Biological Forum – An International Journal **13(4): 913-917(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Performance of Gene Pyramided Rice Lines for Blast and Sheath Blight Resistance

Velprabakaran Senthilvel[1], Vanniarajan Chockalingam[1], Renuka Raman[2], Shanmugasundaram Rangasamy[3], Thiruvudainambi Sundararajan[4] and Ramalingam Jegadeesan[5*]

[1]Department of Plant Breeding and Genetics, Agricultural College & Research Institute, Tamil Nadu Agricultural University, Madurai, (Tamil Nadu), India.
[2]Department of Biotechnology, Agricultural College & Research Institute, Tamil Nadu Agricultural University, Madurai, (Tamil Nadu), India.
[3]Department of Soils and Environment, Agricultural College & Research Institute, Tamil Nadu Agricultural University, Madurai, (Tamil Nadu), India.
[4]Department of Plant pathology Agricultural College & Research Institute, Tamil Nadu Agricultural University, Madurai, (Tamil Nadu), India.
[5]Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, (Tamil Nadu), India.

(Corresponding author: Ramalingam Jegadeesan)*
(Received 28 September 2021, Accepted 17 November, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Rice is the major cereal among all other food crops in the world. It was majorly affected by various biotic stresses. Blast and sheath blight imposes 20-25% of yield penalty. The elite and sustainable method for valuable management of disease resistance development is to develop resistant cultivars. The phenotypic and genotypic evaluations were used in the marker assisted crop enhancement. The present study was carried out to inculcate blast (*Pi54)* and sheath blight resistant QTLs *(qSBR11-1, qSBR11-2 and qSBR7-1)* resistant lines into high yielding Co51 cultivar from donor parent Tetep. The homozygous lines (F_5) showed significant yield potential and resistant attributes. Presence of resistance genes were confirmed through linked DNA markers (Pi54 MAS, RM209, RM224 and RM336) and the desirable plants were assessed for the morphological evaluation. Six selected improved lines exhibited good agronomic performance and good grain quality. The better resistant lines will be forwarded for future crop improvement.

Keywords: Marker assisted selection (MAS), Rice, blast, Sheath blight resistance.

INTRODUCTION

Rice (*Oryza sativa* L.) is unique and important food crops for more than half of the world's population (Velprabakaran *et al.,* 2020). In the world rice production scenario, the top most producer of rice (142.3 million tones) is china followed by India (110.4 million tones) which secured second position (FAO, 2018). In India, rice is shared by 46-48% of total grain production and it related income source of common people (Ramalingam *et al.,* 2020: Mew *et al.,* (1987). Rice production is maximum caused by many stresses like pest and diseases, which are major threats to food production. This threats to be enveloped and productivity could be uplifted by inculcating the resistant QTLs/genes, which are highly available in the unexploited wild (W) species and germplasm into highly adapted popular cultivars. The newer improvement in molecular breeding and marker assisted breeding approaches can enrich the applications of resistant lines improvement. Many local cultivars and improved lines (IL) have been developed for the sustainable benefit of farming community for multiple stresses through marker-assisted gene stacking and selection strategies (Sundaram *et al.,* 2008; Ramalingam et *al.,* 2017). Rice blast caused by *Magnaporthe grisea*, is considered as an important disease of rice which limits the yield factors. (Variar *et al.,* 2009: Pinta *et al.,* (2013). At host-pathogen interface, Avr-gene when recognized by the plant, R-gene triggered rapid and robust suite of cellular defense, to get manifested as hypersensitive responses at the infection area. Around 100 number of blast resistance genes have been genetically mapped and 22 of them have already been cloned Sharma *et al.,* 2012.

Sheath blight (SB) is another most important biotic stress of rice next to blast (Sushmitha *et al.,* 2020). Mapping populations that are derived from relatively resistant species as a parent can be used to identify new QTLs related to sheath blight resistance. These new QTLs can be combined with known resistant QTLs to generate rice varieties with higher resistance to sheath blight and blast Many QTLs have been identified in the background of Tetep and linked to molecular markers through QTL approach (Pinson *et al.,* 2005). The present study was carried to evaluate the resistance of both diseases through bioassay screening. The developed RILs would be highly useful for future stress breeding program.

MATERIALS AND METHOD

The experimental material consisted of Recombinant Inbreed Lines (RILs) derived from Co51 × Tetep. Tetep was used as the donor parent for both blast and sheath blight resistance and high yielding popular rice variety Co51 was the recipient. The detail of linked marker used in this study is given in (Table 1). Out of 755 plants, phenotypically 214 individual plants were selected and six plants are genotypically confirmed. The selected six genes pyramided RILs (F_5) with high yielding were screened for blast and sheath blight resistance. For blast screening, different blast isolates were collected from various rice growing regions (Melur-IS(MLR)-3, Ariyalur-IS(ARL)-1, Poonjuthi-IS(PNT)-6, Melur-IS(MLR)-8 and Singampuneri-IS(SNG)-2) and its virulence nature was indentified . Among the isolates, IS(SNG)-2 showed more virulence compared to other strains. After nine days of inoculation under green house condition, blast lesions were scored and evaluated on a (0-9) scale based on the IRRI (SES) 2002.

For sheath blight screening, improved Recombinant Inbred Lines (RILs) with parental lines (Co51 and Tetep) were transplanted in separate pots and kept under controlled artificial net house condition. For artificial disease screening the method was developed. Pathogen was multiplied in autoclaved young stem pieces (2-3 inches in length) of *Typha angustata* soaked with 1% peptone solution for 7 days. 4-5stem bits colonized with fungal and *sclerotia* were then kept in between the hills in the central region of the hill (5-10 cm) above the water line and tied with plastic band to maintain humidity in the (Bhaktavatsalam *et al.,* 1978) . The observations were recorded and the entries were scored after 21-24 days of artificial inoculation, (Table 3) Percent diseased leaf area for each inoculated seedlings was measured by 1-9 scale and was scored to rate the resistance capacity of rice plants to bacterial leaf blight according to scoring method adopted by (IRRI, 2002).

The young leaves from 21 days old seedling were collected. The total genomic DNA was extracted using modified CTAB (Cetyl trimethyl ammonium bromide) method as described by Doyle (1991). Isolated DNA was diluted with double distilled water and stored at -40ºC for subsequent marker analysis. The diluted 50 ng/µl DNA template was used for the PCR amplification. The amplified products were resolved by electrophoresis on a 1.5% agarose gel in 0.5x TBE buffer to determine whether PCR amplification was successful. The combinations were used to amplify the DNA of the lines and parents method as earlier discussed by Williams *et al.,* (2001) who used DNA markers for confirmation of blast and sheath blight resistance, The details of linked markers are listed in the (Table 1) All the RILs with parents were evaluated and selected plants were forwarded to phenotypic and yield performance screening.

Table 1: Details of molecular markers used for foreground selection.

Targeted trait	Gene/QTL	Marker	AT	Chr	Reference
Blast	***Pi54***	Pi54	**65**	**11**	Ramkumar *et al.,* (2011)
Sheath blight	***qSBR11-1***	RM224	**55**	**11**	Channamallikarjuna *et al.,* (2010)
	qSBR11-2	RM209	**55**	**11**	Channamallikarjuna *et al.,* (2010)
	qSBR7-1	RM336	**55**	**7**	Channamallikarjuna *et al.,* (2010)

RESULTS AND DISCUSSION

A total number of 755 lines were phenotypically evaluated and selected 214 lines were analyzed for the presence of resistance (R) genes using linked molecular markers of *Pi54, RM209, RM224 and RM336*). Six plants were selected based on the presence of resistance genes. These plants were evaluated for both blast and sheath blight screening. The selected recombinant inbreed lines were assessed for superior agronomic characters. *viz.,* days to fifty percent flowering, plant height, flag leaf length , number of productive tillers, panicle length, 1000 seed weight and single plant yield (Table 2) . The pyramided RILs showed similar and good yield attributed traits which is able compare with recipient parent (Co51). (Channamallikarjuna *et al.,* 2010) analysed the phenotypic characterization in improved stress resistant lines in rice.

Table 2: Phenotypic characterization of selected improved RILs with parents.

Genotype	Days to fifty percent flowering	Plant height (cm)	Flag leaf length (cm)	Flag leaf Width (cm)	Number of productive tillers	Panicle length (cm)	1000 grain weight (gm)	Single plant yield (gm)
RIL#0034	88.00	97.50	31.00	0.90	19.00	22.70	17.70	31.80
RIL#0101	92.00	118.00	34.00	0.88	22.00	25.50	18.00	25.50
RIL#0114	85.00	101.00	33.40	1.18	18.00	19.50	17.30	29.00
RIL#0197	93.00	99.40	33.30	1.01	18.00	22.00	17.85	23.00
RIL#0015	85.00	89.30	29.30	1.10	17.00	22.40	17.80	27.50
RIL#0236	91.00	89.70	35.00	1.21	19.00	24.00	19.00	27.40
Co51	87.00	118.50	34.00	1.19	18.00	24.50	18.50	28.00
Tetep	95.00	131.50	36.00	0.90	16.00	28.50	16.00	25.50
Mean	89.33	103.53	33.33	1.03	18.22	23.43	17.79	27.18
CD	4.04	3.89	1.71	0.04	0.85	0.6	1.18	2.13
CV	2.63	2.65	3	2.6	2.12	1.99	2.53	1.84

The improved six Recombinant Inbred Lines (RILs) with parents of Co51 X Tetep, donor lines and susceptible check were evaluated for their reactions to identify predominantly available races of blast and sheath blight strains under artificial screening condition. A representative picture is given in Fig. 1.

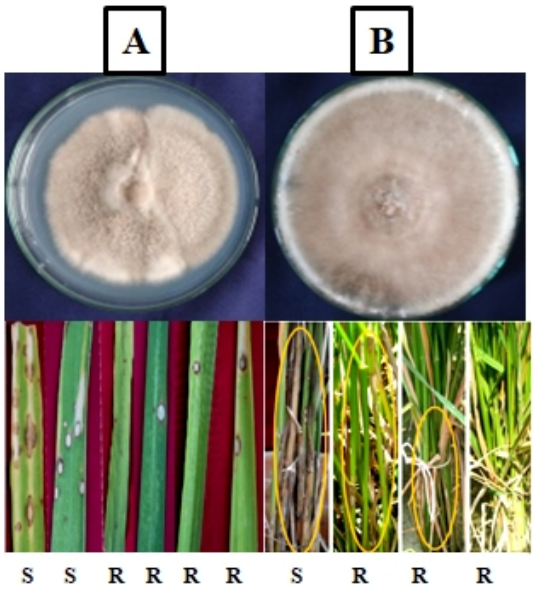

Fig. 1. Screening of RILs harboring (*Pi54, qSBR11-1, qSBR11-2 and qSBR7-1)* against *Magnaporthe grisea* (A) and *Rhizoctonia solani* (B).

In blast bioassay screening, mean lesion lengths of plants with resistant QTL (*Pi54*) in RILs and parental population were evaluated (Table 3). As expected, all the selected RIL lines showed the resistance attributes. Among these selected lines RIL#0101,0015 and 0236 showed good resistance (i.e., plants with *Pi54* QTL in the lines) which was showed higher levels of resistance with a mean lesion length of less than 3.0 cm for all the races. The recipient parent Co51 showed highly susceptible character and it showed more than 14.5 cm of lesion length and categorized as highly susceptible to blast disease. The donor parent, Tetep showed high level of resistance when compared with other lines. According to Ramalingam *et al.*, (2020), crosses from Tetep with ADT43 and ASD16, the BC3F2 resistant population showed high resistance compared to recipient checks it proved that *Pi54*gene /QTL inculcated lines showed more effective resistance when compare with other gene combinations.

In the sheath blight screening of improved resistant RILs (F5) the selected RIL lines showed the resistance QTLs (*qSBR11-1, qSBR11-2 and qSBR7-1)* and parents, Compared to all the six RILs, Three lines (RIL#0034,0236 and 0101) showed better resistance, The typha stem/leaf bits method (Bhaktavatsalam *et al.*, 1978) was followed with virulent, IS (CBE)-3 isolate was used for the bioassay pure *Rhizoctonia solani* Coimbatore isolate Fig. 2). Results of the artificial pathological screening showed that the donor parent, Tetep having resistance to sheath blight infection and improved RILs were categorized under MR class. The combined disease resistance of both diseases (Blast and Sheath blight) were observed on RIL#0236 and RIL#0101.Moderate resistance for sheath blight was notice in back cross derived progenies (Vidya *et al.*, 2018). The improved recombinant lines with resistance to both blast and sheath blight are now in yield trials to assess the stability performance.

Table 3. Phenotypic disease score of Recombinant Improved Lines (RILs) against blast and sheath blight resistance.

Sr. No.	Lines	Blast screening			Sheath blight screening		
		Lesion length (cm)	Scale	Resistance	RLH (%)	Scale	Resistance
1.	**Co51**	14.5	9	HS	63.21	9	HS
2.	**Tetep**	0	0	I	8.42	1	R
3.	**RIL#0034**	2.10	1	R	19.58	3	MR
4.	**RIL#0101**	1.11	1	R	22.03	3	MR
5.	**RIL#0114**	2.85	1	R	26.50	3	MR
6.	**RIL#0197**	2.32	1	R	33.15	3	MS
7.	**RIL#0015**	1.15	1	R	23.15	5	MR
8.	**RIL#0236**	1.11	1	R	22.03	3	MR

Blast scoring: *Blast – 0–1 (highly resistant), 2–3 (resistant), 4 (moderately resistant), 5–6 (moderately susceptible), 7 (susceptible), and 8–9 (highly susceptible).*
Sheath blight scoring *0 (Immune) =<1 % 1 (resistant) =1-20% 3 (moderately resistant) = 21-30% 5 (moderately susceptible= 31-45 7 (susceptible),9 (highly susceptible)= 46 and above*

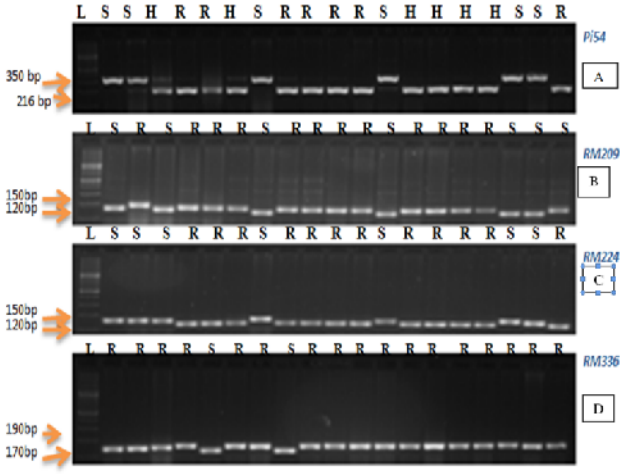

Fig. 2. Agarose gel electrophoresis pictures depicting the presence and absence of (A) *Pi54* (B) *RM209* (C) *RM224* (D) *RM336* alleles. M – 100-bp ladder, R, resistant; H, heterozygote; S, susceptible in RILs and parents.

Acknowledgement. This study was financially supported by DBT under a ATGC research grant (127-SP32889), New Delhi, India. Thanks to all the advisory committee members, Agricultural college and research institute, AC&RI, Madurai.
Conflict of Interest. None.

REFERENCES

Bhaktavatsalam, G., Satyanarayana, K., Reddy, A. P. K., & John, V. T. (1978). Evaluation for sheath blight resistance in rice. *Int Rice Res Newsl Int Rice Res Inst*.

Channamallikarjuna, V., Sonah, H., Prasad, M., Rao, G. J. N., Chand, S., Upreti, H. C., & Sharma, T. R. (2010). Identification of major quantitative trait loci qSBR11-1 for sheath blight resistance in rice. *Molecular Breeding*, *25*(1): 155-166.

Doyle, J. (1991). DNA protocols for plants. In *Molecular techniques in taxonomy* (pp. 283-293). Springer, Berlin, Heidelberg.

IRRI, I. (2002). Standard evaluation system for rice: International Rice Research Institute. *PO Box*, *933*, 1099.

Mew, T. W. (1987). Current status and future prospects of research on bacterial blight of rice. *Annual review of phytopathology*, *25*(1): 359-382.

Pinson, S. R., Capdevielle, F. M., & Oard, J. H. (2005). Confirming QTLs and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines.

Pinta, W., Toojinda, T., Thummabenjapone, P., & Sanitchon, J. (2013). Pyramiding of blast and bacterial leaf blight resistance genes into rice cultivar RD6 using marker assisted selection. *African Journal of Biotechnology*, *12*(28).

Ramalingam, J., Raveendra, C., Savitha, P., Vidya, V., Chaithra, T. L., Velprabakaran, S., & Vanniarajan, C. (2020). Gene pyramiding for achieving enhanced resistance to bacterial blight, blast, and sheath blight diseases in rice. *Frontiers in plant science*, *11*, 1662.

Ramalingam, J., Savitha, P., Alagarasan, G., Saraswathi, R., & Chandrababu, R. (2017). Functional marker assisted improvement of stable cytoplasmic male sterile lines of rice for bacterial blight resistance. *Frontiers in plant science*, *8*, 1131.

Sharma, T. R., Rai, A. K., Gupta, S. K., Vijayan, J., Devanna, B. N., & Ray, S. (2012). Rice blast management through host-plant resistance: retrospect and prospects. *Agricultural Research*, *1*(1): 37-52.

Sundaram, R. M., Vishnupriya, M. R., Biradar, S. K., Laha, G. S., Reddy, G. A., Rani, N. S & Sonti, R. V. (2008). Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica*, *160*(3), 411-422.

Sushmitha, D., Badri, J., Prakasam, V., Bhadana, V. P., Eswari, K. B., Laha, G. S.,& Ram, T. (2016). Identification and agro-morphological characterization of rice genotypes resistant to sheath blight. *Australasian Plant Pathology*, *45*(2): 145-153.

Variar, M., Cruz, C. V., Carrillo, M. G., Bhatt, J. C., & Sangar, R. B. S. (2009). Rice blast in India and strategies to develop durably resistant cultivars. In *Advances in genetics, genomics and control of rice blast disease* (pp. 359-373). Springer, Dordrecht.

Velprabakaran, S., Rajeswari, S., Jeyaprakash, P., & Raveendran, M. (2020). Quantitative characterization on indigenous rice cultivars through Agro-Morphometric traits. *Journal of Pharmacognosy and Phytochemistry*, *9*(3): 367-370.

Vidya, V., & Ramalingam, J. (2018). Marker Assisted Selection for Sheath Blight, Blast and Bacterial Blight Resistance in Two Popular Rice Varieties. *Madras Agricultural Journal*, *105*(march (1-3)), 1.

Williams, M. C., Wenner, J. R., Rouzina, I., & Bloomfield, V. A. (2001). Effect of pH on the overstretching transition of double-stranded DNA: evidence of force-induced DNA melting. *Biophysical Journal*, *80*(2): 874-881.

How to cite this article: Senthilvel, V.; Chockalingam, V.; Raman, R.; Rangasamy, S.; Sundararajan, T. and Jegadeesan, R. (2021). Performance of Gene Pyramided Rice Lines for Blast and Sheath Blight Resistance. *Biological Forum – An International Journal*, *13*(4): 913-917.