AATCC Review

09 May 2025: Received
25 June 2025: Revised
05 July 2025: Accepted
06 August 2025: Available Online

https://aatcc.peerjournals.net/

Original Research Article

Open Access

Marker Assisted breeding for Bacterial blight resistance and yield enhancing gene to improve Pranahitha, a local popular elite fine cultivar with good cooking quality

K Alekya*[1], B Laxmi Prasanna[2], M Balram[3], N Balram[4], V. Srujana[5] and K Suryam[6]

[1]*S.V. Agricultural College, Tirupati, ANGRAU, India*
[2]*Department of Genetics and Plant Breeding, Rice Research Unit, ARI, Rajendranagar, Hyderabad, India*
[3]*Department of Genetics and Plant Breeding, College of Agriculture, Rajendranagar, India*
[4]*Department of Plant Pathology, Regional Agricultural Research Station, Jagtial, India*
[5]*Department of Genetics and Plant Breeding, College of Agriculture, Rajendranagar, India*
[6]*Technical Assistant, Regional Agricultural Research Station, Jagtial, India*

ABSTRACT

Biotic stresses are a major threat to rice production. Among the biotic stresses, bacterial leaf blight (BB) is one of the major diseases affecting rice grain production. The present investigation was conducted to evaluate genotypic and phenotypic effects of 50 breeding lines from a cross (Pranahitha//ISM/MTU1010 NIL) at the Regional Agricultural Research Station, Jagtial. 50 advanced breeding lines were screened through foreground selection for confirmation of the presence of target trait-specific genes, viz., for BB (Xa21) and yield gene (Gn1a). It was carried out with the co-dominant marker pTA 248 for checking the presence of Xa21 and the Gn1a INDEL marker-3 for checking the presence of Gn1a. Out of 50 advanced breeding lines, 36 lines possess the resistant allele of the Gn1a gene and 21 lines possess the Xa21 gene. Phenotypic screening of 50 F4 breeding lines identified 29 resistant lines, 10 moderately resistant lines, seven susceptible lines, and four highly susceptible lines. The results obtained in the present study indicate the success of combining marker-assisted breeding with phenotypic selection. Analysis of variance among advanced breeding lines revealed the presence of significant difference for days to fifty percent flowering, plant height, panicle length, number of productive tillers, number of filled grains per panicle, number of unfilled grains per panicle, total number of spikelets per panicle, 1000 seed weight and grain yield per plant between parents and breeding lines.

Keywords: *Rice, marker assisted selection, Bacterial blight resistant gene, yield enhancing gene, Phenotypic screening.*

INTRODUCTION

Rice is the world's second most important crop after wheat, which feeds half of the population. Among the biotic stresses, which cause yield reduction in rice, Bacterial leaf blight (BB) is one of the serious diseases caused by the pathogen *Xanthomonas oryzae* pv. *Oryzae (Xoo)*, responsible for significant yield reduction in rice. This disease is prevalent in almost all paddy-growing regions across the states in India, reported firstly by (34). The most destructive phase of the disease is the "Kresek" or wilt resulting from early systemic infection. The symptom of the disease at the seedling stage is known as kresek (28). Bacterial Blight disease can be managed through chemical control, biological control, and by introducing genetic resistance through plant breeding. Biological control for BB has not received much attention due to pathogen variation, thus preventing the development of suitable biological agents for control. Also, negative impact is seen by the usage of chemical control for BB disease, and excessive chemical sprays have a negative impact on consumer health and farmers as well as the environment.

*Corresponding Author: ***K Alekya***

DOI: https://doi.org/10.21276/AATCCReview.2025.13.03.574

Developing disease-resistant cultivars against BB is the only effective method to control the disease. Deployment of gene-conferred host plant resistance provides a cheap, effective, environment environment-friendly approach to regulate plant diseases and minimize the losses. Molecular markers usage accelerates resistance breeding efforts, as the plants can be selected on the basis of molecular marker alleles instead of their phenotypes. Although more than 42 genes for bacterial leaf blight resistance have been identified and mapped in both *indica* and *japonica* rice. Out of them only 25 percent genes have been cloned including *Xa21* (33), *Xa1* (41), *xa13* (6), *xa5* (14 and 16), *Xa26/Xa3* (36 and 39), *Xa27* (10), *Xa25* (24), *Xa7* (38) and *Xa10* (37) for bacterial blight resistance in rice. The rice BB disease resistance gene *Xa21* is widely used in breeding programs as it has having broad spectrum of resistance. Several tested chemicals or antibiotics could not control the BB infestation completely. Consequently, the most effective and sustainable approach is the development and deployment of BB-resistant rice varieties introgressed with resistant genes.

Enhancing the rice grain yield potential is a major challenge in areas where rice yield is stagnating. Yield stagnation is mainly due to adverse or unsuitable climatic conditions, pressure of insects, diseases, and weeds, and inadequate crop and soil management. The era of the green revolution has significantly improved rice yield productivity. However, with the growing population and decreasing arable land, rice scientists must find new ways to improve rice productivity.

Molecular breeding would improve the efficiency of breeding methods by introgressing genes and selecting plants having yield genes that could directly contribute to significant grain yield improvement. A large number of QTLs related to rice grain yield, such as *Gn1a* (3), *OsSPL14* (17; 26), *APO1* (13), *DEP1* (12), *OsARG* (25), *TAWAWA1* (40), *OsEBS* (7), *DST* (23) and *PAY1* (42) had been functionally characterized by positional cloning. Of these genes, the *Gn1a* (grain number) gene, a significant QTL mapped on chromosome number 1, directly affects the plant yield by increasing the number of spikelets per panicle. Although significant studies have been made in crop improvement through phenotypic selections for agronomically important traits, considerable difficulties are often encountered during this process, mainly due to genotype-environment interactions. Marker-assisted selection accelerates line development in breeding programs with greater efficiency. Pranahitha is a medium-duration (135-140 days) local popular fine-grain rice variety with a yield capacity of 6200 kg/ha, having resistance to gall midge, with good cooking quality, and suitable for irrigated conditions. It was released during the year 2012 by the Regional Agricultural Research Station (RARS), Polasa, Jagtial. Hence, Pranahitha is used to improve its resistance to the major disease, bacterial leaf blight (BB), coupled with high yield. Considering the imminent need to improve BB along with yield, we attempted to deploy the strategy of marker-assisted pedigree breeding (MAPB) to transfer *Xa21* and *Gn1a* into the genetic background of Pranahitha.

MATERIAL AND METHODS

Plant material

Donor parents/Male parents include ISM (*Xa21*) and MTU1010NIL (*Gn1a*). ISM is a medium duration (135-140 days) variety having a yield capacity of 5500-6000 kg/ha. Three genes pyramided line (*Xa21*, *xa13,* and *xa5*) exhibit a high level of resistance to BB disease, showing a high yield advantage over Samba Mahsuri. Recently, it has been confirmed to have a low glycemic index (50.99). It was released during year 2008 by the Indian Institute of Rice Research (ICAR-IIRR), Hyderabad. MTU1010 is a short duration semi dwarf Mega variety (120-125 days) with long slender grain type, cultivated in many rice-growing states during wet and dry seasons. It is tolerant to BPH, resistant to blast, but susceptible to BB. MTU1010 is introgressed with the *Gn1a* gene through marker-assisted backcross breeding by the International Rice Research Institute (IRRI), Philippines & Indian Institute of Rice Research (IIRR), Hyderabad, and MTU1010 NIL possessing *Gn1a* was developed. The female parent is Pranahitha, a medium-duration (135-140 days) fine-grain rice variety with a yield capacity of 6200 kg/ha, having resistance to gall midge, with good cooking quality, and suitable for irrigated conditions.

Isolation and characterization of the bacterial blight pathogen

The bacterial cultures of the virulent isolate, DX-020 of *Xanthomonas oryzae pv. Oryzae (Xoo)*was isolated from the infected leaf samples on modified Wakimoto's medium, which was collected from Hyderabad, Telangana, were maintained on Hayward's agar media at 28^{0}C for 96 hours. After the incubation period, the bacterial cells were harvested and diluted with 10ml of sterile distilled water to get a final concentration of approximately 10^{8-9}cfu/µl (29).

The pathogenicity of the bacterial pathogen was confirmed on the susceptible rice variety MTU1010.

STRATEGY FOR MARKER-ASSISTED PEDIGREE BREEDING

A cross was carried out between Pranahitha and Improved Sambha Mahsuri (ISM) having the *Xa21* gene, and resultant F_1s were confirmed. True F_1s were crossed with MTU 1010 NIL, having *Gn1a*. The F_1s were confirmed for their heterozygosity using target-resistant gene-specific markers. The true F_1 plants (*i.e.,* heterozygous F_1 plants) were selfed to develop F_2s. Homozygous positive F_2 plants were identified through foreground selection, using the target QTLs/gene-specific markers. DNA marker pTA 248 (5'-AGACGCGGGAAGGGTGG TTCCCGGA -3', 5'-AGACGCGGGTAATCGAAAGATGAAA-3') was used to select the plants that were homozygous for the *Xa21* gene. DNA marker *Gn1a* INDEL marker -3 (5'- GATCTAGATGCT CCAAAGTCC-3 5'- CTGTACGTACGTGCACGTAG-3') was used to select the plants that were homozygous for *Gn1a* gene and advanced further by selfing through pedigree breeding to F_4 generation, based on selection for phenotypic characters specific for Pranahitha. DNA isolation was carried out through modified method of (8). Twenty one promising homozygous positive F_4 lines, which closely resembled Pranahitha in terms of plant type and grain type, were selected and further evaluated for their resistance/tolerance to target stresses as well as for key agro-morphological traits.

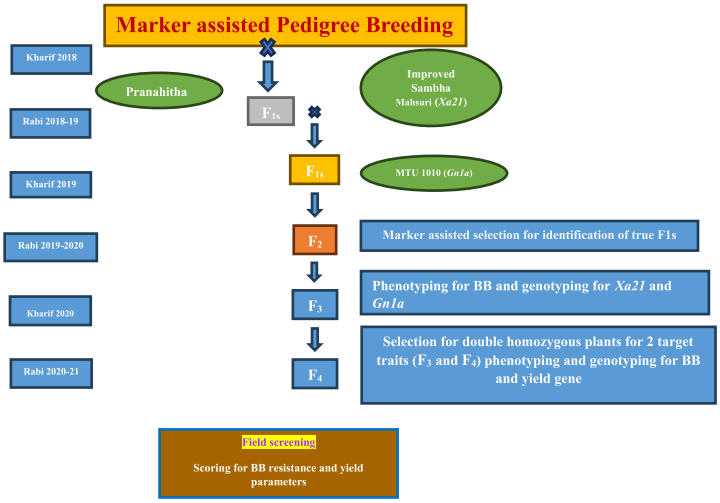

Figure 2. Strategy for development of marker assisted pedigree breeding

Bacterial leaf blight screening and evaluation

The selected target F_4 plants, along with the parents, were screened in the field at RARS, Jagtial. Homozygous F_4 lines were inoculated with bacterial culture at maximum tillering and flag leaf stages by following the leaf clipping method described by (18). Plant inoculation was carried out by clipping the tip (about 1 to 2 cm) of the fully expanded uppermost leaf with scissors that had been dipped into the inoculum. The lesion length on leaves was measured at 15 days after inoculation. Scoring was done as per SES, IRRI, 2013 (Table 1).

Evaluation of agro-morphological traits in the improved breeding lines of Pranahitha

During Rabi 2020-21, selected F_4 lines possessing *Xa21*, *Gn1a,* and the parents MTU1010 and ISM were transplanted into the experimental farm of RARS, Jagtial in a randomised block design of spacing 15cm 20cm in soil with optimum levels of macro and micro-nutrients.

Data was recorded for selected agro morphological traits such as days to 50% flowering (DFF), Plant height (PH), Panicle length (PL), Number of productive tillers/plant (NPT), Total spikelets/panicle, Number of filled grains/panicle, Number of unfilled grains/panicle, 1000-grain weight (g) and Grain yield per plant (g).

Statistical analysis

The data collected was subjected to statistical analysis using SAS 9.2 (SAS version 9.2 software packages; SAS Institute, Inc.; Cary, NC) software for coefficient of variation (CV), Critical Difference (CD), Standard Error (SE), and Analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

Combining the BB gene *Xa21* and the yield gene *Gn1a* into the genetic background of Pranahitha

Phenotypic evaluation of improved breeding lines for BB

A total of 50 F_4 breeding lines, along with parents, were screened for bacterial blight resistance using bacterial cultures of a virulent local isolate of the BB pathogen, DX-020. A total number of 500 plants (10 plants/entry) lines were inoculated with bacterial culture at the maximum tillering stage by following the leaf clipping inoculation method described by (18). Along with F_4 plants, a resistant check, ISM, and a BB susceptible check, MTU1010 was screened for bacterial blight resistance under field conditions. BB Scoring was done at 15 days after inoculation. Scoring was done as per the SES scale, IRRI, 2013. Out of the 50 F_4 lines evaluated against BB, twenty-nine breeding lines showed a resistant reaction with a disease score of 3 (Table 2). Eleven lines, *viz*., showed moderately resistant reaction with a disease score of 5. Susceptible check, MTU1010 showed a highly susceptible reaction with a disease score of 9. Resistant check, Improved Samba mahsuri showed a highly resistant reaction with a disease score of 1.

Genotypic characterization of advanced breeding lines for introgression of the BB gene, *Xa21,* and *Gn1a* gene

The efficiency of selection will be greatly increased by the employment of molecular markers, especially through 'foreground selection' where markers are used for the identification of target genes or QTL to accelerate phenotypic screening (11). In the present study, the co-dominant marker, pTA248, was used to assess the presence of the major bacterial blight resistant gene (*Xa21*). Functional/closely linked co-dominant markers are available for the major bacterial blight resistance gene, *Xa21* (pTA248). It was used to screen the improved breeding lines to identify the homozygous one for the respective target genes. As the marker, pTA248 is tightly linked with *Xa21* at a genetic distance of ~ 0.1 cM (31) it is a good choice to use in marker assisted breeding programmes.

A total number of 250 plants (5 plants each from the entry/line) were tested for confirmation of the target gene *Xa21* specific for bacterial blight resistance. Out of them thirty two lines were identified to be homozygous positive for the target trait, BB and the remaining eighteen lines were identified as homozygous negative (Figure 6.1). Among the breeding lines that are tested for *Gn1a* gene, thirty six breeding lines were identified to be homozygous positive for grain number gene *Gn1a* and remaining fourteen lines were identified to be homozygous negative. (Figure 6.2).

Though the gene pyramiding with more than one gene found effective (1), single gene (*Xa21*) conferring durable resistance was observed with the studies (32, 2, 22 and 30). These lines could perform better in farmer's field with respect to bacterial blight resistance. With reference to *Gn1a* gene, similar results were obtained by the earlier studies (9, 19, 35 and 27) in which the reliability of the gene has been observed.

Mean performance of yield and yield contributing characters in rice

Analysis of variance among advanced breeding lines revealed the presence of significant difference for days to fifty percent flowering, plant height, panicle length, number of productive tillers, number of filled grains per panicle, number of unfilled grains per panicle, total number of spikelets per panicle, 1000 seed weight and grain yield per plant between parents and breeding lines (Table 3.0).

Female parent Pranahitha (JGL11727) attained 50 % flowering after 107 days of sowing. Mean days to 50% flowering of improved breeding lines (possessing *Xa21* and *Gn1a*) ranged from 90 days (KAR-17) to 108 days (KAR-13) with an average of 98. Seventeen breeding lines *viz*., KAR-1, KAR-3, KAR-7, KAR-8, KAR-9, KAR-14, KAR-17, KAR-20, KAR-21, KAR-22, KAR-24, KAR-25, KAR-27, KAR-29, KAR-31, KAR-37 and KAR-47 flowered significantly earlier as compared to female parent Pranahitha. Results obtained were found to be similar to (22, 4 and 1). The mean plant height of female parent Pranahitha (JGL11727) was observed to be 117 days. Mean plant height values of the breeding lines (F_4 population) ranged from 87 cm (KAR-20) to (KAR-34) 114 cm with an average value of 100.5 cm. Forty breeding lines *viz*., were significantly shorter (<117 cm) than female parent Pranahitha (JGL11727) *viz*., KAR-1, KAR-3, KAR-5, KAR-6, KAR-7, KAR-8, KAR-9, KAR-11, KAR-12, KAR-13, KAR-14, KAR-15, KAR-17, KAR-18, KAR-19, KAR-20, KAR-21, KAR-23, KAR-24, KAR-25, KAR-26, KAR-27, KAR-29, KAR-30, KAR-31, KAR-32, KAR-33, KAR-35, KAR-36, KAR-37, KAR-38, KAR-39, KAR-40, KAR-42, KAR-43, KAR-44, KAR-45, KAR-46, KAR-47 and KAR-49. Similar observations of significant variation in plant height were found in (2, 22, and 30). Lower plant height results in lodging resistance in the farmer's field.

The panicle length of female parent Pranahitha (JGL11727) was observed to be 24.9 cm. Mean panicle length was 27.9 cm with a range of 24.65 (KAR-1) to 31.3 cm (KAR-2). Twelve breeding lines *viz.,* KAR-2, KAR-4, KAR-9, KAR-10, KAR-16, KAR-22, KAR-30, KAR-35, KAR-41, KAR-45, KAR-46 and KAR-50 showed significantly higher panicle length than Pranahitha (JGL11727). Similar to the present study, significant variations in terms of panicle length were found with (2, 22 and 4) *et al.* 2018. As panicle length is directly correlated with grain yield, the breeding lines with better panicle length may perform better in farmers' fields. The mean number of productive tillers of female parent Pranahitha (JGL11727) was found to be eight (8). The mean productive tillers of improved breeding lines ranged from eight (8) (KAR-11) to 13 (KAR-30 and KAR-38), with an average of 11. Thirty three improved breeding lines *vi*KAR-1, KAR-5, KAR-6, KAR-9, KAR-14, KAR-15, KAR-16, KAR-17, KAR-18, KAR-19, KAR-20, KAR-22, KAR-23, KAR-24, KAR-26, KAR-27, KAR-28, KAR-29, KAR-30, KAR-31, KAR-33, KAR-34, KAR-35, KAR-36, KAR-38, KAR-39, KAR-40, KAR-41, KAR-42, KAR-44, KAR-45, KAR-46 and KAR-47 *viz*., were found to be significantly higher in terms of number of productive tillers than Pranahitha (JGL11727).

In the present study, observations of significant variations regarding productive tillers per plant were found to be similar to (20 and 4). The number of filled grains per panicle of female parent Pranahitha (JGL11727) was recorded as 194. The mean number of filled spikelets per panicle varied from 121(KAR-15) to 317 (KAR-1), with an average of 219. Fourteen lines showed a significantly higher number of filled grains per panicle than the female parent, *viz*., KAR-1, KAR-2, KAR-4, KAR-6, KAR-9, KAR-10, KAR-12, KAR-22, KAR-24, KAR-27, KAR-30, KAR-31, KAR-32, and KAR-47. Similarly, significant variation in terms of filled grains per panicle was found with (5, 20, 2, 9, 19 and 4). The number of unfilled grains per panicle of female parent Pranahitha (JGAL11727) was recorded as 29. The mean number of unfilled spikelets per panicle varied from 13 (KAR-15) to 120 (KAR-11), with an average of 66.5. Four breeding lines, *viz*., KAR-15, KAR-34, KAR-44, and KAR-49, showed significantly lower numbers of unfilled grains per panicle. The average number of spikelets per panicle of the female parent was recorded as 198. The mean number of spikelets per panicle varied from 134 (KAR-15) to 366 (KAR-1), with an average of 250. Eight improved breeding lines *viz*., KAR-1, KAR-2, KAR-4, KAR-6, KAR-11, KAR-22, KAR-27 and KAR-32 showed significantly higher number of spikelets per panicle. Similar observations in terms of total spikelets per panicle were found with (5, 4, 15 and 27). 1000-grain weight of female parent Pranahitha (JGL11727) displayed a mean of 16.14 g. Mean 1000-grain weight values of breeding line (F_4 generation) were varied from 14.2 g (KAR-10) to 27.15 g (KAR-49) with a mean range of 20.6 g. Seven breeding lines, *viz*., KAR-1, KAR-7, KAR-12, KAR-17, KAR-46, KAR-47, and KAR-49, showed significantly higher grain weight than the female parent. Similar observations of significant variations with regard to 1000-grain weight were found with (5, 30, 22 and 20). Mean grain yield per plant of female parent Pranahitha (JGL11727) was observed to be 30.1 g. Mean grain yield per plant values of breeding line (F_4 generation) were varied from 21.35 g (KAR-7 and KAR-39) to 48.45 g (KAR-19) with a mean range of 34.9 g. Twenty two lines *viz*., KAR-1, KAR-4, KAR-6, KAR-9, KAR-14, KAR-17, KAR-18, KAR-19, KAR-20, KAR-21, KAR-22, KAR-24, KAR-25, KAR-28, KAR-30, KAR-31, KAR-32, KAR-35, KAR-46, KAR-47, KAR-48 and KAR-49 showed significantly higher grain yield than female parent. Results were on par with the findings of (5, 20, 2, 9, 21, 27, 35 and 15). Seven lines performed better, having higher yields with a high level of resistance to bacterial leaf blight disease (Table 4.0)

CONCLUSIONS

In the present study, in the F4 generation, out of 50 breeding lines evaluated, 21 breeding lines were found to possess both *Xa21* and *Gn1a* resistance alleles and eight lines had only the *Xa21* resistance allele, and 15 lines had only the *Gn1a* resistance allele. Seven lines performed better, having higher yields with a high level of resistance to bacterial leaf blight disease. Thus, the present study has proven that the Marker-assisted selection coupled with phenotypic selection is successful, as these improved breeding lines with higher yield levels could perform better in the farmer's field with a good level of bacterial leaf blight resistance.

Future perspective:

The identified breeding lines carrying both *Xa21* and *Gn1a* genes can be advanced for multilocation trials and released as high-performing varieties with durable resistance to bacterial leaf blight and improved grain yield.

These lines can serve as valuable genetic resources for pyramiding additional biotic (e.g., blast, sheath blight) and abiotic stress tolerance (e.g., drought, salinity) traits through further marker-assisted selection.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the PJTSAU, Hyderabad, for the financial support to conduct research activities on the improvement of rice varieties for adaptation to biotic stresses. The author and co-authors in this work contributed significantly by providing relevant research information to ensure a detailed view of this article.

Conflict of interest

Authors declare no conflict of interest

FUNDING DECLARATION

This research received no external funding support

Figure 1. Transplanted Field

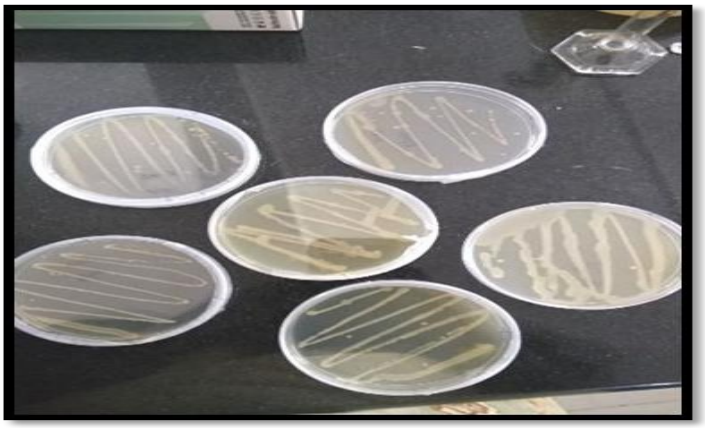

Figure 3. BB culture plates and bacterial blight inoculum

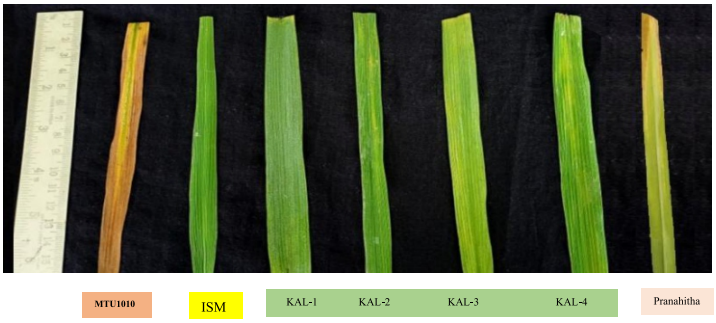

Figure: 4. Phenotypic screening for bacterial blight resistance using Xoo (DX020) culture. ISM- Resistant check; MTU1010 - Susceptible check; Improved breeding lines possessing Xa21 (F4 Generation)

*Figure 5. Panicles of Gn1a introgressed lines of cross: Pranahitha*ISM (Xa21) *MTU1010 (Gn1a)*

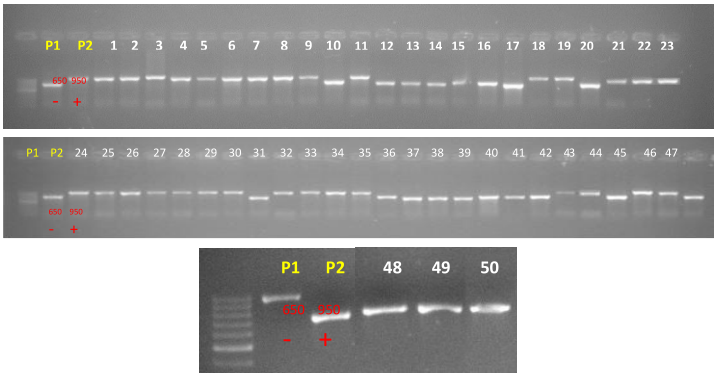

Figure 6.1 Segregation pattern of F4 population for the marker pTA248 for Xa21gene L=100bp Ladder, P1=MTU1010, P2=ISM

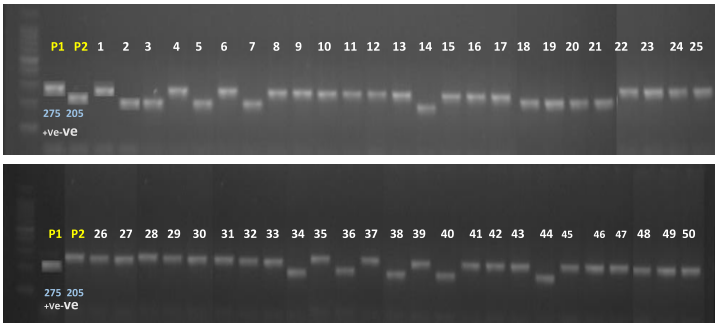

Figure 6.2 Segregation pattern of F4 population for the marker Gn1aINDEL marker-3 for Gn1agene L=100bp Ladder, P1=MTU1010, P2=ISM

Table. 1. Standard Evaluation System, IRRI scale (2013) for bacterial leaf blight

Scale	Rating	% leaf area diseased
1	Highly resistant	1-5
3	Resistant	6-12
5	Moderately resistant	13-25
7	Susceptible	26-50
9	Highly susceptible	51-100

Table. 2. Scoring details of twenty-nine breeding lines which showed resistance obtained through screening for BB resistance as per IRRI-SES scale (IRRI 2013)

S. No	Parents and Checks	Reaction against BB	
		DX020	
		Score	HR/R/MR/S/HS
1	MTU1010	9	HS
2	ISM	1	HR
3	Pranahitha	5	MR
	Improved breeding lines (F_4)	Score	HR/R/MR/S/HS
1	KAL1	3	R
2	KAL 2	3	R
3	KAL 3	3	R
4	KAL 4	3	R
5	KAL 5	3	R
6	KAL 6	3	R
7	KAL 7	3	R
8	KAL 8	3	R
9	KAL 9	3	R
10	KAL 11	3	R
11	KAL 18	3	R
12	KAL 19	3	R
13	KAL 21	3	R
14	KAL22	3	R
15	KAL 23	3	R
16	KAL 24	3	R
17	KAL 25	3	R
18	KAL 26	3	R
19	KAL 27	3	R
20	KAL 28	3	R
21	KAL 29	3	R
22	KAL 31	3	R
23	KAL 32	3	R
24	KAL 33	3	R
25	KAL 34	3	R
26	KAL 42	3	R
27	KAL 43	3	R
28	KAL 45	3	R
29	KAL 46	3	R

Table 3.0 Mean performance of yield and yield contributing characters in rice

Entry	Days to50 % flowering (DFF)	Plant height (cm)	Panicle length (cm)	No. of tillersper plant	Number offilled grains per panicle	Number of unfilled grains per panicle	Total numberof spikelets per panicle	1000-grain weight (g)	Total yield per plant (g)
KAL-1	101	89	24.6	11	317	49	366	20.1	48.4
KAL-2	103	107.5	31.3	10	233	60	293	17.2	27.3
KAL-3	97	100.6	25.5	10	168	49	217	18.6	28.2
KAL-4	104	110.5	30.1	10	246	62	308	18.2	46.2
KAL-5	102	102	28.6	11	179	41	220	18.6	33.3
KAL-6	102	106.1	28.6	11	241	58	299	19.2	41
KAL-7	100	98.1	26.2	10	186	45	231	20.2	21.3
KAL-8	101	97.3	25.5	10	186	44	230	19.7	32.4
KAL-9	100	102	29.9	11	228	56	284	17.1	38.2
KAL-10	102	109	30.1	7	210	39	249	14.2	33
KAL-11	102	104.5	25.3	8	181	120	301	15.9	26.1
KAL-12	101	100.8	27.1	10	216	38	254	26.1	32.1
KAL-13	108	100.6	25	10	161	37	198	17.7	23.2
KAL-14	100	98.3	23.4	11	195	71	266	18.1	39.2
KAL-15	103	92.1	24.8	12	121	13	134	15.1	26.3
KAL-16	106	107.8	30	12	166	26	192	14.2	27.2
KAL-17	90	103	28.6	11	147	91	238	22.3	42.1
KAL-18	102	106.8	28.3	11	186	52	238	19.1	38.1
KAL-19	104	104	26.9	12	194	41	235	19.3	40.2
KAL-20	101	87.65	26.6	11	203	28	231	18	38.2
KAL-21	98	89	24.8	10	194	41	235	15.1	36.2
KAL-22	97	107.5	28.8	11	262	37	299	18.1	47.2
KAL-23	102	90	24.8	11	129	37	166	15.5	26.1
KAL-24	100	104	27.5	11	215	30	245	18.2	42.1
KAL-25	101	91.5	24.7	10	185	35	220	16.7	37.4
KAL-26	102	97.3	27.3	11	168	45	213	17.7	34
KAL-27	98	101.4	26.3	10	213	72	285	15.3	23.3
KAL-28	102	110.5	26.6	11	189	26	215	15.4	37.6
KAL-29	99	98.5	25.5	12	168	26	194	14.4	31
KAL-30	104	101.3	28.9	13	211	62	273	16	37
KAL-31	100	105	25.5	12	228	28	256	18	42.3
KAL-32	103	97.1	24.8	10	218	21	239	16	39.1
KAL-33	104	102.1	26.5	11	177	45	222	15.1	25.8
KAL-34	102	114.9	28.1	12	183	15	198	19.4	34.2
KAL-35	104	103	29.3	11	170	25	195	18.7	40.1
KAL-36	104	100	26.1	11	189	57	246	17.1	25.3
KAL-37	101	99.6	25.4	10	194	60	254	16.3	22
KAL-38	102	99.1	28.4	13	192	35	227	15	23
KAL-39	103	102.3	27.3	12	181	69	250	18.9	21.3
KAL-40	102	96	26.3	12	190	30	220	18.7	26.1
KAL-41	106	110.3	31.1	11	194	32	226	15.1	30
KAL-42	102	101.6	28.6	11	160	35	195	18.9	32.7
KAL-43	106	101.6	28	9	168	30	198	16	27.1
KAL-44	103	105.8	28.6	12	166	20	186	18	33.6
KAL-45	103	100.8	30.6	11	200	24	224	14.4	28.2
KAL-46	104	100.4	31.1	12	201	24	225	24.9	44
KAL-47	99	102.5	26.1	11	214	34	248	21.3	35
KAL-48	106	107.6	26.5	10	186	55	241	19.2	37.6
KAL-49	104	96.6	27	9	199	17	216	27.1	37.3
KAL-50	102	108.3	30.3	11	141	49	190	15	25
Pranahitha	107	117	24.9	8	194	29	223	16.1	30.1
MTU1010	92	99.1	26	10	218	21	239	24.8	32
ISM	97	97.2	19.9	9	170	28	198	14.4	20.7
CV %	2.59	4.64	7.06	11.16	3.14	8.70	13.28	10.51	7.02
CD	5.27	9.48	3.88	2.37	12.21	7.46	61.81	3.78	4.70
S.E d	2.62	4.72	1.93	1.18	6.07	3.71	30.76	1.88	2.34

CV % - Coefficient of Variation, CD - Critical Difference and S.E d - Standard Error

Table 4. Performance of lines with bacterial blight resistance and higher yield

S.No	Entry	BB Score	BB resistant gene(*Xa21)*	Yield gene (*Gn1a)*	Single Plant Yield (g)
1	KAL-1	1	Homozygous +ve	Homozygous +ve	48.2
2	KAL-22	1	Homozygous +ve	Homozygous +ve	47.2
3	KAL-4	1	Homozygous +ve	Homozygous +ve	46.2
4	KAL-46	1	Homozygous +ve	Homozygous +ve	44.0
5	KAL-31	1	Homozygous +ve	Homozygous +ve	42.3
6	KAL-24	1	Homozygous +ve	Homozygous +ve	42.1
7	KAL-6	1	Homozygous +ve	Homozygous +ve	41.0

References

1. Abhilash Kumar V, Balachiranjeevi CH, Bhaskar Naik S, Rambabu R, Rekha G, Harika G, Hajira SK, Pranathi K, Anila M, Kousik M, Vijay Kumar S, Yugander A, Aruna J, Dilip Kumar T, Vijaya Sudhakara Rao K, Hari Prasad AS, Madhav MS, Laha GS, Balachandran SM, Prasad MS, Viraktamath BC, Ravindra Babu V, Sundaram RM (2016) Development of gene-pyramid lines of the elite restorer line, RPHR-1005 possessing durable bacterial blight and blast resistance. Front. Plant Sci. 7:1195.

2. Arunakumari K, Durgarani CV, Satturi V, Sarikonda KR, Chittoor PDR, Vutukuri B, Laha GS, Nelli AK, Gattu S, Jamal M, Prasadbabu A, Hajira S, Sundaram RM (2016). Marker assisted pyramiding of genes conferring resistance against bacterial blight and blast diseases into Indian rice variety MTU1010. Rice Sci. 23(6): 306-316.

3. Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka, M (2005). Cytokinin oxidase regulates rice grain production. Sci. 309(5735): 741-745.

4. Balachiranjeevi CH, Naik BS, Kumar AV, Harika G, Swamy MHK, Hajira SK, Kumar DT, Anila M, Kale RR, Yugender A, Pranathi K, Koushik MBVN, Suneetha K, Bhadana VP, Hariprasad AS, Laha GS, Rekha G, Balachandran SM, Madhav MS. , Senguttuvel P, Fiyaz AR, Viraktamath BC, Giri A, Swamy BPM, Jauhar A, Sundaram RM (2018). Marker-assisted pyramiding of broad-spectrum bacterial blight resistance genes into an elite maintainer line. Plos One. 13(10): 0201271.

5. Chen S, Lin XH, Xu CG, Zhang Q (2001). Improvement of bacterial blight resistance of 'Minghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection. Crop Sci. 40(1): 239-244.

6. Chu Z, Yang B, Fu H, Xu C, Li Z, Sanchez A, Park YJ, Bennetzen JL, Zhang Q and Wang, S (2006). Targeting *xa13*, a recessive gene for bacterial blight resistance in rice. Theor Appl Genet. 112(3):455-461.

7. Dong X, Wang X, Zhang L, Yang Z, Xin X, Wu S, Sun C, Liu J, Yang J, Luo X (2013). Identification and characterization of *OsEBS*, a gene involved in enhanced plant biomass and spikelet number in rice. Plant Biotechnol J.11 (9): 1044-1057.

8. Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11-15.

9. Feng X, Wang C, Nan J, Zhang X, Wang R, Jiang G, Yuan Q, Lin S (2017). Updating the elite rice variety Kongyu 131 by improving the *Gn1a* locus. *Rice*.10 (1): 35.

10. Gu K, Yang B, Tian D, Wu L, Wang D, Sreekala C, Yang F, Chu Z, Wang G, White FF, Yin Z (2005). R gene expression induced by a type-III effector triggers disease resistance in rice. Nature. 435(7045):1122-1125.

11. Hospital F, Charcosset A (1997). Marker-assisted introgression of quantitative traitloci. Genet. 147: 1469-1485.

12. Huang X, Qian Q, Liu Z, Sun H, He S, Luo D, Xia G, Chu C, Li J, Fi X (2009). Natural variation at the *DEP1* locus enhances grain yield in rice. Nat Genet.41 (4): 494-497.

13. Ikeda K, Ito M, Nagasawa N, Kyozuka J Nagato Y (2007). Rice ABERRANT PANILE ORGANIZATION1, encoding an F- box protein, regulates meristem fate. Plant J. 51 (6): 1030-1040.

14. Iyer A, Sand Mc, Couch SR (2004) The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance. MPMI.17(12): 1348-1354.

15. Jamaloddin M, Durga Rani CV, Swathi G, Anuradha C, Vanisri S, Rajan CPD, Raju KS, Bhuvaneshwari V, Jagadeeswar R, Laha GS, Prasad MS, Satyanarayana PV, Cheralu C, Rajani G, Ramprasad E, Sravanthi P, Kumar APN, Kumari AK, Yamini KN, Mahesh D, Rao SD, Sundaram RM, Madhav MS (2020). Marker assisted gene pyramiding for bacterial blight and blast resistance into mega rice variety "Tellahamsa". *PLoS One*. 15 (6): e0234088.

16. Jiang GH, Xia ZH, Zhou YL, Wan J, Li DY, Chen, RS (2006). Testifying the rice bacterial blight resistance gene *xa5* by genetic complementation and further analyzing *xa5* (*Xa5*) in comparison with its homolog TFIIAgamma1. Mol Genet. Genomics. 275(4): 354-366.

17. Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X, Qian Q, Li J (2010). Regulation of *OsSPL14* by OsmiR156 defines ideal plant architechture in rice. *Nat Genet*. 42 (6):541-544.

18. Kauffman HE, Reddy APK, Hsieh SPY, Merca SD (1973). An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Dis rep*. 57 (6): 537-541.

19. Kim SR, Ramos J, Ashikari M, Virk PS, Torres EA, Nissila E, Hechanova SL, Mauleon R, Jena, KK (2018). Introgression of a functional epigenetic *OsSPL14*^{WFP} allele into elite indica rice genomes greatly improved panicle traits and grain yield. *Sci Rep*. 8(1):3833.

20. Kottapalli KR, Narasu ML, Jena, KK (2010). Effective strategy for pyramiding three bacterial blight resistance genes into fine grain rice cultivar, Samba Mahsuri, using sequence tagged site markers. Biotechnol Lett. 32(7):989-996.

21. Kumar KR, Kumaravadivel N (2018). Marker-assisted selection for biotic stress (Bacterial leaf blight and gall midge) tolerance in BC_4F_4 generation of rice (*Oryza sativa* L.). EJPB. 9(1): 275-282.

22. Laxmi Prasanna B, Dangi KS, Sundaram RM, Damodar Raju CH, Jagadeeshwar R, Rekha G, Sinha, P (2018). Screening of breeding lines of MTU1010 for their resistance against bacterial blight and blast. IJCMAS. 7(7): 4077-4084.

23. Li S, Zhaob B, Yuanb D, Duanb M, Qianc Q, Tangb L, Wanga B, Liua X, Zhanga J, Wanga J, Suna J, Liud Z, Fengd Y, Yuanb L, PandLia C (2013). Rice zinc finger protein *DST* enhances grain production through controlling *Gn1a/OsCKX2* expression. *Proc of the Natl Acad Sci.* 110(8): 3167-3172.

24. Liu Q, Yuan M, Zhou YA, Li X, Xiao J, Wang S (2011). A paralog of theMtN3/saliva family recessively confers race-specific resistance to *Xanthomonas oryzae* in rice. *Plant, Cell and Environment*. 34 (11): 1958-1969.

25. Ma X, Cheng Z, Qin R, Qiu Y, Heng Y, Yang H, Ren Y, Wang X, Bi J, Zhang X, Wang J, Lei C, Guo X, Wu F, Jiang L, Wang H, Wan J (2012). *OsARG* encodes an arginase that plays critical roles in panicle development and grain production in rice. The Plant Journal. 73 (2): 190-200.

26. Miura K, Ikeda M, Matsubara A, Song XJ, Ito M, Asano K, Matsuoka M, Kitano H, Ashikari, M (2010). *OsSPL14* promotes panicle branching and higher grain productivity in rice. *Nat Genet*. 42 (6):545-549.

27. Mohapatra S, Pandit E, Mohanty SP, Barik SR, Pawar S, Nayak DK, Subudhi HN, Das Land Pradhan, SK (2018). Molecular and phenotypic analyses of yield components QTLs in IR64 backcross progenies and popular high yielding rice varieties of India. *ORYZA*. 55(2):271-277.

28. Ou SH (1985). Common wealth Mycological Institute. *Rice diseases*. Kew.

29. Preece TF, Rhodes ME, Skinner FA (1982). *Progression of bacterial disease within plants*. Bacterial and plants, edsAcademic Press, London. PP:71-83.

30. Rekha G, Abhilash Kumar V, Viraktamath BC, Pranathi K, Koushik MBVN, Laxmi Prasanna B, Backiyalakshmi C, Sinha P, Ravindra RK, Bhaskar S, Hajira SK. , Blachiranjeevi CH, Swapnil K, Rambabu R, Harika G, Punniakotti m E, Anila M, Mahadev HK, Dilipkumar T, Yugander A, Chaitra K, Praveen M, Madhavi KR, Prasad MS, Laha GS, Neeraja CN, Balachandran SM, Senguttuvel P, Fiyaz RA, Badri J, Giri A, SubbaRao LV, Ravindra Babu V, Sundaram RM (2018). Improvement of blast resistance of the popular high-yielding, medium slender-grain type, bacterial blight rice variety, Improved Samba Mahsuri by marker-assisted breeding. J. *Biochem. Biotechnol.* 27(4): 463-472.

31. Ronald PC, Albano B, Tabien R, Abenes L, Wu K, McCouch SR (1992). Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. *Mol Genet Genomics*. 236(1):113-120.

32. Singh S, Sidhu JS, Huang N, Vikal Y, Li Z, Brar DS, Dhaliwal HS, Khush GS (2001). Pyramiding three bacterial blight resistance genes (*xa5*, *xa13*and*Xa21*) using marker-assisted selection into indica rice cultivar PR106. Theor Appl Genet. 102 (6): 1011-1015.

33. Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald, P (1995). A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science. 270(5243): 1804-1806.

34. Srinivasan MC, Thirumulachar MJ, Patel MK (1959). Bacterial blight of rice. Curr Sci. 28:469-470.

35. Sundaram RM, Madhav S, Neeraja CN, Balachandran SM, Mangrauthia SK, Barbadikar KB, Divya PS, Subba Rao LV, Hariprasad AS (2018). Multi-trait improvement in rice through marker-assisted breeding. Oryza.55(special issue): 24-37.

36. Sun X, Cao Y, Yang Z, Xu C, Li X, Wang S, Zhang, Q (2004). *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *Oryzae* in rice, encodes an LRR receptor kinase-like protein. Plant J. 37 (4): 517-527.

37. Tian D, Wang J, Zeng X, Gu K, Qiu C,Yang X, Zhou Z, Goh M, Luo Y, Hori MM, White FF, Yin Z (2014). The rice TAL effector-dependent resistance protein *Xa10* triggers cell death and calcium depletion in the endoplasmic reticulum. The Plant Cell. 26 (1):497-515.

38. Utami DW, Lestari P, Koerniati S (2013). A relative expression of *Xa7* gene controlling bacterial leaf blight resistance in Indonesian local rice population (*Oryzasativa*L.). J Crop Sci. Biotechnol. 16: 1-7.

39. Xiang Y, Cao Y, L Xu, C Q, Li X, Wang S (2006). *Xa3*, conferring resistance for rice bacterial blight and encoding a receptor kinase-like protein, is the same as *Xa26*. Theor. Appl. Genet. 113 (7):1347-1355.

40. Yoshida A, Sasao M, Yasuno N, Takagi K, Daimon Y, Chen R, Yamazaki R, Tokunaga H, Kitaguchi Y, Sato Y, Nagamura Y, Ushijima T, Kumamaru T, Iida S, Maekawa Kyozuka, J (2013). *TAWAWA*, a regulator of rice inflorescence architechture, functions through the suppression of meristem phase transition. PNAS USA. 110(2): 767-772.

41. Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX, Kono I, Kurata N, Yano M, Iwata N, Sasaki T (1998). Expression of *Xa1*, a bacterial blight resistance gene in rice, is induced by bacterial inoculation. PNAS USA. 95 (4):1663-1668.

42. Zhao L, Tan L, Zhu Z, Xiao L, Xie D, Sun C (2015). *PAY1* improves plant architecture and enhances grain yield in rice. Plant J. 83(3):528-536.