RESEARCH ARTICLE

Marker-assisted introgression of a major broad spectrum blast resistance gene *Pi54* into a popular rice variety Warangal Samba (WGL-14)

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Abstract

Warangal Samba (WGL-14) is a widely known medium slender-grain rice variety that matures within 135-140 days. This variety has been extensively cultivated in Telangana, India, due to its high yield potential of 7-7.5 t/ha and its favourable cooking qualities. However, it is highly susceptible to rice blast disease caused by the fungus *Magnaporthe oryzae*. This research sought to improve the blast resistance of WGL-14 by incorporating the *Pi54* gene, a widely recognized blast resistance gene, using (MABB) along with selection based on phenotypic traits. NLR-145, a high-yielding rice variety containing the *Pi54* gene, was used as the donor parent and crossed with WGL-14 to produce F_1 plants. The Pi54MAS marker, which is specific to the *Pi54* gene, was employed for foreground selection in F_1, BC_1F_1, BC_2F_1 and BC_3F_1 generations, while background selection involved 80 parentals polymorphic SSR markers and phenotypic traits observed in each backcross generation. At BC_3F_5 generation, 28 lines were selected for their strong resistance to blast, high yields, plant type, grain characteristics and other traits comparable to the recurrent parent. These 28 lines were tested in yield trials during the wet seasons from 2018 to 2020 at the Regional Agricultural Research Station (RARS) in Warangal. At Advanced Varietal Trials (AVT), 3 lines namely WGL-1467, WGL-1472 and WGL-1473 were identified as having strong blast resistance and superior agronomic traits, closely resembling the original WGL-14.

Keywords

grain yield; *Pi*54; rice blast resistance; Warangal Samba

Introduction

Rice (*Oryza sativa* L.) serves as a staple food for more than half of the global population. Over the years, rice cultivation has expanded significantly in India, growing from 30.81 million ha in 1950-51 to 43.86 million ha in 2014-15, representing a 142 % increase in area (1). Concurrently, rice production surged nearly fivefold, from 20.58 million tonnes to 104.86 million tonnes during the same period (1). As the world's largest rice producer, India contributes 40 % of

global rice exports (2). Projections suggest that by 2030, rice production in India could reach 160 million tonnes and further rise to 259 million tonnes by 2050, driven by growing population demands (3).

However, increasing rice production is hindered by several challenges, with one of the most significant being rice blast disease, which is caused by the fungal pathogen *Magnaporthe oryzae* (teleomorph: *Pyricularia oryzae*) (4). This disease is among the most destructive rice diseases globally, capable of reducing yields by over 50 % during severe outbreaks, posing a major threat to food security (5, 6). In India, rice blast was first documented in 1919, resulting in an estimated 4 % yield loss (7). Over time, this disease has intensified, particularly in Eastern India, where it causes up to 50 % yield losses (4). One of the most effective strategies to combat rice blast is through the development of host-plant resistance (8, 9). Over the years, plant breeders have developed numerous blast-resistant rice varieties using traditional breeding methods (6). However, due to the lengthy breeding cycles and limited selection efficiency, there has been a shift towards Marker-Assisted Selection (MAS) for developing resistant varieties (10). To date, around 130 major resistance genes have been identified, with 30 of them molecularly cloned (11, 12). The *Pi54* gene offers broad-spectrum resistance against various *Magnaporthe oryzae* isolates, including PLP-1 and 37-1, which are prevalent in the North-Western Himalayas. Hz-1 poses a threat in Eastern India, while PB-1primarily affects Basmati rice in North India. These strains impact rice production in different regions of India (13). The mechanism of *Pi54* involves inducing callose synthesis, which forms a physical barrier to block fungal penetration. The gene encodes a protein with NBS-LRR domains and a unique zinc finger motif (14). Functional markers for the *Pi54* have been developed (15) and this gene has been successfully incorporated into various rice cultivars and hybrid parental lines through marker-assisted selection (16-21). This study aimed to introduce the broad-spectrum blast resistance gene *Pi54* into the widely grown and popular rice variety WGL-14 through Marker-Assisted Backcross Breeding (MABB), while also selecting for grain type and other agro-morphological traits based on phenotype.

Materials and Methods

Plant material

Warangal Samba (WGL-14), a popular rice variety known for its high yield medium duration (135-140 days), medium slender grains with a yield potential of 7-7.5 t/ha. It was developed from a cross between BPT-5204 and IRC5984/BPT3291 and was released in 2005. WGL-14 was used as the recipient parent in this study. The donor parent, NLR-145, which contains the major blast resistance gene *Pi5*4, was developed from a cross between CICA-4 and IR-625-23-3-1/Tetep. Additionally, Taichung Native 1 (TN1) was utilized as a susceptible control in screening backcross-derived lines for resistance to blast disease.

Crossing scheme

A **Marker-Assisted Backcross Breeding (MABB)** approach was employed to introduce a major blast resistance gene

Pi54 into WGL-14. The experiment was started during *kharif* 2012 at Regional Agricultural Research Station (RARS), Professor Jayashankar Telangana Agricultural University (PJTAU), Telangana, India. NLR-145 was used as the male parent and crossed with Warangal Samba (WGL-14) to generate F_1s (Fig. 1). The true F_1s were backcrossed to Warangal Samba (WGL-14) to obtain BC_1F_1s, which were then screened with PCR based molecular marker linked to the target gene for selection of plants possessing the resistance allele of *Pi54* in heterozygous condition. The positive BC_1F_1 plants were then screened for selection of maximum recurrent parent genome recovery. The backcrossing progressed up to the BC_3 generation and from there, plants were advanced through the BC_3F_1, BC_3F_2, BC_3F_3, BC_3F_4 and BC_3F_5 generations following the pedigree method.
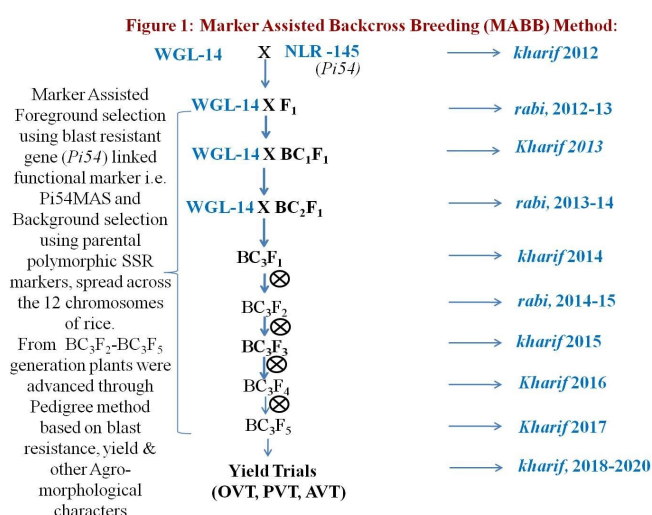

Fig. 1. Crossing Scheme and flow chart adopted in marker-assisted introgression of a major broad-spectrum blast resistance gene *Pi54* into a popular rice variety Warangal Samba (WGL-14) through marker-assisted backcross breeding method (MABB).

Marker assisted selection for Blast Resistance and Recurrent Parent Genome (RPG) recovery

DNA was isolated from the parents and backcross progenies by following the protocol (22) with few modifications. The PCR based functional marker Pi54MAS (15) (Table S1) was used to confirm the presence of the resistant allele of *Pi54* in the F_1- generation and subsequent backcross generations. For selection of maximum recurrent parent genome recovery (RPGR) in BC_1F_1, BC_2F_1 and BC_3F_1 plants were screened using parental polymorphic SSR markers spread across all the 12 chromosomes of rice (Table S2). The PCR mixture contained 50 ng template DNA, 5 pmoles of each primer, 0.05 mM dNTPs, 1X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/mL gelatin) and 1 unit of Taq DNA polymerase (Fermentas, Lithuania) in a reaction volume of 10 µL. The thermal cycling conditions included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec, extension at 72 °C for 1 min and a final extension at 72 °C for 7 min. The PCR amplified product of Pi54MAS was resolved on a 1.2 % Seakem LE agarose gel (Lonza, USA), while the PCR products of SSR markers used for background selection were resolved on 3.5 % Seakem LE agarose gels containing 0.5 mg/mL ethidium bromide in 0.5x TBE buffer and visualized under UV light.

(RPG %) was calculated by using the formula: = (R+1/2H) x 100/P

Where R = Number of markers homozygous for RP allele, H = Number of heterozygous markers, Total number of SSR markers utilized for background selection.

Screening for Blast Resistance

A highly virulent strain of *Magnaporthe oryzae* was obtained from the Rice Research Institute, Rajendranagar, Hyderabad, Telangana, India, to evaluate the blast resistance of donor and recurrent parents, as well as the BC_3F_5 lines and Taichung Native 1 was used as control throughout the trials. This screening was conducted under *in vivo* conditions using the uniform blast nursery method i.e. during *rabi* season from November to January, where the minimum temperatures were recorded below 15 °C and which was favourable for the initiation and spread of blast disease at the Regional Agricultural Research Station (RARS), Warangal. The pathogen cultures were prepared and maintained following established protocols (3). At the four-leaf stage, seedlings were inoculated with a fungal conidial suspension at a concentration of 1×10^6 cfu/mL, with high humidity maintained to promote disease development. One week after inoculation, blast lesions were evaluated and plants were scored for blast resistance using a 0-9 scale based on the IRRI-SES guidelines (23).

Evaluation of Agronomic Traits

Seedlings that were 30 days old from the backcross-derived lines were transplanted into the main field at a spacing of 15 x 20 cm, alongside the donor and recurrent parent plant. During 2018, at the BC_3F_5 generation, 28 lines developed from the cross between WGL-14 and NLR-145 were selected and advanced to the observation varietal trial (OVT). These lines were chosen based on their resistance to blast, high grain yield (GY) and key agro-morphological traits such as days to 50 % flowering (DFF), panicle length (PL), plant height (PH), 1000-seed weight (SW), number of grains per panicle (NGP) and grain type resembling the recurrent parent. From the 28 OVT lines, 8 lines were further advanced to the preliminary varietal trial (PVT) during 2019 based on their performance for the desired traits. Of these, 5 lines were promoted to the advanced varietal trial (AVT) during 2020 based on their blast resistance and other agro-morphological traits similar to WGL-14. Standard agronomic practices were implemented to ensure healthy crop growth and evaluations were carried out during the wet seasons of 2018, 2019 and 2020. Data on the specified traits were collected from each entry, which was conducted using a randomized complete block design (RCBD) with 2 replications.

Statistical Analysis

Descriptive statistics were calculated using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). To identify significant differences among the pooled trait means, Fisher's protected least significant difference (LSD) test was applied with a significance level of $p \leq 0.05$, utilizing the "Agricolae" R package (v.4.2.3). Correlation and Path Analysis - effects plots were created using the "Corrplot" and "Agricolae" R packages (24).

Results

Marker-Assisted Selection for Blast Resistance

In the cross between WGL-14 and NLR-145, F_1 plants were tested for the presence of the major blast resistance gene *Pi54* using the functional marker *Pi54*MAS (15), allowing for the identification of true F_1 plants exhibiting a heterozygous amplification pattern. Among the 125 F_1 plants, eight were confirmed to carry the *Pi54* gene in heterozygous form and were backcrossed with WGL-14 to generate 196 BC_1F_1 plants. Out of these, 98 BC_1F_1 plants were identified as heterozygous for *Pi54 (Fig. 2a)*. From these 98 plants, 15 were selected based on their plant type, grain type and other agro-morphological traits similar to WGL-14. These plants were then screened for background selection using 80 parentals polymorphic SSR markers distributed across all 12 rice chromosomes (Table S2). One BC_1F_1 plant, SN54 (Fig. 2b), was identified with a 73 % recurrent parent genome recovery and was backcrossed with WGL-14 to produce BC_2F_1 plants. The same marker-assisted selection (MAS) process was used for selection of BC_2F_1 and BC_3F_1 plants, resulting in the identification of a BC_3F_1 plant, SN54-11-143, with 93.6 % RPG recovery. This plant was self-pollinated to produce 1365 BC_3F_2 plants, of which 175 were homozygous dominant for *Pi54*. These 175 plants were advanced to BC_3F_3, BC_3F_4 and BC_3F_5 generations based on resistance to blast, high yield, plant type, grain type and other agro-morphological traits resembling the recurrent parent. The list of total plants screened and putative plants identified with positive *Pi54* gene in improved WGL-14 blast resistance lines of each backcross generations (Table S3). At BC_3F_5 generation, 28 lines were identified for their strong blast resistance (Table 1), high yield and agro-morphological traits similar to WGL-14.

In this study, we used a PCR-based functional marker, Pi54MAS, for foreground selection of the *Pi54* gene, which ensured 100 % selection accuracy by reducing recombination events between the marker and the gene. The effectiveness of MAS relies on the accurate identification of foreground markers and during the MABB process; we successfully identified positive plants with no false positives. For background selection, we employed 80 parentals polymorphic SSR markers, providing extensive coverage per chromosome (around 5.3 markers per chromosome), which improved upon earlier studies. Special emphasis was placed on chromosome 11, where the *Pi54* gene is located, with 25 markers used for background selection following methodology. This strategy minimized linkage drag around the target gene. Elite lines WGL-1473, WGL-1467 and WGL-1472 were identified with 95.6 % recurrent parent genome recovery, with the donor chromosome segment confined to a small region, less than 5.8 Mb from the *Pi54* gene (data not shown).

Blast Resistance of Selected Backcross-Derived Lines of WGL-14

The donor parent, NLR-145, which carries the *Pi54* gene, exhibited strong resistance to rice blast, with a disease score of '0'. In contrast, the recurrent parent, WGL-14, showed extensive disease lesions, covering more than 75 % of its leaf

Table 1. Screening of BC₃F₅ lines against rice blast at uniform blast nursery and lines mean performance for studied traits at OVT during 2018.

Sl. No.	Name of the Entry	Gene status	Blast score	Disease reaction	Days to 50% flowering	Plant Height (cm)	Panicle length (cm)	No. of grains per panicle	Grain yield (kg/ha)	1000 seed weight (g)	Grain type
1	WGL-1467	*Pi54*	1	R	105	110.5	25.0	236	7160	16.7	MS
2	WGL-1469	*Pi54*	1	R	104	108.4	26.1	216	6468	16.9	MS
3	WGL-1462	*Pi54*	1	R	106	119.3	25.2	212	6442	17.1	LS
4	WGL-1473	*Pi54*	1	R	104	111.4	24.0	231	6363	17.3	MS
5	WGL-1466	*Pi54*	1	R	105	111.6	25.5	216	6231	16.9	MS
6	WGL-1457	*Pi54*	2	R	104	121.2	26.7	342	6211	15.0	MS
7	WGL-1465	*Pi54*	1	R	105	115.2	24.8	256	6010	17.9	MS
8	WGL-1458	*Pi54*	2	R	104	116.7	26.7	244	6006	17.7	MS
9	WGL-1474	*Pi54*	1	R	106	115.6	27.7	230	5955	17.5	MS
10	WGL-1464	*Pi54*	1	R	105	113.0	24.2	224	5775	17.6	MS
11	WGL-1459	*Pi54*	2	R	104	112.7	25.6	230	5692	17.2	MS
12	WGL-1454	*Pi54*	2	R	111	118.3	26.6	207	5628	17.0	MS
13	WGL-1463	*Pi54*	1	R	104	128.2	27.2	264	5622	15.9	MS
14	WGL-1451	*Pi54*	2	R	106	117.2	28.6	201	5622	20.4	LS
15	WGL-1461	*Pi54*	1	R	105	115.0	26.2	243	5615	16.6	MS
16	WGL-1456	*Pi54*	2	R	108	121.0	28.2	242	5429	17.4	LS
17	WGL-1449	*Pi54*	2	R	103	129.3	26.9	337	5423	14.7	MS
18	WGL-1450	*Pi54*	2	R	104	118.1	26.2	193	5384	17.8	MS
19	WGL-1471	*Pi54*	1	R	105	120.4	26.5	202	5327	17.0	MS
20	WGL-1468	*Pi54*	1	R	104	110.4	25.2	207	5320	17.9	MS
21	WGL-1460	*Pi54*	2	R	105	111.2	26.2	214	5288	17.1	MS
22	WGL-1470	*Pi54*	1	R	110	114.7	27.4	209	5147	17.0	MS
23	WGL-1475	*Pi54*	1	R	103	108.0	24.8	193	5122	18.1	MS
24	WGL-1448	*Pi54*	2	R	104	107.3	24.4	183	5090	18.2	MS
25	WGL-1453	*Pi54*	2	R	106	114.8	26.7	183	5045	16.7	MS
26	WGL-1452	*Pi54*	2	R	104	112.8	27.3	227	4942	17.6	MS
27	WGL-1472	*Pi54*	1	R	106	116..4	27.3	238	4910	17.5	MS
28	WGL-1455	*Pi54*	2	R	105	117.0	25.0	221	4295	16.8	MS
29	WGL-14[A]	-	7	HS	108	117.3	15.6	228	4481	15.6	MS
30	NLR-145[B]	*Pi54*	1	R	105	116.3	24.6	196	3674	17.6	MS
31	TN1[C]	-	9	HS	110	125.2	26.2	193	5122	18.1	MS
	CV %				*3.80*	*6.63*	*5.43*	*5.77*	*12.53*	*5.77*	
	LSD (0.05)				*2.62*	*5.48*	*2.33*	*4.96*	*40.15*	*1.89*	

OVT: Observation varietal trial, A: Recipient parent, B: Donor parent, C: Susceptible check, R: Resistant, HS: Highly susceptible, MS: Medium slender, LS: Long Slender
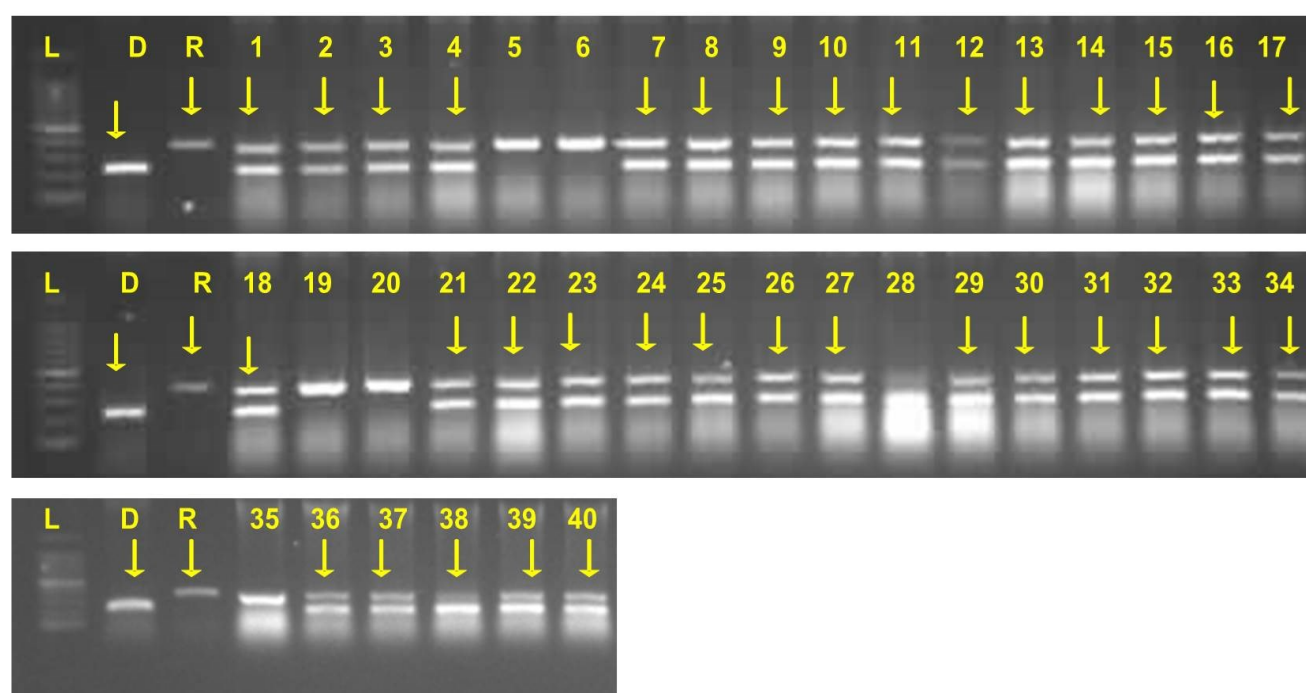

Fig. 2a. Foreground selection of BC₁F₁ plants for *Pi54* gene using functional marker Pi54MAS. 296 BC₁F₁ plants derived from the cross between WGL-14 and NLR-145 were screened for the presence of *the Pi54* gene by using the PCR-based functional marker Pi54MAS. M: molecular weight marker (100 bp ladder), D: donor parent (NLR-145), R: recipient parent (WGL-14). The lane numbers (1 to 40) shown on the gel indicate BC₁F₁ plants. The arrow indicates positive plants for the *Pi54* gene.
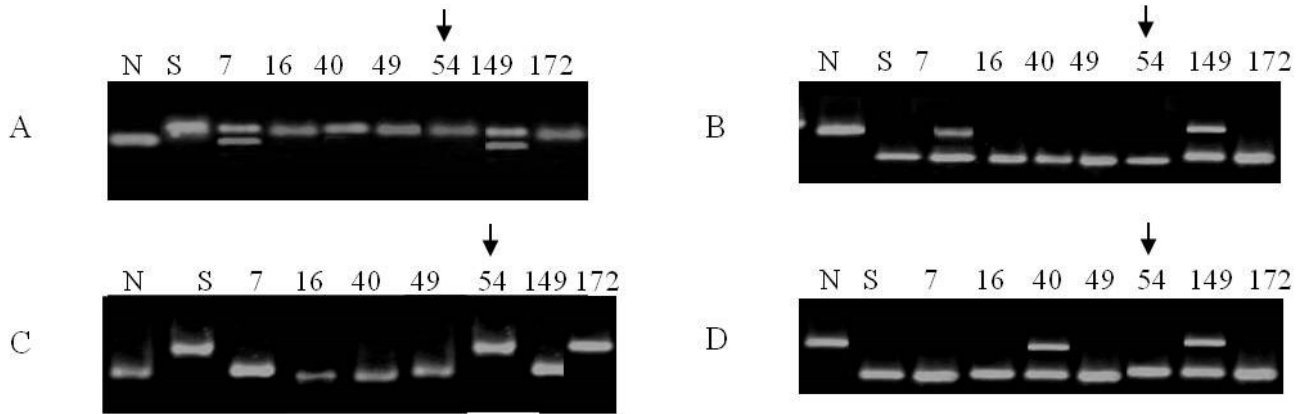
Fig. 2b. Background selection using parental polymorphic SSR markers among the positive BC$_1$F$_1$ plants. A few representative markers from Chr. 11 near the target genes (i.e. *Pi54*) and their amplification pattern are shown in this figure. Figure A to D represents the amplification pattern of RM224, RM26937, RM26002 & RM26969, respectively. The arrow indicates the plants showing homozygosity at the respective marker loci concerning the recurrent parent allele for the SSR markers. The numbers on the top of each gel represent the BC$_1$F$_1$ plant numbers. M- Molecular weight marker (100bp ladder), N-NLR-145 and S -WGL-14 (Warangal Samba).

area and received a disease score of '7'. All the 28 BC$_3$F$_5$ lines, however, demonstrated a high level of resistance to rice blast, with no visible lesions on their leaves and scores of '1' or '2' (Table 1). The lines tested during advanced varietal trials consistently displayed strong blast resistance, with the improved WGL-14 lines, WGL-1473, WGL-1467 and WGL-1472 showing significantly better resistance compared to the original WGL-14 (Fig. 3). Importantly, the presence of the resistance gene did not negatively affect the yield of the improved WGL-14 lines.

Yield and Agronomic Characterization of Improved Lines

The 28 selected BC$_3$F$_5$ entries were evaluated in an observation varietal trial (OVT) alongside the donor and recipient parents. They were assessed for yield, blast resistance and important agro-morphological traits, including (DFF), plant height (PH), panicle length (PL), 1000 seed weight (SW), number of grains per panicle (NGP) and grain type, during the 2018 *kharif* season (Table 1). From these 28 OVT lines, eight lines namely WGL-1458, WGL-1465,

WGL-1466, WGL-1467, WGL-1469, WGL-1471, WGL-1472 and WGL-1473 were demonstrated yields comparable to or exceeding the recipient parent WGL-14 and were advanced to the preliminary varietal trial (PVT) in 2019 (Table 2). Out of these, five lines namely WGL-1466, WGL-1467, WGL-1469, WGL-1472 and WGL-1473 were selected for advancement to the advanced varietal trial (AVT) stage (Table 3).

The overall performance of these five lines was evaluated for grain yield and associated traits across OVT, PVT and AVT trials (Table 4). The days to 50 % flowering (DFF) for these lines ranged from 106 to 108 days, averaging 107 days, which was three days earlier than WGL-14 with 110 days. The average plant height (PH) was 114.09 cm, ranging from 111.60 to 115.97 cm, which was close to WGL-14 (height of 115.37 cm). Panicle length (PL) averaged 25.57 cm, ranging from 25.00 to 28.17 cm, outperforming WGL-14 with average of 22.57 cm. The (NGP) averaged 227.52, with a range of 217 to 236 grains, compared to WGL-14 with 221.33 grains. Seed weight (SW) averaged 16.96 g, ranging from
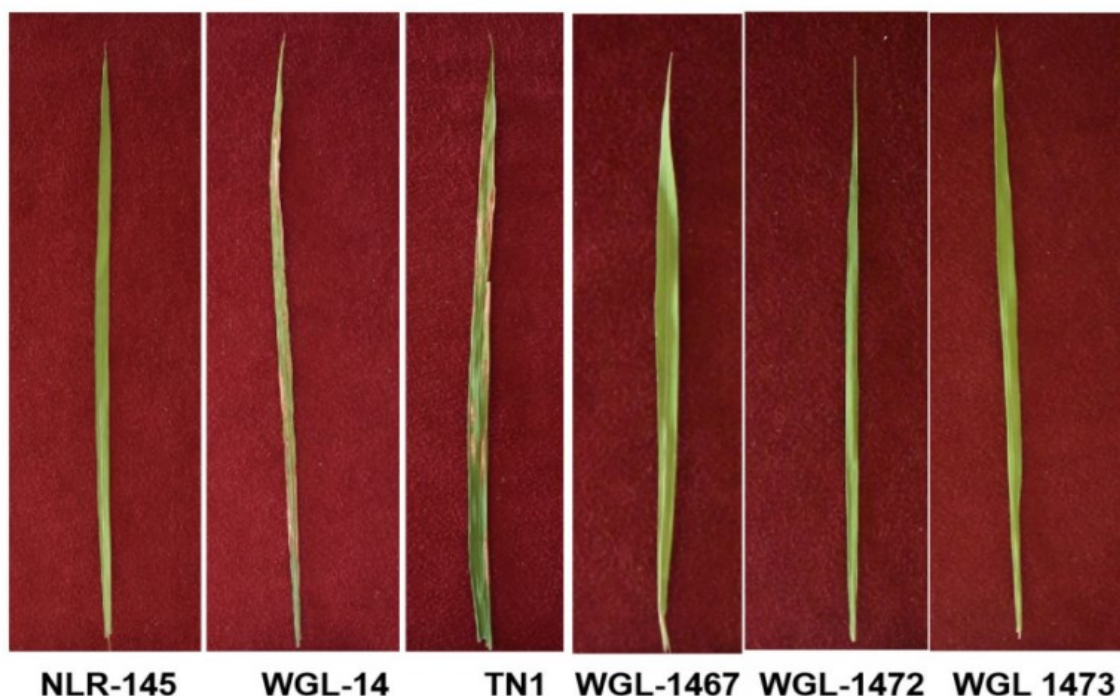

Fig. 3. Rice blast disease reaction of NLR-145: donor parent for blast resistance, WGL-14: receipt parents, TN1: susceptible check for blast resistance while WGL-1467, WGL-1472 and WGL-1473 are WGL-14 of improved lines having *Pi54* gene.

Table 2. Lines mean performance for studied traits at preliminary varietal trial (PVT) during 2019.

S. No	Name of the Entry	Days to 50% flowering	Plant Height (cm)	Panicle length (cm)	No. of grains per panicle	Grain yield (kg/ha)	1000 seed weight (g)	Grain type
1	WGL-1458	110	113.9	28.4	236	6143	16.7	MS
2	WGL-1465	108	115.6	26.1	231	5109	17.5	MS
3	WGL-1466	108	117.2	25.4	229	4607	17.8	MS
4	WGL-1467	108	115.5	25.4	223	4309	17.7	MS
5	WGL-1469	109	114.8	25.4	233	5243	17.3	MS
6	WGL-1471	108	113.4	26.5	220	4207	17.0	MS
7	WGL-1472	108	115.2	26.2	221	5155	16.9	MS
8	WGL-1473	109	111.7	25.5	227	4359	17.1	MS
9	WGL-14[A]	112	114.4	26.0	217	3680	16.2	MS
10	NLR-145[B]	114	95.7	24.3	156	2321	20.8	LB
	CV%	5.13	5.50	3.86	3.79	10.88	4.32	
	LSD (0.05)	2.02	1.42	1.63	2.67	36.31	1.21	

A: Recipient parent, **B**: Donor parent.

Table 3. Lines mean performance for studied traits at advanced varietal trial (AVT) during 2020.

S. No	Name of the Entry	Days to 50% flowering	Plant Height (cm)	Panicle length (cm)	No. of grains per panicle	Grain yield (kg/ha)	1000 seed weight (g)	Grain type
1	WGL-1466	108	117.2	25.4	231	4607	16.9	MS
2	WGL-1467	108	115.5	25.4	229	6637	16.7	MS
3	WGL-1469	109	114.8	25.4	227	5243	16.9	MS
4	WGL-1472	108	115.2	26.2	222	6627	15.6	MS
5	WGL-1473	109	111.7	25.5	221	5071	15.7	MS
6	WGL-14[A]	112	114.4	26.1	219	5412	15.6	MS
7	NLR-145[B]	114	95.7	24.3	197	2321	15.7	LB
	CV%	6.21	3.60	5.31	2.16	10.88	5.71	
	LSD (0.05)	1.53	2.67	3.69	4.12	29.76	1.66	

A: Recipient parent, **B**: Donor parent.

Table 4. Combined OVT, PVT and AVT trails mean performance of studied traits in five improved WGL-14 lines.

S. No	Name of the Entry	Days to 50% flowering	Plant Height (cm)	Panicle length (cm)	No. of grains per panicle	Grain yield (kg/ha)	1000 seed weight (g)	Grain type
1	WGL-1466	107.00	115.33	25.43	225.33	5148.33	17.20	MS
2	WGL-1467	107.00	113.83	25.27	229.33	6035.33	17.03	MS
3	WGL-1469	107.33	112.67	25.63	225.33	5651.33	17.03	MS
4	WGL-1472	107.00	115.20	25.73	233.00	5564.00	16.80	MS
5	WGL-1473	107.33	111.60	25.00	226.33	5264.00	16.70	MS
6	WGL-14[A]	110.67	115.37	22.57	221.33	5264.00	15.80	MS
7	NLR-145[B]	114.63	95.71	24.35	197.36	2321.36	15.71	LB
	CV%	5.36	3.60	6.11	2.99	15.36	6.36	
	LSD (0.05)	2.17	3.13	2.97	3.71	36.99	1.31	

A: Recipient parent, **B**: Donor parent.

16.70 to 17.63 g, exceeding WGL-14 with 15.80 g. The average grain yield was 5487.83 kg/ha, ranging from 5148.33 to 6035.33 kg/ha, which was significantly higher than WGL-14s yield of 5264 kg/ha.

At the AVT stage during *kharif* 2020, 3 lines namely WGL-1469, WGL-1472 and WGL-1473 were selected for further development (Fig. 4). A comparison of grain types for the donor (NLR-145), recipient (WGL-14) and the 3 improved WGL-14 blast-resistant lines during the 2020 AVT stage was shown in (Fig. 5).Notably, the improved lines namely WGL-1467, WGL-1472 and WGL-1473 recorded yield increases of 14.64 %, 5.69 % and on par with recurrent parent respectively, compared to WGL-14. The frequency distribution of these 3 lines is presented in (Fig. 6). Stability analysis was carried out at 6 locations of Telangana State during *kharif* 2021 and recorded an average yield of 6348 kg/ha for WGL-1467 and 5880 kg/ha for WGL-1473 (data not shown).

Correlation and Path Analysis

Correlation analysis is a straight forward and efficient method for deriving insights from large datasets (25). It reveals the strength of relationships between different traits. The combined correlation analysis from the OVT, PVT and AVT trials for yield and related traits in the improved WGL-14 blast-resistant lines is shown in (Fig. 7). Grain yield exhibited a strong, positive and significant correlation with plant height (r = 0.71*) and the number of grains per panicle (r = 0.74**), along with a moderately significant association with panicle length (r = 0.53*). However, grain yield showed a strong, negative and significant correlation with days to 50 % flowering (r = 0.75**). These findings suggest that the observed correlations may result from the direct and indirect effects of these traits on grain yield. Our results indicate that a higher number of grains per panicle lead to increased grain yield, implying that selection should prioritize lines with more grains per panicle. Additionally, increases in plant height and
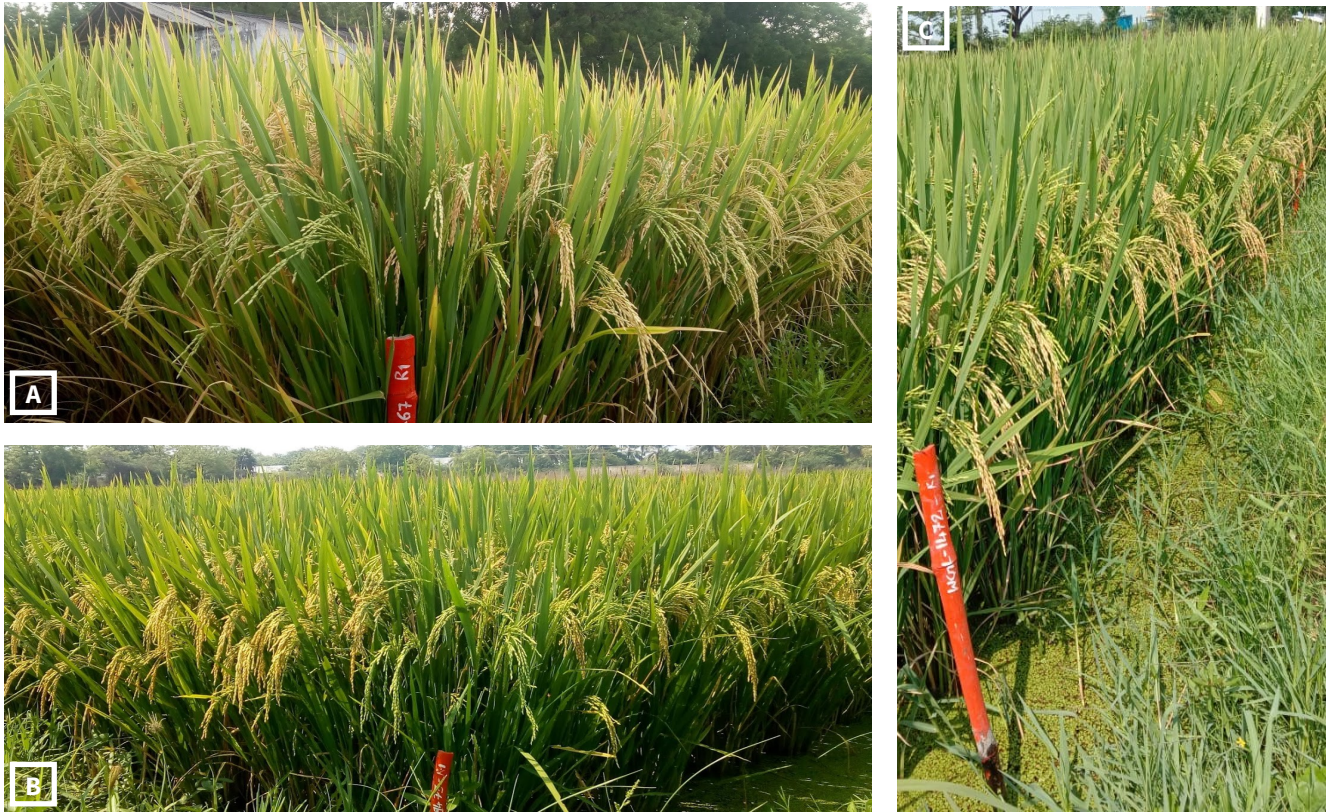
Fig. 4. Plant type of three improved WGL-14 blast-resistant lines at advanced varietal stage (AVT). A: WGL-1467, B: WGL-1472, C: WGL-1473.

Fig. 5. Grain types of the donor (NLR-145), recipient (WGL-14) parents and improved WGL-14 blast-resistant lines at advanced varietal stage (AVT) during 2020.
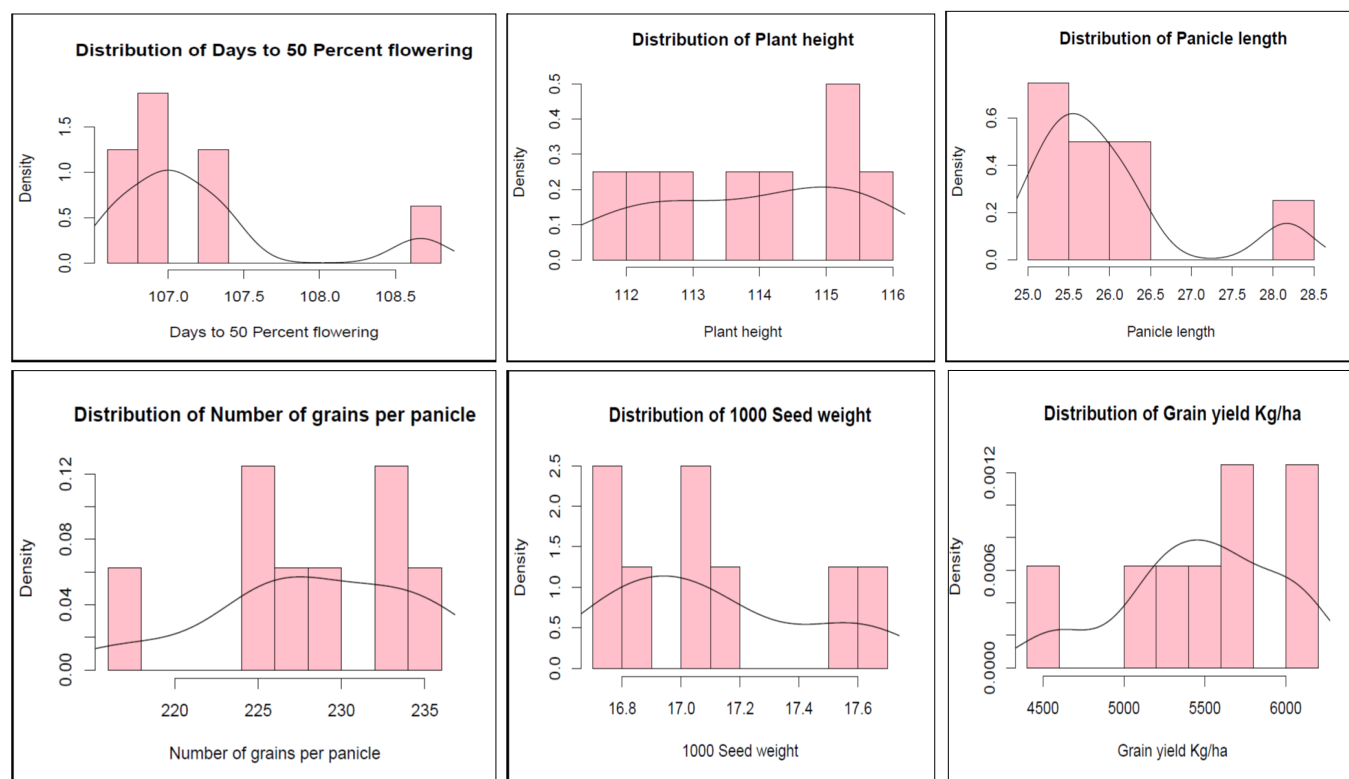
Fig. 6. Pooled trails frequency distribution of grain yield and related traits in five selected WGL -14 improved blast resistance lines.

panicle length also contribute to higher grain yield. suggesting that early-maturing lines tend to yield higher grain yield and may avoid drought stress (26). Significant positive and negative associations were also identified among other traits, as shown in (Fig. 7) and similar results have been previously reported (24).

Path analysis determined that the correlation between paddy grain yield and yield related traits can be broken down into direct and indirect effects at phenotypic levels are presented in (Fig. 8). The residual effects estimated value of 0.03 supports existing traits sufficiency in rationalizing grain yield variation in the improved WGL-14 blast-resistant lines. Among the examined traits, number of grains per panicle (1.76) exhibited the strongest positive direct impact on grain yield, followed by 1000 seed weight (1.11). These traits displayed a consistent positive correlation with grain yield.

However, plant height and panicle length showed a notable negative direct effect of -0.64 and -0.81 respectively. But these traits are exhibited a statistically significant positive relationship with grain yield. This discrepancy is attributed to the more indirect effects of panicle length, 1000 seed weight and number of grains per panicle for trait plant height. Whereas, indirect influence of plant height, 1000 seed weight and number of grains per panicle for trait panicle length. In addition, trait days to 50 % flowering also displayed exceptional pattern of a moderate positive direct effect on grain yield (0.74), while significantly negatively correlated with it. This was attributed to days to 50 % flowering indirect association with a strongly negative indirect effect of number of grains per panicle (-0.70), 1000 seed weight (-0.67), plant height (-0.62) and panicle length (-0.45).
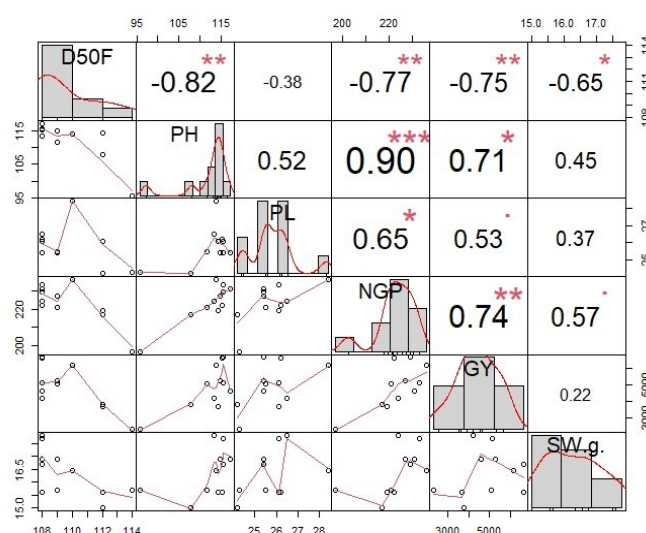

Fig. 7. Pooled correlogram (The upper panels display numerical values that indicate whether the correlations are positive or negative), frequency distribution (diagonal panels) and scatter plots (lower panels) of grain yield and related traits in improved WGL-14 blast resistance lines. *, ** and *** indicate significance at p ≤ 0.05, p ≤ 0.01 and p ≤ 0.001. **D50F**: Days to 50 % flowering, **PH**: Plant height, **PL**: Panicle length, **NGP**: Number of grains per panicle, **GY**: Grain yield, **SW**: 1000 seed weight.

Discussion

Climate changes leading to an increase in disease prevalence, making many popular rice cultivars susceptible to biotic stresses (21). Among various biotic stresses, rice blast is the most devastating, with the potential to cause yield losses of up to 100 % (27). To overcome these challenges and boost rice production, developing high-yielding cultivars with enhanced resistance genes is crucial. This would improve both the yield and resistance of the plants to various diseases (28). Deploying host plant resistance has proven to be an effective strategy for controlling rice blast disease, which severely impacts both rice varieties and hybrids (29).
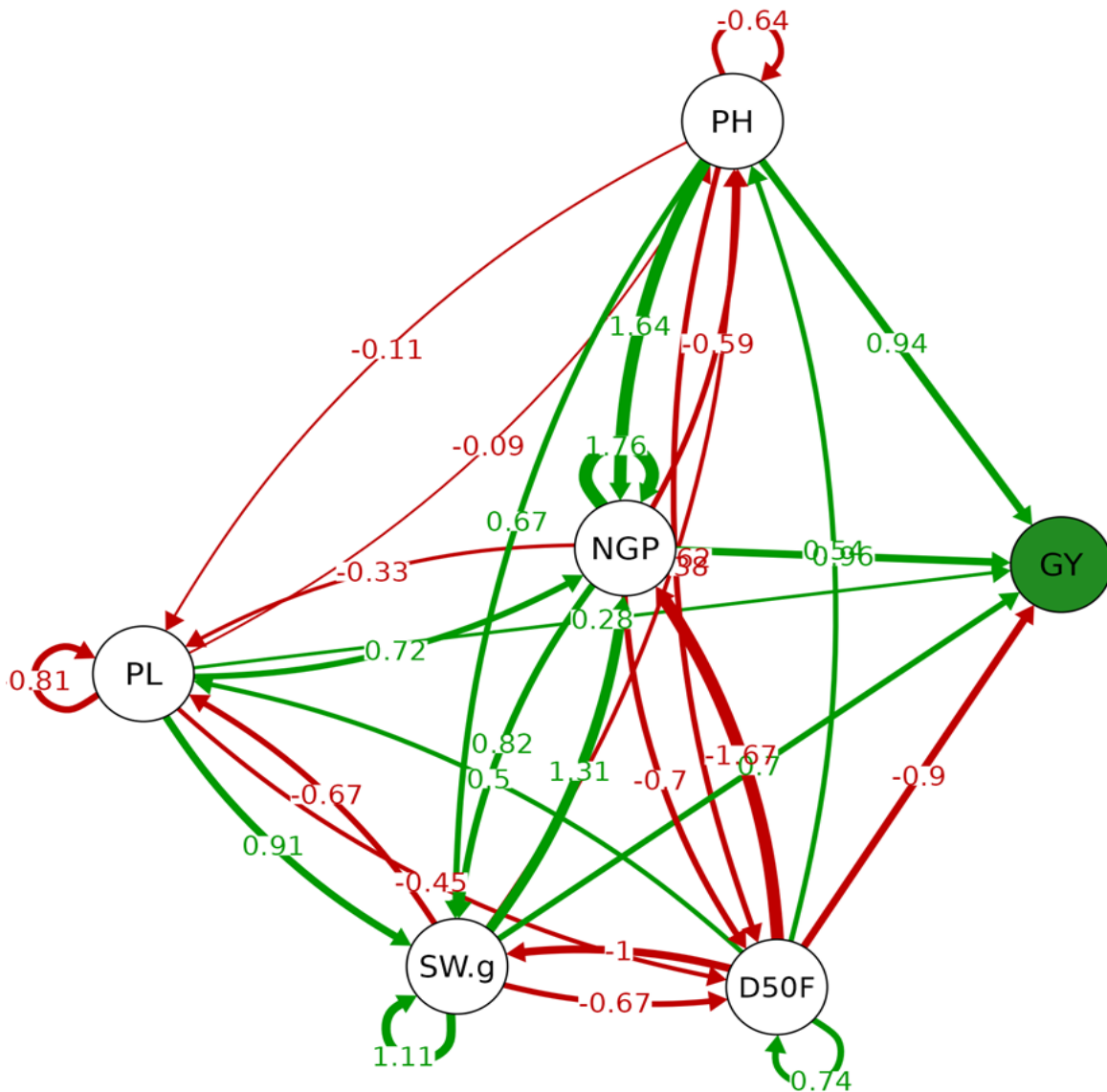
Fig. 8. Phenotypic path coefficient plot of yield-related traits on grain yield in improved WGL-14 blast resistance lines. Path coefficients indicate effects that cascade from first to second-order traits and finally to grain yield. U-turn arrows represented direct effects, while connections to other characteristics indicated indirect effects. Green arrows denoted positive effects, and red indicated negative effects. The strength of the impact was shown by arrow thickness and colour intensity. Residual effects = 0.03. GY: Grain yield, D50F: Days to 50 % flowering, PH: Plant height, PL: Panicle length, NGP: Number of grains per panicle, SW: 1000 seed weight.

WGL-14 is well-known for its high yield and medium slender grain type with excellent cooking qualities, but it is highly susceptible to rice blast. In contrast, NLR-145, the donor parent, is a high-yielding variety with desirable agro-morphological traits and contains the dominant *Pi54* gene (30), known for its broad-spectrum blast resistance across India (20, 31). The dominant, broad-spectrum blast resistance gene *Pi54* has proven to be very effective against the majority of rice blast isolates across India (9). Marker-assisted backcross breeding (MABB) has gained favour in rice breeding for transferring blast resistance genes, due to the availability of reliable markers linked to major *Pi* loci (10). In this study, we aimed to improve WGL-14 for blast resistance through MABB, coupled with phenotypic selection for agro-morphological traits. Similar methods have been used successfully in the earlier studies (32). WGL-14 is one of the national checks in the AICRP Hybrid rice trials as well as in medium slender grain type varietal trials. Hence, the present study distinguishes from previous research on *Pi54* introgression through MABB.

In this study, we used a PCR-based functional marker, Pi54MAS, for foreground selection of the *Pi54* gene (15), which ensured 100 % selection accuracy by reducing recombination events between the marker and the gene. The success of MAS depends on the accurate selection of foreground markers (33) and we were able to precisely identify positive plants without false positives during the MABB process. The advantages of using functional markers *Pi54* for selection have been highlighted in previous studies on blast screening (10, 18, 20). Numerous studies have demonstrated the effectiveness of introgressing *Pi54* into elite rice varieties (17, 21, 34, 35) and hybrid lines through marker-assisted breeding (18, 20).

For background selection, we employed 80 parentals polymorphic SSR markers, providing extensive coverage per chromosome (around 5.3 markers per chromosome), which improved upon earlier studies (36, 37). Special emphasis was placed on chromosome 11, where the *Pi54* gene is located, with 25 markers used for background selection (38). This strategy minimized linkage drag around the target gene. Elite

lines WGL-1473, WGL-1467 and WGL-1472 were identified with 95.6 % recurrent parent genome recovery, with the donor chromosome segment confined to a small region, less than 5.8 Mb, near the *Pi54* gene (data not shown). Similarly, previous studies have demonstrated the effectiveness of background selection in improving RPG recovery. Some studies reported significant enhancement in RPG recovery, reaching 95.04 % in the BC_2F_2 generation (39). Earlier results showed successful use of polymorphic SSR markers to achieve maximum RPG recovery in the BC_3F_1 stage (28).

The agronomic performance of three improved rice lines, namely WGL-1467, WGL-1472 and WGL-1473, demonstrated notable yield increases along with durable resistance to blast disease. The higher yields and superior quality traits observed in these lines are likely attributable to the inheritance of yield-contributing traits from the recurrent parent. These traits, such as yield, agro-morphological characteristics and quality, are typically governed by polygenes, which are distributed across the genome (28). Earlier report showed significant yield improvements in newly developed lines, with a recorded 36 % higher yield compared to DRR 9B (40).

In path coefficient study we identified highly positive significant correlated traits such as number of grains per panicle and 1000 seed weight has a direct influence on grain yield. Similar results of significant positive direct effects of traits 1000-grain weight and number of grains per panicle on grain yield is reported in (41). This directly influenced traits may be leads the best genotypes selection criteria. Some discrepancy is attributed in direct influenced traits due to the more cumulative indirect effects. Our findings are evident that these indirect traits (days to 50 % flowering, plant height, panicle length, number of grains per panicle, 1000 seed weight) can serve as valuable indicators for selecting paddy plants with enhanced grain yield. Prioritizing these traits during the selection process holds the potential to increase the likelihood of developing paddy genotypes for improved grain yield.

Conclusion

Through successive backcrossing and careful selection based on both genetic markers and agro-morphological traits, we successfully introgressed the *Pi54* blast resistance gene from the donor parent NLR145 into WGL-14. The use of a functional marker, Pi54MAS, ensured accurate foreground selection throughout the breeding process. Moreover, employing background selection by using a higher number of parental polymorphic markers to minimize linkage drag and facilitated the retention of desirable traits from the recurrent parent. Elite breeding lines in the genetic background of WGL-14, specifically WGL-1467, WGL-1472 and WGL-1473 have been developed with enhanced grain yield and blast resistance. These lines were selected for their superior agronomic traits, making them ideal candidates as donors in future rice crop improvement programs. The genetic enhancement of these lines, particularly for disease resistance and yield traits, positions them as valuable assets in breeding programs aimed at improving rice cultivars for both productivity and disease resistant.

Authors' contributions

This work was carried out in collaboration among all authors. YH executed the research work, designed methodology and drafted the final manuscript. VV, BSC, KRD, GN, KRP, NL, AS participated in the field trials and laboratory works. RSK, SM, DA performed screening tests for blast resistance. CR, UN, RUR, PRRR conducted agronomic trails. YH supervised the whole research work. YH, BE, BM analysed the data, prepared figures and tables, results interpreted and drafted manuscript. YH, PJMR, CDR reviewed the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: "All authors hereby declare that all experiments have been examined and carried out and there is **"**None" ethical issues in the present investigation.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used chatGPT service, in order to reduce plagiarism, to improve English language and readability. After using this tool/ service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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