



RESEARCH ARTICLE

Integrating genotypic and phenotypic approaches for blast resistance selection in rice (*Oryza sativa* L.) segregating populations through marker-assisted selection

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Abstract

The present study aimed to develop blast-resistant homozygous recombinant lines through marker-assisted selection (MAS) in segregating populations derived from the cross between Pusa Samba 1850 and CO55. Unlike backcross-based approaches, selection was focused on F₂ derived recombinants and their advancement to homozygosity. Foreground selection using gene-specific markers for *Pi54*, *Pi1* and *Pita* enabled the precise identification of resistant genotypes. In the F₂ population, a limited number of plants were identified as homozygous for single, double and triple gene combinations. Selected plants were advanced to F₃ generation, where fixation of resistance genes was confirmed. Phenotypic screening revealed variation in blast resistance despite genetic fixation, indicating genotype-environment interaction. Three recombinant lines showed strong resistance against blast disease and these lines were further advanced to the next generation. Genetic variability analysis showed high heritability (80–100 %) and significant genetic advance for key traits. Correlation and path analysis identified number of productive tillers (NPT) and thousand seed weight (TSW) as major contributors to yield. Superior recombinant lines such as 7–36, 11–28 and 107–25 exhibited strong blast resistance and desirable agronomic performance. The study demonstrates that MAS combined with pedigree selection is an effective strategy for developing improved homozygous recombinant lines with durable blast resistance.

Keywords: blast resistance; gene pyramiding; genetic variability; marker-assisted selection; recombinant lines; rice

Introduction

Rice (*Oryza sativa* L.) is a primary staple crop sustaining more than half of the global population and it plays a central role in ensuring food security, particularly across Asia (1, 2). However, rice productivity is severely constrained by biotic stresses, among which blast disease, caused by the filamentous ascomycete *Magnaporthe oryzae*, remains the most destructive. The pathogen infects all aerial parts of the plant and can result in yield losses ranging from 50–90 % under conducive environmental conditions, posing a major threat to stable rice production (3, 4).

Genetic resistance through the deployment of major resistance (R) genes represents the most effective and environmentally sustainable strategy for managing blast disease (5). To date, more than 100 R genes and over 350 quantitative trait loci (QTLs) associated with blast resistance have been identified, with a subset of genes such as *Pi54*, *Pi1* and *Pita* being widely utilised in rice

improvement programmes (6). Among these, *Pi54* confers broad-spectrum resistance across diverse pathogen populations, *Pita* provides race-specific resistance *via* gene-for-gene interactions and *Pi1* contributes to resistance against multiple blast races. The complementary nature of these genes makes them ideal candidates for pyramiding strategies aimed at achieving durable resistance.

Despite the availability of numerous resistance genes, the rapid evolution of *M. oryzae* populations frequently leads to the breakdown of resistance in varieties carrying single R genes. This highlights the inherent limitation of single-gene deployment and underscores the need for more robust breeding strategies. In this context, gene pyramiding, involving the accumulation of multiple resistance genes within a single genotype, has emerged as a key approach for enhancing resistance durability and broad-spectrum effectiveness. The simultaneous presence of multiple R genes reduces the probability of pathogen adaptation and ensures more stable performance across environments (7).

The integration of molecular marker technologies with conventional breeding has significantly accelerated the efficiency of gene pyramiding. Marker-assisted selection (MAS) enables precise tracking of target genes and facilitates the identification of desirable recombinants in early segregating generations. In particular, MAS applied in F_2 and subsequent generations enhances selection efficiency by allowing simultaneous screening for multiple resistance loci while promoting recombination and fixation of favourable allelic combinations. When complemented with rigorous phenotypic screening, this approach ensures that selected genotypes not only possess the desired genetic constitution but also express stable resistance under field conditions.

The elite rice variety CO55, developed by Tamil Nadu Agricultural University, is characterised by short duration, high yield potential and desirable grain quality traits. However, its susceptibility to blast disease restricts its adaptability and stable performance in disease-prone environments. Introgression and pyramiding of key resistance genes such as *Pi54*, *Pi1* and *Pita* into this genetic background offers a promising strategy to enhance its resilience without compromising agronomic performance. The present study utilises the cross between Pusa Samba 1850, a genotype possessing desirable grain quality traits and blast resistance gene (*Pi54*, *Pi1*, *Pita*) and CO55 to generate segregating populations for recombination breeding. By integrating MAS with phenotypic evaluation in F_2 and subsequent generations, the study aims to identify superior recombinants and develop homozygous lines carrying pyramided blast resistance genes along with improved yield attributes. This approach emphasises the development of novel recombinant genotypes through selection and fixation, providing valuable genetic resources for sustainable rice improvement.

Materials and Methods

A high-yielding but blast-susceptible rice variety, CO55, was used as the recipient parent in this study, while Pusa Samba 1850 served as the donor parent for blast resistance genes (*Pi54*, *Pi1* and *Pita*). To achieve synchronised flowering for hybridisation, staggered sowing was carried out at the Paddy Breeding Station, Tamil Nadu Agricultural University (TNAU), Coimbatore. Approximately 25 days old seedlings were transplanted into the main field following standard rice cultivation practices recommended by International Rice Research Institute (IRRI). The experiment was conducted across multiple cropping seasons (kharif, rabi and summer seasons) from December 2023 to January 2026 and all agronomic practices, including nutrient management, irrigation, weed control and plant protection measures, were uniformly applied as per IRRI (1996) guidelines.

A cross was made using CO55 as the female parent and Pusa Samba 1850 as the male parent to generate F_1 hybrid progenies. The

F_1 plants were subjected to genotyping using MAS to identify true heterozygous hybrids carrying target resistance genes. The confirmed F_1 hybrids were selfed to generate an F_2 segregating population, which served as the primary population for selection. The segregation patterns observed in the F_2 population occasionally deviated from expected Mendelian ratios, potentially due to gametic or zygotic selection, differential survival of genotypes, linkage drag surrounding resistance gene loci, or sampling variation arising from finite population size. Such segregation distortion has been widely documented in rice breeding populations and may affect the transmission of target alleles during MAS. To assess the extent of deviation, observed and expected segregation ratios were compared using chi-square (χ^2) goodness-of-fit analysis and the significance of deviations was determined statistically. Foreground selection was carried out in the F_2 generation to identify desirable recombinants possessing single and multiple gene combinations. Selected plants were advanced across generations to ensure fixation of pyramided resistance genes and the development of genetically stable homozygous recombinant lines. An overview of the breeding scheme is presented in Fig. 1.

DNA extraction

Genomic DNA was extracted from young leaf tissues of individual plants using the modified Cetyltrimethylammonium bromide (CTAB) method (8). The DNA quality and concentration were assessed using agarose gel electrophoresis (AGE) and spectrophotometric analysis, followed by dilution to a working concentration of approximately 30–50 ng μL^{-1} for PCR amplification. A DNA sample with high purity was stored at -20°C .

Molecular analysis

In foreground selection, gene-specific markers were used to identify breeding lines carrying the *Pi54*, *Pi1* and *Pita* resistance genes (Table 1). The *Pi54*-MAS, RM 224 and *Pita* markers were successfully used to identify positive plants for blast resistance. Polymerase chain reaction amplification was carried out in a 10 μL reaction volume containing 1 $\times$ PCR buffer, 1 μL DNA, 1 μL (0.5 μL forward + 0.5 μL reverse primer), 4 μL master mix and 4 μL sterile water.

The PCR reactions were performed in a thermal cycler with an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing temperature 55°C for 30 sec (*Pi54* gene), 59°C for 40 sec (*Pita* gene), 60°C for 30 sec (*Pi1* gene) and extension at 72°C for 1 min. A final extension was performed at 72°C for 7 min.

The amplified products were resolved on 2.5 % AGE, stained with ethidium bromide (EtBr) and visualised under ultraviolet (UV) illumination using a gel documentation system. The allele sizes were scored based on band presence or absence and plants were classified according to their genotypic profiles for blast resistance genes.

Table 1. Detailed information on simple sequence repeats (SSR) markers tightly linked to or gene specific primers associated with blast resistance genes

Gene	Gene-specific marker/ linked marker	Chromosome number	Marker sequence (5'-3')	Resistant band size (bp)	Annealing temperature ($^\circ\text{C}$)	References
<i>Pi54</i>	Pi54MAS	11	F- CAATCTCCAAAGTTTTCAGG R- GCTTCAATCACTGCTAGACC	216	55	(9)
<i>Pi1</i>	RM224	11	F- ATCGATCGATCTTCACGAGG R- TGCTATAAAAGGCATTCCGGG	140	60	(10)
<i>Pita</i>	Pita	12	F- AGTCGTGCGATGCGAGGACAGAAAC R- GCATTCTCCAACCTTTTGATGCAT	861	59	(11)

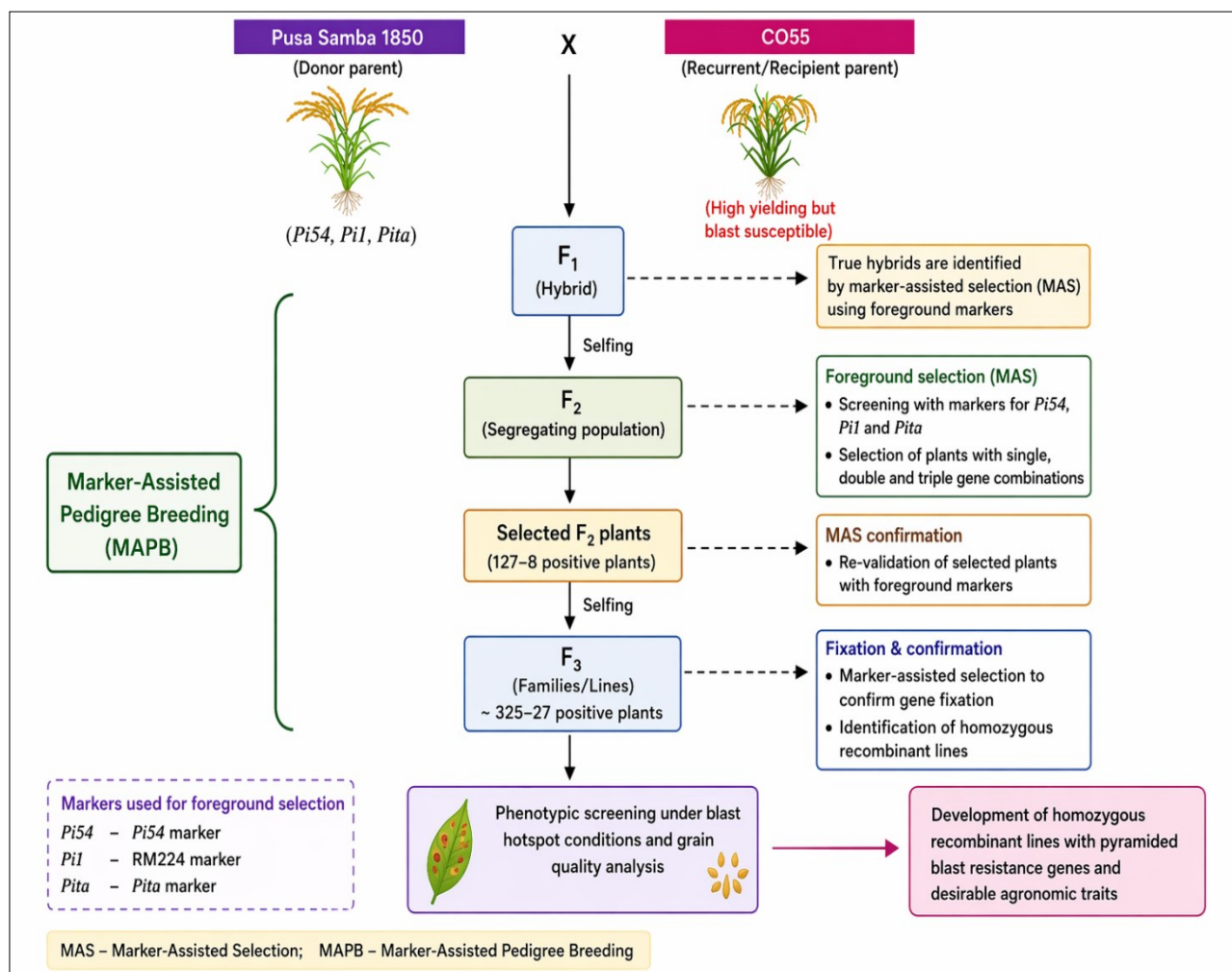


Fig. 1. Schematic representation of the marker-assisted pedigree breeding (MAPB) integrated with marker-assisted selection (MAS) for developing blast-resistant homozygous recombinant rice lines. F_1 hybrids derived from Pusa Samba 1850 $\times$ CO55 were confirmed using foreground markers and selfed to generate the F_2 segregating population. Marker-assisted selection was employed to identify desirable recombinants, which were advanced to the F_3 generation for gene fixation and development of homozygous recombinant lines. Phenotypic screening was conducted under blast hotspot conditions along with evaluation of grain quality and agronomic traits.

Segregation data were analysed using chi-square (χ^2) goodness-of-fit tests to evaluate conformity with expected Mendelian segregation ratios. The chi-square statistic was calculated using the formula $\chi^2 = \sum [(O - E)^2 / E]$, where O represents the observed frequency and E represents the expected frequency. Statistical significance was assessed at the 5 % probability level ($p < 0.05$). All statistical analyses were performed using R software version 4.5.2. Agronomic traits of the selected lines were evaluated using a randomised complete block design (RCBD).

Results

Foreground selection

The total number of five F_1 plants carrying a heterozygous combination of alleles at the *Pi54*, *Pi1* and *Pita* loci were identified using simple sequence repeats (SSR) molecular markers (*Pi54*-MAS, RM 224 and *Pita*) (Fig. 2). The cross of CO55 with Pusa Samba 1850 resulted in F_2 segregating population. A total of 127 F_2 plants were subjected to genotyping and identified 8 plants that were homozygous for all three genes (*Pi54*, *Pi1* and *Pita*). Although the population size was moderate, it proved adequate for recovering the desired triple homozygous recombinants. Under the assumption of independent segregation, the expected frequency

of plants homozygous for all three target loci in the F_2 generation is $1/64$. Therefore, approximately two triple homozygous individuals would be expected among 127 plants. The recovery of eight such plants indicates that the population size was sufficient for effective identification and selection of the target gene combinations through MAS. Nevertheless, larger populations may further enhance the probability of recovering desirable recombinants and capturing greater genetic variation. Ten plants possessed the homozygous *Pi54* + *Pi1* combination, while nine plants carried the *Pi1* + *Pita* combination. Single-gene homozygous included 11 plants for *Pi54*, 7 for *Pi1* and 5 for *Pita* (Fig. 3). Hence, it was concluded that 8 selected plants from F_2 were advanced to the F_3 generation, whereas 77 plants were completely susceptible to all blast-resistance genes. Screening of 325 F_3 plants, identified 27 plants were found to be completely homozygous for all three blast resistance genes. Additionally, in two gene combinations, 19 plants with *Pi54*+*Pi1*, 10 with *Pi1*+*Pita* and 6 with *Pi54* +*Pita* are homozygous.

Segregation analysis of marker genotypes in F_2 population

Segregation of marker genotypes in the F_2 populations derived from different cross combinations was analysed using the chi-square (χ^2) test to evaluate conformity with the expected Mendelian segregation ratio of 1:2:1. The χ^2 test results for all

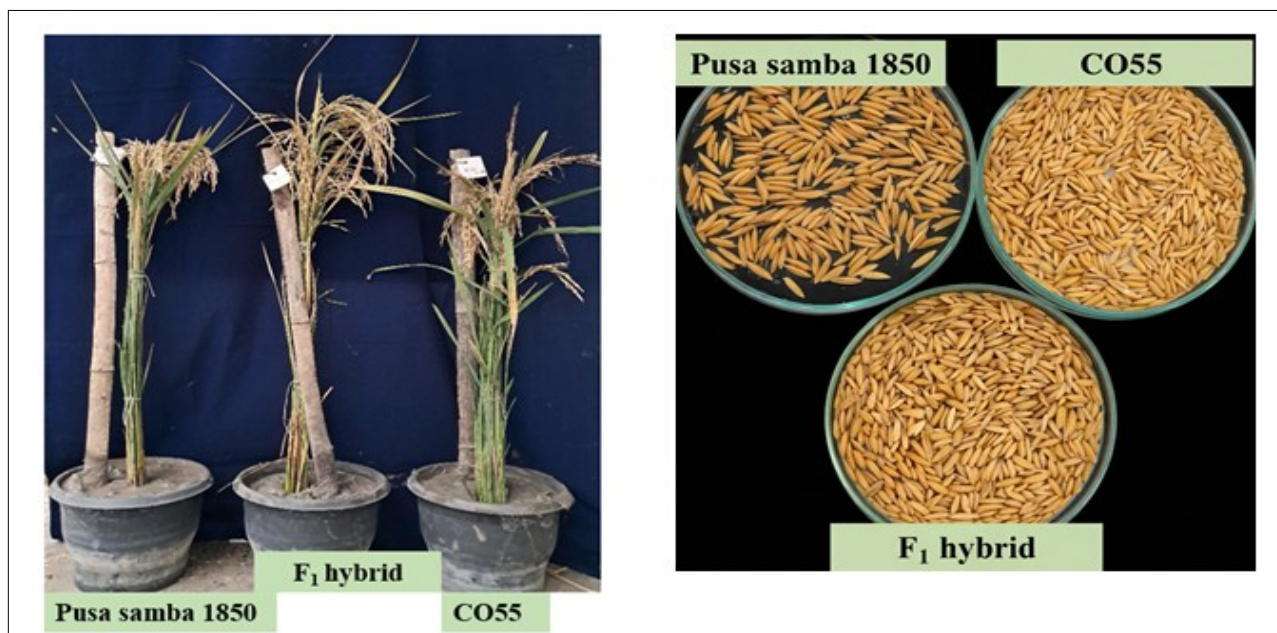


Fig. 2. Comparative evaluation of morphological and grain traits among parental lines and F_1 hybrid. The left panel illustrates the plant phenotype of Pusa Samba 1850, CO55 and the derived F_1 hybrid under controlled growth conditions. The right panel represents the grain morphology of Pusa Samba 1850, CO55 and the corresponding F_1 hybrid, highlighting variations in grain size, shape and density, indicative of phenotypic expression and heterotic effects.

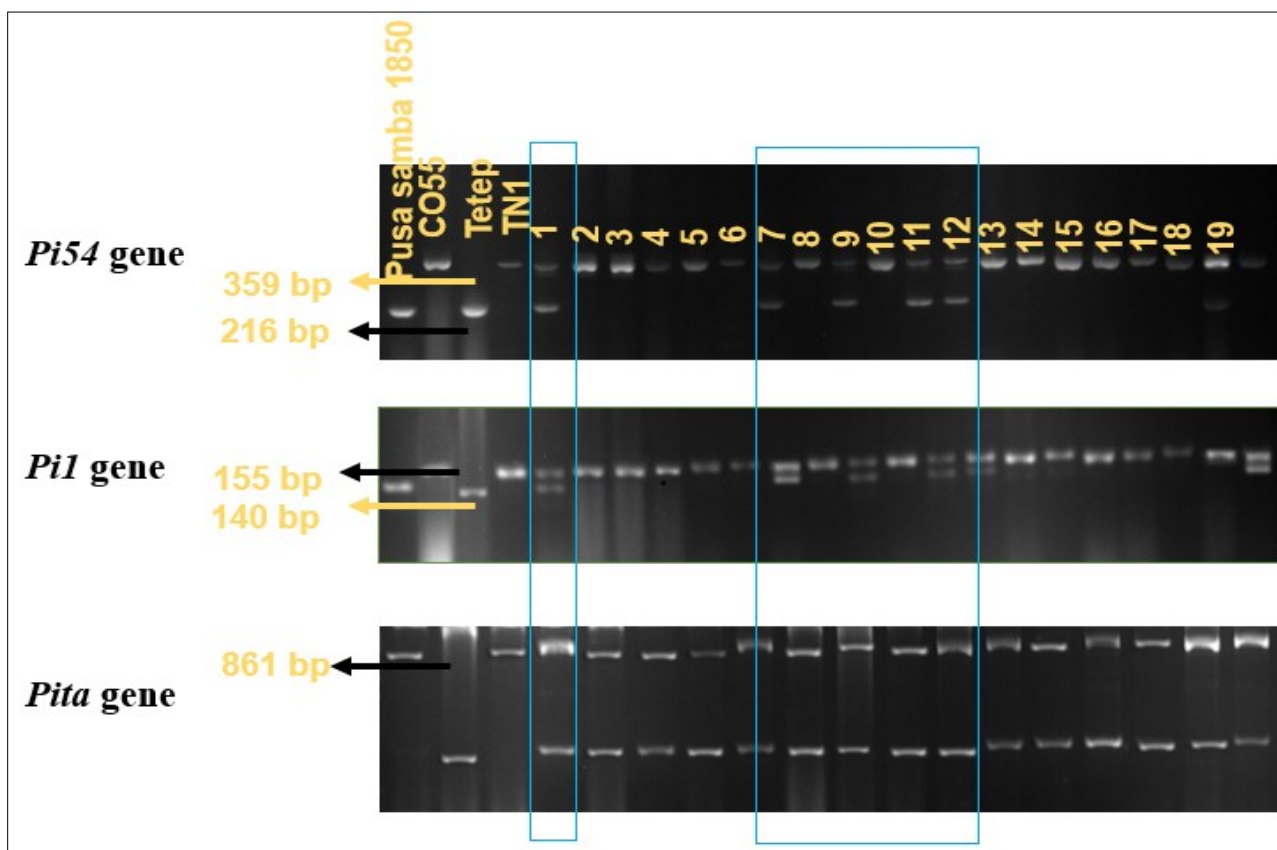


Fig. 3. Marker profiling of the backcross progenies of the cross Pusa Samba 1850 x CO55 in F_2 generation indicating foreground selection for the genes *Pi54*, *Pi1* and *Pita*.

markers across the crosses are presented in Table 2.

In the cross Pusa Samba 1850 x CO55, the blast resistance markers *Pi54*-MAS (*Pi54*), RM224 (*Pi1*) and *Pita* (*Pita*) showed significant deviation from the Mendelian ratio, with χ^2 values of 14.24, 138.53 and 157.49, respectively. This deviation may be attributed to segregation distortion arising from linkage of resistance genes with other genomic regions or differential survival of genotypes. Hence, it concluded that the consistently

high χ^2 values across all crosses indicate segregation distortion in the F_2 populations.

Gene profiling of blast resistance genes

The distribution of blast resistance genes (*Pi54*, *Pi1* and *Pita*) among the F_3 lines of cross Pusa Samba 1850 and CO55 was assessed using gene-specific markers (Fig. 4). Among the three genes analysed, *Pi54* and *Pi1* were widely distributed across the majority of genotypes, indicating their successful introgression and

Table 2. Segregating analysis of marker genotypes in segregating population of cross Pusa Samba 1850 x CO55

Marker (gene)	Observed frequency			Total plants	χ^2 (1: 2: 1)
	Homozygous resistant (RR)	Heterozygous (Rr)	Homozygous susceptible (rr)		
Pi54- MAS(<i>Pi54</i>)	28	49	50	127	14.24**
RM224 (<i>Pi1</i>)	17	21	89	127	138.53**
Pita (<i>Pita</i>)	22	13	92	127	157.49**

RR: homozygous resistant genotype; Rr: heterozygous genotype; rr: homozygous susceptible genotype. Chi-square (χ^2) values were calculated based on the expected Mendelian segregation ratio of 1:2:1. * and ** indicate significant deviation from the expected ratio at the 5% ($P < 0.05$) and 1% ($P < 0.01$) probability levels, respectively.

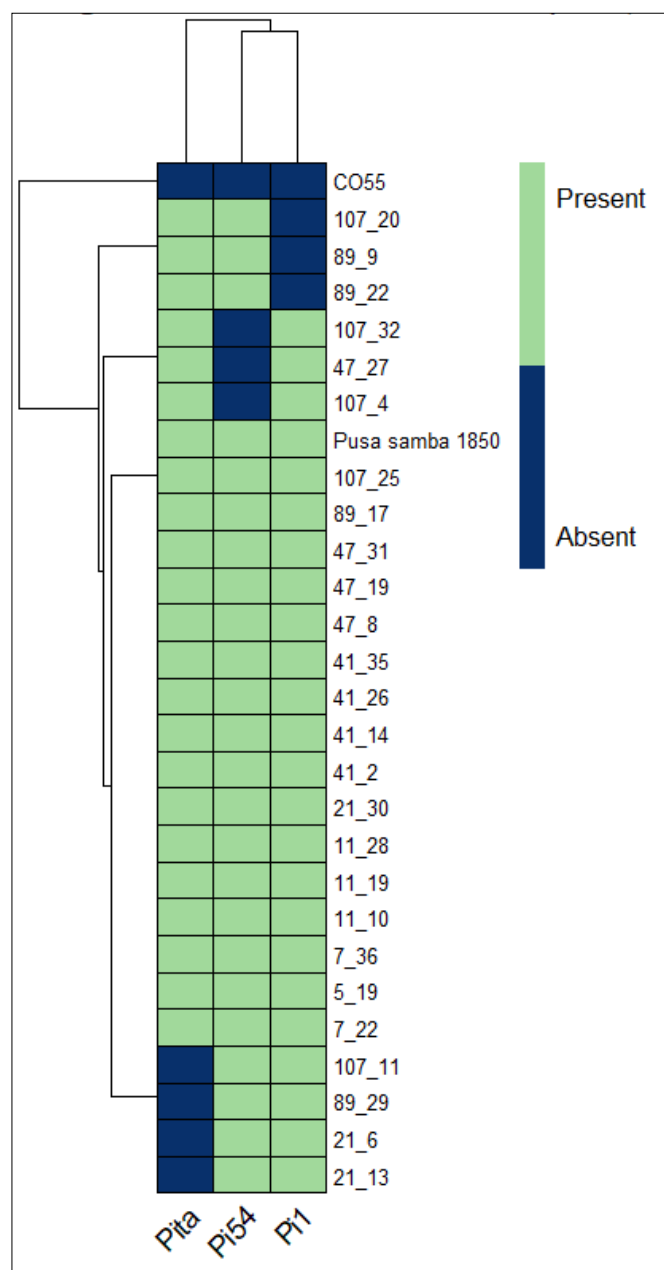


Fig. 4. Hierarchical clustering heatmap showing the distribution of blast resistance genes (*Pi54*, *Pi1* and *Pita*) among F_3 recombinant lines of cross Pusa Samba 1850 and CO55. Green colour indicates the presence of the gene, while blue indicates absence. Genotypes are grouped based on similarity in gene profiles.

stable inheritance. In contrast, the *Pita* gene showed limited presence and was absent in several lines, suggesting incomplete incorporation in certain progenies.

The clustering pattern grouped genotypes into distinct clusters based on gene combinations. Genotypes possessing multiple resistance genes (*Pi54* + *Pi1* + *Pita*) were clustered together, reflecting higher genetic similarity and potential for

broad-spectrum resistance. In contrast, genotypes lacking one or more genes formed separate clusters, indicating variability in resistance gene composition.

Notably, improved lines such as selected progenies exhibited a higher frequency of gene pyramiding, confirming the effectiveness of MAS in combining multiple blast resistance genes. The recurrent parent and susceptible checks were grouped separately due to the absence or lower number of resistance genes. Overall, these demonstrate significant variation in the distribution of blast resistance genes among genotypes and highlight the successful development of lines with multiple gene combinations for enhanced blast resistance.

Genetic variability and trait association analysis for efficient selection and crop improvement

The analysis of variability parameters revealed considerable differences among the agronomic traits days to fifty percent flowering (DFF), days to maturity (DM), flag leaf length (FLL), flag leaf width (FLW), number of grains per panicle (NGP), number of productive tillers (NPT), plant height (PH), panicle length (PL), thousand seed weight (TSW) and single plant yield (SPY). The phenotypic coefficient of variation (PCV) was consistently higher than the genotypic coefficient of variation (GCV) for all traits, indicating environmental influence on trait expression. Moderate to high PCV and GCV values were observed for traits such as FLW, PH and SPY, suggesting substantial genetic variability and good scope for selection, whereas traits like DFF and DM exhibited relatively low variability (Fig. 5).

The heritability estimates were uniformly high across all traits, ranging from approximately 80 % to nearly 100 %, indicating strong genetic control. The highest heritability was recorded for PH and DFF, suggesting minimal environmental influence. The genetic advance as percent of mean (GAM) varied among traits, with higher values observed for FLW, PH and TSW, indicating the effectiveness of selection. Moderate GAM was recorded for NGP and PL, while lower values were observed for DFF, DM and SPY (Fig. 5).

The correlation analysis revealed several significant associations among yield and its component traits. Single plant yield (SPY) showed a positive correlation with NPT ($r = 0.32^{***}$) and TSW ($r = 0.27^{***}$), indicating their contribution to yield improvement (Fig. 6). However, SPY showed weak or negative associations with DFF and DM, suggesting that earliness may not directly enhance yield. A strong positive correlation was observed between DFF and DM ($r = 0.85^{***}$), indicating synchronised flowering and maturity. Plant height (PH) exhibited significant positive associations with NPT ($r = 0.66^{***}$), PL ($r = 0.45^{***}$) and FLL ($r = 0.50^{***}$), suggesting its contribution to yield components. Similarly, PL showed a strong positive correlation with FLL ($r = 0.51^{***}$), while NGP was positively associated with PH ($r = 0.66^{***}$) and FLL ($r = 0.38^{***}$), highlighting their importance in yield determination. In contrast, NPT showed a negative association with PH ($r = -0.21^{***}$) and NGP ($r = -0.32^{***}$),

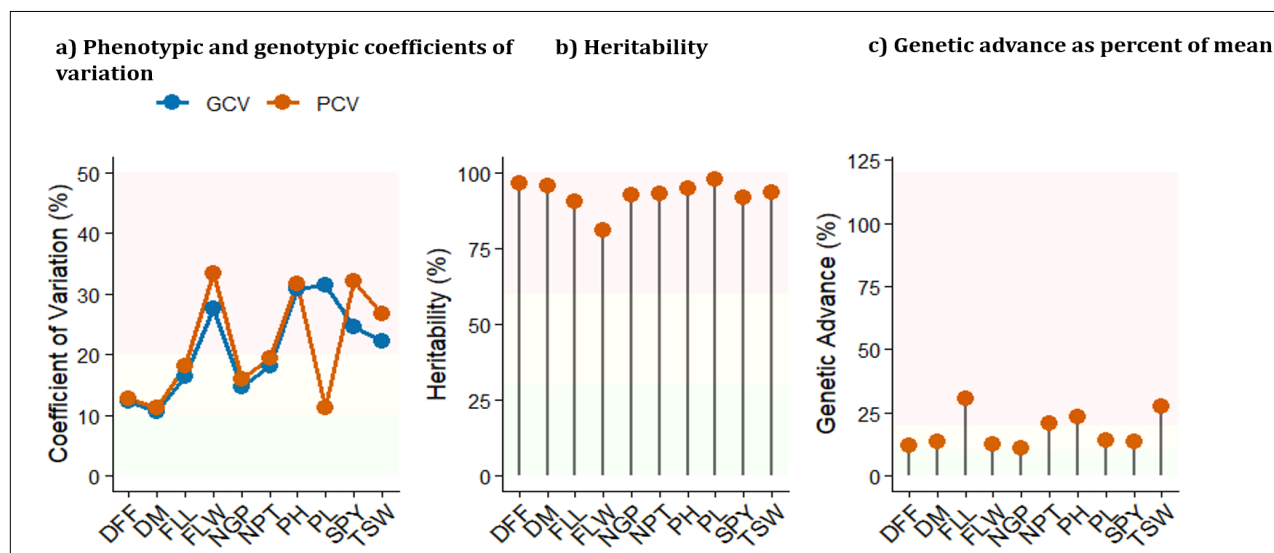


Fig. 5. Genetic variability, heritability and genetic advance of yield and its component traits. a: Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) expressed as percentages for DFF (days to 50 % flowering), DM (days to maturity), FLL (flag leaf length), FLW (flag leaf width), NGP (number of grains per panicle), NPT (number of productive tillers), PH (plant height), PL (panicle length), TSW (thousand seed weight) and SPY (single plant yield); b: Broad-sense heritability (h^2 , %) of the studied traits, indicating the proportion of total phenotypic variance attributable to genetic factors. (c) Genetic advance as percent of mean (GAM), reflecting the expected improvement under selection. *Error bars represent variability estimates and traits with high heritability coupled with high GAM indicate the predominance of additive gene action and greater effectiveness of selection.

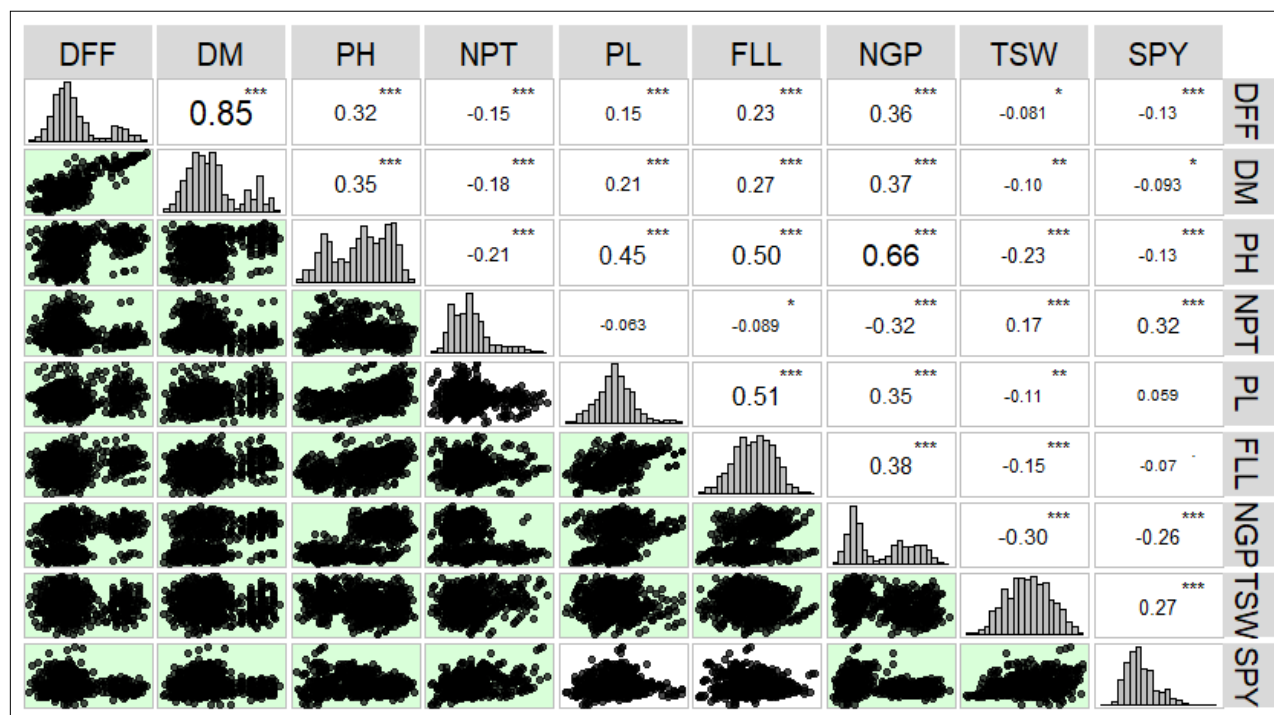


Fig. 6. Pairwise correlation matrix and distribution of yield and its component traits. The matrix summarises the distribution and inter-relationships among DFF (days to 50 % flowering), DM (days to maturity), PH (plant height), NPT (number of productive tillers), PL (panicle length), FLL (flag leaf length), NGP (number of grains per panicle), TSW (thousand seed weight) and SPY (single plant yield). The diagonal panels depict the frequency distribution (histograms) of individual traits. The lower triangle displays scatter plots indicating pairwise relationships, while the upper triangle presents Pearson's correlation coefficients (r) along with their significance levels. Asterisks denote significance (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). Positive correlations are highlighted in green, whereas negative correlations are indicated without shading.

indicating a trade-off between tiller number and grain number per panicle. Thousand seed weight (TSW) also exhibited weak negative correlations with traits such as PH ($r = -0.23^{***}$) and NGP ($r = -0.30^{***}$), suggesting complex relationships among yield components.

The path coefficient analysis provided further insights into the direct and indirect effects of traits on SPY (Fig. 7). Among all traits, NPT exhibited the highest positive direct effect (0.25), followed by TSW (0.19) and DM (0.14), indicating their major contribution to yield.

Days to fifty percent flowering (DFF) showed a smaller positive direct effect (0.10), while NGP had a low but positive direct influence. Traits such as PH and PL had low direct effects but contributed positively to yield indirectly through NGP, FLL and NPT, indicating their supportive role. Similarly, FLL exerted positive indirect effects via its strong association with PL and PH. However, NGP showed a negative indirect effect via NPT (-0.32), suggesting a trade-off between grain number and tiller production. Thousand seed weight

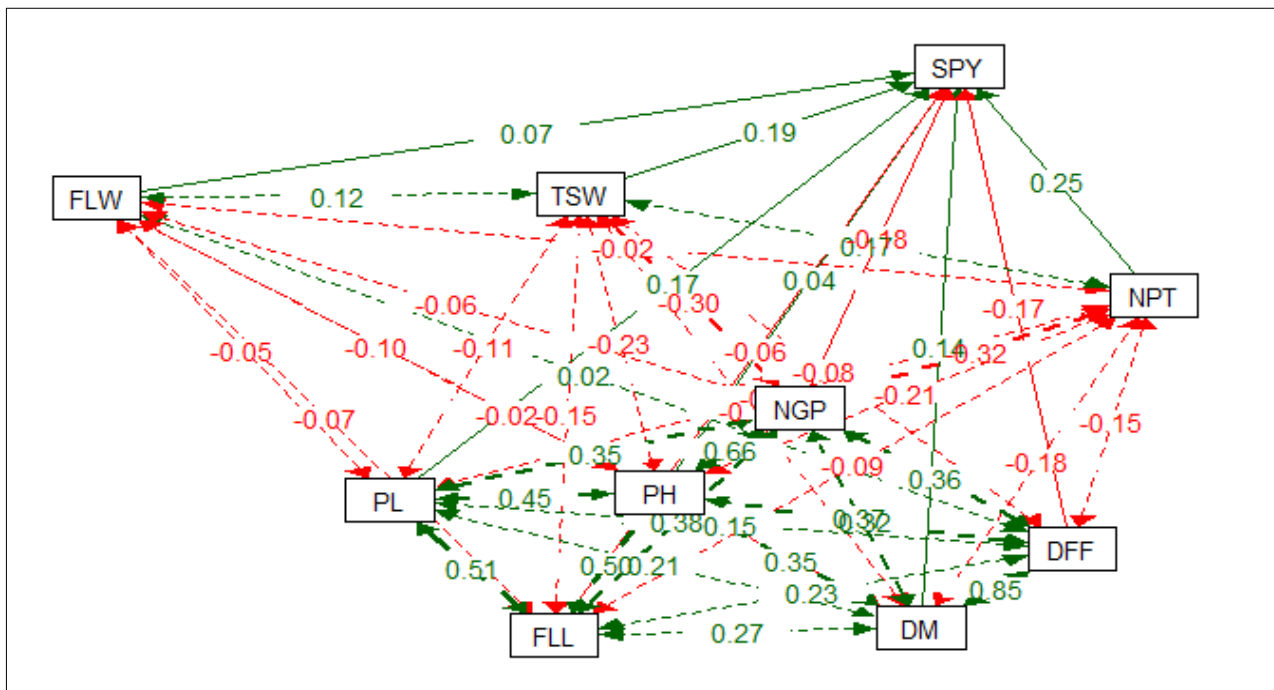


Fig. 7. Path coefficient analysis depicting direct and indirect effects among yield and its component traits. The network illustrates relationships among DFF (days to 50 % flowering), DM (days to maturity), PH (plant height), NPT (number of productive tillers), PL (panicle length), FLL (flag leaf length), FLW (flag leaf width), NGP (number of grains per panicle), TSW (thousand seed weight) and SPY (single plant yield). Solid green arrows represent positive effects, whereas dashed red arrows indicate negative effects. Numerical values along the paths denote the magnitude of standardised path coefficients, reflecting the strength of direct and indirect influences among traits.

also exhibited negative indirect effects through NGP and other traits, reflecting complex trait interactions. Flag leaf width showed predominantly negative indirect effects, indicating a limited direct role in yield improvement.

Phenotypic screening for blast resistance

Phenotypic screening for blast disease was conducted in the pyramided F_3 homozygous lines derived from the cross Pusa Samba

1850 x CO55 under natural field conditions at the Gudalur blast hotspot region, Tamil Nadu, India, during the rabi season (Fig. 8, Table 3). The experiment was carried out with two replications under environmental conditions highly favourable for blast disease development. The Gudalur region during the rabi season is characterised by cool temperatures ranging from 18–28 °C, high relative humidity (80–95 %), frequent morning dew, prolonged leaf

Table 3. Phenotypic screening of pyramided homozygous F_3 lines for blast disease resistance in Pusa Samba 1850 x CO55

S. No	F_3 lines	<i>Pi54</i>	<i>Pi1</i>	<i>Pita</i>	Infection scoring	Response
1	5–7	R	R	S	4	Moderately susceptible
2	5–19	R	R	R	4	Moderately susceptible
3	7–22	R	R	R	4	Moderately susceptible
4	7–36	R	R	R	1	Highly resistant
5	11–10	R	R	R	5	Moderately susceptible
6	11–19	R	R	R	6	Susceptible
7	11–28	R	R	R	1	Highly resistant
8	21–6	R	R	S	3	Moderately resistant
9	21–13	R	R	S	3	Moderately resistant
10	21–30	R	R	R	4	Moderately susceptible
11	41–2	R	R	R	6	Susceptible
12	41–14	R	R	R	4	Moderately susceptible
13	41–26	R	R	R	4	Moderately susceptible
14	41–35	R	R	R	4	Moderately susceptible
15	47–8	R	R	R	4	Moderately susceptible
16	47–19	R	R	R	5	Moderately susceptible
17	47–27	S	R	R	5	Moderately susceptible
18	47–31	R	R	R	6	Susceptible
19	89–9	R	S	R	3	Moderately resistant
20	89–17	R	R	R	6	Susceptible
21	89–22	R	S	R	5	Moderately susceptible
22	89–29	R	R	S	5	Moderately susceptible
23	107–4	S	R	R	4	Moderately susceptible
24	107–11	R	R	S	4	Moderately susceptible
25	107–20	R	S	R	6	Susceptible
26	107–25	R	R	R	2	Resistance
27	107–32	S	R	R	3	Moderately resistant
28	Pusa Samba 1850	R	R	R	1	Highly resistant
29	CO55	S	S	S	6	Susceptible

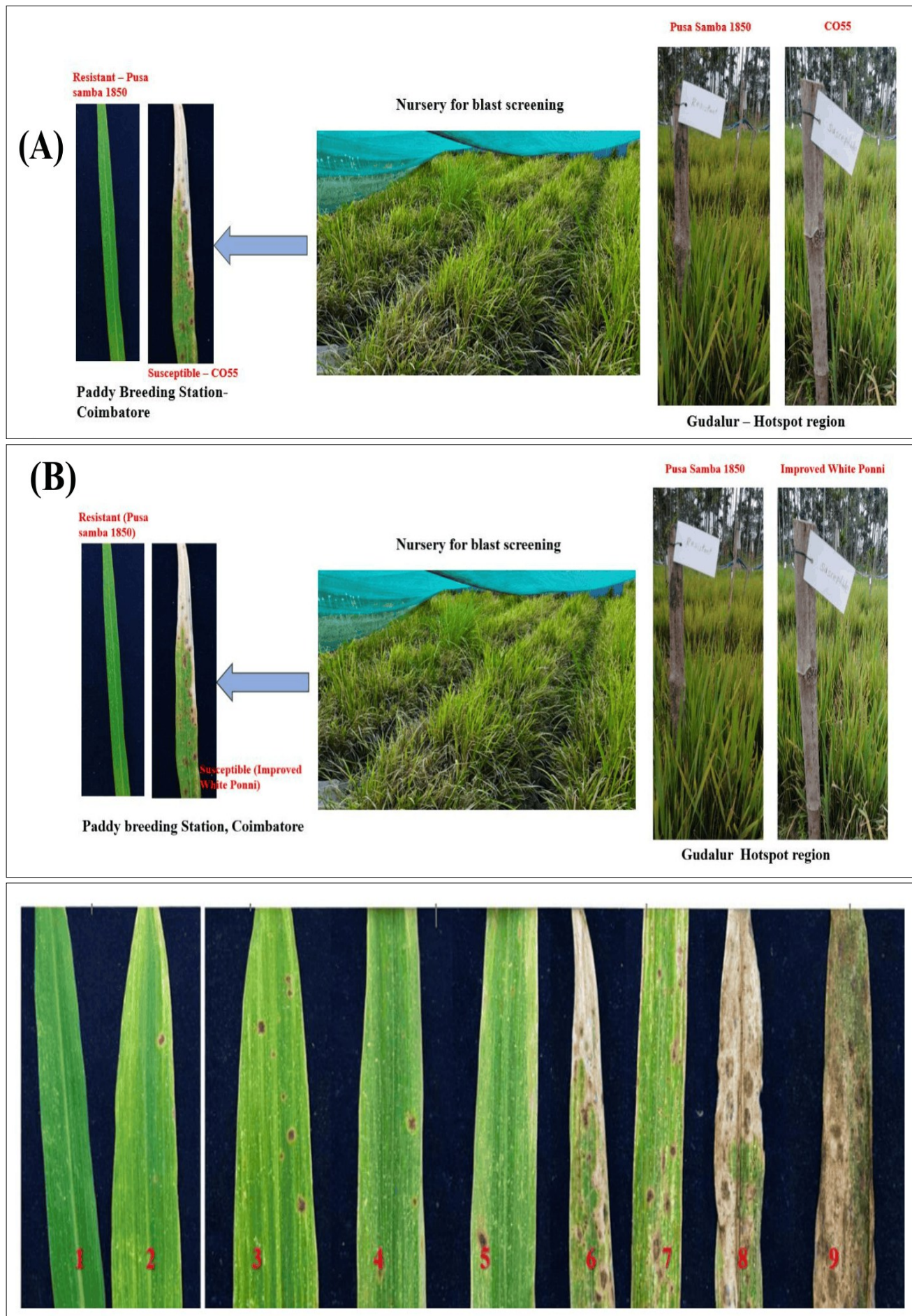


Fig. 8. Screening and evaluation of blast disease severity in rice F_3 lines of cross Pusa Samba 1850 and CO55 under field and hotspot conditions. A: Blast screening nursery established under hotspot conditions at Gudalur, showing resistant (Pusa Samba 1850) and susceptible (CO55) checks are used; B: Representative rice leaves illustrating blast disease severity (1-Pusa Samba 1850, 2- F_3 line 7-36, 3- F_3 line 11-28, 4- F_3 line 21-30, 5- F_3 line 89-17, 6- F_3 line 47-27, 7- F_3 line 107-11, 8- F_3 line 107-32, 9-Recurrent parent CO55).

wetness, intermittent rainfall and persistent cloud cover, creating ideal conditions for the growth and spread of *M. oryzae*. Disease pressure was provided by the natural pathogen population prevalent in the hotspot region (Gudalur). Disease severity was assessed using the standard evaluation system (SES) for rice (IRRI) on a 0–9 scale, where lower scores indicate a higher level of resistance.

All selected F_3 homozygous lines were confirmed to be homozygous for the three blast resistance genes (*Pi54*, *Pi1* and *Pita*); however, despite the fixation of these genes, variation in blast response was observed among the lines. Infection scores ranged from 1–6, indicating differential expression of resistance. Two F_3 lines, 7–36 and 11–28, recorded an infection score of 1 and were categorised as highly resistant, demonstrating strong resistance against blast disease. Moderate resistance was observed in line 107–25, which recorded an infection score of 3 and were classified as moderately resistant. The variation in disease reaction among the homozygous lines may be attributed to differences in genetic background, gene interactions and environmental influences affecting the expression of blast resistance.

Discussion

The present study demonstrates the successful application of MAS for pyramiding multiple blast resistance genes (*Pi54*, *Pi1* and *Pita*) into the genetic background of the high-yielding variety CO55 using Pusa Samba 1850 as the donor parent. The successful identification of F_2 and F_3 individuals carrying gene combinations confirms the efficiency of foreground selection using gene-specific SSR markers (*Pi54*-MAS, RM224 and *Pita*). The recovery of eight triple-gene homozygous F_2 plants and their advancement to F_3 generation, followed by identification of 27 homozygous recombinant inbred F_3 lines, highlights the effectiveness of MAS in accelerating gene pyramiding compared to conventional breeding approaches (12, 13).

The observed segregation distortion in the F_2 population, as indicated by significant deviation from the expected 1: 2: 1 ratio for all three markers, may be attributed to several biological and genetic factors (14). These include linkage of resistance loci with deleterious alleles, differential gametic or zygotic selection or reduced viability of certain genotypic classes. Similar segregation distortion has been widely reported in inter-varietal rice crosses involving resistance gene introgression, particularly when exotic or genetically distant donors are involved. Such distortion, while deviating from Mendelian expectations, often reflects underlying selection pressure favouring specific allele combinations linked to fitness or disease resistance.

Gene profiling of the F_3 population comprising 30 F_3 lines (5–7, 5–19, 7–22, 7–36, 11–10, 11–19, 11–28, 21–6, 21–13, 21–30, 41–2, 41–14, 41–26, 41–35, 47–8, 47–19, 47–27, 47–31, 89–9, 89–17, 89–22, 107–4, 107–11, 107–20, 107–25, 107–32, Pusa Samba 1850 and CO55) revealed a differential distribution of blast resistance genes, with *Pi54* showed the highest frequency and most stable introgression, followed by *Pi1*, while *Pita* was present in comparatively fewer lines. A total of 17 lines possessed all three blast resistance genes (*Pi54*, *Pi1* and *Pita*), demonstrating successful gene pyramiding and indicating a high potential for durable and broad-spectrum blast resistance through the combined action of multiple resistance mechanisms. Ten lines carried two-gene combinations, including three lines with *Pi54* +

Pita, three lines with *Pi1* + *Pita* and four lines with *Pi54* + *Pi1*, which are expected to provide moderate to high levels of resistance. No genotype carrying only a single resistance gene was identified among the selected lines. The recurrent parent CO55 lacked all three target genes, whereas the donor parent Pusa Samba 1850 possessed all three resistance genes. Clustering analysis further supported these findings, as triple-gene carriers grouped distinctly, indicating greater genetic similarity and effective pyramiding. Overall, *Pi54* emerged as the most important gene due to its widespread presence, strong effect and known broad-spectrum resistance, while *Pi1* contributed additively to resistance durability. The comparatively low frequency of *Pita* suggests possible challenges in its stable introgression, emphasising the need for continued selection to fix all target genes in advanced generations for achieving durable blast resistance.

The genetic variability analysis revealed substantial phenotypic and genotypic variation among traits, with higher PCV than GCV for all traits indicating environmental influence on trait expression (15). However, the generally high heritability estimates (80–100 %) across most traits suggest that additive genetic effects predominate, making selection highly effective (16). Traits such as PH, FLW and SYP exhibited both high heritability and high genetic advance, indicating that direct selection for these traits would result in substantial genetic gain (17). Conversely, traits like days to flowering and DM showed low genetic advance despite high heritability, suggesting limited scope for selection due to restricted variability (18).

Correlation and path coefficient analyses provided deeper insight into yield component relationships. The positive association of single plant yield with number of productive tillers and thousand seed weight indicates that these traits are primary contributors to yield improvement (19). The strong positive correlation between flowering and maturity suggests synchronised developmental phases, which may be advantageous for uniform crop establishment but may limit independent selection for earliness. The negative association of productive tillers with PH and grain number per panicle indicates a physiological trade-off, reflecting competition for assimilates among yield components. Path analysis further confirmed that number of productive tillers and thousand seed weight exerted the highest direct effects on yield, establishing them as key selection indices in breeding programs (15). In contrast, traits such as PH and PL influenced yield indirectly, mainly through their effects on other yield components.

Phenotypic screening under natural or controlled blast pressure validated the functional expression of introgressed resistance genes (20). Although all selected F_3 lines carried the targeted resistance gene combinations, variation in disease reaction was observed, ranging from highly resistant to moderately resistant classes. This suggests that gene pyramiding alone may not guarantee uniform resistance expression and that background genetic effects, gene interactions and possible environmental modulation play significant roles in determining phenotypic resistance. Recombinant lines 7–36 and 11–28 exhibited superior performance, recording an infection score of 1 and showing highly resistant reactions. Moderate resistance was observed in lines such as 107–25 which recorded an infection score of 3 and were classified as moderately resistant.

Overall, the study highlights the effectiveness of combining marker-assisted gene pyramiding with phenotypic and biometrical selection to develop rice lines with improved blast resistance and desirable agronomic performance. The identification of high-yielding, multi-gene resistant F₃ lines demonstrates the potential of integrated breeding strategies in accelerating the development of elite rice cultivars with durable disease resistance and yield stability.

Conclusion

The present study demonstrated the successful application of marker-assisted gene pyramiding for improving blast resistance in rice by introgressing the *Pi54*, *Pi1* and *Pita* genes into the CO55 background using Pusa Samba 1850 as the donor parent. Foreground selection using gene-specific simple sequence repeats (SSR) markers enabled precise identification of F₂ and F₃ individuals carrying single, double and triple gene combinations, leading to the recovery of stable homozygous lines with stacked resistance genes. A total of 27 homozygous recombinant lines carrying pyramided blast resistance genes were identified in the F₃ population of this cross. Although segregation distortion was observed in the F₂ population the successful identification and advancement of homozygous lines carrying all three resistance genes confirmed the effectiveness of the breeding strategy. Overall, *Pi54* emerged as the most important gene due to its widespread presence, strong effect and known broad-spectrum resistance, while *Pi1* contributed additively to resistance durability. Genetic variability analysis showed sufficient scope for selection, with high heritability and genetic gain for key traits. Correlation and path analysis identified the number of productive tillers and thousand seed weight as major contributors to yield. Phenotypic screening of pyramided lines revealed varied expression of blast resistance, with some lines (7–36, 11–28 and 107–25) demonstrating high resistance levels. Overall, combining MAS with phenotypic screening proved to be an effective approach for developing blast-resistant, high-yielding rice varieties.

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Authors' contributions

SR contributed to the conceptualisation, methodology, investigation, formal analysis and preparation of the original draft manuscript. RJ contributed to conceptualisation, supervision, manuscript review and editing. AL was involved in investigation, validation, manuscript review and editing. MS contributed to manuscript review, editing and supervision. GC was responsible for validation and formal analysis. RV contributed to supervision, manuscript review and editing. All authors read and approved the final version of the manuscript.

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During the preparation of this research article, the authors used Grammarly to enhance the clarity, grammar and academic language of the manuscript. The tool was used solely for language editing purposes and did not contribute to the scientific content, interpretation, or conclusions of the study. All authors have critically reviewed and approved the final version of the manuscript and take full responsibility for its content.

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