

## Field evaluation of marker assisted backcross families introgressed with *Pi1* and *Pi54* in Intan rice variety

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**Abstract:** Rice blast caused by *Magnaporthe oryzae* is one of the most destructive diseases in all rice growing regions of India. Host plant resistance is the best option for the management of blast disease. Broad spectrum blast resistance genes *Pi1* and *Pi54* were transferred to popular but blast susceptible rice variety, Intan. Homozygous BC<sub>1</sub>F<sub>3</sub> backcross families were developed using Intan as recurrent parent and Tetep as donor of *Pi1* and *Pi54* genes through marker-assisted backcross breeding. Homozygous plants in BC<sub>1</sub>F<sub>3</sub> families were identified using genic/tightly linked markers, RM206 (0.6 cM) for *Pi54* and RM224 (0.0 cM) for *Pi1*. The present study aimed to evaluate the homozygous backcross families carrying *Pi1*, *Pi54* and *Pi1+Pi54* combination for blast resistance, yield and yield related traits under field conditions. During evaluation, marker assisted backcross families showed resistant reactions to leaf and neck blast disease (0-3 grade) under field as well as artificial inoculation conditions. The analysis of morpho-agronomic performance showed that the majority of the homozygous plants were found to be on par with Intan for grain yield and grain features. Grain yield per plant was found to be positively correlating with days to 50 per cent flowering, number of tillers, number of productive tillers, panicle length and filled grains per panicle and test weight.

**Key words:** Blast resistance, *Magnaporthe oryzae*, Marker-assisted selection, Rice

### Introduction

Rice blast disease caused by *Magnaporthe oryzae* B.Couch., (*Pyricularia oryzae*) (Couch and Kohn, 2002) is one of the most devastating fungal diseases that infect all parts of rice plant but causes the greatest losses when necks and panicles are infected. At global level, the severe yield loss of up to 85 per cent has been reported due to the incidence of blast alone (Divya *et al.*, 2014). Several fungicides are recommended to control blast disease but they generate additional costs in rice production and contaminate environment and food items. Therefore, the use of resistant varieties is thought to be one of the most economically and environmentally efficient ways of crop protection from the diseases.

There are about 102 genes reported for blast resistance and 35 genes have been cloned (Wang *et al.*, 2017). Among *Pi* genes, *Pi1* and *Pi54* are reported to show broad spectrum resistance against many isolates of *M. oryzae* in India (Sharma *et al.*, 2010; Ramkumar *et al.*, 2011). The *Pi54* gene was originally identified and cloned from a rice variety Tetep, mapped to chromosome 11L and was reported to govern resistance against predominant races of the blast pathogens in India and Tetep also carries *Pi1* on chromosome 11 which is highly effective against the *M. oryzae* population found in the tropical area of South Asia and South-East Asia (Sharma *et al.*, 2010). Considering the importance of genes *Pi1* and *Pi54* for blast resistance across India, these genes were chosen for rice improvement in the present research study.

The marker-assisted selection (MAS) is considered to be efficient, reliable and feasible in comparison with conventional plant breeding to develop disease resistant varieties by avoiding the prolonged selection process (Collard and Mackill, 2008). Marker-assisted backcross breeding (MABB) is a breeding

approach, where a trait is precisely introgressed from a donor parent into the genetic background of a recurrent parent with elite characters using molecular markers and is considered ideal for the improvement of varieties (Collard *et al.*, 2005).

The primary components of rice grain quality include appearance, eating, cooking, milling and nutritional qualities. Amylose content (AC) is considered as the most important character for predicting rice cooking and processing behaviour. The ratio of amylose to starch, estimated as AC, changes from cultivar to cultivars and rice varieties are assembled based on their AC into waxy (0 to 2 %), very low (3 to 9 %), low (10 to 19 %), intermediate (20 to 25 %) and high with more than 25 per cent amylose content. Intan has high AC of 25.6 per cent (Mahanta and Bhattacharya, 2010). Rice grains with a high AC show high volume expansion and are very much suitable for puffing purposes (Kamaraddi and Prakash, 2015).

Intan is a medium slender *indica* variety and popular among farmers and consumers across Karnataka because of its higher yield (45-50 q/ha) and excellent puffing quality (Kamaraddi and Prakash, 2015). However, Intan has now become highly susceptible to blast disease. So, there is a need to improve Intan for blast resistance and bring back this variety to for cultivation. Considering the popularity of Intan, broad spectrum resistance gene *Pi1* and *Pi54* were introgressed for blast resistance by applying MABB and backcross families were developed in the Department of Biotechnology, University of Agricultural Sciences, Dharwad. Backcross families (BC<sub>1</sub>F<sub>3</sub>'s) carrying *Pi1*, *Pi54* and *Pi1+Pi54* combination genes were evaluated for blast resistance, yield and yield attributing traits at Agricultural Research Station (A.R.S.), Mugad and are also for grain quality parameters.

## Material and methods

### Plant material

Rice variety Intan, a popular but blast susceptible was used as recurrent parent and donor parent Tetep (carries *Pi1* and *Pi54*) was used to introgress *Pi1* and *Pi54* into Intan and BC1F3 homozygous backcross families were developed using genic/tightly linked markers RM206 (0.6 cM) for *Pi54* and RM224 (0.0 cM) for *Pi1* through marker-assisted backcross breeding (MABB) (Debnath, 2016).

In the present study homozygous backcross families carrying major blast resistance genes *Pi1*, *Pi54* and *Pi1 + Pi54* combination in blast susceptible rice variety Intan were evaluated for blast resistance and yield and yield related traits at A.R.S., Mugad in uniform blast nursery. Homozygous families were sown in uniform blast nursery under augmented block design at A.R.S. (15°27' N, 74°55' E; 687 m above sea level), Mugad, University of Agricultural Sciences (UAS), Dharwad in *kharif* 2018. Each screening bed was surrounded by two rows of HR-12 as susceptible checks to facilitate uniform inoculum and high disease pressure. High nitrogenous fertilizer was used to enhance the disease pressure @ 150:60:60 kg NPK/ha.

### Evaluation of backcross families for blast resistance

Blast disease resistance screening was done in uniform blast nursery at A. R. S., Mugad during the *kharif* season 2018. The homozygous plants with blast resistance genes *Pi1*, *Pi54* and *Pi1+Pi54* combination were field evaluated during vegetative as well as reproductive stages. Disease scores were recorded at differential intervals using standard evaluation system developed by International Rice Research Institute, 0-9 scale (Anon., 1996) for leaf blast. The lines with 0-3 were considered as resistant, 4-5 as moderately resistant and 6-9 rated as susceptible. Disease scoring of neck blast was recorded by following the standard scoring system developed by IRRI, Standard Evaluation System for Rice (Anon., 2002). Neck blast severity was recorded as a percentage of the infection on the neck of the rice panicle at physiological maturity. Where, scores of 0-3 were considered as resistant (0-10 %), 5 as moderately resistant (11-25 %), 7 as susceptible (26-50 %) and 9 as highly susceptible (> 50 %).

### Screening of backcross families for blast resistance under artificial inoculation condition

The homozygous backcross families were subjected to artificial inoculation for screening against blast disease at greenhouse conditions. Thirty days old identified homozygous plants were inoculated with a suspension culture of *M. oryae* isolate with the spore concentration of  $1 \times 10^5$ /ml (Kim *et al.*, 2005). Plants were covered with polythene bags after spraying to maintain 90 per cent relative humidity. Plants were observed after 15 days of spraying to check the disease incidence and were assessed per the 0-5 scale (Mackill and Bonman, 1992).

### Evaluation of backcross families for yield and yield attributing parameters

Homozygous families with blast resistance genes *Pi1*, *Pi54* and *Pi1+Pi54* combination were field evaluated at A.R.S.,

Mugad during *kharif* 2018. The morphological observations of both parents and total of 173 homozygous families were recorded by following the guidelines for distinctness, uniformity and stability (DUS) (Anon., 2007). Different characters *viz.*, days to 50 per cent flowering (DFF), plant height (PH, cm), number of tillers (NT), number of productive tillers (NPT), panicle length (PL, cm), filled grains per panicle (FGPP), 1000 seed weight (test weight) (TW, g) and yield per plant (YPP, g).

### Evaluation of backcross families for grain parameters

Observation on grain length and breadth was measured by using a Digital Vernier Caliper (Mstutoyo, Japan) having a 0.05 mm precision. Grain type was graded according to the classification given by Ramaiah (1969). The experiment was replicated thrice.

### Statistical analysis

The recorded data was calculated for the mean, range, coefficient of variation (CV) and critical difference (CD). The phenotypic correlation coefficients among 8 traits (yield and yield attributing traits) were computed to know the nature of association among characters.

## Results and discussion

### Evaluation of homozygous backcross families carrying genes *Pi1*, *Pi54* and *Pi1 + Pi54* combination for blast resistance

Homozygous families were screened along with checks (Intan, Tetep and HR12) for blast resistance under field condition as explained in material and methods. Leaf blast incidence was noticed in susceptible checks Intan and HR12 with 6 and 8 grades respectively. Donor parent Tetep showed resistant reaction (grade 0). Majority of the homozygous plants carrying genes *Pi1*, *Pi54* and *Pi1+Pi54* combination in the background of Intan were free from leaf blast infections. However, only specks were observed in few homozygous plants (Table 1a).

In recipient parent Intan, 78 per cent of plants showed neck blast infection with disease score of 5-7 grade and donor Tetep showed resistant reaction without any neck blast symptoms. Susceptible check, HR12 showed highest infection with 9 grades. Maximum number of homozygous plants free from neck blast infection, however, few homozygous plants, 16 out of 173 plants with different gene combinations showed resistant reaction to neck blast with disease score of 3. It was noticed

Table 1. Field evaluation of backcross families with *Pi1*, *Pi54* and *Pi1+Pi54* combination for blast resistance

Table 1a. Leaf blast disease reactions observed in homozygous plants along with parents

Homozygous plants with gene	Total plants	Resistant (0 to 3 grade)	Moderately resistant (4 to 5 grade)	Susceptible (6-9 grade)
<i>Pi54</i>	51	48	3	0
<i>Pi1</i>	38	38	0	0
<i>Pi1+Pi54</i>	84	79	5	0
Checks:				
Intan	50	0	15	35
HR12	50	0	28	22
Tetep	50	50	0	0

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Table 1b: Neck blast disease reactions observed in homozygous plants along with parents

Homozygous plants with gene	Total plants	Plants with no incidence (0 grade)	Resistant (3 grade)	Moderately resistant (5 grade)	Susceptible (7-9 grade)
<i>Pi54</i>	51	33	12	6	0
<i>Pi1</i>	38	34	2	1	0
<i>Pi1+Pi54</i>	84	80	2	2	0
Checks:					
Intan	50	-	-	20	30
HR12	50	-	-	9	41
Tetep	50	45	5	-	-

Neck blast disease scale: 0-9 scale was used for evaluation, where, scores of 0-3 were considered as resistant (0-10 %), 5 as moderately resistant (11-25 %), 7 as susceptible (26-50 %) and 9 as highly susceptible (> 50 %)

that, the plants pyramided with *Pi1+Pi54* genes showed higher resistance levels than plants carrying individual genes indicating *Pi1* and *Pi54* genes in combination offers higher degree of resistance than individual genes at A.R.S., Mugad (Table 1b). This region is considered as one of the 'hot spots' for rice blast disease in Karnataka, with lots of variability in *M. oryzae* isolates (Prashanthi *et al.*, 2010). Resistance reaction of homozygous plants indicated the tight linkage of markers (RM206 (0.6 cM) for *Pi54* and RM224 (0.0 cM) for *Pi1*) with the resistance trait and effectiveness of the selection strategy applied in deriving these plants. Tetep with broad-spectrum resistance genes *Pi54* and *Pi1*, which is extremely effective against *M. oryzae* population in North-Western Himalayan region and Southern India (Srinivasacharya *et al.*, 2002; Singh *et al.*, 2012).

### Screening of backcross families for blast resistance under artificial inoculation condition

Homozygous families were subjected to artificial disease inoculation with *M. oryzae* isolate isolated from Intan. After 15 days of inoculation, the recipient parent Intan showed spindle shaped lesions indicating susceptible blast reaction with disease score 4-5. The leaves were dried before panicle emergence. The donor parent Tetep showed resistance reaction without any lesions. The homozygous families were free from blast infection and were scored 0. This confirms the effectiveness of introgressed genes for blast resistance in Intan background.

### Evaluation of backcross families carrying *Pi1*, *Pi54* and *Pi1+Pi54* combination for yield and yield attributing traits

The morphological observations of Intan, Tetep and homozygous backcross families were recorded as described in material and methods. Total 173 homozygous plants were evaluated for agronomic traits *viz.*, days to 50 per cent flowering (DFF, days), plant height (PH, cm), number of tillers per plant (NT), number of productive tillers per plant (NPT), panicle length (PL, cm), number of filled grains per panicle (FGPP), 1000 seed weight (test weight) (TW, g) and single plant yield (YPP, g) were recorded. Twelve best homozygous plants were selected by considering the characters like disease resistance, yield and yield attributing

traits and were further analyzed. The data on yield and yield related traits of selected twelve homozygous plants of BC<sub>1</sub>F<sub>3</sub> families in comparison with the recipient Intan are presented in Table 2. Most of the homozygous plants were statistically on par with Intan grain yield and grain features.

There was an ample variation in the families for DFF with a minimum of 88 days (Plant code: 16-14-12-8) to a maximum of 97 days (Plant code: 16-14-4-7), whereas, the recurrent parent Intan with 120 days for DFF. DFF of all the best selected homozygous plants were found to be

showing early maturity when compared with Intan. The PH of Intan was recorded 112cm whereas in homozygous plants, it ranged from 106.02 to 163.09 cm with mean height of 130.34 cm. The PL of homozygous plants was more than recurrent parent Intan (27 cm), it ranges from 25.19 to 33.09 cm with an average of 29.14 cm. Nine plants were found to be superior over Intan PL (27 cm) out of 12 best selected homozygous plants. Significant increase in PL helps in getting higher yields. NPT ranged from 4 to 24 with mean of 16 tillers. FGPP recorded; it ranges from 199 to 370 with mean of 251 grains per panicle. All 12 best selected homozygous plants were found to be on par with Intan FGPP (302). There was significant difference in the YPP levels of homozygous plants which ranged from 24.05 (Plant code: 16-14-4-7 carrying *Pi1*) to 50.23 g (18-11-2-1 carrying *Pi1+Pi54* pyramid) with general mean of 40.86 g compared to 50 g in original Intan, indicating the efficiency of selection. Interestingly, plant 18-11-2-1 carrying *Pi1+Pi54* combination recorded significant superiority over Intan for PL, FGPP and YPP (Table 2). The increase in the yield performance of some of the families was primarily brought about by the increase in PL and FGPP. The pooled grain yield performance of selected homozygous plants was found to be statistically on par with Intan (50 g/plant).

### Evaluation of backcross families for grain parameters

In 12 homozygous plants, kernel length (L), kernel breadth (B) and L/B ratios were significantly better grain traits, which are on par with Intan. L/B ratio ranged from 2.14 to 3.22 as compared to 2.89 for Intan. The lines possessed medium slender grain type similar to Intan except for four plants (Plant codes: 16-14-4-7 carrying *Pi1*, 16-14-9-7 carrying *Pi1*, 16-14-7-3 carrying *Pi1+Pi54* and 18-17-7-1 carrying *Pi1+Pi54* combination) were found to be long slender grain type (Table 3). Evaluation of the homozygous plants along with the parents for agronomic performance indicated the similarity of the backcross families to Intan for most of the traits studied (Tables 2 and 3).

### Correlation of grain yield with other characters

The phenotypic correlation coefficients among 8 traits were computed to know the nature of association among characters. Grain yield showed significant positive association with DFF,

Table 2. Performance of best selected backcross families carrying genes *Pi1*, *Pi54* and *Pi1+Pi54* combination for yield and yield related traits

Sl. No.	Code	Gene	DFF	PH	PL	NT	NPT	FGPP	TW	YPP
1	16-14-12-8	<i>Pi54</i>	88	122.49*	34.69*	15	15	231†	23.00†	39.65
2	16-4-13-3	<i>Pi54</i>	95	117.39*	26.40†	24†	24†	199	20.97	47.63†
3	16-14-4-7	<i>Pi1</i>	97	163.09*	33.74*	4	2	370*	20.80	24.05
4	16-14-9-7	<i>Pi1</i>	87	129.70*	28.09*	19†	17	302†	25.26†	45.86†
5	16-8-5-12	<i>Pi1</i>	83	117.46*	25.18†	17	17	222†	19.00	42.78
6	18-17-18	<i>Pi1+Pi54</i>	92	120.30*	25.36†	15	15	24†	22.14	40.00
7	16-14-7-3	<i>Pi1+Pi54</i>	95	159.23*	29.32*	16	16	245†	22.48	45.98†
8	16-8-5-6	<i>Pi1+Pi54</i>	84	134.52*	29.52*	14	14	253†	22.67	38.33
9	18-17-7-1	<i>Pi1+Pi54</i>	92	133.12*	31.56*	17	17	272†	20.57	42.95
10	18-11-1-8	<i>Pi1+Pi54</i>	90	130.33*	29.01*	18†	18†	256†	21.32	44.64†
11	18-11-2-1	<i>Pi1+Pi54</i>	89	106.02†	28.49*	16	16	285†	21.48	50.23*
12	18-11-2-3	<i>Pi1+Pi54</i>	91	126.78*	28.62*	17	17	205†	21.71	30.67
P <sub>1</sub>	Intan	-	120	112.00	27	25	25	302	26.00	50.00
P <sub>2</sub>	Tetep	<i>Pi54, Pi1</i>	78	150.00	26	11	11	185	16.50	29.30
Mean			92.12	130.34	29.14	17	16	251	21.48	40.86
Minimum			88.00	106.02	25.19	4	4	199	15.72	24.05
Maximum			97.00	163.09	33.09	24	24	370	25.26	50.23
C.V. (%)			10.57	13.12	10.07	9.11	8.90	13.85	3.15	19.34
C.D. @ 5 %				5.48	2.13	3.31	2.54	2.44	28.73	2.16
										4.64

P<sub>1</sub> - Intan (Recipient), P<sub>2</sub> - Tetep (Donor), † - On par with Intan, \* - Superior over Intan at P < 5 %, DFF - Days to 50 % flowering (days), PH - Plant height (cm), PL - Panicle length (cm), NT - No. tillers, NPT : No. productive tillers, FGPP - Filled grains per panicle, TW - 1000 seed weight (g), YPP - Yield per plant (g)

Table 3. Grain characters of selected backcross families with *Pi1*, *Pi54* and *Pi1+Pi54* combination

Sl. No.	Code	Gene	Kernel length (L, mm)	Kernel breadth (B, mm)	L/B ratio	Grain type
1	16-14-12-8	<i>Pi54</i>	6.46*	2.18†	2.97*	Medium- Slender
2	16-4-13-3	<i>Pi54</i>	5.51	2.30*	2.40†	Medium-Slender
3	16-14-4-7	<i>Pi1</i>	6.59*	2.18†	3.02*	Long- Slender
4	16-14-9-7	<i>Pi1</i>	6.63*	2.17†	3.05*	Long- Slender
5	16-8-5-12	<i>Pi1</i>	5.37	2.52*	2.14	Medium-Slender
6	18-17-18	<i>Pi1+Pi54</i>	5.50	2.25*	2.45†	Medium- Slender
7	16-14-7-3	<i>Pi1+Pi54</i>	6.69*	2.22*	3.02*	Long- Slender
8	16-8-5-6	<i>Pi1+Pi54</i>	6.02†	2.40*	2.51†	Medium- Slender
9	18-17-7-1	<i>Pi1+Pi54</i>	5.49	2.11	3.22*	Long- Slender
10	18-11-1-8	<i>Pi1+Pi54</i>	6.09†	2.39*	2.55†	Medium- Slender
11	18-11-2-1	<i>Pi1+Pi54</i>	5.54	2.19†	2.53†	Medium- Slender
12	18-11-2-3	<i>Pi1+Pi54</i>	5.67	2.27*	2.50†	Medium- slender
P <sub>1</sub>	Intan	-	6.34	2.20	2.89	Medium- Slender
P <sub>2</sub>	Tetep	<i>Pi54, Pi1</i>	5.56	2.22	2.50	Medium - Slender
C.D. @ 1 %	0.31	28.73	0.17	-	-	
C.V. (%)	11.06	13.85	0.15	-	-	

P<sub>1</sub> - Intan (Recipient), P<sub>2</sub> - Tetep (Donor), † - On par with Intan, \* - Superior over Intan at P < 1 %

Table 4. Phenotypic correlation between yield traits of backcross families with *Pi1*, *Pi54* and *Pi1+Pi54* combination

Trait	DFF	PH	PL	NT	NPT	FGPP	TW	YPP
DFF	1							
PH	-0.1921	1						
PL	0.0416	0.3744*	1					
NT	0.4125	-0.6903	0.1036	1				
NPT	0.4069	-0.6990	-0.4685	0.9944**	1			
FGPP	0.0656	0.5758*	0.5448**	-0.5108	-0.5624	1		
TW	0.6215*	-0.3043	0.1744	0.4537*	0.4043*	0.2549	1	
YPP	0.3268	-0.6242	0.3085	0.8028**	0.8011**	0.0275	0.4778*	1

DFF : Days to 50 % flowering (days)

NPT : No. productive tillers

PH : Plant height (cm)

FGPP : Filled grains per panicle

PL : Panicle length (cm)

TW : 1000 seed weight (g)

NT : No. tillers YPP : Yield per plant (g)

\* : Significant at 1 % level

\*\* : Significant at 5 % level

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NT, NPT, PL, FGPP and TW. There has been a highly significant positive correlation between YPP and, NT and NPT and it results in negative correlation with PH (Table 4). Thompson *et al.* (2003) have reported a significant and high positive association of grain yield with number of panicles, number of fertile spikelets per panicle and test weight.

## Conclusion

The backcross population and homozygous lines pyramided with blast resistance genes (*Pi1* +*Pi54*, *Pi54* and *Pi1*) may serve as valuable genomic resources in future rice breeding programmes for blast resistance.

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