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RESEARCH ARTICLE

Molecular characterization and phenotypic selection for blast resistance and yield enhancement in rice (*Oryza sativa* L.)

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Abstract

Gurjari, a widely cultivated high-yielding potential variety popular among the farmers of Gujarat, is highly susceptible to the blast disease. To improve its blast resistance, GNR-9 and Tetep, which carry major broad-spectrum resistance genes, were used as donors in a marker-assisted breeding program, combined with phenotypic selection for key agro-morphological traits. Foreground selection in F_3 generation confirmed the presence of blast resistance genes *Pikh*/*Pi54*, *Pitp*(*t*) and *Pita-2*/*Pi67* in 36 of 178 breeding lines. These 36 lines, along with 4 checks, were further evaluated in a Randomized Block Design (RBD) during *Kharif* 2022 for variability, correlation, Principal Component Analysis (PCA) and Multi-Trait Genotype-Ideotype Distance Index (MGIDI) for selecting the best lines and molecular screening of blast resistance. Analysis of variance revealed significant variation for all 13 traits, with moderate GCV for plant height, productive tillers, 100-grain weight, grain yield, straw yield and harvest index. Grain yield showed positive correlations with panicle length, grains per panicle, 100-grain weight, straw yield and harvest index. PCA explained 82.13 % of total variation across traits, while MGIDI identified four high-performing lines; 22KSMF4-8, 22KSMF4-5, 22KSMF4-6 and 22KSMF4-14. Molecular screening of F_4 progenies identified nine F_4 derivatives carrying three blast resistance genes, with two (Gurjari × GNR-9) exhibiting superior grain yield and quality over *Gurjari*, making them promising candidates for developing high-yielding, blast-resistant varieties.

Keywords: blast; correlation; marker-assisted breeding; Principal Component Analysis and MGIDI; variability

Introduction

Rice (*Oryza sativa* L.) is the primary and most valuable staple food crop in the world. By 2050, rice output must increase by 42 % from its current level to feed the world's expanding population. Numerous biotic and abiotic factors can greatly affect rice yield. The impact of various biotic stresses on crop yields is estimated to cause reductions of around 52 %. Among these stresses, diseases such as bacterial blight, blast, sheath blight and tungro disease contribute to nearly 31 % of the overall yield loss. (1). Rice blast is a significant disease affecting rice cultivation worldwide (2). It is caused by *Pyricularia oryzae* Cavara (teleomorph: *Magnaporthe oryzae*) and is among the most destructive diseases, leading to yield losses of up to 50 % (3). The pathogen infects various parts of the rice plant, including leaves (leaf blast), panicles (panicle blast), nodes (node blast) and necks (neck blast), resulting in substantial yield losses in rice-producing regions. The pathogen exhibits high genetic variability, causing blast-resistant varieties to become susceptible within a short time after commercial cultivation. Therefore, it is crucial to identify durable blast-resistant cultivars and explore new resistance sources against the prevailing virulent strains (4).

Conventional breeding for disease resistance is labor-intensive, time-consuming and largely influenced by environmental factors. In contrast, molecular breeding, particularly Marker-Assisted Selection (MAS) is environment-independent, more efficient, precise and relatively easier to implement. Several genes, such as *Pi-1(t)*, *Pi2*, *Pi9*, *Pi20(t)*, *Pi27(t)*, *Pi39(t)*, *Pi40(t)* and *Pikh*, have been reported to provide Broad-Spectrum Resistance (BSR). Additionally, certain genes, including *Pia*, *Pib*, *Pi1*, *Pi-km*, *Pi-t*, *Pil2(t)* and *Pi9(t)*, are known to confer race-specific resistance (RSR) (5-7). The major resistance gene *Pi54*, derived from the rice landrace *Tetep*, was the third *R*-gene cloned for blast resistance after *Pib* and *Pit16*. *Pi54* confers broad-spectrum resistance against various strains of *Magnaporthe oryzae*. The rice cultivar Tetep has been found to be resistant to most of the pathogenic races occurring in India (8). MAS utilizing DNA markers enhances the precision and efficiency of breeding while reducing overall costs in the

selection process. Markers closely associated with resistance genes can be utilized to monitor the inheritance of specific target genes or alleles in segregating populations, a process known as foreground selection. SSR markers linked to target genes can be effectively utilized for introgression of desired genes from one genetic background to another and for pyramiding multiple genes or alleles from different donor genotypes. The current study aims to incorporate the blast resistance genes *Pi-1(t)*, *Pikh/Pi54*, *Pitp(t)* and *Pita-2/Pi67* from *Tetep* and *GNR-9* to enhance the resistance of the rice cultivar *Gurjari*.

The selection of lines with target trait introgression and desired agro-morphological traits which result in the creation of cultivars is necessary for the success of any MAS (9). Selection within a segregating generation can partially achieve the goal of enhancing polygenic traits such as yield. This requires field evaluation in replicated trials. A thorough understanding of the genetic nature of the target trait, along with careful selection of parents to generate and predict genetic variability in subsequent generations, is essential for the success of a breeding program. Assessing genetic variability through parameters such as genotypic and phenotypic coefficients of variation (GCV, PCV), heritability (h^2) and genetic advance as a percentage of the mean (GAM) is crucial for accurately evaluating the extent of genetic variation within a population. Understanding the degree of correlation between yield and its associated traits is crucial for identifying and selecting promising cultivars (10-12).

As a result, multivariate analytic methods such as PCA and MGIDI can be used as a model instrument for testing and identifying the causes of variance (13-15). PCA, for example, reduces the dimensionality of a data set by decreasing the number of variables while preserving as much information as possible. It utilizes orthogonal transformation to convert a set of potentially correlated variables into a set of uncorrelated variables, referred to as principal components. Breeders frequently seek to generate an ideotype, which is a genotype that combines many traits for optimal performance. The goal of ideotype design is to improve crop performance by considering multiple attributes at the same time while selecting genotypes (16).

The goal of the investigation was to screen F_3 and F_4 segregating generations for blast resistance using molecular markers; analyses of genetic variability, correlation, PCA and MGIDI for yield and its components in F_4 progenies; and identify blast-resistant lines with high grain yield and superior grain quality compared to Gurjari.

Material and Methods

Experimental material

In the present investigation, 178 F_3 progenies were derived from two crosses *viz.,* Gurjari × GNR-9 and Gurjari × Tetep. F_3 progenies including 100 progenies of Gurjari × GNR-9 cross and 78 progenies of Gurjari × Tetep cross were used to reveal the presence of blast resistance genes linked to the earlier reported molecular markers. Gurjari is derived from Asha × Kranti. It is a well-established and high yield potential variety popular among the farmers of Gujarat but is highly susceptible to the blast disease. Tetep, a local Vietnam landrace, is a tall type, resistant to blast and sheath blight disease. Tetep has been found resistant to most of the blast pathogenic races occurring in India. GNR-9 (Lalkada Gold) is derived from IR28 × Lalkada and its features are non-lodging, bio-fortified (enriched with protein) and resistant to blast disease.

Location, experimental site and environments

The field experiment was conducted at the Regional Rice Research Station, Navsari Agricultural University, Vyara, located at 21º 04' N latitude and 73º 03' E longitude. The research station is positioned at an elevation of 69 meters above mean sea level and falls within the South Gujarat agro-climatic zone, which is characterized by high rainfall. The experimental trials were carried out during the summer and *kharif* seasons of 2022 to evaluate the F_3 and F_4 segregating generations.

Genomic DNA isolation

Genomic DNA of all F_3 and F_4 progenies along with parents were isolated from tender fresh leaves by using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method described (17) with some modifications. The quantity and quality of the purified DNA were assessed using 0.8 % agarose gel electrophoresis and a Nano Drop spectrophotometer (Thermo Fisher Scientific, USA). The DNA samples were subsequently diluted with nuclease-free water for PCR amplification.

Parental polymorphism and screening of segregating populations

A set of nine primers linked to the blast resistance genes present in Tetep and GNR-9 were used to screen the parents to identify polymorphic markers (Table 1). Only primers that showed polymorphism between the parents were used for screening the F_3 and F_4 progenies for blast resistance. PCR reactions for SSR primers were carried out in a reaction volume of 10 µL containing, 5 µL Emerald AmpGT PCR master mix, 2 µL RNase-free water, 1 µL forward primer, 1 µL reverse primer and 1 µL DNA. The reagents were mixed thoroughly with a mini centrifuge. Then PCR tubes were placed in the thermal cycler for amplification. All the PCR reactions were performed in 200 µL thin-walled PCR tubes. Amplification was carried out using a thermal cycler (BIORAD, USA) and PCR program was set up a follow: initial denaturation was carried out at 94 °C for 5 minutes, followed by 35 cycles consisting of denaturation at 94 °C for 45 seconds, primer annealing at different temperatures (ranging from 54 °C to 58 °C) for 45 seconds and extension at 72 °C for 2 minutes. The process concluded with a final extension at 72 °C for 10 minutes. The amplified products were analyzed by horizontal electrophoresis on 3 per cent agarose gel stained with ethidium bromide (0.5 µg/mL) at 100 V for 2 hrs and 30 minutes. A 100-bp ladder was used to assess the size of PCR products. The gel was visualized under UV in the gel documentation system (Gel Doc™ XR⁺ Imaging system).

SSR marker analysis

A total of 178 F_3 lines along with parental lines were screened using markers listed in Table 1. The aim of this was to identify the genotype showing the allele as that of the resistant parent (Tetep and GNR-9). The plants showing similar alleles as that of Tetep and GNR-9 as well as heterozygous plants were identified and were selfed and F_4 seeds derived from such plants advanced further or used for field evaluation of F_4

Table 1. List of primers used during the investigation

Sr. No.	Gene	Ch. No.	Marker	Primer sequence (5' to 3')	References
1	*Pi-1(t)*	11	RM1233	F- GTGTAAATCATGGGCACGTG R- AGATTGGCTCCTGAAGAAGG	(18)
2	*Pi-1(t)*	11	RM224	F- ATCGATCGATCTTCACGAGG R- TGCTATAAAAGGCATTCGGG	(19)
3	*Pik^h/ Pi54*	11	TRS33	F-AAGAAGAAGCGTACGCATGAAT R- GTCCTGGAGGGGAGGAGA	(20)
4	*Pik^h/ Pi54*	11	RM144	F- TGCCCTGGCGCAAATTTGATCC R- GCTAGAGGAGATCAGATGGTA GTGCATG	(21)
5	*Pik^h/ Pi54*	11	TRS26	F- GGAGAGCCAATCTGATAAGCA R-CAACAAGAGAGGCAAATTCTCA	(20)
6	*Pik^h/ Pi54*	11	RM206	F- CCCATGCGTTTAACTATTCT R- CGTTCCATCGATCCGTATGG	(20)
7	*Pitp (t)*	1	RM246	F- GAGCTCCATCAGCCATTCAG R- CTGAGTGCTGCTGCGACT	(22)
8	*Pita-2/ Pi67*	12	YL87/ YL155	F- CTACCAACAAGTTCATCAAA R- AGCAGGTTATAAGCTAGGCC	(23, 24)
9	*Pita-2/ pi67*	12	RRS8	F- GGAGAAGATGGAGCAATTCG	(24)

progenies along with checks for various variability parameters and blast resistance.

Phenotyping for yield and yield attributing traits of F_4 progenies

Based on agro-morphological traits and molecular screening results, a subset of 36 F_3 progenies with 4 checks (Gurjari, GNR-9, Tetep and Lalkada) was carefully selected for further evaluation to study variability parameters and blast resistance. A total of 40 lines including 36 blast resistant F_4 progenies and 4 checks planted in RBD during *Kharif* 2022 for evaluating variability parameters and molecular screening of blast resistance (Supplementary Table 1). Mean values were used for statistical analysis and the traits assessed to obtain information included Days to 50 % Flowering (DFF), Days to Maturity (DM), Plant Height (cm) (PH), Panicle Length (cm) (PL), Productive Tillers per Plant (PTP), Grains per Plant (GP), 100-grain weight (g) (100 GW), Straw Yield per Plant (g) (SYP), Harvest Index (%) (HI), Kernel Length (mm) (KL), Kernel Breadth (mm) (KB) and the L/B ratio (L/B), Grain Yield per Plant (g) (GYP).

Data analysis

Variance analysis and variability parameters for all morphological and quality attributes were calculated using the package in R software. A categorization scheme for variations was proposed (25). For the categorization of GCV and PCV a method based on a range of values was used, as follows: 0 – 10 (Low), 10 - 20 (Moderate), > 20 (High). The Broad-sense heritability (H^2b) was categorized following the procedure given (26). For the categorization of H^2b, a method based on a range of values was used, as follows: low H^2b had a < 30 % value, medium H^2b values range between 30 and 60 % and high H^2b values above 60 %. The expected genetic advance as per cent of mean was categorized as suggested (26) as follows: 0 - 10 (Low), 10 - 20 (Medium) and 20 (High). Correlation analysis was performed for each environment using the corr plot function of the “metan” package in R software. Additionally, PCA and biplot diagrams were developed using Grapes software. The MGIDI was analysed using the R Package ‘metan’ (27). Normalisation, factor analysis, ideotype planning and computing genotype distance to ideotype were the four procedures used to create the MGIDI index (16).

Results and Discussion

Genotypic characterization of F_3 progenies for introgression of blast genes

The parental lines Gurjari, Tetep and GNR-9 were initially screened using nine primer pairs associated with four distinct blast resistance genes present in Tetep and GNR-9 to assess polymorphism. Among the nine primers screened in the parental survey (RM1233, RM224, TRS26, TRS33, RM144, RM206, RM246, RRS8 and YL87/155), three markers RM206 (linked to *PiKh*/*Pi54*), YL87/155 (linked to *Pi67*) and RM246 (linked to *Pitp*(*t*)) exhibited polymorphism between the parents, while the remaining markers were monomorphic. RM206 marker that has been reported to be linked to the blast resistant gene *Pik^h/Pi54* on chromosome 11 in rice (20). Marker RM206 amplified two distinct alleles: one specific to Gurjari and the other shared by Tetep and GNR-9. Among the 178 F_3 plants screened with RM206, 73 progenies exhibited the Gurjari allele, 64 carried the allele from Tetep and GNR-9, while 41 were heterozygous, amplifying both alleles (Supplementary Fig. 1). The YL87/155 marker, associated with the blast resistance gene *Pi67* on chromosome 12 in rice (24), is a dominant marker that amplified a single allele in F_3 plants, representing the allele of Tetep and GNR-9. Among the 178 F_3 plants screened, 87 progenies exhibited the Tetep and GNR-9 allele, while 91 resembled Gurjari (Supplementary Fig. 2). The RM246 marker, linked to the blast resistance gene *Pitp(t)* on chromosome 1 in rice (22), amplified two distinct alleles in F_3 plants from the Gurjari × Tetep and Gurjari × GNR-9 crosses: ~128 bp for Gurjari and ~106 bp for Tetep and GNR-9. Among the 178 F_3 plants screened, 85 carried the Gurjari allele, 46 exhibited the Tetep and GNR-9 allele and 47 were heterozygous, amplifying both alleles (Supplementary Fig. 3). The status of blast resistance genes in F_3 progenies is presented in Supplementary Table 2. Among the 178 progenies screened, 16 carried all three resistance genes, while 20 exhibited a combination of two resistant genes and one in a heterozygous state. Based on agro -morphological traits (resemblance to Gurjari) and molecular screening results, a subset of 36 F_3 [(15 Gurjari × GNR-9), (21 Gurjari × Tetep)] progenies, along with four checks, were carefully selected for further evaluation of variability parameters and blast resistance.

Phenotypic evaluation of F4 Lines for yield and yield related traits

Mean performance of different traits

Descriptive statistics of morpho and quality traits were visualized using a box plot (Fig. 1). Among the 36 selected F4 breeding lines, the line 22KSMF4-4 exhibited significantly earlier flowering (64 days) and maturity (96 days) compared to all four check varieties. Additionally, two breeding lines, 22KSMF4-29 (83 cm) and 22KSMF4-4 (91.6 cm), displayed significantly shorter plant height compared to the checks (92.3 cm). Notably, several lines, including 22KSMF4-31, 22KSMF4-14, 22KSMF4-36, 22KSMF4-2 and 22KSMF4-8, recorded significantly longer panicle lengths than the checks. Moreover, the breeding lines 22KSMF4-22, 22KSMF4-20, 22KSMF4-26, 22KSMF4-25, 22KSMF4-14 and 22KSMF4-27 produced significantly higher numbers of productive tillers than the checks. Four breeding lines, namely 22KSMF4-36, 22KSMF4-1, 22KSMF4-12 and 22KSMF4-31, demonstrated a higher number of grains per panicle compared to the checks. Furthermore, two lines, 22KSMF4-1 and 22KSMF4-33, exhibited significantly higher 100-grain weights. Six breeding lines-22KSMF4-14, 22KSMF4-31, 22KSMF4-6, 22KSMF4-36, 22KSMF4-34 and 22KSMF4-2 were significantly superior in grain yield compared to the checks. Additionally, the breeding lines 22KSMF4-10, 22KSMF4-26, 22KSMF4-8, 22KSMF4-5, 22KSMF4-24 and 22KSMF4-25 were identified as having long and bold kernels with a high length-to-breadth (L/B) ratio.

Genetic variability

Variability components were estimated for yield and their attributes in F_4 progenies as presented in Table 2. The PCV values were slightly higher than the GCV values for all thirteen traits, indicating some influence of environmental factors. However, the small differences between GCV and PCV suggest that the environmental impact on these traits was minimal. Moderate GCV was recorded for PTP, PH, SYP, HI and GYP. The results indicated the presence of inherent variation for these traits and its further improvement is possible by applying judicious selection to the individual traits; while days to 50 per cent flowering, days to maturity, grains per panicle, panicle length, 100-grain weight, kernel length, kernel breadth and L/B ratio recorded lower value of GCV indicating the need to create variability either hybridization or by selection. These types of finding were also reported for SYP, PH and HI (28-30); for grain yield per plant and productive tillers per plant (31, 12). High heritability estimates accounted for the traits *viz.,* DFF, DM, PH and 100 grain weight showing the least environmental influence. Heritability estimates, when combined with genetic advance as a percentage of the mean, provide a more reliable prediction of yield under phenotypic selection than heritability estimates alone. Higher heritability, combined with a high genetic advance as a percentage of the mean, was observed for plant height and 100-grain weight indicating the role of additive gene effects and less effect of environmental factors on the expression of the traits. Thus, the improvement of these traits could be achieved through direct phenotypic selection. Similar kinds of results were reported for HI and 100 grain weight (29, 10, 12).

Correlation coefficient analysis

The correlation matrix is presented in Fig. 2. The correlation studies indicated that GYP was positive and significantly correlated with the yield components like PL, GP, 100 grain weight, SYP and HI. Therefore, enhancing all the traits may contribute to a simultaneous improvement in GYP. Hence, these traits can be considered key yield-attributing factors. This is very helpful to plant breeders for practicing selection based on phenotypic expression of the linked traits for the improvement in grain yield. These results are in accordance with the findings reported earlier (32-35) for GP, PL, 100 grain weight, SYP and HI.

Table 2. Variability, heritability and genetic advance as per cent of mean for rice yield and its component traits

Sr. No.	Traits	Components of variance			Coefficient of variation		Heritability broad sense (%)	GAM
		σ^2_g	σ^2_p	σ^2_e	GCV (%)	PCV (%)		
1	Days to 50 % flowering	60.96	69.35	8.39	9.22	9.83	87.90	17.79
2	Days to maturity	56.37	65.95	9.57	6.53	7.06	85.48	12.44
3	Plant height (cm)	264.59	324.73	60.14	14	15.51	81.48	26.04
4	Panicle length (cm)	1.41	4.18	2.76	5.1	8.77	33.89	6.12
5	Productive tillers per plant	1.24	2.39	1.14	16.6	23.04	52.06	24.71
6	Grains per panicle	203.17	459.76	256.59	13.28	19.98	44.19	18.19
7	100 grain weight (g)	0.08	0.09	0.01	12.25	12.83	91.13	24.09
8	Grain yield per plant (g)	3.46	11.13	7.67	13.59	24.39	31.07	15.61
9	Straw yield per plant (g)	5.72	13.5	7.78	15.71	24.14	42.35	21.06
10	Harvest index (%)	23.05	49.83	26.77	11.06	16.25	46.27	15.50
11	Kernel length (mm)	0.05	0.13	0.08	3.43	5.41	40.14	4.47
12	Kernel breadth (mm)	0.01	0.04	0.03	4.52	8.77	26.61	4.81
13	L/B ratio	0.03	0.08	0.05	5.77	9.43	37.47	7.27

Where σ^2_g , σ^2_p and σ^2_e are the genotypic, phenotypic and environmental variance, respectively. GCV % and PCV % are the genotypic and phenotypic coefficients of variation, respectively.

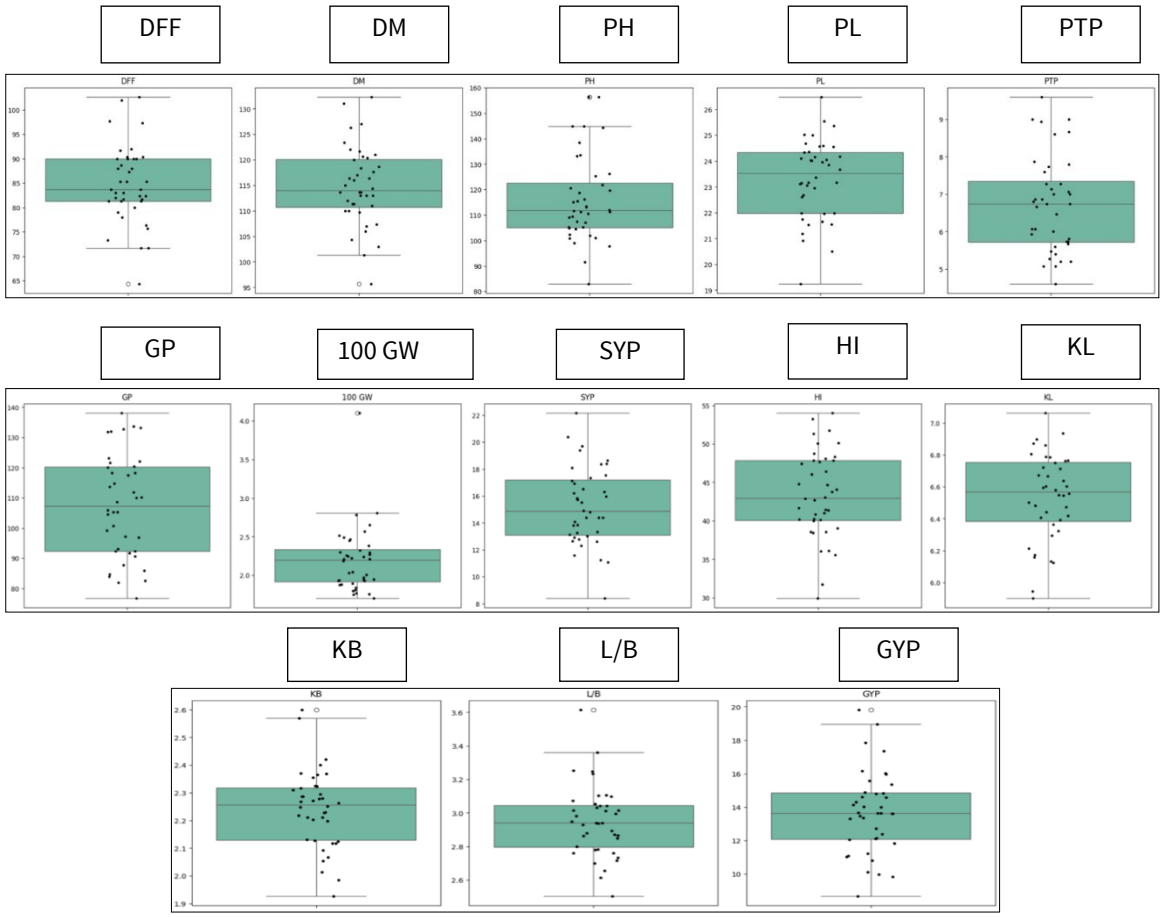


Fig. 1. Box plots displaying mean performance of the studied traits.

DFF - Days to 50% Flowering; DM - Days to Maturity; PH - Plant Height; PL - Panicle Length; PTP - Productive Tillers per Plant; GP - Grains per Plant; 100 GW - 100-Grain Weight; SYP - Straw Yield per Plant; HI - Harvest Index; KL - Kernel Length; KB - Kernel Breadth; L/B - L/B ratio; GYP - Grain Yield per Plant

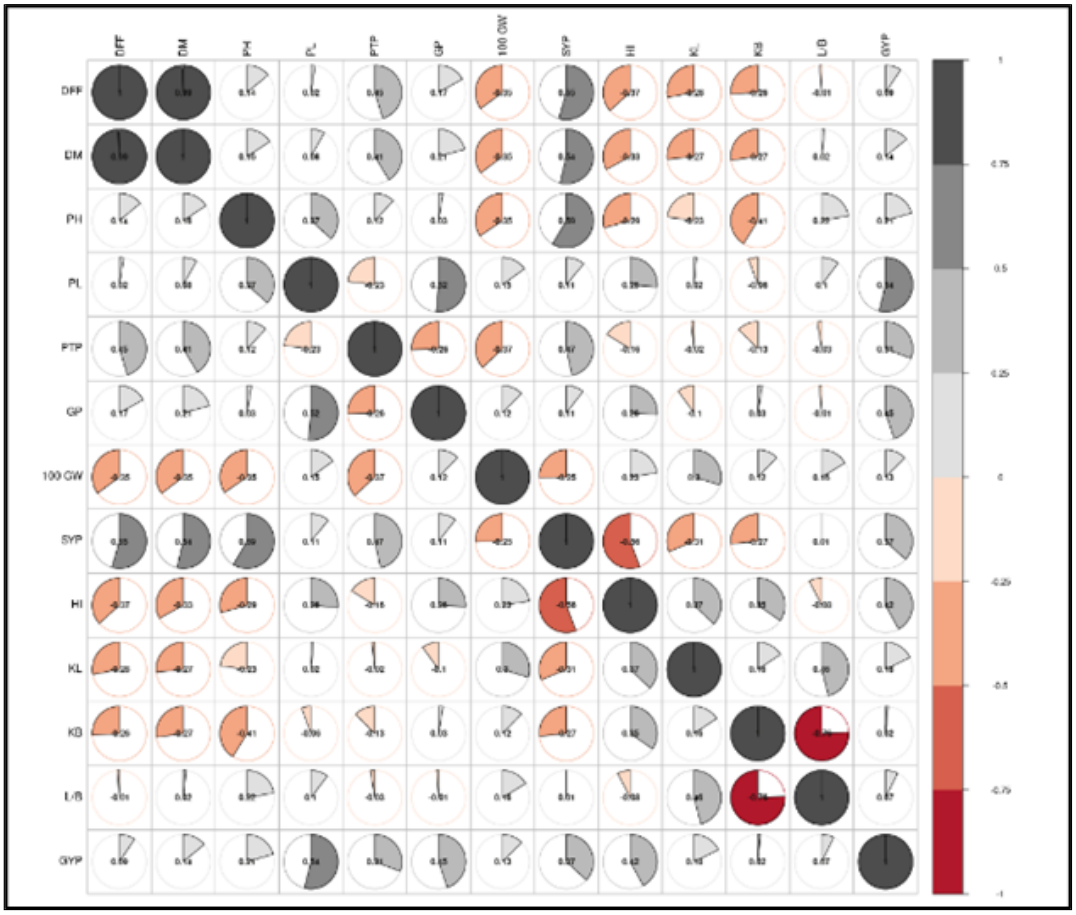


Fig. 2. Correlation matrices of the studied traits.

Principal component and biplot analysis

PCA, a sophisticated multivariate data analysis tool, was specifically utilized in this study to simplify and interpret complex, high-dimensional datasets. We applied PCA to enhance our ability to differentiate between the measured traits based on their relationships. This method enabled the identification of key traits that contributed the most to overall variability, providing deeper insights into trait interactions. Among the 13 principal components (PCs), five components exhibited Eigenvalues greater than 1, accounting for 82.13 % of the cumulative variability for the traits under investigation (Supplementary Table 3) and Fig. 3A.

The cumulative contribution rate was 82.13 %. Principal Component I (PC I) had an Eigenvalue of 3.76, contributing 29 % of the total variability. Germplasm in PC I had the most significant positive impact on DFF, DM and SYP. Principal Component II (PC II) exhibited an Eigenvalue of 2.407, explaining 18.51 % of the variability. The germplasm in PC II showed positive effects on PL, GP, 100GW, HI, L/B and GYP. Principal Component III (PC III) had an Eigenvalue of 1.91, accounting for 14.69 % of the variability. Germplasm in PC III positively influenced KB. Principal Component IV (PC IV) showed an Eigenvalue of 1.42, contributing 10.92 % of the total variability, with a favorable impact on PTP and KL. Principal Component V (PC V) had an Eigenvalue of 1.175, explaining 9.03 % of the variability, with positive results for PH (Supplementary Table 3). Germplasm lines exhibiting maximum positive PC scores and common presence in PC1, PC2, PC3, PC4 and PC5 are lines 22KSMF4-6, 22KSMF4-8, 22KSMF4-14, 22KSMF4-19, 22KSMF4-23 and 22KSMF4-31 (Supplementary Table 4). Selecting these lines can contribute significantly to the further development of new high yielding with grain quality varieties. The cos^2 (squared cosines or squared coordinates) values are used to assess the quality of variable representation on the factor map. A high cos^2 value signifies a strong representation of the variable on the principal component, whereas a low cos^2 value indicates that the variable is not well represented by the PCs (Fig. 3B).

The PC-1-2 biplot in Fig. 3C illustrates trait variability, inter-trait correlations and genotype dispersion. Most traits displayed relatively long vector lengths, except for L/B, KL and KB, suggesting significant variability. The acute angle (<90°) between the vectors indicates a strong positive correlation among the traits. So, the traits like GYP, PL, GP, HI, 100 GW and SYP were positively correlated with each other. Similarly, DFF, DM, PH, PTP and SYP exhibited a positive correlation among themselves. Conversely, an obtuse angle (>90°) between the vectors signifies a negative correlation, indicating that GYP was negatively correlated with KB. Similarly, the traits SYP, HI, KL, KB, 100GW, DFF, DM and L/B were negatively correlated among themselves. The genotypes 22KSMF4-4, 22KSMF4-10, 22KSMF4-31, 22KSMF4-36, 22KSMF4-27, 22KSMF4-20 and 22KSMF4-16 exhibited the highest diversity for various traits, as they were positioned far from the origin (Fig. 3D).

Selection of high yielding and good grain quality genotypes using MGIDI

There are various drawbacks to PCA that can make it difficult to pick high yielding genotypes. These include subjective interpretation, difficulties managing missing data, inadequate dimensionality reduction, the inability to consider interaction effects and lack of statistical rigor. To address these constraints, it is critical to incorporate additional analytical approaches. PCA can be integrated with quantitative indicators such as the MGIDI to help identify short duration, high yielding with good grain quality genotypes. The MGIDI is an ideal and innovative method for genotypic selection due to its ability to address multicollinearity and eliminate the need for assigning economic weights (27).

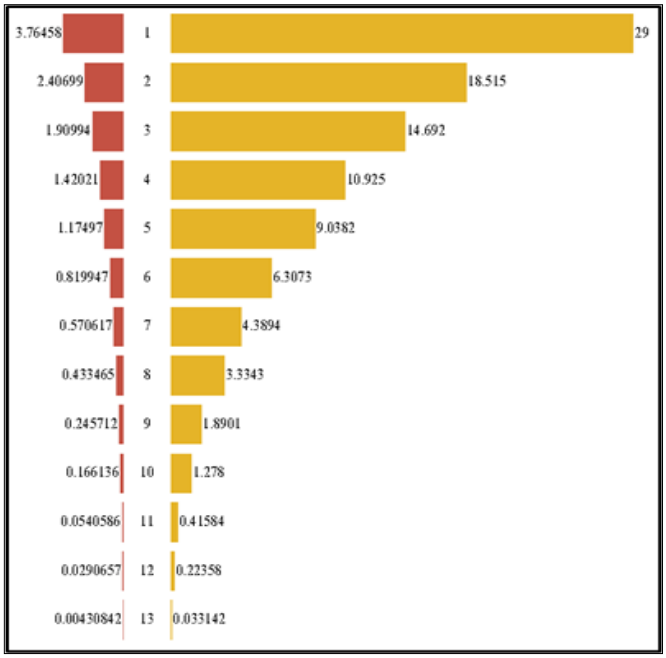
Selection of genotypes using MGIDI

The MGIDI index identified four lines 22KSMF4-8, 22KSMF4-5, 22KSMF4-6 and 22KSMF4-14 as high-performing for multiple traits, demonstrating significant potential for simultaneously improving the 13 measured traits in rice breeding programs (Fig. 4A). These genotypes were particularly notable for traits such as early flowering, short stature and early maturity. Among them, 22KSMF4-14, positioned near the cut-off point indicated by the red line, displayed intriguing characteristics warranting further investigation, as suggested (16). Successful applications of this selection index had been demonstrated in evaluating ideal yield and yield-related traits across various crops including guar (36), wheat (37) and brinjal (38). These different studies demonstrated the effectiveness of multivariate selection indices for simultaneous trait selection. The MGIDI is the most efficient index for choosing genotypes with desirable features, demonstrating its relevance and usefulness in crop development (16). These selected derivatives serve as the foundation for establishing recombinant populations through judicious crossings, ensuring maximum genetic diversity for the breeding of novel rice lines.

Strength and weakness

Fig. 4B illustrates the relative strengths and weaknesses of the examined genotypes, as determined by total of five factors (FA1, FA2, FA3, FA4 & FA5) each factor's contribution to the MGIDI score for each genotype. MGIDI serves as a valuable graphical tool that highlights the strengths and weaknesses of genotypes, offering insights into how they perform in traits that require enhancement. A strength-weakness analysis revealed that FA1 had the greatest influence on 22KSMF4-14, FA2 on 22KSMF4-8 and FA3 on 22KSMF4-6. FA4 contributed most significantly to 22KSMF4-5, while FA5 had the highest impact on 22KSMF4-14. A similar methodology was employed to assess the performance of 13 strawberry cultivars (16). In another study, (36) utilized MGIDI to identify promising guar genotypes with high gum and seed yield across three seasons. Likewise, MGIDI as an effective tool for enhancing selection methods in breeding climate-resilient maize hybrids, evaluating their performance under varying moisture and drought conditions (39). Furthermore, MGIDI was applied in quinoa to analyze the effects of different plant spacing strategies (40).

In our study, MGIDI is applied to upland cotton, providing a comprehensive framework for identifying



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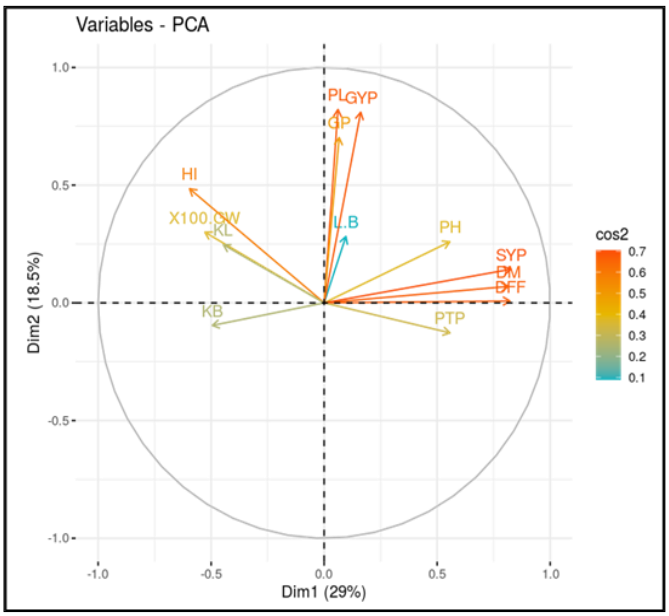

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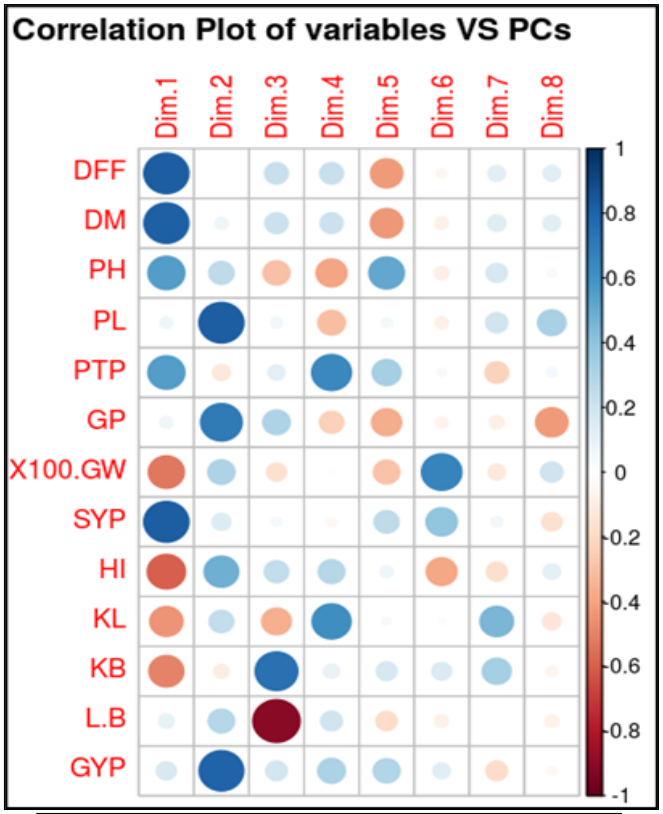
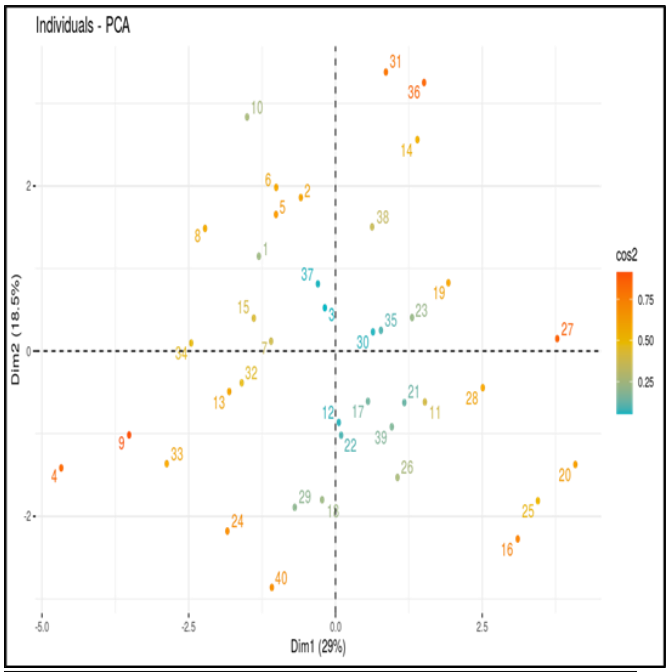


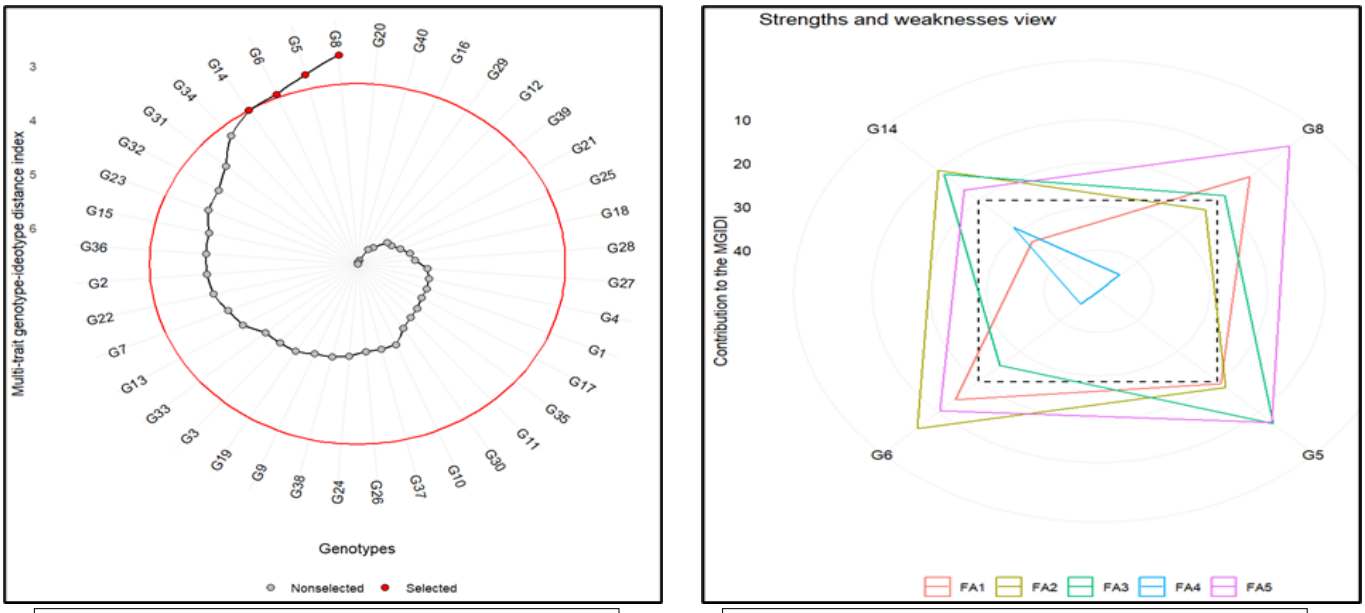
B



D

Fig. 3. Summary of Panels (A) Depict butterfly bar charts showing the variable percentage contribution of each Principal Component (PC) as well as the eigenvalue. (B) Quality of representation of different traits (cos2). Panels (C) and (D) display biplots involving PC1 and PC2, illustrating the allocation of 13 traits and 40 lines respectively.

Scattered numbers across the plot indicates serial number of lines enlisted in materials and methods.



(A) Selection of genotypes **(B) Strengths and Weaknesses**

Fig. 4. The MGIDI analysis of lines ordering is presented in ascending order (A, B). The genotypes with the highest rankings and selection are highlighted in red. The central red circle indicates the cut-off point, determined by the selection pressure (A). The percentage contribution of each factor in the generated MGIDI index illustrates the strengths and weaknesses of each lines (B). The closer a factor's indices are to the ideotype, the lower the fraction of explanation, indicating proximity to the outer boundary.

genotypes with both high yield and superior quality traits, which are well-suited for hybrid development. The detailed examination of strengths and weaknesses yielded useful insights, emphasising the importance of selecting the best rice genotype with superior quantitative traits. These selected genotypes stood out as promising candidates for future breeding projects, establishing MGIDI as a revolutionary technique for improving rice varieties with early maturity and yield attributes.

Molecular screening of F_4 progenies for blast resistance

Among the 36 selected F_4 progenies, a single plant was chosen from homozygous blast-resistant lines, while three plants were selected from heterozygous blast-resistant lines for further molecular analysis of blast resistance genes. A total of 76 F_4 plants, including 27 from *Gurjari* × *Tetep* and 49 from *Gurjari* × *GNR-9*, were selected from 36 progenies for molecular screening, along with four check varieties (*Gurjari, GNR-9, Tetep* and *Lalkada*). The molecular analysis was conducted using markers RM206, RM246 and YL87/155, linked to *PiKh/Pi54*, *Pitp(t)* and *Pi67*, respectively (Supplementary Table 5).

Molecular screening with RM206 revealed that 29 plants exhibited genetic similarity to Gurjari, 32 carried the allele of Tetep and GNR-9 and 20 were heterozygous, amplifying both alleles (Fig. 5). Similarly, screening with RM246 identified two plants resembling Gurjari, 48 carrying the Tetep and GNR-9 allele and 26 as heterozygous (Fig. 6). Analysis using YL87/155 showed that 51 plants exhibited genetic similarity to Tetep and GNR-9, while 25 resembled Gurjari (Fig. 7). Among the 76 F_4 plants derived from 36 progenies, 17 plants possessed all three blast resistance genes, while 14 plants carried two resistance genes with one in a heterozygous state.

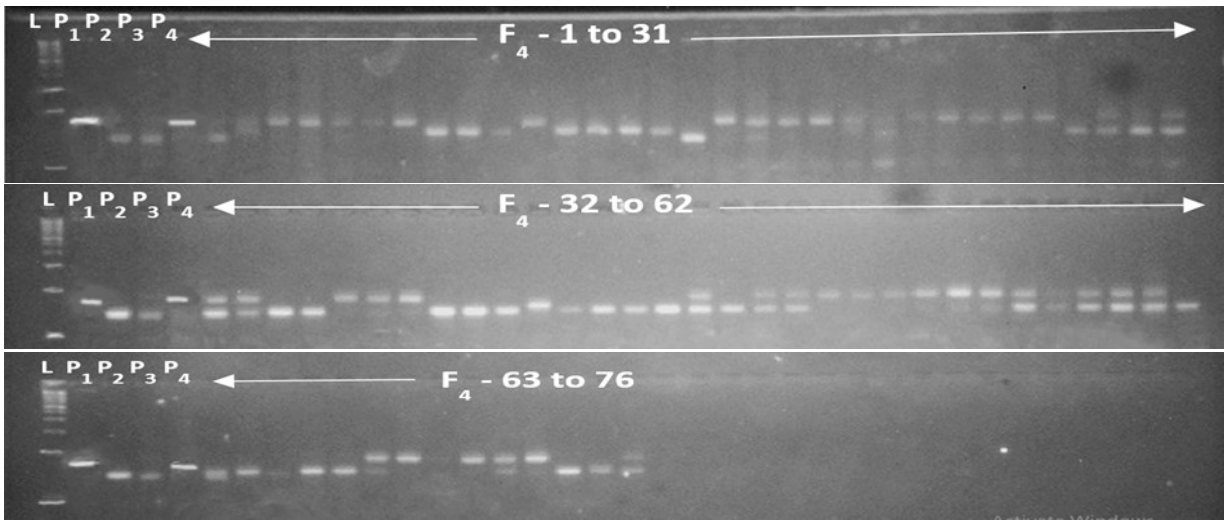


Fig. 5. Genotyping of F_4 progenies for blast resistant gene *Pi54*/*Pik*^{h} along with parents using marker RM206. Where, L: 100 bp ladder, P_1: Parent 1 (Gurjari), P_2: Parent 2 (Tetep), P_3: Parent 3 (Lalkada), P_4: Parent 4 (GNR-9), F_4: 76 F_4 plants selected from a cross Gurjari × Tetep and Gurjari × GNR-9 in F_3 population.

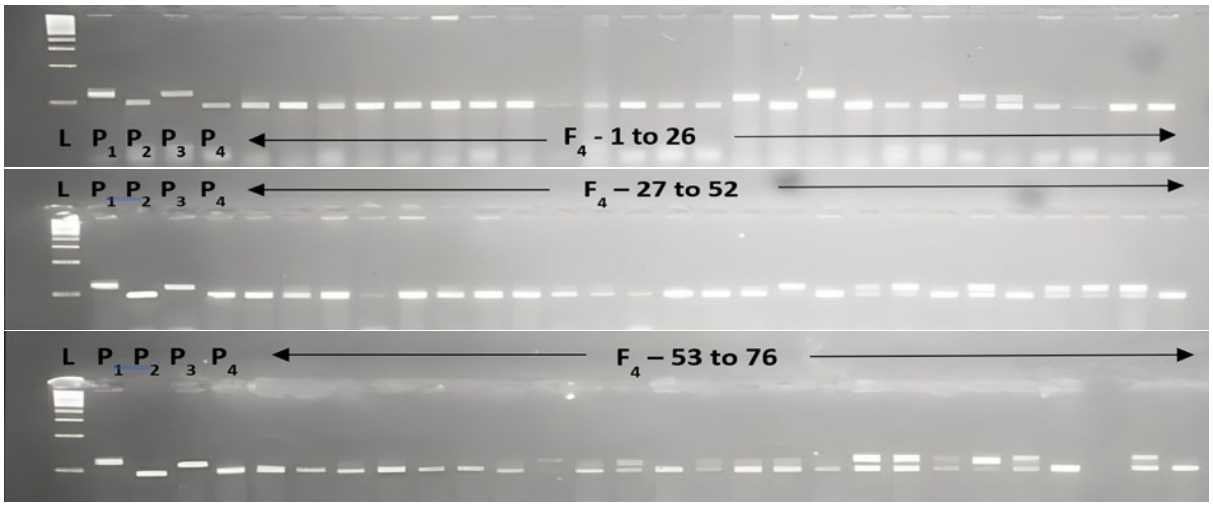


Fig. 6. Genotyping of F_4 progenies for blast resistant gene *Pitp(t)* along with parents using marker RM 246. Where, L: 100 bp ladder, P_1: Parent 1 (Gurjari), P_2: Parent 2 (Tetep), P_3: Parent 3 (Lalkada), P_4: Parent 4 (GNR-9), F_4: 76 F_4 plants selected from a cross Gurjari × Tetep and Gurjari × GNR-9 in F_3 population.

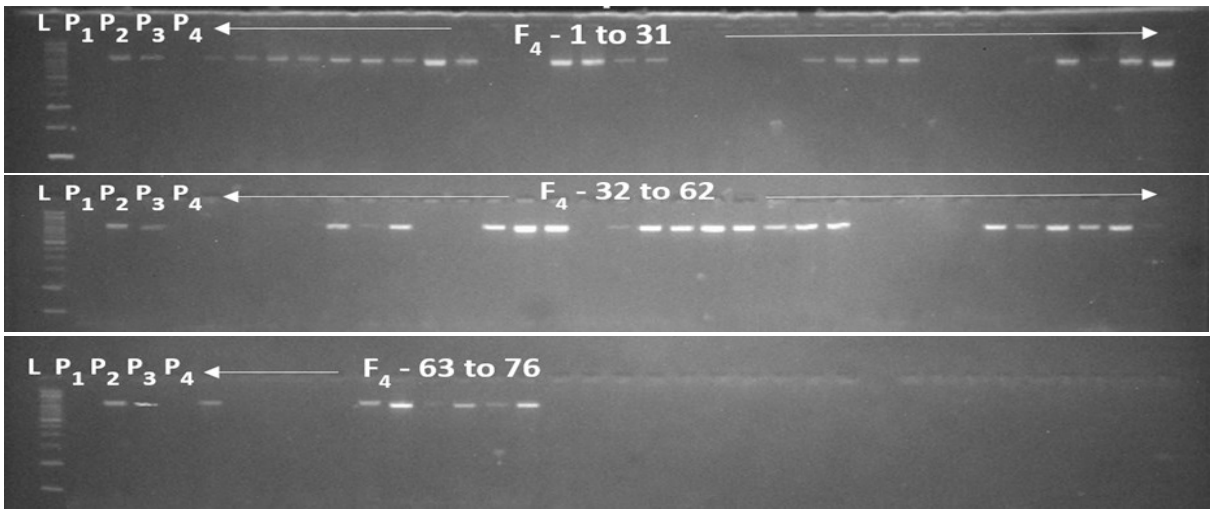


Fig. 7. Genotyping of F_4 progenies for blast resistant gene *Pi67* along with parents using marker YL87/155. Where, L: 100 bp ladder, P_1: Parent 1 (Gurjari), P_2: Parent 2 (Tetep), P_3: Parent 3 (Lalkada), P_4: Parent 4 (GNR-9), F_4: 76 F_4 plants selected from a cross Gurjari × Tetep and Gurjari × GNR-9 in F_3 population.

Conclusion

Molecular screening of F_3 and F_4 progenies identified nine F_4 lines (22KSMF4-1, 22KSMF4-2, 22KSMF4-6, 22KSMF4-8, 22KSMF4-9, 22KSMF4-16, 22KSMF4-17, 22KSMF4-22 and 22KSMF4-32) carrying all three blast resistance genes (*PiKh/Pi54*, *Pitp(t)* and *Pi67*). Among them, 22KSMF4-6 and 22KSMF4-8 (Gurjari × GNR-9) exhibited superior yield potential while maintaining resistance to blast, outperforming all checks. These lines hold promise as potential donors for blast resistance in breeding programs and could be developed into high-yielding, disease-resistant varieties for cultivation in blast-prone regions of Gujarat, following extensive multi-location and multi-year evaluations.

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Authors' contributions

CPC was the primary drafter of the manuscript, contributed to writing, review, figure and editing and performed data analysis. MCS was responsible for data curation, data analysis and investigation. VPP, KM, VBP and RKP designed the overall study, established the methodology and provided project administration and supervision. DS and HPV assisted in drafting the original manuscript and contributed to its review and editing. All authors reviewed and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: None; The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical issues: None

References

1. Pradhan SK, Barik SR, Nayak DK, Pradhan A, Pandit E, Nayak P, et al. Genetics, molecular mechanisms and deployment of bacterial

blight resistance genes in rice. Critical Reviews in Plant Sciences. 2020;39(4):360–85. https://doi.org/10.1080/07352689.2020.1801559

2. Ou SH. Rice disease. 2nd edn. CAB International, Wallingford, Oxon, UK. 1985.

3. Sagar V, Dhawan G, Gopalakrishnan S, Vinod KK, Ellur RK, Mondal KK, et al. Marker-assisted introgression of genes governing resistance to bacterial blight and blast diseases into an elite Basmati rice variety, "Pusa Basmati 1509". Euphytica. 2020;216:1–18. https://link.springer.com/article/10.1007/s10681-019-2549-4

4. Sester M, Raveloson H, Tharreau D, Becquer T. The difference in blast development in upland rice grown on Andosol vs Ferralsol. Journal of Crop Protection. 2020;115:40–6. https://doi.org/10.1016/j.cropro.2018.09.010

5. Ashikawa I, Hayashi N, Yamane H, Kanamori H, Wu J, Matsumoto T, et al. Two adjacent nucleotide-binding site leucine-rich repeat class genes are required to confirm Pikm-specific rice blast resistance. *Genetics.* 2008;180:2267–76. https://doi.org/10.1534/genetics.108.095034

6. Bryan GT, Wu KS, Farrall L, Jia YL, Hershey HP, McAdams SA, et al. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene Pi-ta. *Plant Cell*. 2008;12:2033–45. https://doi.org/10.1105/tpc.12.11.2033

7. Chen XW, Li SG, Ma YQ, Li HY, Zhou KD, Zhu LH. Marker-assisted selection and pyramiding for three blast resistance genes, Pid(t)1, Pi-b, Pi-ta2, in rice. Shengwu Gongcheng Xuebao - Chinese Journal of Biotechnology. 2008;20(5):708–14. https://europepmc.org/article/med/15973995

8. Padmanabhan SY. Blast resistance in India. International Rice Research Institute. Rice blast workshop. Los Baños, Laguna, Philippines. 1979:49-61.

9. Singh UM, Dixit S, Alam S, Yadav S, Prasanth VV, Singh AK, et al. Marker-assisted forward breeding to develop a drought, bacterial leaf blight and blast-resistant rice cultivar. The Plant Genome. 2022;15(1):e20170. https://doi.org/10.1002/tpg2.20170

10. Balat JR, Patel VP, Visat ML, Bhagora RN. Variability analysis in F_2 population of rice (*Oryza sativa* L.) for yield and related traits. International Journal of Pure and Applied Bioscience. 2018;6(1):1021–7. https://krishikosh.egranth.ac.in/items/6b0ed2b6-cd61-4131-bf64-70209327ad02

11. Patel HR, Patel VP, Patel PB, Rathod AJ, Pampaniya AG. Genetic variability, correlation and path analysis for grain yield and component traits in F_3 segregating population of rice (*Oryza sativa* L.). International Journal of Chemical Studies. 2018;6(2):2327–31.

12. Prajapati MR, Bala M, Patel VP, Patel RK, Sushmitha US, Kyada AD, et al. Analysis of genetic variability and correlation for yield and its attributing traits in F_2 population of rice (*Oryza sativa* L.). Electron Journal Plant Breeding. 2022;13(3):983–90. https://ejplantbreeding.org/index.php/EJPB/article/view/4354

13. Grzesiak M, Hordyńska N, Maksymowicz A, Grzesiak S, Szechyńska-Hebda M. Variation among spring wheat (*Triticum aestivum* L.) genotypes in response to drought stress. II-Root system structure. *Plants*. 2019;8(12):584. https://doi.org/10.3390/plants8120584

14. Al-Ashkar I, Alderfasi A, El-Hendawy S, Al-Suhaibani N, El-Kafafi S, Seleiman MF. Detecting salt tolerance in doubled haploid wheat lines. Agronomy. 2019;9(4):211. https://doi.org/10.3390/agronomy9040211

15. El-Hendawy S, Al-Suhaibani N, Al-Ashkar I, Alotaibi M, Tahir MU, Solieman T, et al. Combining genetic analysis and multivariate modeling to evaluate spectral reflectance indices as indirect selection tools in wheat breeding under water deficit stress conditions. Remote Sensing. 2020;12(9):1480. https://doi.org/10.3390/rs12091480

16. Olivoto T, Nardino M. MGIDI: Toward an effective multivariate selection in biological experiments. Bioinformatics. 2021;37(10):1383–9. https://doi.org/10.1093/bioinformatics/btaa981

17. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry. 1990;19:11-5. https://worldveg.tind.io/record/33886/

18. Fuentes JL, Correa-Victoria FJ, Escobar F, Prado G, Aricapa G, Duque MC, et al. Identification of microsatellite markers linked to the blast resistance gene Pi-1(t) in rice. Euphytica. 2008;160:295–304. https://link.springer.com/article/10.1007/s10681-007-9497-0

19. Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theoretical and Applied Genetics. 2000;100:1121–28. https://link.springer.com/article/10.1007/s001220051395

20. Sharma TR, Madhav MS, Singh BK, Shanker P, Jana TK, Dalal V, et al. High-resolution mapping, cloning and molecular characterization of the *Pi-kh* gene of rice, which confers resistance to *Magnaporthe grisea*. Molecular Genetics and Genomics. 2005;274(6):569-78. https://link.springer.com/article/10.1007/s00438-005-0035-2

21. Fjellstrom R, Conaway-Bormans CA, McClung AM, Marchetti MA, Shank AR, Park WD. Development of DNA markers suitable for marker-assisted selection of three Pi genes conferring resistance to multiple *Pyricularia grisea* pathotypes. Crop Science. 2004;44(5):1790–98. https://doi.org/10.2135/cropsci2004.1790

22. Barman SR, Gowda M, Venu RC, Chattoo BB. Identification of a major blast resistance gene in the rice cultivar 'Tetep'. Plant Breeding. 2004;123(3):300–2. https://doi.org/10.1111/j.1439-0523.2004.00982.x

23. Jia Y, Redus M, Wang Z, Rutger N. Development of a SNLP marker from the Pi-ta blast resistant gene by tri-primer PCR. Euphytica. 2004;138:97–105. https://link.springer.com/article/10.1023/B:EUPH.0000047079.42768.4d

24. Joshi S, Dhatwalia S, Kaachra A, Sharma KD, Rathour R. Genetic and physical mapping of a new rice blast resistance specificity Pi-67 from a broad-spectrum resistant genotype Tetep. Euphytica. 2019;215(1):1–11. https://link.springer.com/article/10.1007/s10681-018-2332-y

25. Sivasubramanian S, Menon M. Heterosis and inbreeding depression in rice. Madras Agricultural Journal. 1973;60(7):1139–40. https://www.cabidigitallibrary.org/doi/full/10.5555/19741623222

26. Johnson HW, Robinson H, Comstock RF. Estimates of genetic and environmental variability in soybean. Journal of Agronomy. 1955;47(1):314-8. https://www.cabidigitallibrary.org/doi/full/10.5555/19561600791

27. Olivoto T, Lúcio AD metan: An R package for multi-environment trial analysis. Methods in Ecology and Evolution. 2020;11:783–9. https://doi.org/10.1111/2041-210X.13384

28. Singh M, Kumar K, Singh RP. Study of coefficient of variation, heritability and genetic advance in hybrid rice. Oryza. 2007;44:160–2. https://www.indianjournals.com/ijor.aspx?target=ijor:oryza&volume=44&issue=2&article=016

29. Sravan T, Rangare NR, Suresh BG, Kumar SR. Genetic variability and character association in rainfed upland rice (*Oryza sativa* L.). Journal of Rice Research. 2012;5(1):2. https://sarr.co.in/wp-content/uploads/03-JRR-Vol-5_-Sravan-et-al.pdf

30. Kahani F, Hittalmani S. Genetic analysis and traits association in F_3 intervarietal populations in rice under aerobic condition. Journal of Rice Research. 2015;3:152. https://www.researchgate.net/profile/Farhad-Kahani/publication/284717209

31. Meena AK, Suresh J, Mahanthesha M, Meena HP. Genetic variability, heritability and genetic advance for yield and yield components in rice (*Oryza sativa* L.). The Ecoscan. 2015;9(4):1053–6. https://www.researchgate.net/publication/325736406

32. Priyanka GP, Senguttuvel M, Sujatha N, Sravanraju P, Beulah P, Naganna P, et al. Correlation between traits and path analysis coefficient for grain yield and other components in direct-seeded aerobic rice (*Oryza sativa* L.). Advanced Research Journal of Crop Improvement. 2016;7(1):40–5. https://www.researchgate.net/profile/Brajendra-Parmar/publication/335965289

33. Sameera SK, Srinivas T, Rajesh AP, Jayalakshmi V, Nirmala PJ. Variability and path coefficient for yield and yield components in rice. Bangladesh Journal of Agricultural Research. 2016;41(2):259–71. https://banglajol.info/index.php/BJAR/article/view/28229

34. Tulasi G, Padma V, Reddy DVV, Rao PR, Rao SD. Correlation and path coefficient analysis for grain yield and other component traits in rice genotypes. International Journal of Agricultural Science and Research. 2016;6(5):363–70. https://www.researchgate.net/profile/D-Reddy-10/publication/324588078

35. Nath S, Kole PC. Genetic variability and yield analysis in rice. Electronic Journal of Plant Breeding. 2021;12(1):253–8. https://ejplantbreeding.org/index.php/EJPB/article/view/3508

36. Benakanahalli NK. A framework for identification of stable genotypes based on MTSI and MGIDI indexes: An example in guar (*Cymopsis tetragonoloba* L.). Agronomy. 2021;11(6):1221. https://doi.org/10.3390/agronomy11061221

37. Meier C, Marchioro V, Meira D, Olivoto T, Klein L. Genetic parameters and multiple-trait selection in wheat genotypes. Pesquisa Agropecuária Tropical. 1953;*51*:e67996. https://www.scielo.br/j/pat/a/9VFXs5WbXgjJpmyNj8PjjRK/

38. Uddin S, Billah M, Afroz R, Rahman S, Jahan N, Hossain M, et al. Evaluation of 130 eggplant (*Solanum melongena* L.) genotypes for future breeding program based on qualitative and quantitative traits and various genetic parameters. Horticulturae. 2021;7(10):376. https://doi.org/10.3390/horticulturae7100376

39. Singamsetti A, Zaidi PH, Seetharam K, Vinayan MT, Olivoto T, Mahato A, et al. Genetic gains in tropical maize hybrids across moisture regimes with multi-trait-based index selection. Front Plant Science. 2023;14:1147424. https://doi.org/10.3389/fpls.2023.1147424

40. Ahmed SR, Ali Z, Ijaz I. Multi-trait selection of quinoa ideotypes at different levels of cutting and spacing. Sustainability. 2023;15(14):11446. https://doi.org/10.3390/su151411446

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