

VALIDATION OF MAS DERIVED LINES FOR INTROGRESSED GENE AGAINST BLAST AND BLB RESISTANCE IN SOUTHERN CHHATTISGARH

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Abstract: The experiment was carried out at SGCARS, Jagdalpur, IGKV Raipur, Chhattisgarh to validate Marker Assisted Selection (MAS) derived genotypes, from ICAR-IIRR, Hyderabad, against blast and bacterial leaf blight resistance and access recurrent parent recovery. BPT 5204 (Samba Mahsuri) the recurrent parent 01 (RP 1) for the four test genotype recorded average plot yield of 4.00 kg/ha placing second in the experiment. When the recurrent parent 02 (Improved Sambha Mahsuri) was taken into account, genotype RP-Patho-1-2-15 recorded higher plot yield (4.23 kg). RP-Patho-1-2-15 and RP-Patho-3-56-11 were similar to the recurrent parent with heading span of 78 and 79 DAS accordingly while RP-Patho-3-73-6 was six days in advance (70 days) to the recurrent parent (76 days). The entire test Near Isogenic Lines (NILs) with a plant height of 79-85 cm were similar to the recurrent parent (82 cm). Blast and bacterial leaf blight resistance gene carrying genotypes RP-Patho-2-18-5 and RP-Patho-2-16-4 gave plot grain yield 3.77kg, which out yielded recurrent parent 02 but lesser than recurrent parent 01. Incidence of blast reported in Tetep (1-2%), C 101 LAC (8-10%) and average (5-8%) in all NILs. Blast resistance genes *Pi 1* carrying genotypes RP-Patho-1-2-15 and RP-Patho-1-6-5, the infestation the comparatively higher (Score 6) than those with *Pi 54* (RP-Patho-3-56-11 and RP-Patho-3-73-6, RP-Patho-3-56-11 and RP-Patho-3-73-6 (Score 4). However, dual genetic resistance background i.e. blast and bacterial blight resistance genes *Xa 21+ Pi-54*, provided excellent resistance even in hot spot centre for the disease. There was no incidence of Bacterial Leaf Blight (BLB) in all the isogenic line including donor and recurrent parents which may be because of plant defence system or incompatible environmental condition for disease prevalence.

Keywords: NILs, Marker Assisted Selection, Recurrent parent, Blast, Bacterial Leaf Blight

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the vital cultivated crop, which provides food for more than half of the world's population and constitutes a major source of calories for urban and rural inhabitants (Singh *et al.*, 2015, Kumar *et al.*, 2015a), regrettably, whose production is constrained by substantial number of fungal, bacterial and viral origin diseases. Rice blast (caused by *Magnaporthe grisea*) and bacterial leaf blight (BLB, caused by *Xanthomonas oryzae* pv. *Oryzae*, Xoo) are two most destructive diseases leading to severe yield losses in rice production worldwide (Zhan *et al.*, 2012). Bacterial leaf blight (BLB) is one of the most devastating diseases affecting entire rice acreages and causes severe yield losses of up to 74-81% depending upon crop stage, cultivar susceptibility index and the environmental phenomenon (Srinivasan *et al.*, 2005). Rice blast, caused by the filamentous ascomycete fungus *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*), is another major threats for rice production and leads to significant yield loss, as high as 70-80% during an epidemic (Khush *et al.*, 2009). The most effective approach to prevent the two diseases is the genetic improvement using resistant varieties however, narrow genetic diversity in existing gene pool is problematic in breeding for adaption to these major biotic stresses (Sattari *et al.*, 2014). Therefore, exploitation of host plant resistance is emerging as most effective, economical and environmentally safe measure for

controlling paddy blast and bacterial leaf blight in combination with pathological management. So far, 73 blast resistance genes and 31 BLB resistance genes have been identified (Ballini *et al.*, 2008, Ruan *et al.*, 2008, Cheema *et al.*, 2008, Sujatha *et al.*, 2011) and some of them have been incorporated into modern rice varieties (Sundaram *et al.*, 2008, 2009) through marker assisted selection (MAS). Among these, few genes like *Pi-1*, *Pi-2*, *Pi-9*, *Pi-54* etc. (for blast resistance) and *Xa5*, *Xa 13*, *Xa21*, etc. (for bacterial leaf blight resistance) are being extensively used in rice breeding programmes globally as are highly effective and having tightly linked molecular markers.

Marker Assisted Backcross Selection (MABS) has previously been used in rice breeding to incorporate *Sub1* gene of mega-variety Swarna to a submergence tolerant variety and IR64 *SUB1* for developing a new submergence tolerant rice variety ASS996-*SUB1* (Neeraja *et al.* 2007, Septiningsih *et al.* 2009, Luu *et al.* 2012). It was also used to incorporate *badh2* and *Wx* gene from Basmati into *Manawthukha* for cooking quality parameters (Yi *et al.* 2009), and *Pup1* under Phosphorus deficient lowland/irrigated conditions into Situ Bagendit and Batur (Chin *et al.* 2011). Rice salt tolerance on BT7 cultivar, FL478 was used as a donor parent of *Saltol* QTL (Linh *et al.* 2012) and three resistance genes (*Xa4* + *xa5* + *Xa21*) to bacterial leaf blight were transferred from an indica donor (IRBB57) to Korean rice *Mangeumbyeo* (Suh *et al.* 2013). More recently Fatimah *et al.*, (2014)

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successfully transferred *hd2* gene in rice for early heading date. With these background, some of the ICAR-IIRR (Formerly DRR) Hyderabad developed MAS breeding lines, obtained under AICRIP programme, possessing high level of resistance against blast (conferred by *Pi1* or *Pi54*) and bacterial blight (conferred by *Xa21*) was evaluated for trait verification and for substantial equivalence with the recurrent parents in southern Chhattisgarh province.

MATERIALS AND METHODS

The Study Material

The breeding materials were obtained from Indian Institute of Rice Research, (ICAR-IIRR, formerly DRR), Hyderabad under AICRIP programme in Advanced Varietal Trial 01- Near Isogenic Lines (AVT-1-NILs). The trial was constituted with 6 test entries, 2 recurrent parents and 2 donor parents. Entry RP-Patho-1-2-15 and RP-Patho-1-6-5 possess *Pi-1*, resistant to leaf blast. Entry RP-Patho-3-56-11 and RP-Patho-3-73-6 possess *Pi-54*, conferring resistance to leaf blast (DRR, 2014). Entry RP-Patho-2-18-5 and RP-Patho-2-16-4 were derived from backcross between Improved Samba Mahsuri (recurrent parent) and Tetep (donor parent) possessing combined resistance to leaf blast and bacterial leaf blight as they possess the resistance genes *Pi-54* and *Xa21* respectively (DRR, 2014, Table 01).

Experiment Conduction and Statistical Analysis

The trireplicated field experiment was conducted at Rice Research Block of S. G. College of Agriculture and Research Station, Jagdalpur, Chhattisgarh, India in Kharif 2013-14 with 5x2.6m (Net plot) plot size, Randomized Complete Block Design. Standard agronomic package was followed to raise the crop. Restriction selection index was constructed based on previous research review and four quantitative parameters were selected for genetic evaluation. The observation was recorded on net plot basis (heading date and plot yield), unit plot basis (panicles/sq M) and arithmetic mean basis among random selections. Days to 50 percent flowering was recorded when half of plant flowered among the plot. Panicle count was made with one square meter square at maturity. The disease scoring was made visually giving the score 1-9. Statistical analysis was done with SPARK 2.

RESULTS AND DISCUSSION

Rice is oldest domesticated cereal crop imparting diet to three billions globally (Shrivastava *et al.*, 2014) of which irrigated rice account for 55 percent of area and 75 percent of production (Kumar *et al.*, 2015b). Era of input oriented agriculture and changing climate have led this important crop susceptible to many diseases mainly Blast and BLB and scenario became more critical when narrowed crop genetic

base and expending pathogen biotype failed the monogenic plant breeding approach. Recently, pyramiding of more than one major resistance gene has been proven to deliver durable resistance (Rajpurohit *et al.*, 2010). Since, conventional breeding tools are inefficient for gene pyramiding, particularly in recessively inherited resistance, such as *xa5* and *xa13*, marker assisted selection (MAS) enables to address these limitations by the evaluation of the expression status of resistance gene(s).

Genotypic response referring to Recurrent and Donor parent

The test entries were evaluated at Jagdalpur centre, hot spot location for rice blast disease, under natural field conditions. The experimental yield ranged from 2.67 kg/plot (Improved sambha Mahsuri) to 4.23 kg/plot (RP-Patho-1-2-15). The Recurrent parent 01 (BPT 5204) was the earliest to flower with 50% flowering in 91 DAS followed by donor parent 01 (92 DAS) while RP-Patho-1-2-15 and RP-Patho-3-73-6 recorded to be late flowering genotypes with 113 DAS days to 50 percent flowering. The experimental mean for blooming period was 101 DAS. RP-Patho-3-73-6, BPT 5204 and RP-Patho-1-2-15 were short in plant height (70, 76 and 78 cms respectively) while Tetep was recorded to be tallest (115 cms) (Table 02) whereas, the mean plant height was reported to be 84.5 cms. Panicles count was significantly higher for BPT 5204, RP-Patho-3-73-11 and RP-Patho-1-6-5 (348, 324 and 312 correspondingly) while RP-Patho-1-2-15 and Tetep exhibited comparative lower count. The grain yield was in perfect direction with panicles number. BPT 5204 (Samba Mahsuri) the recurrent parent 1 (RP 1) for the four test genotype recorded average plot yield of 4.00 kg/ha placing second in the experiment. None of the entry recorded on par with recurrent parent 01 statically however, all the genotypes no significant difference was observed with respect to grain yield. When the recurrent parent 02 (Improved Samba Mahsuri) was taken into account, genotype RP-Patho-1-2-15 recorded higher plot yield (4.23 kg) and was at par. Flowering duration wise, RP-Patho-1-2-15 and RP-Patho-3-56-11 were similar to the recurrent parent with flowering of 78 and 79 DAS accordingly while RP-Patho-3-73-6 was six days in advance (70 days) to the recurrent parent (76 days). All the test isogenic lines with a plant height of 79-85 cm were similar to the recurrent parent (82 cm). Blast and bacterial leaf blight resistance gene carrying genotypes RP-Patho-2-18-5 and RP-Patho-2-16-4 gave plot grain yield 3.77 kg, which out yielded recurrent parent 02 but lesser than recurrent parent 01. However flowering was delayed (96 DAS and 99 DAS) and plant height was increased in both the isogenic lines (83 cms and 81 cms). The same trend was continued with panicle count. The donor lines Tetep (DP 1) and C 101 LAC (DP 2) showed optimal adaption to Southern Chhattisgarh rice growing ecology. Average plant height of Tetep was 91 cms and attained 50 percent

flowering by 98 DAS. Since vegetative span was lengthened, panicle count (300) and plot yield also increased accordingly (3.43). Taking into account another donor parent (Tetep), it exhibited faster growth rate and attained 115cm plant height by 92 DAS of preflowering period. Compare to DP 2 produced lesser panicle population (264) and plot grain yield (3.15kg).

Resistance breeding by deploying target genes has always been a topic of interest and intense research for breeders and molecular biologist (Sundram *et al.*, 2008, Zhan *et al.*, 2012). Pyramiding major genes to enhance resistance spectrum in commercial cultivars has been proven by many workers (Hittalman *et al.*, 2000, Chen *et al.*, 2001, Zhan and Cheng, 2001, Narayanan *et al.*, 2002, 2004, Joseph *et al.*, 2004, Neerja *et al.*, 2007, Chen *et al.*, 2008, 2009, Fujita *et al.*, 2010). Marker-assisted selection for genes and disease evaluation for genetic verification can be conducted in laboratory conditions during seedling stage at first followed by field evaluation to access the environmental play. The subsequent selection for complex quantitative traits such as combining ability, grain quality and grain yield can be carried out in the fields during heading and maturity stage. Combined laboratory and field evaluation at early and late stages make MAS breeding more efficient and assured than conventional breeding (Zhan *et al.*, 2012). Once disease resistance genes are standardised by MAS, rice breeding can be focused on combining and restoring the agronomic traits (Lang *et al.*, 2008, Pinta *et al.*, 2013). The biphasic selection in breeding programme is promising to overcome the defects of conventional hybridization breeding in which restorer genes and resistance genes usually have low recombinant frequency (Cao *et al.*, 2003) that requires immense effort to identify the individuals combining both the resistance and restoring ability in research programme.

Scoring for Blast and Bacterial Leaf Blight

Morphologically all the isogenic lines were parallel to the respective recurrent parent, blast prevalence was modest in both Recurrent Parents. Incidence of blast reported in Tetep (1-2%), C 101 LAC (8-10%), all isogenic lines (5-8%). Blast resistance genes *Pi 1* carrying genotypes RP-Patho-1-2-15 and RP-Patho-1-6-5, the infestation was comparatively higher (Score 6) however those with *Pi 54* (RP-Patho-3-56-11 and RP-Patho-3-73-6). Similarly RP-Patho-3-56-11 and RP-Patho-3-73-6, having the blast resistance gene, (*Pi 54*) showed average disease incidence (Score 4). However, dual genetic resistance background i.e. both blast and bacterial blight resistance genes *Xa 21* + *Pi-54*, provided excellent resistance even in hot spot centre for the disease. In previous studies multiple resistance gene pyramiding has been reported by Chen *et al.*, 2009 and Zhan *et al.*, 2012. In genotype RP-Patho-2-16-4 the disease was scaled to be 01, which was quite similar to recurrent parent 02 (Improved Sambha Mahsuri). Visual evaluation of

donor parents revealed average disease incidence (score 4). There was no incidence of Bacterial Leaf Blight in all the isogenic line including donor and recurrent parents which may be because of plant defence system or incompatible environmental condition for disease prevalence. Plant adopts several strategies to defend against pathogens, including the production of antibacterial chemicals, which are either preformed (i.e. already present in plant tissue in variable amounts) or induced following infection (e.g. *de novo* synthesized phytoalexins) (Sattari *et al.*, 2014). Many of the phytoalexins or pre-formed chemicals belong to the phenolic group (Latif, 2007). Phenolic compounds are secondary metabolites synthesized in plants and play a role in plant defence against pathogens through antimicrobial properties, involvement in cell wall reinforcement, modulation and induction of plant responses (Aly, 2002). Generally, when a plant is infected, its phenolic content increases in response to defence reaction (Ma, 2002). In addition, biochemical resistance is another complex but equally vital mechanism in plant pathogen defence specifically accumulation of peroxidase, catalase and other pathogenesis related proteins.

Recovery of Recurrent Parent

Almost all the genotypes exhibited stable recovery of one or both recurrent parents indicating stabilization of population allelic recombination. Perusing radar chart of recurrent parent recovery (Fig 01) reproductive behaviour were similar to BPT 5205 and Improved Sambha Mahsuri for all genotypes except RP-Patho-1-2-15 and RP-Patho-2-16-4 but it may attribute to G x E interactions rather than genetic segregation. Similarly, for above ground plant canopy length all test genotypes were relatively more closure to both the recurrent parents which shows comparative expression of photosynthates source sink balance in Near Isogenic Lines (NILs). However, donor parent i.e. Tetep, plant height was significantly higher might be due to regional quantitative adaptability. Looking for sink strength, BPT 5205 recorded significantly higher potential of dry matter accumulation than remaining population due to genetic establishment in given environment. Parallel observation was recorded for RP-Patho-3-56-11, RP-Patho-3-73-6 and RP-Patho-2-18-5 showing potent leaf blast and bacterial leaf blight resistance for Southern province of Chhattisgarh. Ultimately, when crop yield was taken into account, the normal distribution curve shifted towards the recurrent parent 01 and the entire test entries lied between 1750 kg/ha to 3075 kg/ha. Summarily, sustainable yield potential was realized despite of target disease incidence all genotypes may be considered as resistance genotypes.

The varietal improvement for resistance to major prevalent and destructive diseases is necessary for sustainable grain yield. Past attempts to achieve Bacterial Leaf Blight (BLB) resistance is not very

much encouraging, largely due to disease variability level in growing areas (Sreewongchai, 2010). Pyramiding disease resistant genes into a single genetic background might be expected to give more durable disease resistance, as more resistant genes are incorporated into single genotypes (Koide, 2010). Till date, as many as 24 major genes of host plant resistance which have been identified and used in rice improvement programme (Rao, 2002). The BLB resistance becomes quantitative when using NILs with four resistance genes (R gene) (*Xa4*, *x5*, *xa13*, and *Xa21*) that express a higher level and more durable resistance. Further, the resistant genes to BLB, *Xa4*, and *xa13*, links to microsatellites markers RM144 and RM122, respectively, and *x5* links to STS marker (RG136) (Sattari *et al.*, 2014).

CONCLUSION

Many disease resistant varieties have been developed but they have not been widely adopted as tolerant

varieties lack many of desirable traits of the widely grown mega varieties and hence replacement of these megavarieties with modern cultivars cannot be possible. However, these megavarieties despite having many agronomically desirable characters, often susceptible to biotic and abiotic stress. With advances in molecular biology, breeding for resistance genes has become wise and efficient strategy in crop improvement. In present investigation evaluation was made of DRR bred materials for hot spot of paddy blast. All the genotypes were similar to recurrent parent with respect to flowering duration, canopy length, and plot grain yield and disease reaction indicating the recovery of crossed material to desired genetic background after introgression of dual resistance gene and equally and effectively even in hot spot centre for the disease. No incidence of bacterial leaf blight (BLB) was observed and the reason may be excellent resistant source or environmental cause.

Table 1. Composition of Entries (Near Isogenic Lines) for Recurrent Parent Recovery Analysis (RPRA)

S No	Genotype	Cross Combination	Resistance Gene	Grain Type
01.	RP-Patho-1-2-15	BPT 5204*2/C 101 LAC	Blast Resistance Gene <i>Pi-1</i>	MS
02.	RP-Patho-1-6-5	BPT 5204*2/C 101 LAC	Blast Resistance Gene <i>Pi-1</i>	MS
03.	RP-Patho-3-56-11	BPT 5204*2/ Tetep	Blast Resistance Gene <i>Pi-kh (Pi-54)</i>	MS
04.	RP-Patho-3-73-6	BPT 5204*2/Tetep	Blast Resistance Gene <i>Pi-kh (Pi-54)</i>	MS
05.	RP-Patho-2-18-5	Improved Sambha Mahsuri/Tetep	Blast and Bacterial Blight Resistant Gene <i>Xa21+Pi-kh (Pi-54)</i>	MS
06.	RP-Patho-1-2-15	Improved Sambha Mahsuri/Tetep	Blast and Bacterial Blight Resistant Gene <i>Xa21+Pi-kh (Pi-54)</i>	MS
07.	BPT 5204	Recurrent Parent 01 (RP 01)		
08.	Improved Sambha Mahsuri	Recurrent Parent 02 (RP 02)		
09.	Tetep	Donor parent 01 (DP 01)		
10.	C 101 LAC	Donor Parent 02 (DP 02)		

Source: DRR Annual Report, 2014.

Table 2. Ancillary Traits Data following Restriction Selection Indices

	Plant Height (cm)		Days to 50 percent Flowering		Panicles/sqm		Grain Yield/Plot	
	Mean	SE	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
RP- Patho-1-2-15	78	4.98	113	0.88	257	12.42	4.23	0.15
RP- Patho-1-6-5	90	3.84	108	0.33	312	24.12	3.87	0.41
RP- Patho-3-56-11	79	4.33	113	0.83	324	12.2	3.7	0.3
RP- Patho-3-73-6	70	4.97	107	1.16	331	12.44	3.03	0.35
RP- Patho-2-18-5	83	4.33	99	0.88	286	7.26	3.87	0.26
RP- Patho-2-16-4	81	3.18	96	0.81	272	5.81	3.87	0.23
BPT 5204 (RP 1)	76	4.63	91	0.67	348	10.1	4	0.17
Improved Samba Mahsuri (RP 2)	82	2.72	94	0.58	276	8.95	2.67	0.09
Tetep (DP 1)	115	4.53	92	0.57	264	6.96	3.17	0.34

C 101 LAC (DP 2)	91	3.53	98	0.33	300	10.44	3.43	0.2
Critical Difference	8.74		2.39		37.54		0.68	
Standard Error (difference)	4.13		1.13		17.73		0.32	
Standard Error (mean)	2.92		0.79		12.54		0.23	
Coefficient of Variation (%) (Relative measure of dispersion)	5.97		1.37		7.31		11.01	

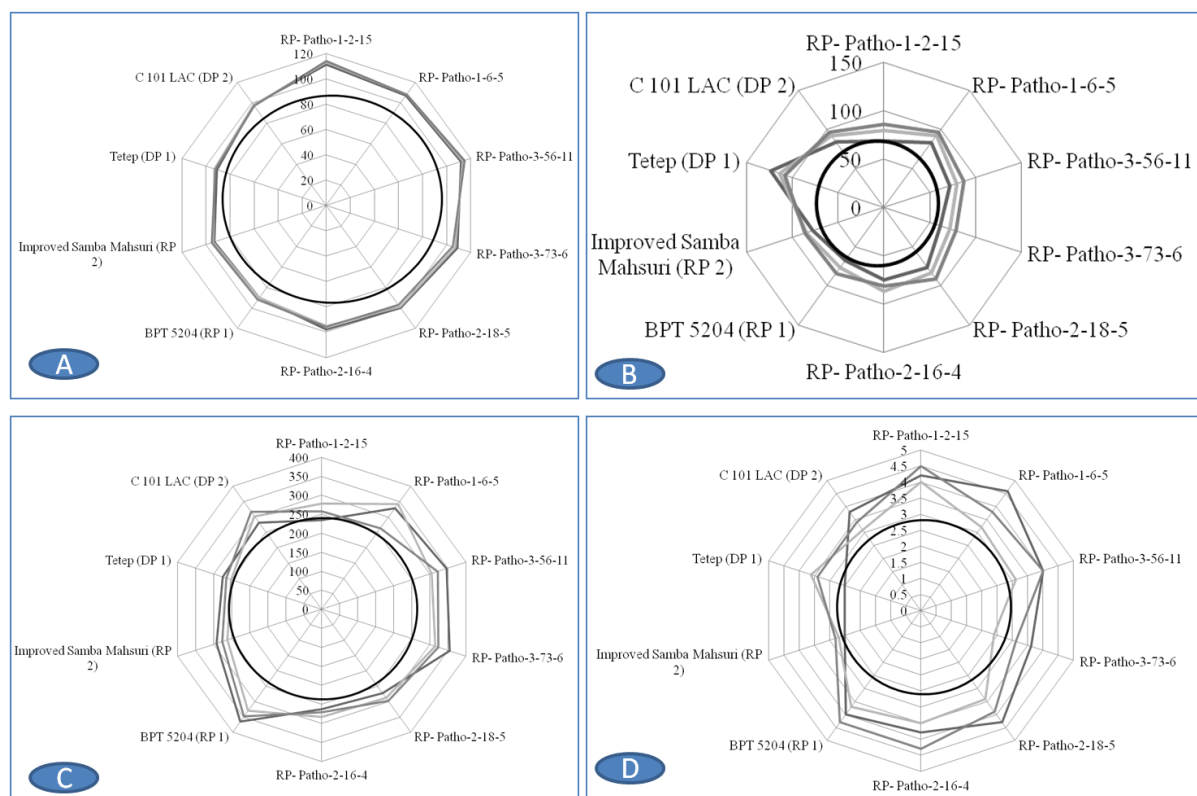


Fig 1. Radar chart of Recurrent parent recovery pattern

*A- Days to 50 percent flowering, B-Plant height, C-Panicle per square meter, D-Grain Yield

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