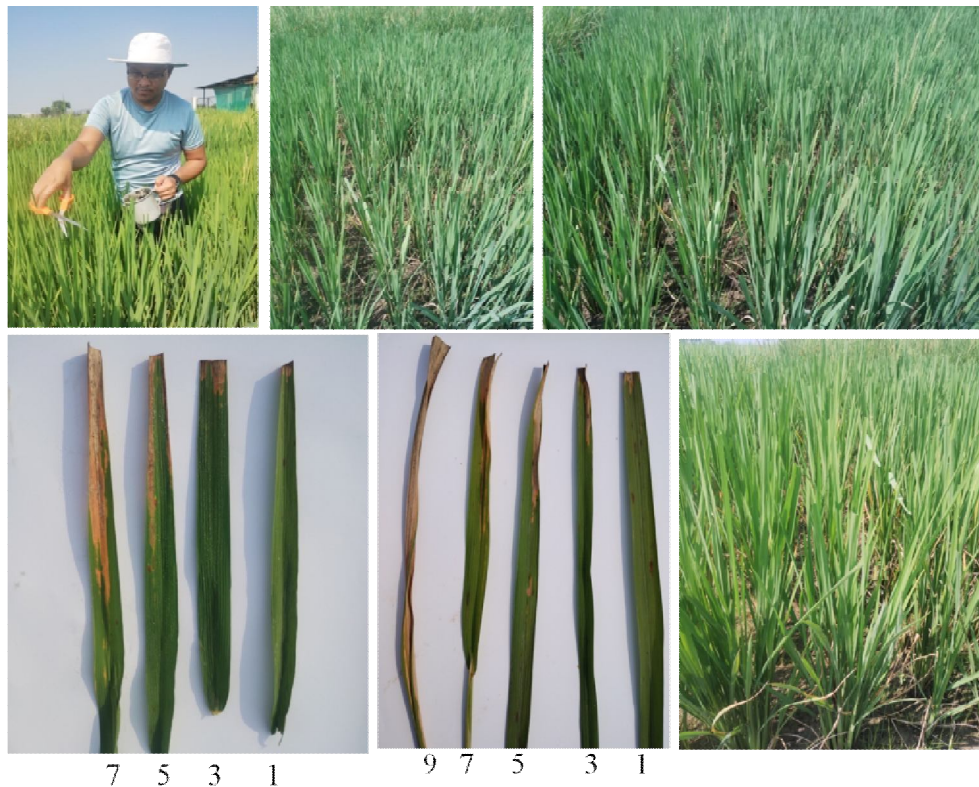
The BC<sub>2</sub>F<sub>2</sub> plants were evaluated in the field of the Department of Genetics and Plant Breeding, IGKV, Raipur, in *Kharif* (Wet season), 2023, for resistance to bacterial leaf blight. The Dhamtari isolate of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was obtained from the Department of Plant Pathology, IGKV, Raipur (kotasthane and Gaikyad 2021). The isolate was sub-cultured from the stock culture and stored at 4°C until needed. Cultures were grown on Wakimoto's medium slants at 30°C for three days (Table 2). Inoculum was prepared by suspending the bacterial mass in sterile distilled water and diluting it to a concentration of approximately 10<sup>9</sup> cells/ml (Pradhan *et al.*, 2022). The freshly prepared inoculum was used for inoculation.

The leaf clip inoculation method developed at the All India Coordinated Rice Improvement Project (AICRIP) was deployed at the maximum tillering stage of the screening population (Kauffman *et al.*, 1973). The top of each plant's fresh leaves

were cut with a sterilized scissor dipped in freshly made bacterial solution, as shown in Fig. 3, early in the morning. Observations were noted 21 days after inoculation by the percentage of diseased leaf area (DLA) followed by the standard evaluation system (SES, IRRI, 2013). The lesion length and total leaf length were measured and graded using a 1-9 scale, and were rated as HR, R, MR, S and HS. Scoring of the disease was done as shown in Table 3.

**Table 2: Composition of Wakimoto's media (1000 ml solution).**

S.N.	Component	Quantity
1.	Peeled Potato	300g
2.	Sucrose	20g
3.	Peptone	5g
4.	Sodium dihydrogen phosphate	1.87g
5.	Calcium nitrate	0.5g
6.	Agar-Agar (Bacteriological grade)	17g
7.	Distilled water	1000 ml
8.	pH	6.8



**Fig 3. Screening of the F<sub>2</sub> population for the Bacterial leaf blight Resistance**

**Table 3. Standard evaluation system scale for bacterial leaf blight damage scoring**

<b>SES score</b>	<b>Diseased leaf area in %</b>	<b>Description</b>
1	1-5%	Highly Resistant
3	6-12%	Resistant
5	13-25%	Moderately Resistant
7	26-50%	Susceptible
9	51-100%	Highly Susceptible

Bacterial blight damage score was done as per SES (IRRI, 2013)

### **Statistical Analysis**

The chi-square is a test of statistical significance which is used to test the significance of difference between observed and expected frequencies. Chi square test was developed by Karl Pearson (Singh 2013). The predicted genetic ratio's significance was tested using chi square ( $\chi^2$ ) analysis in order to estimate the number of genes that segregate in the population using the formula below.

$$\chi^2 = \sum (O_i - E_i)^2 / E_i$$

Where;

$O_i$  = Observed frequencies

$E_i$  = Expected frequencies

### **Result and Discussion**

### Validation and parental polymorphism survey

Marker-assisted selection requires co-segregation of gene with molecular marker, polymorphic marker between parents, and confirmation of disease resistance before crossover programme begins. The investigation involved testing a donor parent in Kharif 2021 for target resistance genes using previously published molecular markers, providing detailed information on linkage group and allele size on Table 3.

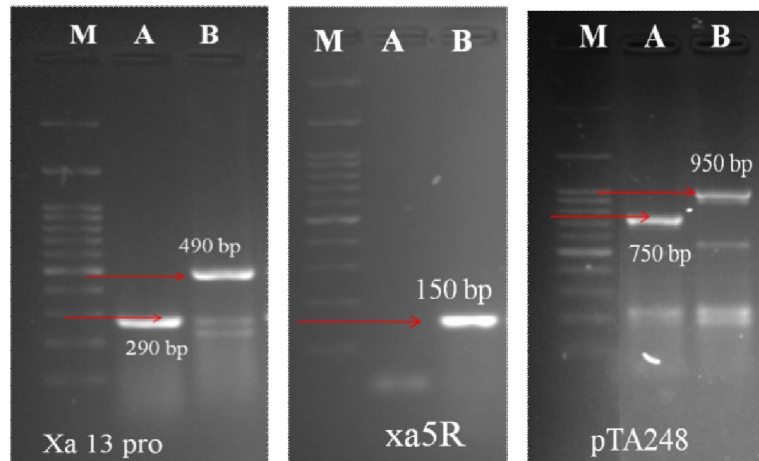
The three primers, pTA248 for the Xa21 gene, xa13 prom for the Xa13 gene, and xa5R marker for the Xa5 gene, were shown to be polymorphic across donor and recurrent parents. These markers were all used for the foreground selection.

The primer pair Xa13prom produced a 490bp fragment in the resistant parent (ISM) and a 290bp fragment in the recurrent parent (R1853-105-1-82-1 and BPT 241). The primer pair pTA248 amplified segments of 950bp in the resistant parent ISM, but only 700bp in the susceptible parents R1853-105-1-82-1 and BPT 241. The primer pair xa5R produced a 150bp fragment only in the resistant parent (ISM) and no fragment in the recurrent parents, R1853-105-1-82-1 and BPT 241 (Fig 4).

The study found that gene-based markers accurately identified gene-positive plants at all phases of MABB, distinguishing between resistant and susceptible lines and separating resistance alleles in homozygous or heterozygous conditions.

**Table 3. Details of the molecular markers used for foreground selection**

SN	Gene	Marker	Chr. No	Amplicon size (bp)	Trait	Reference
1	Xa21	pTA248	11	950	BLB resistance	Huang <i>et al.</i> , (1997)
2	xa13	Xa13prom	8	490	BLB resistance	Hajira <i>et al.</i> , 2016
3	xa5	xa5R	5	150	BLB resistance	Sundaram <i>et al.</i> , 2014



M= 100 bp, A-R1853-105-1-82-1, B-ISM,

**Fig 4 Marker validation in the parents for target genes with gene specific markers**

#### **Inheritance analysis**

For the purpose of genetic resistance analysis, two susceptible cultivars (R1853-105-1-82-1 and BPT 2411) were crossed with a highly resistant cultivar (ISM), and the segregating pattern was evaluated in the  $F_2$  populations. The parents,  $F_1$ s and  $F_2$ s have been inoculated with Dhamtari isolate at the maximum tillering stage and disease scoring was performed 21 days later. The  $F_1$  and  $F_2$  plants were divided into five groups based on SES (IRRI, 2013) using mean leaf lesion length (Table 2).

In the current investigation, the disease severity of the resistant parent was less whereas the susceptible parent R1853-105-1-82-1 and BPT 2411 exhibited a greater disease severity due to the absence of resistance genes while the cultivar Improved Samba Mahsuri was resistant due to the presence of resistance genes *Xa21*, *xa13*, and *xa5*. The early indications of bacterial leaf blight, linear yellow to straw-colored stripes with wavy borders, were noticed with varying intensities in cultivars R1853-105-1-82-1 and BPT 2411. Resistance parents had a considerably lower area under the disease progression curve than susceptible parents.

The  $F_1$  plants from the cross BPT 2411  $\times$  Improved Samba Mahsuri were resistant to BLB while the  $F_2$  population of the same cross displayed distinct BLB reactions due to gene segregation. For segregation analysis, the Highly Resistant (HR),

Resistant (R), and Moderately Susceptible (MS) plants were placed in the resistant group, while the Susceptible (S) and Highly Susceptible (HS) plants were placed in the susceptible group.

### **Inheritance analysis of R1853-105-1-82-1 × Improved Samba Mahsuri**

The 464 F<sub>2</sub> plants from the cross R1853-105-1-82-1 × ISM were assessed individually and categorized into two classes: resistant and susceptible, among them, 191 resistant and 273 susceptible. The chi square value was 1.25, which was non-significant at the 5% level of significance, suggesting that the observed data were consistent with the expected ratio (Table 4).

The F<sub>1</sub> progeny of this cross showed susceptible reaction but its F<sub>2</sub> progeny segregated in 7:9 (Resistant: Susceptible) segregation ratio. It revealed the presence of two recessive genes for resistance in the population. The resistance was governed by two recessive genes *xa5* and *xa13*. From the three introgressed genes only two recessive genes *xa5* and *xa13* were present in the parental gene combinations and the dominant gene *Xa21* was absent in the parental gene combinations. Therefore only two recessive genes *xa5* and *xa13* were expressed in the population and resistance was governed by these genes. Similar findings of 7:9 segregation ratio were reported by Natarajkumaret al. (2008) and Ijaz et al. (2008). They find the presence of two recessive genes for resistance *xa5* and *xa13*. Kihupiet al. (2001) also reported the similar segregation ratio of 7:9, which has been governed by single recessive gene.

**Table 4. Genetic analysis of BLB resistance in the cross R1853-105-1-82-1 × ISM**

Genotype	Host response	Total no of plants	O	E	Phenotypic ratio	$\chi^2$
ISM	R					
R1853-105-1-82-1	S					
F <sub>1</sub>	S					
F <sub>2</sub>	R	464	191	203	7 R	1.25
	S		273	261	9 S	

Where,  $\chi^2$  0.05, 1=3.84. Note: R = Resistant, S = Susceptible, O = Observed value, E = Expected value.

### Inheritance analysis of BPT 2411× Improved Samba Mahsuri

In these F<sub>2</sub> populations 928 plants were evaluated individually and divided into two categories: resistant and susceptible. Among them, 782 were found resistant and 146 susceptible. The chi square value was 2.14, which was non-significant at the 5% level of significance, indicating that, the observed data were consistent with the expected ratio (Table 5). The F<sub>1</sub> progeny of this cross exhibited the same resistance response as the resistant parent ISM, while the F<sub>2</sub> progeny segregated in 55:9 (Resistant: Susceptible) segregation ratio. It showed the existence of three resistance genes in the population. The resistance was governed by one dominant gene *Xa21* and two recessive genes *xa5* and *xa13*. It confirmed the presence of all three resistance genes in the population.

**Table 5. Genetic analysis of BLB resistance in the cross BPT 2411 × ISM**

Genotype	Host response	Total no of plants	O	E	Phenotypic ratio	$\chi^2$
ISM	R					
BPT 2411	S					
F <sub>1</sub>	R					
F <sub>2</sub>	R	928	782	797.5	55 R	2.14
	S		146	130.5	9 S	

Where,  $\chi^2$  0.05, 1=3.84. Note: R = Resistant, S = Susceptible, O = Observed value, E = Expected value.

Marker assisted backcross breeding (MABB) method made easy and precise for the development of durable resistance cultivars through pyramiding broad spectrum resistance genes and also to study the inheritance of these genes. The effective use of a resistance gene requires a thorough examination of the character's inheritance pattern. Breeding techniques change depending on the character's inheritance patterns. Furthermore, the number of populations must be developed in segregating generations to capture the resistance gene is determined by the gene's inheritance pattern. It is

therefore important to research the genetics of that attribute before continue with the breeding program.

Yoshimura *et al.* (1983) used four Philippine races to study the inheritance of resistance in the DV-85 x TN-1 cross. They discovered a dominant and recessive gene for resistance. These findings revealed that the *Xa21* and *xa13* gene combination was preferable in both the parent and segregating generations in order to capitalize on the synergistic benefits of this combination in reducing BLB infections. Similar results have been reported by Deshmukh (2017) and Pradhan *et al.* (2022). They fined 55:9 segregation ratio and reported that the segregation ratio was governed by three genes, one dominant (*Xa21*) and two recessive (*xa5* and *xa13*) genes.

#### **Conclusion :-**

The research analyzed the inheritance patterns of three BLB genes (*xa5*, *xa13*, and *Xa21*) for resistance in rice. The results showed a 7:9 segregation ratio in the R1853-105-1-82-1×Improved Samba Mahsuri cross, indicating the presence of two recessive genes (*xa5* and *xa13*). The 55:9 segregation ratio in the BPT 2411×Improved Samba Mahsuri cross indicated resistance governed by one dominant gene (*Xa21*) and two recessive genes (*xa5* and *xa13*). It confirmed the presence of all three resistance genes in the population. The developed lines will be utilized as breeding materials for the development of BLB-resistant varieties.

#### **ACKNOWLEDGEMENT**

We express our gratitude to the Department of Genetics and Plant Breeding and Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) for technical and financial support during the course of Ph.D. research work of main author.

**Conflicts of Interest:** The authors declare no conflict of interest.

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