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Research Article

Improving rice blast resistance by stacking of two broad spectrum resistance gene *Pi9* and *Pi54* in cultivar CO 51 through Marker Assisted Selection

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Abstract
Blast disease is a highly destructive disease and causes significant yield losses in rice. To address this issue, this study was chosen and focused on stacking of the *Pi9*along with CO 51+*Pi54*, which provide broad-spectrum resistance to blast pathogen. This study demonstrated that stacking of *Pi9* along with CO 51+*Pi54*, against blast disease. An advanced breeding line was evaluated for blast disease resistance in a natural hotspot region over two growing seasons (*kharif* 2021 and *kharif* 2022). All 36 progenies revealed genetic potential of the stacked genes and exhibiting resistance to blast disease *kharif* 2021 and *kharif* 2022. The recurrent parent, CO 51 is moderately resistant while CO 51 with *Pi9*+*Pi54* genes displayed a blast score 0<2, indicating strong resistance. Average yield of single plant has been increased 0.28% to 35.05% in NILs of CO 51. Maximum single plant yield of 35.05 % was recorded in progeny #20x6-5-11-121 which was followed by the progeny #20x6-5-11-125 (31.65%).

Keywords*:* Rice, Blast disease, gene stacking, *Pi9*, *Pi54* and marker assisted selection

INTRODUCTION

Plant defense mechanisms are activated to engage in an “arms race” with invading pathogens (Arora *et al.,* 2021). Plants employ multiple strategies to survive against biotic stresses, such strategy is the resistance gene-mediated host defense, which helps combat pests and diseases (Yan and Talbot, 2016). In rice plant, numerous diseases caused by fungi, bacteria, viruses, nematodes, *etc.*, have been identified, with estimates reaching up to 70 different diseases (Zhang *et al.,* 2009).The yield of rice is significantly affected by various factors, including both abiotic and biotic stress. The intensity of biotic stress has been increasing rapidly in recent times due to climate change(Yugander *et al.,* 2017).Abiotic stress, such as drought, salinity, heavy metals, and extreme temperatures, can lead to substantial yield losses by affecting seedling growth, tillering ability, and grain filling(Akram *et al.,* 2019). Biotic stresses, such as blast disease, bacterial leaf blight (BLB), gall midge, and brown plant hopper (BPH), are also responsible for significant yield reductions. Studies have reported yield losses ranging from 20% to 40% in the case of BLB and 50% or more in the case of blast disease (Khush and Jena, 2009;Vasudevan *et al., 2*014). Blast disease caused by *Magnaporthe oryzae (M.oryzae*) is ranked as the most significant among the top 10 fungal diseases affecting rice production (Dean *et al.,* 2012;Qiu *et al.,* 2022). It is a highly destructive disease that can impact the entire lifespan of the rice plant, leading to yield reductions of up to 30% and even 70%-90% under severe epidemic conditions(Dean *et al.,* 2012). The variability in pathogenicity of the rice blast pathogen makes it difficult to control and manage. Blast pathogen *M. oryzae* is capable of causing up to 100% yield losses by infecting lesions on leaves, stems, necks, panicles, and seeds (Prasad *et al.,* 2012).Rice serves as a major energy source for over 4 billion people worldwide and plays a crucial role in global food security (Kumar *et al.,* 2018). To meet the growing demand for food due to an increasing population, it is necessary to improve rice cultivars and varieties that are tolerant to biotic stresses such as blast disease. The development of rice varieties with stacked or pyramided major resistance genes against biotic stresses like the blast pathogen has the potential to increase rice grain production(Roychowdhury *et al.,* 2012).

According to Chen *et al.* (2018), the development of host resistance is the most effective and economical approach to control blast disease. Plant breeders and biotechnologists have made significant efforts in breeding rice varieties with resistance against blast disease by evaluating the function of resistance genes (R genes). Although the introgression of one or more R genes can improve rice blast resistance, the variability of the pathogen can lead to the breakdown of resistance in improved varieties (Kou and Wang, 2010). Therefore, pyramiding multiple R genes with broad-spectrum and durable resistance against various races of the blast pathogen may provide effective resistance (Fukuoka *et al*., 2012). Marker-Assisted Backcross Breeding (MABB) facilitates the introgression of two or more resistant genes into popular cultivars from suitable donors (Singh *et al.,* 2019). In 1996, Tanksley and Nelson successfully introgressed Quantitative Trait Loci (*QTLs*) from unadapted germplasm into elite breeding lines(Tanksley and Nelson, 1996). Rice varieties possessing multiple resistant genes or *QTLs* targeting specific traits can exhibit strong resistance and contribute to food sustainability.

To date, around 102 R genes(Devanna *et al.,* 2022) and nearly 500 QTLs (Ashkani *et al.,* 2016) against rice blast have been identified. Among them, 38 R genes have been cloned and characterized at the molecular level, including *Pi37, Pit, Pish, Pi35, Pi64, Pi-b, pi21, Pi63/Pikahei-1, Pi9, Pi2, Piz-t, Pi-d2, Pi-d3, Pi25, Pid3-A4, Pi50, Pigm, Pi36, Pi5, Pii, Pi56, Pi54, Pikm, Pb1, Pik, Pik-p, Pia, Pi1, Pi54rh, Pi-CO39, Pi54of, PiK-h, Pike, Piks,* and *Pi-ta* (Devanna **et** *al.,* 2022). According to Devanna *et al.* (2022), among the identified R genes, *Pi9, Pi54, pi21, Pi50, Pi7, Pi57, Pigm,* and *Ptr* are considered the most effective broad-spectrum resistance genes against blast disease. Gene, *Pi9* a major resistant gene, was isolated from the wild species *Oryza minuta* and has shown broad-spectrum resistance against a wide range of *M. oryzae* isolates (Sitch *et al.,* 1989; Amante-Bordeos *et al*., 1992). The gene *Pi54,* former *pi-kh*, was isolated from the Tetep cultivar and is located on chromosome 11L (Sharma *et al.,* 2005). Gene, *Pi54* exhibits resistance against major *M. oryzae* isolates (Rai *et al.,* 2011; Dixit *et al.,* 2020). Both *Pi9* and *Pi54* have durable and broad-spectrum resistance against the blast pathogen *M. Oryzae (*Kumar *et al.,* 2018;Thulasinathan *et al.,* 2020). Therefore, the stacking or pyramiding of *Pi9* and *Pi54* genes may offer broad-spectrum and durable resistance against a wide range of blast pathogen races. The present study focuses on the introgression of two R genes, namely *Pi9* and *Pi54* into the CO 51 genetic background, followed by the evaluation of selected progenies against blast disease. This pathogen is widely prevalent in Tamil Nadu.

MATERIALS AND METHODS

Parent materials: In the present study, the CO51 cultivar, which is a short-duration (110-115 days), fine-grain, and high-yielding rice genotype, was notified for cultivation in 14 different states in India. CO51, being the recurrent parent, exhibits moderate resistance to blast disease due to the presence of the *Pi54* resistant allele on chromosome 11 at 24.2 Mb (Ashkani *et al*., 2016; Devanna *et al*., 2022; Thulasinathan *et al*., 2020). The aim of this study is to enhance the resistance of CO51 against the blast pathogen by introducing the *Pi9* gene along with to the *Pi54* allele. For this purpose, an intermittent breeding line named 562-4 (a near-isogenic line of CO43 X VRP1) that carries the *Pi9* gene was used as the donor to stack the major R genes into the genetic background of CO51.

Breeding strategy to develop advance breeding lines: The initial step involved was crossing the recurrent parent CO 51 with the donor parent, 562-4, to create F_1 hybrid plants. Genomic DNA (gDNA) was then extracted from the F_1 plants using a modified CTAB method(Ausubel *et al.,* 1992). To confirm the authenticity of the F_1 plants, markers associated with the genes were utilized. Specifically, the functional marker NBS4, linked to *Pi9*, and Pi54MAS, linked to *Pi54*, were employed. In $F_{1,}$ selected plant harbouring targeted genes, the desired markers were selected for the development of Near Isogenic Lines (NILs) carrying both *Pi9* and *Pi54* genes.

Genotyping: The *Pi9* gene is located on chromosome 6 and exhibits dominant inheritance between the two parents with functional marker of NBS4. On the other hand, the *Pi54* marker, located on chromosome 11, is an InDel marker that shows co-dominant inheritance (Functional marker, Pi54MAS). The Pi54MAS marker is a gene-based functional marker that amplifies a fragment of 216 bp for the Pi54 resistance allele and 359 bp for the susceptible allele (Ramkumar *et al.,* 2011).

For the PCR amplification, a reaction mixture of 25 µl was prepared, consisting of 3 µl (50 ng/µl) of gDNA template, 3 µl of 10X PCR buffer, 1 µl of 2.5 mM dNTPs, 0.5 µl (3 U/µl) of Taq DNA polymerase, 1 µl of each forward and reverse primer, 3 µl of 1.75 mM MgCl2, and 10.25 µl of nuclease-free water. The PCR reaction profile included an initial denaturation at 94°C for 10 minutes, followed by denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, primer extension at 72°C for 1 minute, and a total of 40 cycles. A final extension step was carried out at 72°C for 5 minutes, followed by holding the temperature at 40°C. The specific details of the forward and reverse primer sequences for the marker are furnished (**Table 1**).

Phenotypic screening against blast disease : The experiment involves growing a of 36 genotypes, including the recurrent parent CO 51, donor parent 562-4, and susceptible check CO 39, in a Uniform Nursery Bed (UNB) at the Hybrid Rice Evaluation Centre (HREC) in Gudalur, Tamil Nadu, India. To create a suitable environment for disease evaluation, the susceptible check CO 39 was sown once in every four rows of the Near Isogenic Lines (NILs), and the UNB was bordered with CO 39 plants. Susceptible check parent, CO 39 served as an inoculum source for the blast disease, ensuring a continuous supply of spores for infection.

The assessment of blast disease infection was conducted at two time points, 30 and 45 days after sowing in the UNB. The disease infection was carried out for both the parents and the NILs(**Table 2**). This blast screening was repeated twice within the same natural hotspot during the *kharif* 2021 and *kharif* 2022.

Performance of agronomic traits: Five plants per genotype were evaluated for their yield and related traits *viz*., Number of Tillers per Plant (NTP), Number of Productive Tillers per Plant (NPTP), Days to First flowering (first), Days to fifty percent flowering, Flag Leaf Length (FLL), Flag Leaf Width (FLW), Panicle Length (PL), Grain Length (GL), Grain Width (GW), and Yield Per Plant (YPP).

RESULTS AND DISCUSSION

Breeding strategy for stacking of Pi9 and Pi54 genes into CO 51: Advanced breeding lines developed in the CO 51 genetic background by combining specific *QTLs*/genes through crosses with various donor parents were utilised. Advanced breeding lines (Inter-Mated F_3) IMF_3, harbouring *Pi9* and *Pi54*have been developed (**Fig.1**) Thirty-six superior progenies in IMF_3, has been forwarded into $IMF_{3:4}$ (**Fig. 2**). These lines of IMF_4were then evaluated for their resistance against blast disease.

To confirm the presence of the resistant R gene/allele in the 36 progenies of Near Isogenic Lines (NILs) of CO 51, molecular analysis was conducted. Two markers, NBS4 and Pi54MAS, which are linked to the *Pi9* and *Pi54* genes/alleles respectively, were used. PCR reactions were performed on all the progenies, as well as the recurrent and donor parents. The results of the PCR analysis revealed that all the progenies exhibited a homozygous positive genotype for both the *Pi9* and *Pi54* genes/alleles, confirming their presence in the NILs (**Table 3**).

Evaluation of CO51 Pyramided line against blast pathogen M. oryzea along with parent and check: In the evaluation of the 36 IMF_4 progenies, along with the recurrent parent CO 51, donor parent 562-4, and susceptible check CO 39, the blast disease screening was conducted during *kharif* 2021 and *kharif* 2022. The severity of blast lesions on rice leaves was recorded at 30 days and 45 days after sowing in the UBN. The recurrent parent CO 51 exhibited a mean score of 3.1 in *kharif* 2021 and 3.15 in *karif* 2022, indicating a moderately resistant (MR) reaction to blast disease (**Table 4**). On the other hand, the donor parent

Table 1. Forward and Reverse sequence of functional markers for *Pi9* and *Pi54*

Marker	Forward sequence	Reverse sequence	Product size
NBS4	5'ACTTTGTTGTGCTTGATAAC3'	5'ATGGTGAACGGTATCTGTAT3'	1kb
Pi54MAS	5'CAATCTCCAAAGTTTTCAGG3'	5'GCTTCAATCACTGCTAGACC3'	216, 360

Table 2. Rice blast disease scoring, SES IRRI, 2002

Scale	Affected leaf area	Immune reaction
0	No incidence	Resistant (R)
1	Less than 1% (apical lesions)	
3	1-5% (apical lesions)	
5	6-25% (apical and some marginal lesions)	Moderately Resistant (MR)
7	26-50% (apical and marginal lesions)	Susceptible (R)
9	51-100% (apical and marginal lesions)	

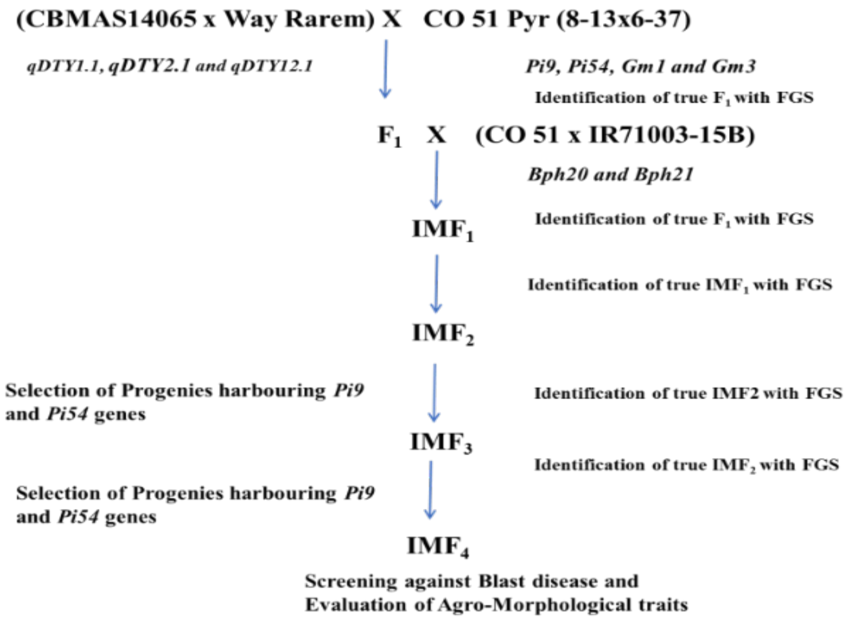

Fig. 1. Breeding strategy followed to develop IMF$_4$ harbouring *Pi9* and *Pi54*

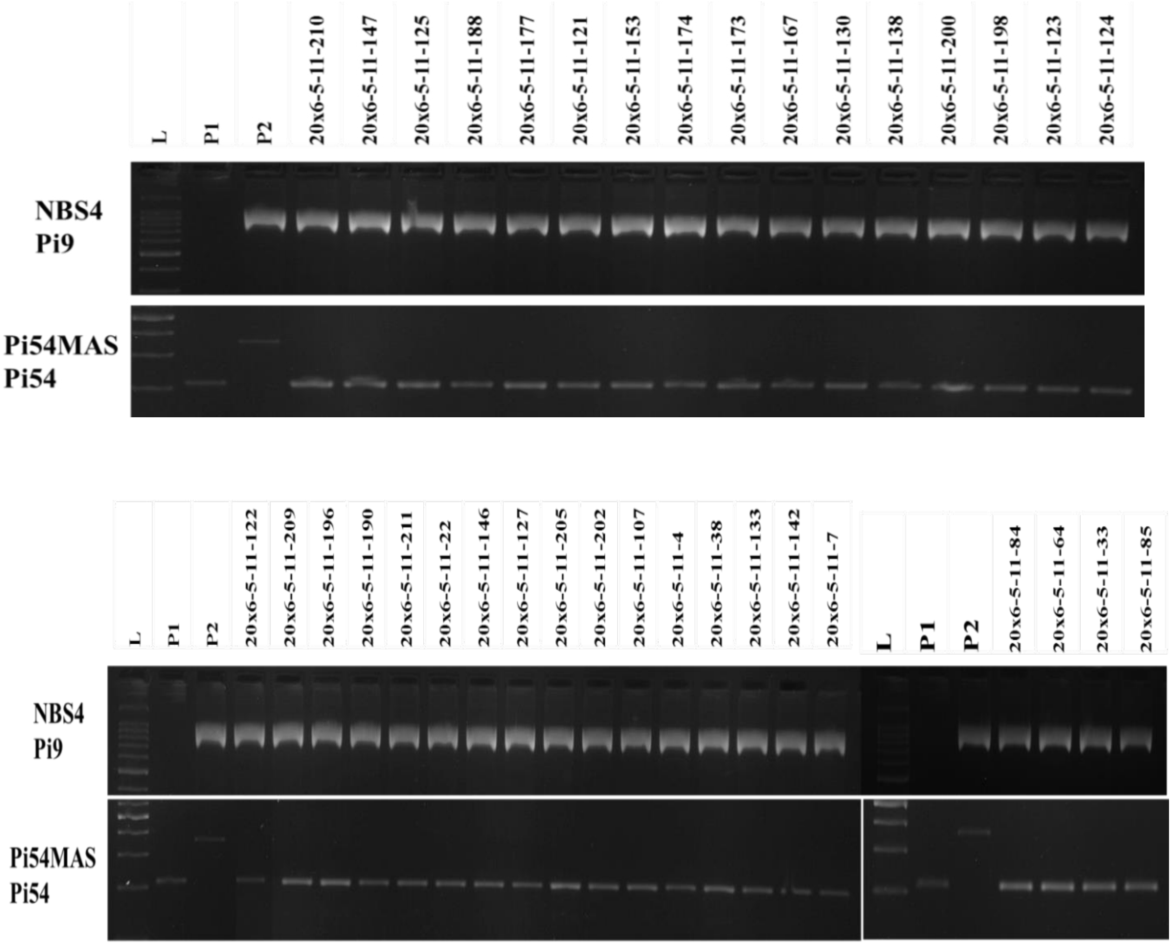

Fig. 2. Genotyping of 36 progenies of IMFF$_{3:4}$ harbouring *Pi9* and *Pi54*

Table 3. Selected progenies of harbouring Pi9 and Pi54 genes in CO 51 genetic background

S. No.	NILs	*Pi9*	*Pi54*	S. No.	NILs	*Pi9*	*Pi54*
1	20x6-5-11-210	+	+	19	20x6-5-11-196	+	+
2	20x6-5-11-147	+	+	20	20x6-5-11-190	+	+
3	20x6-5-11-125	+	+	21	20x6-5-11-211	+	+
4	20x6-5-11-188	+	+	22	20x6-5-11-22	+	+
5	20x6-5-11-177	+	+	23	20x6-5-11-146	+	+
6	20x6-5-11-121	+	+	24	20x6-5-11-127	+	+
7	20x6-5-11-153	+	+	25	20x6-5-11-205	+	+
8	20x6-5-11-174	+	+	26	20x6-5-11-202	+	+
9	20x6-5-11-173	+	+	27	20x6-5-11-107	+	+
10	20x6-5-11-167	+	+	28	20x6-5-11-4	+	+
11	20x6-5-11-130	+	+	29	20x6-5-11-38	+	+
12	20x6-5-11-138	+	+	30	20x6-5-11-133	+	+
13	20x6-5-11-200	+	+	31	20x6-5-11-142	+	+
14	20x6-5-11-198	+	+	32	20x6-5-11-7	+	+
15	20x6-5-11-123	+	+	33	20x6-5-11-84	+	+
16	20x6-5-11-124	+	+	34	20x6-5-11-64	+	+
17	20x6-5-11-122	+	+	35	20x6-5-11-33	+	+
18	20x6-5-11-209	+	+	36	20x6-5-11-85	+	+

+ indicate Homozygous and NILs-Near Isogenic Lines

562-4 showed a mean score of 0.5 in *kharif* 2021 and *kharif* 2022, indicating a resistant (R) reaction to the blast pathogen. The susceptible check, CO 39, exhibited a susceptible reaction with a score of 0.5 in both *kharif* 2021 and *kharif* 2022 (**Table 4 and Fig. 3**).

In *kharif* 2021, among the 36 IMF$_4$ progenies, 10 progenies showed a disease reaction score of 0 (indicating resistance) against blast disease, indicating the presence of *Pi9+Pi54* genes. Eight progenies of IMF$_4$ showed a disease reaction score ranging from 0.1 to 1.0, indicating a R reaction. Eighteen progenies of IMF$_4$ recorded a desirable disease reaction scores (ranging from 1.1 to 2.0), indicating R reaction against the blast pathogen. In *kharif* 2022, out of the 36 IMF$_4$ progenies, 3 progenies revealed SES score, 0, indicating a strong resistance to blast disease, suggesting the presence of *Pi9+Pi54* genes. Furthermore, 15 progenies showed a disease reaction score ranging from 0.1 to 1.0, indicating a R reaction. Eighteen progenies recorded a disease reaction score ranging from 1.1 to 2, indicating a R reaction against the blast pathogen.

Among the identified genotypes, progeny #20x6-5-11-7 exhibited a disease reaction score of 0 in both *kharif* 2021 and *kharif* 2022, indicating strong resistance to the blast pathogen *M. oryzea*. Progeny #20x6-5-11-84 and progeny #20x6-5-11-133 exhibited a disease reaction score of 1 in both *kharif* 2021 and *kharif* 2022, indicating strong resistance to the blast pathogen. Progeny #20x6-5-11-209 recorded a disease reaction score of 2 in *kharif* 2021 and *kharif* 2022, indicating strong resistance to the blast pathogen. Overall, all the NILs harboring *Pi9* and *Pi54* demonstrated strong resistance (blast score 0<2) and displayed the genetic potential of the stacked genes against the blast pathogen in both *kharif* 2021 and *kharif* 2022 of screening in the hotspot region.

CO 51 is a high-yielding rice cultivar with fine grains; however, it exhibits only moderate resistance to blast disease. This breeding program aims to develop CO 51 genotypes with strong resistance against blast pathogen by stacking the *Pi9* and *Pi54* genes using Marker-Assisted Selection (MAS). The resulting NILs of CO 51 exhibited significantly enhanced resistance (blast score 0<2) to blast pathogen compared to the recurrent parent CO 51 (blast score >3). It should be noted that the presence of the *Pi54* allele in CO 51 alone does not provide broad-spectrum resistance against blast pathogen. However, by stacking major R genes with *Pi9* and *Pi54*, the NILs demonstrate resistance against a wide range of blast pathogen strains with durable disease resistance. Backcross breeding is commonly employed to improve specific traits that are lacking in elite crops (Stoskopf *et al.,* 1993). MAS has proven to be a well-developed strategy for transferring or stacking multiple R genes and *Pi* genes into elite cultivars of both *indica* and *japonica* rice, offering a precise, simple, and highly efficient approach to obtain resistance against a broad spectrum of blast pathogens (Fukuoka *et al.*, 2015;Ning *et al.,* 2020).

Table 4. Disease reaction of NILs harboring *Pi9+Pi54* along with parents and check

	kharif 2021			*kharif* 2022		
NILs	**Score I**	**Score II**	**Mean**	**Score I**	**Score II**	**Mean**
CO 51	2.8	3.4	3.1	2.9	3.4	3.15
562-4	0	1	0.5	0	1	0.5
CO 39	7	7	7	7	9	8.0
20x6-5-11-4	1	1	1	1	1	0.8
20x6-5-11-7	0	0	0	0	0	0.0
20x6-5-11-22	0	1	0.5	1	2	1.3
20x6-5-11-33	0	3	1.5	2	1	1.6
20x6-5-11-38	0	3	1.5	1	1	1.0
20x6-5-11-64	1	3	2	2	2	1.9
20x6-5-11-84	1	3	2	2	2	2.0
20x6-5-11-85	0	3	1.5	1	1	1.1
20x6-5-11-107	0	0	0	0	0	0.0
20x6-5-11-121	0	1	0.5	1	2	1.3
20x6-5-11-122	0	3	1.5	2	2	1.9
20x6-5-11-123	0	3	1.5	0	0	0.2
20x6-5-11-124	1	3	2	1	2	1.6
20x6-5-11-125	2	2	2	2	1	1.4
20x6-5-11-127	0	0	0	0	0	0
20x6-5-11-130	1	1	1	1	1	1.2
20x6-5-11-133	1	1	1	1	1	1.0
20x6-5-11-138	0	0	0	0	0	0.2
20x6-5-11-142	0	0	0	0	0	0.2
20x6-5-11-146	0	3	1.5	1	1	1.2
20x6-5-11-147	0	0	0	0	0	0.2
20x6-5-11-153	1	1	1	1	2	1.5
20x6-5-11-167	1	3	2	0	1	0.7
20x6-5-11-173	0	0	0	0	0	0.2
20x6-5-11-174	1	3	2	2	1	1.4
20x6-5-11-177	0	0	0	0	0	0.2
20x6-5-11-188	0	2	1	1	1	0.9
20x6-5-11-190	1	1	1	2	1	1.4
20x6-5-11-196	1	3	2	1	1	0.9
20x6-5-11-198	0	0	0	0	0	0.2
20x6-5-11-200	1	3	2	1	2	1.7
20x6-5-11-202	1	3	2	2	2	1.9
20x6-5-11-205	0	0	0	0	0	0.2
20x6-5-11-209	1	3	2	2	2	2.0
20x6-5-11-210	1	2	1.5	2	1	1.6
20x6-5-11-211	0	3	1.5	1	1	1.0
Mean of NILs	0.6	1.9	1.2	1.2	1.2	1.2
SD	0.974	1.54	1.145	1.186	1.526	1.32
SE	0.158	0.25	0.186	0.192	0.248	0.214

NILs- Near Isogenic Lines; SD- Standard Deviation and SE- Standard Error

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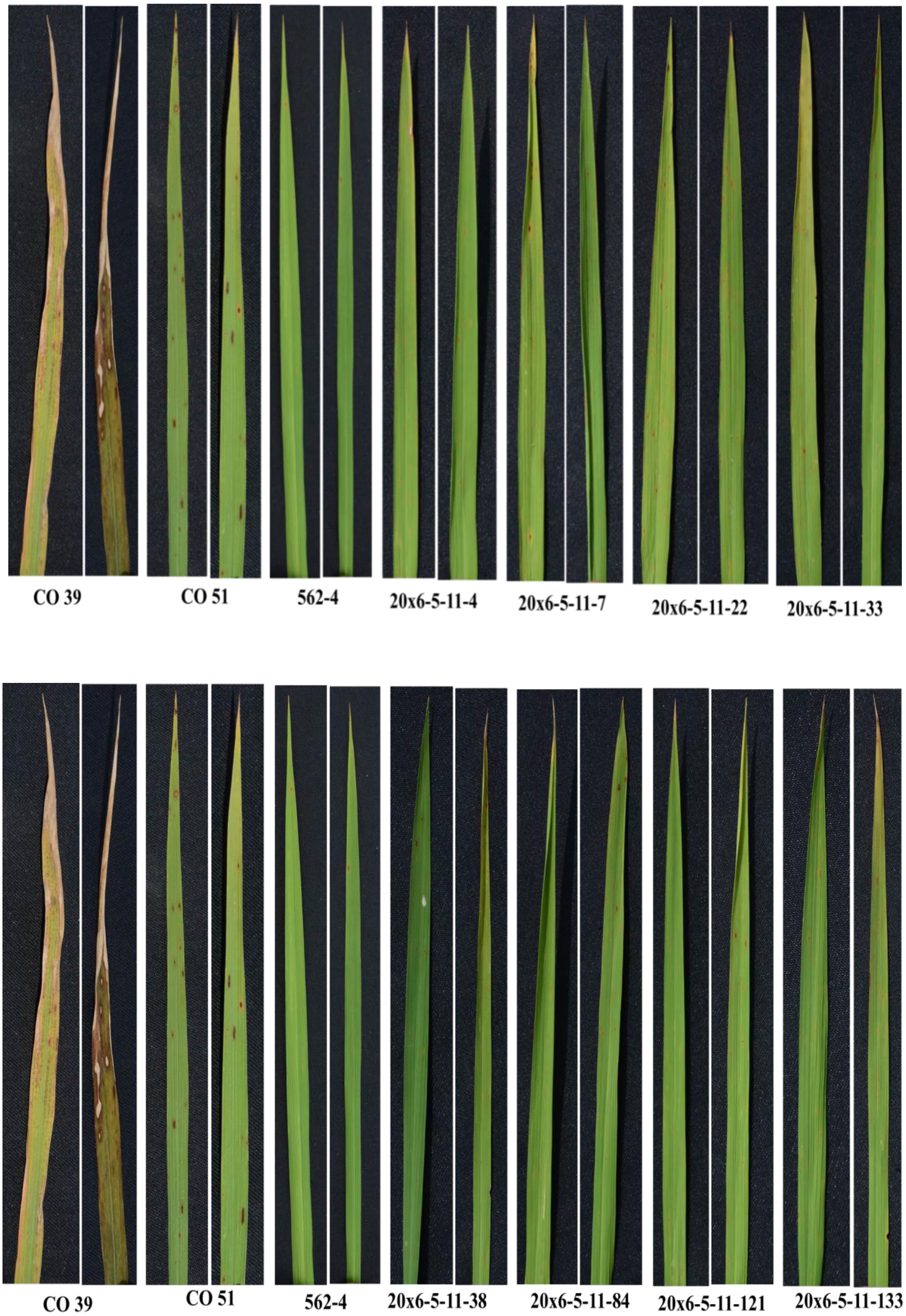

Fig. 3. Phenotypic screening of 36 progenies against blast pathogen

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To facilitate the stacking of major R genes against the blast pathogen, we employed PCR-based functional markers, namely NBS4 andPi54MAS, for foreground selection of the *Pi9* and *Pi54* alleles. These functional markers ensure 100% selection accuracy by minimizing recombination events between the markers and genes. The success of MAS relies on the precise selection of the foreground markers (Hospital *et al.,* 1997). In the NILs harboring the *Pi9* and *Pi54* genes, which confer resistance against a broad range of pathogens, we observed a strong immune reaction against pathogen development. The recurrent parent CO 51, which only harbors *Pi54*, exhibits a moderately resistant disease reaction (disease score >3 in *kharif* 2021 and *kharif* 2022). On the other hand, the NILs of CO 51 progenies, possessing both *Pi54* and *Pi9*, confer a high immune reaction with blast disease scores 0<2 as strong resistant.

Agronomic performance of CO 51 NILs blast R gene pyramided IMF$_4$ population : An advanced breeding line, 36 NILs of IMF$_4$, along with the parents, was evaluated using a Randomized Block Design (RBD). The panicle length of the recurrent parent CO 51 was recorded as 22.82 cm, while the panicle length of the NILs of CO 51 ranged from 28.38 cm to 21.54 cm, with an average of 24.46 cm. Similarly, the grain length of CO 51 was measured as 5.31 mm, and the grain lengths of the NILs of CO 51 varied from 5.25 mm to 5.82 mm, with an average of 5.52 mm. The grain width of CO 51 was recorded as 1.74 mm, while the grain widths of the NILs ranged from 1.80 mm to 1.96 mm, with an average of 1.88 mm. The thousand grain weight in the NILs ranged from 15.50 g to 23.95 g, with an average of 19.88 g, whereas CO 51 had a thousand grain weight of 16.87 g. Agronomic performance of the NILs for various biometrical traits are given in the **table 5**.

Phenotypic traits such as the number of productive tillers per plant, panicle length, thousand grain weight, and grain yield per plant exhibited transgressive segregation in comparison to the CO 51 parent. This transgressive segregation can be attributed to the accumulation of beneficial alleles in the CO 51 genetic background. Previous studies by Basavaraj *et al.* (2021) have reported transgressive segregation for yield and related traits in NILs population derived from crosses between Samba Mahsuri and *O. rufipogon*, highlighting the importance of incorporating resistance/tolerance genes or QTLs for biotic and abiotic stresses through Marker-Assisted Backcross Breeding (MABB) (Basavaraj *et al.,* 2021). The *Pi9* gene sourced from *O. minuta*(tetraploid) has been reported to confer effective resistance to blast pathogen (Rathour *et al.,* 2004; Khanna *et al.,* 2015;Samal *et al.,* 2019). It is worth noting that rice varieties possessing single resistance genes/alleles may quickly encounter pathogen-induced breakdown of resistance (Khush *et al.*, 1989; Mew *et al.,* 1992)

Grain yield per plant, the NILs of CO 51 exhibited a range of 27.32 g to 48.80 g, with an average of 35.69 g, whereas the recurrent parent CO 51 recorded a grain yield per plant of 31.70 g. Average yield of single plant has been increased. Maximum of 35.05 % was recorded in progeny #20x6-5-11-121 followed by the progeny #20x6-5-11-125 as 31.65 %. Among the progenies, progeny #20x6-5-11-121 showed superior performance in terms of number of tillers per plant, number of productive tillers per plant, days to first flowering, days to 50% flowering, panicle length, 1000-grain test weight, and grain yield per plant whereas progeny #20x6-5-11-133 exhibited the least performance in terms of panicle length and grain yield per plant. Similarly, Ellur *et al.* (2016) successfully stacked broad-spectrum resistance genes (*Pi2* and *Pi54*) in Pusa Basmati 1509, resulting in high resistance against various pathogen isolates across India with a 100% incompatible reaction. The *Pi9* and *Pi54* genes trigger an effector-induced immune response against pathogen infection through the nuclear binding site leucine-rich repeats (NBS-LRR) (Qu *et al.,* 2006;Marone *et al.,* 2013;Zhang *et al.,* 2018).These genes have been reported to exhibit a synergistic effect in enhancing resistance against a wider range of pathogen isolates. In our study, the combination of *Pi9* and *Pi54* genes confers highly resistant reactions. When introgressing desirable alleles from wild *Oryza* into cultivated varieties, the expression of the desired allele is often masked by deleterious alleles (Xiao *et al.,* 1998). Therefore, the identification of superior performance in both phenotypic traits and blast resistance across multiple seasons in the NIL progenies compared to the recurrent parent is crucial for the success of MAS.

Identification high yielding IMF$_4$ genotype over recurrent parent CO 51: Several IMF$_4$ progenies, including 20x6-5-11-210, 20x6-5-11-147, 20x6-5-11-125, 20x6-5-11-121, 20x6-5-11-200, 20x6-5-11-123, 20x6-5-11-196, 20x6-5-11-22, 20x6-5-11-107, 20x6-5-11-7, and 20x6-5-11-64, exhibited significantly increased yield compared to the recurrent parent CO 51 (**Table 5 and Fig.4).** These IMF$_4$ progenies showed promising performance in terms of yield, indicating their potential for developing high-yielding varieties.

Developing broad-spectrum and durable resistance to biotic stresses such as the devastating blast disease in Indian agro-climatic zones is a challenging task for plant breeders and geneticists. However, through the stacking of alleles/genes associated with blast resistance, it is possible to confer broad-spectrum resistance to the blast pathogen in early generations of breeding populations using Marker-Assisted Backcrossing (MABB) and gene-specific functional markers. In this study, 36 advanced breeding lines with strong and durable resistance genes (*Pi9+Pi54*) to blast were successfully developed by introgression of two resistance genes that confer resistance to a wide range of pathogens in India. The

Table 5. Agronomic performance of selected NILs harbouring *Pi9* and *Pi54* along with recurrent parent and donor parent

IMF_4	PH (cm)	NTP	NPTP	FLL (cm)	FLW (cm)	Days to first flowering	Days to 50% flowering	PL (cm)	GL (mm)	GW (mm)	1000g weight	GYPP (g)
CO 51	93.97	25.91	24.36	27.75	1.17	92	95	22.82	5.31	1.74	16.87	31.70
562-4	93.21	24.85	23.22	28.62	1.17	97	100	23.54	5.20	1.83	20.74	37.47
20x6-5-11-210	90.40	22.50	20.50	29.90	0.98	90	93	26.34	5.48	1.95	20.15	38.56
20x6-5-11-147	90.10	21.60	17.50	30.64	1.03	93	96	26.35	5.29	1.90	18.44	42.21
20x6-5-11-125	87.30	26.20	23.80	25.64	1.09	95	98	24.82	5.51	1.83	23.28	46.38
20x6-5-11-188	87.50	23.20	22.00	26.84	1.01	90	93	23.65	5.63	1.88	20.27	37.14
20x6-5-11-177	84.60	23.60	2200	25.03	1.08	91	94	23.53	5.60	1.88	20.74	28.36
20x6-5-11-121	88.10	31.00	30.10	31.85	1.15	88	91	28.38	5.62	1.88	23.95	48.80
20x6-5-11-153	95.10	29.40	27.30	29.31	1.05	90	93	26.38	5.53	1.80	19.51	25.62
20x6-5-11-174	89.10	25.70	22.70	27.59	1.09	91	94	24.61	5.67	1.81	19.37	36.60
20x6-5-11-173	92.70	25.80	24.50	29.09	1.04	92	95	25.25	5.50	1.89	20.58	36.49
20x6-5-11-167	85.70	28.50	25.00	26.79	1.10	91	94	24.52	5.53	1.89	17.61	31.13
20x6-5-11-130	78.10	24.30	22.60	28.11	1.07	92	95	23.65	5.69	1.83	17.67	29.54
20x6-5-11-138	83.90	25.20	23.90	26.63	1.11	92	95	25.06	5.54	1.94	20.74	32.32
20x6-5-11-200	81.70	24.30	22.40	26.09	1.19	93	96	24.08	5.48	1.92	22.01	38.94
20x6-5-11-198	91.10	22.90	21.10	26.73	1.08	93	96	23.35	5.43	1.89	20.74	36.86
20x6-5-11-123	82.00	25.00	22.80	26.82	1.18	92	95	24.40	5.34	1.94	19.88	41.01
20x6-5-11-124	82.90	25.10	22.60	28.41	1.09	93	97	23.48	5.54	1.89	19.45	36.52
20x6-5-11-122	83.30	24.80	24.80	27.50	1.11	93	96	23.15	5.52	1.94	20.66	34.39
20x6-5-11-209	86.30	27.90	27.90	29.04	1.12	91	94	25.95	5.76	1.87	19.85	36.00
20x6-5-11-196	92.40	22.30	22.30	29.72	1.12	94	96	24.90	5.51	1.89	20.61	39.84
20x6-5-11-190	89.60	25.20	22.90	29.57	1.18	92	95	23.79	5.60	1.85	18.68	40.84
20x6-5-11-211	83.30	24.30	25.30	27.90	1.14	93	96	24.52	5.44	1.85	15.50	30.22
20x6-5-11-22	87.40	26.00	23.50	27.93	1.08	93	96	24.04	5.68	1.94	20.14	41.63
20x6-5-11-146	89.00	23.00	21.10	29.28	1.07	92	95	25.75	5.50	1.81	21.61	31.12
20x6-5-11-127	83.70	21.20	21.20	29.02	1.09	91	94	26.14	5.68	1.92	18.66	35.71
20x6-5-11-205	90.20	23.80	22.00	28.17	1.00	88	91	25.21	5.45	1.89	22.76	36.45
20x6-5-11-202	90.20	23.80	22.00	28.17	1.00	90	93	25.21	5.45	1.89	22.76	36.45
20x6-5-11-107	87.60	22.30	21.30	28.26	1.17	91	94	22.61	5.56	1.96	17.22	38.53
20x6-5-11-4	86.80	21.90	21.50	25.50	1.08	92	95	24.06	5.41	1.87	19.61	32.75
20x6-5-11-38	83.80	22.60	22.30	29.39	1.15	91	94	25.48	5.69	1.92	18.42	34.26
20x6-5-11-133	88.20	24.20	23.30	28.85	1.17	92	95	21.54	5.82	1.85	16.19	27.32
20x6-5-11-142	89.80	20.90	20.90	29.15	1.14	93	96	24.14	5.37	1.87	19.31	31.79
20x6-5-11-7	86.20	23.40	23.00	26.05	1.16	93	96	23.65	5.62	1.90	22.08	39.98
20x6-5-11-84	87.40	21.30	20.80	27.71	1.06	92	95	23.83	5.25	1.94	20.95	32.05
20x6-5-11-64	88.80	22.70	21.30	29.42	1.11	93	96	23.40	5.64	1.92	22.01	29.83
20x6-5-11-33	91.90	27.00	25.00	28.68	1.06	93	96	23.21	5.58	1.87	17.12	39.81
20x6-5-11-85	89.10	22.80	22.80	29.00	1.11	92	95	24.78	5.41	1.89	19.37	31.50
Mean	87.70	24.38	22.99	28.16	1.10	91.95	94.95	24.46	5.52	1.88	19.88	35.69
SD	3.730	2.259	2.209	1.460	0.055	1.65	1.65	1.286	0.138	0.047	1.938	5.034
SE	0.605	0.367	0.358	0.237	0.009	0.27	0.27	0.209	0.022	0.008	0.314	0.817

PH-Plant Height; NTP-Number of Tillers per plant; NPTP- Number of Productive Tillers per Plant; FLL-Flag Leave Length; FLW-Flag Leave Width; DFF-Days to first Flowering; D50%F- Days to 50% Flowering; PL-Panicle Length; GL-Grain Length; GW- Grain Width; GYPP- Grain Yield per Plant; cm-centi meter and mm-milli meter

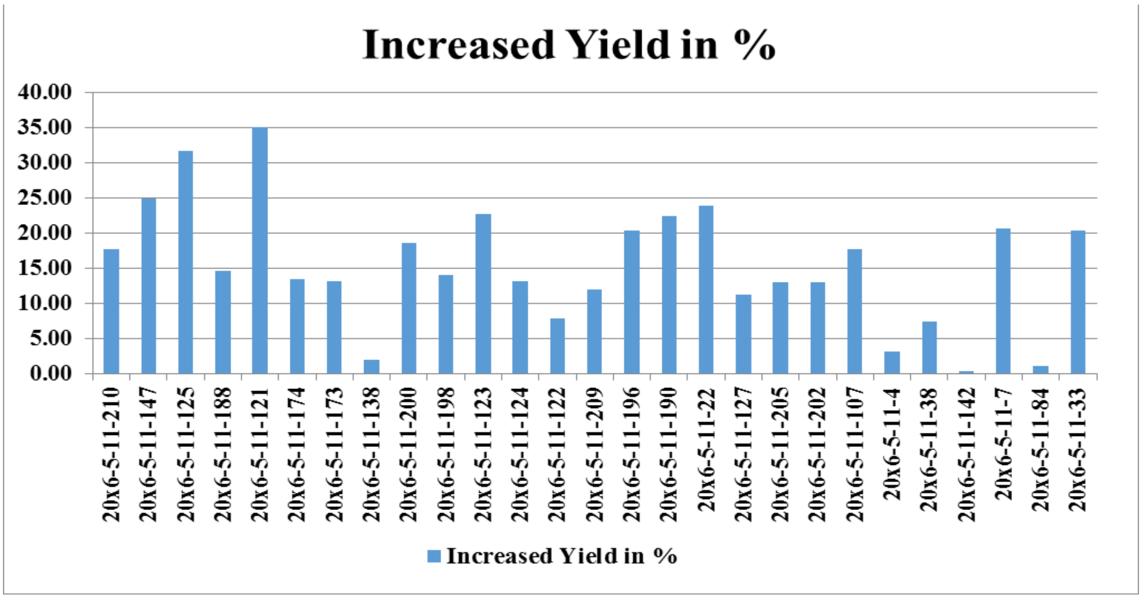

Fig. 4. Representation of yield increase in percentage of NILs as compared with CO 51

resistance of these lines was confirmed through screening in natural hotspots for blast pathogens during *kharif* 2021 and *kharif* 2022.

A NILs of the IMF$_4$ progenies exhibited transgressive segregation, surpassing the performance of the recurrent parent in phenotypic traits such as number of tillers per plant, number of productive tillers per plant, flag leaf length, panicle length, thousand grain weight, and grain yield per plant. This transgressive segregation is a result of the accumulation of beneficial alleles from the donor parent into the recurrent parent's genetic background. Average yield of single plant has been increased in NILs of CO 51. Among the NILs population, progeny #20x6-5-11-121 was selected as the most promising line, displaying high resistance to blast disease and transgressive segregants for yield and its associated traits. This progeny can serve as a valuable genetic stock or genetic resource for resistant breeding programs, particularly in enhancing yield and its related traits.

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