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Research Article

Introgression of durable blast resistance gene Pi-54 into *indica* rice cv. samba mahsuri, through Marker Assisted Backcross Breeding

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Abstract
Blast disease is one of the most significant diseases of rice, where severe infection results in more than 80% reduction in yield. It is caused by *Pyricularia oryzae*. New breeding strategies are essential for developing durable blast resistant varieties. The present study aimed to introgress *Pi-54* gene from highly blast resistant genotype *i.e.* Tetep into elite rice cultivar Samba Mahsuri (BPT 5204), high yielding rice variety with good cooking quality ,but susceptible to blast disease, through Marker-Assisted Backcross Breeding programme (MABB). For foreground selection tightly linked molecular marker specific to *Pi-54* gene (*i.e.* Pi-54MAS) which is located on chromosome 11, and it is utilized at each backcrossed generation to identify plants carrying heterozygous alleles for the targeted resistant gene. A total of 56 background markers were used to estimate the recovery of recurrent parent genome in each backcrossed generations. At BC_2F_2, a single plant carrying the targeted resistant gene *Pi-54* with maximum recovery of recurrent parent genome (~92.80%; plant BT-8-47-22) was selected and forwarded to next generations through the selfing. Ancestry based selection procedure was employed for phenotypic disease screening and agro-morphological traits. Results confirmed that, resistance gene (*Pi-54*) was successfully incorporated into Samba Mahsuri. Six lines *viz.*, BT-8-47-22-6-36, BT-8-47-22-6-55, BT-8-47-22-6-117, BT-8-47-22-6-159, BT-8-47-22-6-203 and BT-8-47-22-6-267 were identified at BC_2F_4 which were possessing high level of resistance to blast and agro-morphological traits similar to Samba Mahsuri. One NIL line (BT-8-47-22-6-203) was found to be better than recurrent parent Samba Mahsuri (BPT 5204) regarding grain yield per plant. This finding will be helpful in developing a blast resistant variety with highest recurrent parent genome recovery in less number of generations through the application of MABB.

Key words
MABB, *Pyricularia oryzae*, *Pi-54*, Samba Mahsuri, Tetep

Introduction

Rice (*Oryza sativa* L) is one of the most significant cereal crop grown all over the world. It is the main food for the 4 billion people in worldwide and for above 70 per cent of the world's 900 million poor. The rice sector is a significant contributor to global food security. Its production and productivity is limited by number of biotic and abiotic factors. Among these blast disease is considered as a major factor due to its distribution and extent of destruction under favourable condition. It is caused by a fungus *Pyricularia oryzae* Cavara (teleomorphic *Magnaporthe oryzae* B. C. Couch) belonging to phylum Ascomycota. The fungus is known to cause lesions on leaves, stems, necks, panicles, seeds, and even roots and it is capable of causing yield losses up to 100 % under favorable conditions across the globe Prasad *et al.*(2012). Management of the incidence and severity of this disease is mainly through fungicides, bio-agents and botanical applications, and cultural practices Miah *et al.*(2013). Unfortunately, these measures are not very effective in all the time and locations. The use of fungicides is expensive, tedious and it affects the sustainable rice production by damaging the ecology and environment. Host resistance offers cost effective and eco-friendly method to manage this deadly disease effectively.

Backcross breeding for the host-plant resistance is an economical strategy and feasible alternative to alleviate this problem in an eco-friendly manner Ragimekula *et al.*(2013). Many resistant varieties have already been bred through conventional back cross breeding method. Conventional backcross breeding programme involves the production of varieties that are like the susceptible parent but included with required resistance genes. However, conventional breeding takes more years to develop resistant variety. The blast resistance has broken down in many rice growing areas in the world. In this milieu, modern molecular techniques make it very easy to use markers to introgress several R-genes into different cultivars from various sources during a backcross breeding programme with accuracy.

Marker Assisted Backcross Breeding (MABB) with DNA markers closely linked to the genotype of the target gene improves the efficiency of conventional plant breeding Chen *et al.*(2005). Generally,

MABB is used to improve the recombinant selection by minimizing the linkage drag Collard and Mackill, (2008). MABB will take much lesser time to develop new resistant variety compared to conventional breeding programme Mackill (2006). The first MABB successfully applied in introgression of QTLs from unadapted germplasm (e.g., land races, wild species) to an elite breeding line by Tanksley and Nelson (1996). Many varieties have developed from this backcross breeding programme by introgressing the agriculturally significant genes Liu *et al*.(2002). Using this approach, Vikas *et al.,* (2012) had successfully pyramided two major blast resistance genes *Pi-54* and *Piz5* into an elite Basmati variety. Recently, Hasan *et al.,* (2015) had successfully introgressed durable blast resistant gene *Pi-54* into a Malaysian *cv.* MR264.

As on today around 100 blast resistance genes are identified Sharma *et al*.(2012);Sharma *et al*.(2010) and among them, *Pi-54* gene (earlier *Pi-kh*) are exhibited resistance to major races of the *P. oryzae* pathogen in India (DRR Progress Report, 2008-16). Considering the earlier work, in this study the rice cultivar 'Tetep' was used as donor mainly for the introgression of blast resistance gene *Pi-54*, through Marker Assisted Backcross Breeding (MABB) programme in permutation with strict phenotypic selection for recovering agro-morphological characters of the recurrent parent 'Samba Mahsuri' (BPT 5204).

Materials and Methods

Samba Mahsuri (BPT 5204), a high yielding popular indica variety with good grain quality was used as recurrent parent and highly blast resistant variety 'Tetep' (*Pi-54* gene) was used as donor parent. F_1 seeds were developed by hybridization between Samba Mahsuri and Tetep. To generate BC_1F_1 seeds, the true F_1's plants are selected and then backcrossed with recurrent parent. The desirable BC_1F_1 plants are identified with maximum recovery of the recurrent parent genome (RPG) and again backcrossed with recurrent parent to develop the BC_2F_1 generation. At BC_2F_1 generation plants are subjected to foreground selection followed by phenotypic selection (disease reaction and both plant type) to identify the heterozygous plants for *Pi-54* gene and with maximum recovery of RPG. To develop the BC_2F_2 populations, these plants (BC_2F_1) are selfed.

The plant genomic DNA was extracted from F_1, BC_1F_1, BC_2F_1 and BC_2F_2 generations, respectively by using the micro-extraction procedure Prabhu *et al.(*1998). Leaf tissue of 3-5 cm size was grinded in spot plate by adding 700 µl of CTAB buffer. After grinding, the sample was kept at 65℃ for 30-40 min. in water bath for incubation. Later equal volume of chloroform with isoamyl alcohol (24:1) was added to the solution in 1.5ml eppendorf tubes. Then the tubes were shaken vigorously for 15 min. to get proper mixing and centrifuged at 13,000 rpm for 15min. The supernatant was transferred to another fresh eppendorf tube by discarding the pellet. Later equal volume of isopropanol was added to the solution. These tubes were kept in -20℃ freezer for 1-2 hour. After removing from the freezer tubes were shaken gently for 5-10 min. and then centrifuged at 13,000 rpm for 10 min. After removing from the centrifuge the supernatant was discarded and the pellet was retained. 120 µl of 70% ethanol was added to the pellet and tapped for 2 min. to mix the contents. Tubes were again centrifuged at 13,000 rpm for 5 min. The supernatant was discarded and the DNA pellet was dried and then dissolved in 300 µl of 1X TE buffer (pH-8.0) for long term storage. Quality of DNA was checked with 0.8% agarose gel electrophoresis at 90 V for 30-45 min and the concentration of DNA was measured by using Nano-Drop (Thermo Fisher, USA).

PCR was performed in a thermal cycler (AB Bio systems). Every 10 µl PCR reaction mixture contained 100 ng of genomic DNA, 2 mM dNTPs, 10 µM each of the primer pair (Genei, India), 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2.5 mM $MgCl_2$ and 1 unit Taq DNA polymerase. PCR comprised by one cycle of denaturation at 94℃ for 5 min, followed by 35 cycles at 94℃ for 30 s, 55℃ for 30 s and 72℃ for 1 min and final extension 72℃ for 10 min. The amplified products were resolved on 3 per cent agarose gel in 1X TAE buffer and stained with ethidium bromide (0.5pg/mL) and finally documented under UV light.

Introgressed Lines (IL's) were evaluated for their reaction of leaf blast disease at the Uniform Blast Nursery (UBN), ICAR-Indian Institute of Rice Research (IIRR), Hyderabad by using standard protocol by Prasad *et al.*(2011). Each entry was sown in 10 cm long row in the upland nursery bed with row spacing of 5 cm. A row of highly susceptible check HR-12 was sown after every five entries and also on the surrounding borders to ensure uniform spread of the disease. At the fourth-leaf stage (about 10-15 days after sowing), the seedlings were sprayed with spores of *P. oryzae* (IIRR-MSP-28 isolate). Pathogen infection and disease pressure was increased by maintaining high

relative humidity (93-99%) by water misting and covering the nursery beds with polythene sheets during night Prasad *et al.*(2012). Disease reaction of the entries was recorded at 15 days after inoculation by using the Standard Evaluation Scale

0-9 (IRRI, 1996) *i.e.* scores of 0-3 were considered as resistant, 4-5 as moderately resistant, 6-7 as moderately susceptible and 8-9 as highly susceptible.

Selected IL's were evaluated for the significant agro-morphological traits in the field along with recurrent and donor parents. The progenies (BC_2F_4) were grown and evaluated at ICAR-IIRR field during *Kharif* -2015. Individual plants from the IL's were evaluated and the data were recorded for various agro-morphological characters *viz.,* yield per plant (Y/P), number of tillers (NT), 1000 grain weight (TW), grain per panicle (GP), panicle length (PL), plant height (PH), days to maturity (DM) and days to 50 % flowering (DFF) for their selection. The procedures for measurement of these traits have been followed as per Sarawgi *et al*. (2013).

Results and Discussion

The F_1s generated from the cross between recurrent parent Samba Mahsuri (BPT 5204) and donor parent Tetep, were evaluated for presence of the targeted resistance gene *Pi-54* by using the gene-linked molecular marker Pi-54 MAS. The 'true' F_1s were identified through gene *Pi-54* amplification pattern. A total 103 F_1 plants were generated and 57 plants were confirmed for their heterozygosity (Table-1, Figure 1) and these were then used as male parent and backcrossed with recurrent parent Samba Mahsuri to generate BC_1F_1 plants.

Out of 347 BC_1F_1 plants 86 were identified as heterozygous for the target gene *Pi-54* through foreground analysis of these plants with the gene-specific linked marker. Among these, one plant *i.e*., #BT-8-47 possessing maximum recovery of the recurrent parent genome (~73.20%; Table 1; Figure 2) was identified by using 56 parental polymorphic SSR markers through background selection and it was backcrossed with recurrent parent BPT 5204 to produce 240 BC_2F_1 plants. Foreground selection among BC_2F_1 plants was done in 59 plants carrying gene *Pi-54* in heterozygous condition, which were then subjected to background selection. A single BC_2F_1 plant (#BT-8-47-22) with maximum RPG (~83.90%) was selected and generated the BC_2F_2 population by selfing. Among 419 plants, 105 plants were observed to be homozygous possessing dominant *Pi-54* gene. Among these plants a single plant was (#BT-8-47-22-6; Table 1; Figure 3) possessing gene and recovered maximum recurrent parent genome (92.80%) was identified through background selection.

Selected line was screened using the 'R' gene linked marker to identify plant that was homozygous for 'R' gene. These plants were forwarded to next generation by selfing and advanced through ancestry scheme involving morphological character based selection till BC_2F_4. Six promising advanced backcross derived lines were identified (*viz.,* BT-8-47-22-6-36, BT-8-47-22-6-55, BT-8-47-22-6-117, BT-8-47-22-6-159, BT-8-47-22-6-203 and BT-8-47-22-6-267) based on yield, disease reaction and other agro morphological traits.

All the six selected positive BC_2F_4 lines along with susceptible check (HR-12), donor (Tetep) and recipient parents (Samba mahsuri) were phenotypically evaluated for disease reaction. The donor parent Tetep, carrying *Pi-54* gene, showed resistance to disease reaction with '0' disease score and the recurrent parent BPT 5204 showed occurrence of disease lesions on leaves more than 80% with a disease reaction of '9'. While all six IL's (*viz.*, BT-8-47-22-6-36, BT-8-47-22-6-55, BT-8-47-22-6-117, BT-8-47-22-6-159, BT-8-47-22-6-203 and BT-8-47-22-6-267) showed complete resistance (0-2 score) to rice blast disease (Table 2; Figure 4).

The selected six ILs (BT-8-47-22-6-36, BT-8-47-22-6-55, BT-8-47-22-6-117, BT-8-47-22-6-159, BT-8-47-22-6-203 and BT-8-47-22-6-267) were evaluated for key agro-morphological traits (Table 3). Results showed that BT-8-47-22-6-203 (IL) had RPG of 92.80% and grain yield slightly higher than (20.5±05 gm) recurrent parent (*i.e.* BPT 5204; 19.9±0.1gm). While other five IL's *viz.,* BT-8-47-22-6-36, BT-8-47-22-6-55, BT-8-47-22-6-117, BT-8-47-22-6-159, and BT-8-47-22-6-267 possesing RPG of 91.1, 92.6, 91.9, 92.5 and 92.0 respectively and showed grain yield per plant corresponding to the recurrent parent. Moreover, IL BT-8-47-22-6-159 (79.7±0.6 cm) was identified significantly taller than recurrent parent Samba Mahsuri (78.7±1.5 cm). A few significant variations were observed with respect to the no. of panicles per plant and panicle length among the six ILs as compared to Samba Mahsuri (Table 3). The IL BT-8-47-22-6-203 was found to be better than Samba Mahsuri because it had higher grain yield per plant and as well as more disease resistant reaction (Figure 5).

The Indica variety Samba Mahsuri (BPT 5204) is popular in India due to its excellent cooking and eating qualities, medium slender grain type and high yield (Reddi *et al.,* 1979). It was initially released in 1986 for farming in Andhra Pradesh. Now, it has spread to eight other states (Bihar, Chhattishgarh, Karnataka, Maharashtra, Telangana, Tamil Nadu, Pudicherry and Uttar Pradesh) of the rice growing area in the country. Thereby it is targeted for incorporating various biotic and abiotic resistant through gene introgression/pyramiding. Recently, strict incidence of blast and neck blast

disease (5-40 %) is a major restriction in this rice variety for its production and productivity (POS, 2014). It is occupying around thirty per cent of the rice area in Andhra Pradesh accounting for ten lakh hectares while another five lakh hectare in other states (The Hindu New Paper, 2014). However, the present study was undertaken with the aim to transfer a durable or broad-spectrum resistance gene *Pi-54* through marker assisted backcross breeding from the cultivar 'Tetep' as the resistant donor. Even though there are some reports about breakdown of resistance conferred by a single blast resistance gene Ramkumar *et al.,(*2011); Khush, (1989) in rice, till date there is no report about breakdown of resistant conferred the *Pi-54* gene across India or abroad. As for recent reports Tetep was showing consistent resistant across country (DRR Progress Report, 2008-16).

Improvement and selection of plant carrying desirable genome (resistant gene) can be speed up through introgression with advent of molecular marker technology Sundaram *et al.(*2008). Microsatellite markers are the most preferable markers for plant breeding application due to well distributed throughout rice genome and hyper variable Miah *et al.*(2013). According to Alam *et al.* (2012) polymorphic markers is an essential step in breeding program as it can distinguish between two different parental genotypes. In the present study a higher number of parental polymorphic markers are used *i.e.*, 57 SSR markers with ~4 polymorphic markers per each chromosome to the better coverage of each chromosome in genetic background selection. Ragimekula *et al.* (2013) stated that selection of primers was best based on repeat number and location on all chromosomes. As compared to the earlier successful reports of target gene *Pi-54*, located on chromosome 11, Hospital, (2001) suggested a higher number of parental polymorphic markers on chromosome 11 for background selection. It had surely resulted in restrictive the linkage drag to the regions close to the target genes (Brinkman and Frey, 1977). In present study, BC_1F_1, BC_2F_1 and BC_2F_2 were noticed with the average RPG of 73.20%, 83.90% and 92.80% respectively and it proved the statement that percentage of RP genome was higher in MABB compared to conventional breeding MABB program applied in this study demonstrated that a few individual plants in three generations showed a full recovery of recurrent genome. The same thing had been observed using the graphical genotypes concept that was first introduced by Van Berloo, (2008).

Till now, a large number of blast resistant varieties were developed to blast disease through MAS *e.g.*, Madhavi *et al.* (2012) developed a variety in the background of Improved Samba Mahsuri, Wen and Gao, (2011) introgressed *Pi9*(t) into the restorer line Luhui17 and Jiefeng *et al.* (2015) developed a line C815S. In the same way, in the background of IR 64 Sreewongchai *et al.* (2010) also developed blast resistance by using the Marker Assisted Selection coupled with phenotypic selection for their agronomical traits. However, the MAS heavily depend upon the strong linkage between the target gene and markers. Narayanan *et al.* (2002) was successfully introgressed blast resistant gene *Piz-5* in the background of IR50 through MABB. The very same MABB/MABC method was exploited by Hari *et al.* (2011), Basavaraj *et al.* (2010) and Gopalakrishnan *et al.* (2008) to improve bacterial blight resistance *i.e.* KMR-3R, Basmati rice variety Pusa RH10 and Pusa Basmati 1 respectively. Hasan *et al.* (2015) introgressed blast resistant gene *Pi-kh* into a Malaysian cultivar, MR264 through Marker Assisted Backcrossing (MABC). In the present finding also the very same research methodology was applied to develop blast resistant variety in Samba Mahsuri.

In this study, six selected IL's, BT-8-47-22-6-36, BT-8-47-22-6-55, BT-8-47-22-6-117, BT-8-47-22-6-159, BT-8-47-22-6-203 and BT-8-47-22-6-267 of Samba Mahsuri possessing high yielding and good cooking quality. The recurrent and donor parents are vary significantly with respect to grain type, the donor parent Tetep with short-bold grain type while, recurrent parent Samba Mahsuri possessed medium-slender grain type. Hence, phenotype-based visual selection for medium-slender grain type was restored, from BC_1F_1 generation onwards a large number of backcross plants, were evaluated and identified the lines possessed with blast resistance and medium-slender grain type. Previously, Rambabu *et al.* (2016), Madhavi *et al.* (2016), Hari *et al.* (2011) and Sundaram *et al.* (2008) followed a strategy of agro- morphology based selection for grain type coupled with polymorphic markers for their selection of target disease resistant (*i.e.* blast and bacterial blight) in improved NIL's of elite cultivars Swarna, ISM, KMR-3R and Samba Mahsuri respectively. Present study is the first time report of successful introgression of blast resistance gene *Pi-54* into Samba Mahsuri through MABB along with phenotypic selection. These selected lines showed strong resistance against virulent isolate IIRR MSP-28 like donor parent Tetep, that's indicate the strong bond between this marker with the trait (Table 2; Figure 4). Also in the improved lines of Samba Mahsuri no apparent yield penalty was observed. Hence, the farming of introgressed lines would be of great advantage to reduce the yield losses due to effect of blast disease in endemic areas. Advanced lines (carrying *Pi-54* gene) of

Samba Mahsuri are showed a similar agro-morphological performance in the field level as a recurrent parent Samba Mahsuri. Results are indicating that the performance of introgression lines were on par with recurrent parent with marginal differences (Table 3; Figure 5).

This is the first report of transfer of *Pi-54* gene conferring resistance to blast in Samba Mahsuri through MABB. The IL's have the desirable recurrent parent characteristics like cooking quality and grain type, coupled with inbuilt resistance to blast disease and more yield compared with 'Samba Mahsuri'. These resistant lines are under multi-location trials for release to the farmers. They can be used as unique source for blast resistance genes in future backcross breeding programmes.

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Table 1. Details of backcross derived plants from the cross Samba Mahsuri (BPT 5204)/Tetep for foreground and background selection.

S. No	Cross combination	Generation	No. of plants analyzed	Foreground Selection	Background genome selection			
				+ve for *Pi54* gene	SSRs analyzed	Polymorphic SSRs, homozygous for R' allele	Recurrent parent genome Recovery (%)	Selected plant based on background selection
1	B X T	F_1	103	57	-	-	-	BT-8
2	B X BT-8	BC_1F_1	347	86	56	41	73.20%	BT-8-47
3	B X BT-8-47	BC_2F_1	240	59	15	6	83.90%	BT-8-47-22
4	B X BT 8-47-22	BC_2F_2	419	105	9	5	92.80%	BT-8-47-22-6

Note: B=BPT 5204, T=Tetep, BT= NILs of BPT 5204 X Tetep

Table 2. Reaction of the six selected pyramided BC_2F_4 lines after inoculation with blast isolate (IIRR MSP-28)

S. No.	Designation	Resistance gene *Pi-54* genotyped by linked marker Pi-54 MAS	Reaction against to blast disease with IIRR MSP-28 isolate		Grain type
			Score*	R/S	
1	BPT 5204	--	8.0±1.0	S	MS
2	TETEP	++	0.3±0.6	R	SB
3	HR-12	--	8.7±0.6	S	LB
4	BT-8-47-22-6-36	++	0.7±0.6	R	MS
5	BT-8-47-22-6-55	++	1.0±1.0	R	MS
6	BT-8-47-22-6-117	++	0.3±0.6	R	MS
7	BT-8-47-22-6-159	++	1.3±0.6	R	MS
8	BT-8-47-22-6-203	++	1.3±0.6	R	MS
9	BT-8-47-22-6-267	++	0.7±0.6	R	MS

'++':- Possessing homozygous resistant allele at the particular gene locus, based on screening with gene linked marker.
'--':- Possessing homozygous susceptible allele at the particular gene locus, based on screening with gene linked marker; 'R'- Resistant; 'S'- Susceptible; 'MS' - Medium Slender and 'SB; Short Bold respectively.
'*':- Mean of the three replications and each replication data was collected from 50 plants.

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Table 3. Details of agronomic performance of the parents and introgressed lines of Samba Mahsuri (BPT 5204) at BC_2F_4 under field conditions

S. No.	Designation	DFF*	DM*	PH* (cm)	PN*	PL* (cm)	GP*	TGW	Y/P**	RPG (%)
1	BPT 5204	124.0±1.0	148.3±1.2	78.7±1.5	11.0±1.0	23.5±0.5	179±1.0	17.9±0.1	19.9±0.2	-
2	TETEP	93.7±1.5	128.0±1.0	94.3±0.6	9.7±0.6	22.8±0.8	160.3±0.6	16.8±0.2	19.2±0.2	-
3	BT-8-47-22-6-36	119.7±0.6	144.7±0.6	77.3±0.6	10.0±1.0	23.3±0.3	178.7±0.6	17.6±0.5	20.0±0.4	91.1
4	BT-8-47-22-6-55	119.0±1.7	142.0±1.0	77.7±1.2	10.0±1.0	23.3±0.6	178.0±1.0	17.0±0.2	20.0±0.3	92.6
5	BT-8-47-22-6-117	118.7±0.6	141.7±1.2	78.3±1.2	10.7±0.6	23.5±0.5	177.3±0.6	17.2±0.8	19.8±0.7	91.9
6	BT-8-47-22-6-159	119.0±2.0	141.7±0.6	79.7±0.6	10.3±0.6	23.2±0.3	177.0±1.0	17.5±0.5	19.7±0.4	92.5
7	BT-8-47-22-6-203	118.3±2.3	141.7±1.2	78.3±1.2	11.7±0.6	23.7±0.3	179.3±0.6	18.0±0.1	20.6±0.4	92.8
8	BT-8-47-22-6-267	118.3±1.5	142.7±1.7	77.7±0.6	10.0±1.0	23.5±0.5	177.07±1.5	17.3±0.6	19.7±0.4	92.0

DFF: Days to 50% flowering, DM: Days to maturity, PH: Mean plant height (cm), PN: No. of panicle per plant, PL: Panicle length (cm), GP: Grain weight (gm), TGW (gm): 1000 grain weight and RPG: Recurrent parent genome recovery (%).
'*':- Mean of the three replications; '**':- Mean of the twelve replications.

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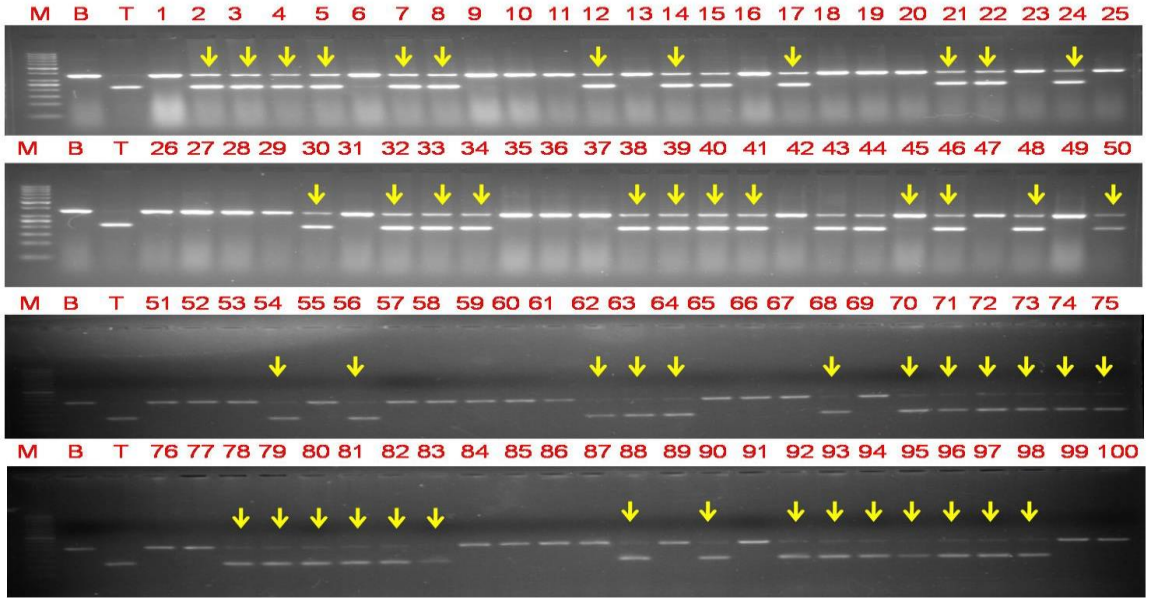

Fig. 1. The gene linked marker Pi-54 MAS was used for screening of *Pi-54* gene in the F_1 plants. The numbers represents the F_1 plants from the cross BPT 5204/Tetep. Gel Lanes M: 50bp molecular weight ladder; B-Recurrent parent 'BPT 5204 (Samba Mahsuri)', T- Donor parent 'Tetep'; 1- 25 - F_1 plants, (line No's 2, 3, 4, 5, 7, 8, 12, 14, 17, 21, 22, 24, 30, 32, 33, 34, 38, 39, 40, 41, 45, 46, 48, 50, 54, 56, 62, 63, 64, 68, 71, 71, 72, 73, 74, 75, 78, 79, 80, 81, 82, 83, 88, 90, 92, 93, 94, 95, 96, 97 and 98 ' heterozygous +ve plants' for *Pi-54* gene).

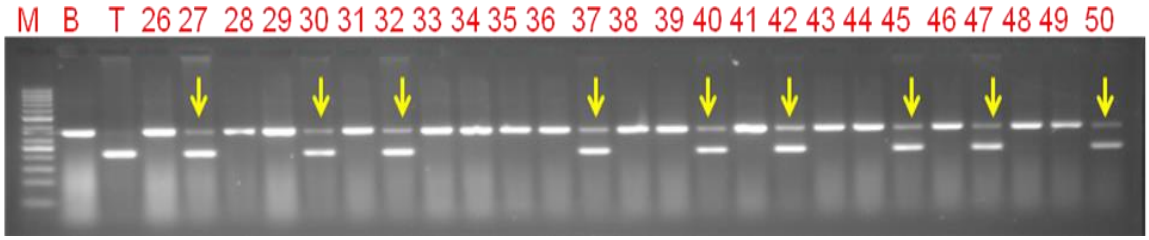

Fig. 2. The gene linked marker Pi-54 MAS was used for screening of *Pi-54* in the BC_1F_1 plants. The numbers represents the BC_1F_1 plants from the cross BPT 5204/Tetep. Gel Lanes M: 50bp molecular weight ladder; B-Recurrent parent 'BPT 5204 (Samba Mahsuri)', T-Donor parent 'Tetep'; 1- 25 - BC_1F_1 plants, (line No's 27, 30, 32, 37, 40, 42, 45, 47 and 50 'heterozygous +ve plants' for *Pi-54* gene).

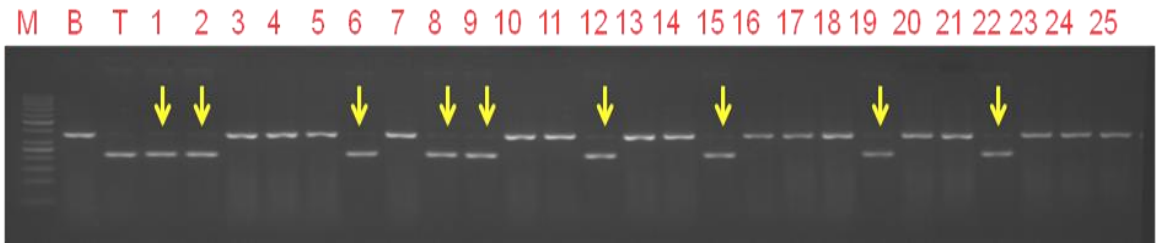

Fig. 3. The gene linked marker Pi-54 MAS was used for screening of *Pi-54* in the BC_2F_2 plants. The numbers represents the BC_2F_2 plants from the cross BPT 5204/Tetep. Gel Lanes M: 50bp molecular weight ladder; B-Recurrent parent 'BPT 5204 (Samba Mahsuri)', T-Donor parent 'Tetep'; 1- 25 - BC_1F_1 plants, (line No's 1, 2, 6, 8, 9, 12, 15, 19 and 22 'homozygous +ve plants' for *Pi-54* gene).

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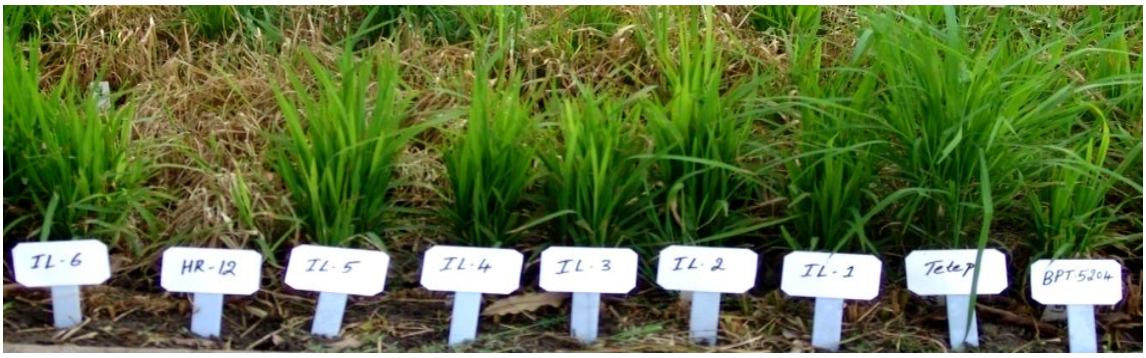

Fig. 4. Screening of selected BC_2F_2 lines against blast disease following UBN Method, HR-12: Susceptible check, Samba Mahsuri (BPT 5204): Recurrent parent (susceptible) and Tetep: Donor parent (highly resistant); IL-1 to IL6 (i.e., BT-8-47-22-6, BT-8-47-22-55, BT-8-47-22-117, BT-8-47-22-159, BT-8-47-22-203 and BT-8-47-22-267) introgressed lines.

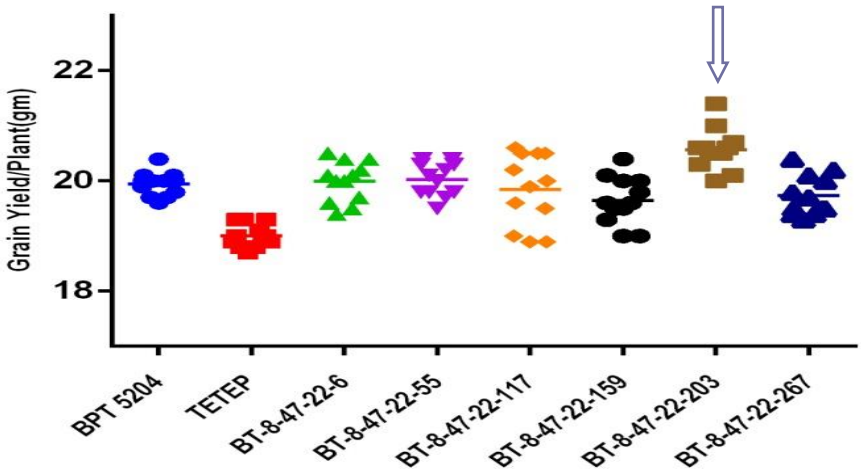

Fig. 5. Scatter plot showing IL BT-8-47-22-6-203 to be better than recurrent parent Samba Mahsuri (BPT 5204) in grain yield per plant and remaining IL's are mostly similar with the recurrent parent, indicating that the performance of introgression lines was on par with recurrent parent with marginal differences.