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Characterization of the mutant lines of Akshaya rice variety for blast resistance

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Abstract

The M_4 generation mutant lines of Akshaya rice variety were characterized for blast resistance in 24 mutagenic treatments treated with gamma rays and EMS. The results of phenotypic screening carried out against blast disease by adopting the uniform blast nursery method at two locations revealed that majority of the mutant lines were moderately resistant to blast as that of the parent. The mutant lines derived from the treatments T9 (20kr + 0.25% EMS), T17 (30 kr) and T19 (40 kr+ 0.1% EMS) recorded blast score of 0-1 showing immune reaction whereas mutant lines derived from 7 other treatments (T1, T2, T7, T14, T15, T16 and T22) scored 8-9 and manifested highly susceptible reaction both at Bapatla and Hyderabad. Based on the results of the polymorphism with the markers tested, it was concluded that the mutation might have occurred in the location between 35.1 Mb (RM208) to 37.6 Mb (RM266) on 2nd chromosome and / or 34.5 Mb (RM 567) to 34.9 Mb (RM280) on 4th chromosome. The results of gene profiling studies revealed that these resistant mutant lines do not possess the tested eight major blast resistant genes.

Key words: Resistance, blast, chromosome, mutant lines, polymorphism

Introduction

The use of resistant cultivars is the most economically viable and effective way of controlling rice blast, but the useful life span of many cultivars is only for few years in disease conducive environments because of the breakdown of resistance in the face of high pathogenic variability of *M. oryzae*. Hence, breeding of cultivars with more durable resistance has become a constant challenge in rice breeding programs. Mutations were traditionally identified on the basis of their morphological properties, but the development of new techniques based on DNA information has made this process quicker and more reliable. The present study was undertaken during *kharif* 2012 at Rice Research Unit (RRU), Bapatla and Indian Institute of Rice Research (IIRR), Hyderabad with an objective of identifying blast resistant lines from the M_4 generation mutant population of Akshaya rice variety.

Material and Methods

The material for the present study comprised of 24 mutagenic treatments along with the control variety Akshaya (BPT 2231) treated with gamma rays (10kr, 20kr, 30 kr and 40kr), ethyl methane sulfonate (0.1%, 0.2%, 0.25% and 0.3%) and their combinations. The M_1, M_2 and M_3 generations were raised at RRU, Bapatla. In the M4 generation, ten single plant progenies of each mutant treatment along with the control were grown during *rabi* 2012-13 at two locations *viz.*, RRU, Bapatla and at Indian Institute of Rice Research, Hyderabad. The phenotypic screening of the mutant lines was carried out against blast disease by adopting the uniform blast nursery method. Uniform Blast Nursery was laid out in 10 X 1 m bed and the soil is pre-treated with FYM and recommended dose of fertilizers. Later commercial sulphuric acid is added to the beds before sowing. The susceptible variety for blast HR 12 was sown as border on all sides of the bed and in between the rows after every ten rows for spreading the

inoculum under natural conditions. The test material was sown in 50cm rows perpendicular to the border rows. Relative humidity is maintained with water sprinklers. The beds are covered with polythene sheets during night to maintain high humidity and to increase the disease pressure on the entries. All necessary precautionary measures were taken up to increase/develop the disease pressure on mutant lines and the scoring for blast symptoms was done by using Standard Evaluation System, SES, (IRRI,1996) at 30 days after sowing at which the susceptible check HR 12 died. The DNA for genotypic screening was isolated from young leaves harvested after 21 days of sowing using C-TAB method as described by Doyle and Doyle (1990).

The genomic DNA of the selected mutant lines (identified through phenotypic screening) was subjected to PCR amplification as per the procedure described by Chen *et al.* (1997). PCR was carried out using a programmable thermocycler (Corbett Research, Australia). The PCR reaction mixture containing 2µl DNA, 8.5 µl water, 1.5 µl Taq buffer, 1 µl dNTP, 0.5 µl forward primer, 0.5 µl reverse primer and 1 µl Taq polymerase (15 µl reaction mixture) was subjected to the polymerase chain reaction. 36 microsatellite markers distributed over 12 chromosomes were used to reveal the genetic polymorphism between resistant and susceptible mutants.

Gene Profiling

In order to identify the blast resistant gene present in the resistant mutant lines that are screened at field level, gene profiling was carried out using eight major blast resistant gene specific markers *viz*., *Pi1, Pi2, Pi9, Pi33, Pi54, Pib, Pita and Pita2* (Table 1). The positive and negative checks along with the untreated control Akshaya were utilized for each gene separately. The negative checks used were BPT-5204, Co-39 and Swarna which do not contain any gene conferring resistance to blast.

The profile of the PCR (PCR conditions)

94ºC: 5 minutes (Initial denaturation)

94ºC: 30 seconds (denaturation)

55ºC: 1 minute (annealing)

72ºC: 1 minute (extension)

72ºC: 10 minutes (final extension)

Agarose gel electrophoresis

A 3% gel was prepared and the PCR product was loaded to check the amplification of SSR markers.

Gel documentation

After the gel run, the gel was visualized under UV light transmitted gel documentation system. The banding pattern was observed and recorded using gel documentation unit (Alpha Infotech, USA).

Results and Discussion

The results of phenotypic screening revealed that majority of the mutant lines were moderately resistant to blast as that of the parent (Table 2). Mutant lines from the treatments T9 (20kr + 0.25% EMS), T17 (30 kr) and T19 (40 kr+ 0.1% EMS) scored 0-1 showing immune reaction whereas mutant lines from 7 treatments (T1, T2, T7, T14, T15, T16 and T22) scored 8-9 and manifested highly susceptible reaction both at Bapatla and Hyderabad.

The genotypic screening was done using three highly resistant mutant lines *viz.,* T9, T17 and T19 treatments and three susceptible lines from T14, T16 and T22 treatments which were identified through phenotypic screening in the Uniform Blast Nursery in both the locations. The control variety Akshaya was also included to confirm the blast resistance. Among the 36 tested SSR primers, 27 primers showed monomorphic banding pattern indicating that the mutation has not occurred in those loci. Five markers *viz.,* RM266, RM280, RM228, RM72 and RM23946 showed considerable polymorphism and the number of alleles detected per primer ranged from 2 (RM228, RM266 and RM72) to 5 (RM23946). The amount of polymorphism reflects the existence of considerable difference in their loci among resistant and susceptible mutant lines. Abedi *et al.* (2012) reported a significant level of polymorphism with four microsatellite markers *viz.,* RM224, RM277, RM463 and RM179 and tested the association between phenotypic results and the molecular data in rice.

The results of amplification pattern with the marker RM266 on second chromosome showed the significant polymorphism among the mutant lines. The amplicons of the resistant mutant lines have similar amplicon size and it differed with that of all the susceptible lines, the amplification of susceptible mutant lines is similar to that of control (Fig.1). The amplification pattern with the primer RM280 on fourth chromosome manifested a clear polymorphism between resistant mutant lines with its parent (Fig.2). The treatment T9 (R1) is differentiated with the other two resistant mutant lines (T17 and T19) but T17 and T19 (R2 and R3) differed significantly with control as well as with susceptible lines. The banding pattern with the flanking marker RM208 to the primer RM266 manifested clear polymorphism among the resistant and susceptible lines (Fig. 3). Zhou *et al*. (2004) also reported the presence of the blast resistant gene *pi g(t)* by using marker RM208.

Similarly, significant polymorphism between resistant and susceptible lines was also evident from the banding pattern of flanking marker RM567 on chromosome 4 (Fig. 4).

Based on the results of the polymorphism with the arkers tested, it was concluded that the mutation might have occurred in the location between 35.1 Mb (RM208) to 37.6 Mb (RM266) on 2 nd chromosome and / or 34.5 Mb (RM 567) to 34.9 Mb (RM280) on 4th chromosome which might have led to the resistant reaction against blast conferred by the mutant lines isolated from treatments T9, T17 and T19. Madamba *et al*. (2009) isolated a gamma ray induced IR 64 mutant G978 that showed enhanced resistance to blast. The mutation was mapped as a quantitative trait locus to a 3.8-Mb region on chromosome 12.

A molecular profile for the resistant mutant lines was carried out using eight major blast genes (*Pi 1, Pi 9,Pi 2, Pi 54, Pi b, Pi ta, Pi 33 and Pi ta 2*) linked markers to confirm whether the resistant mutant lines have any known blast resistant gene. The DNA of these resistant mutant lines (T9, T17 & T19 treatments) and three susceptible lines identified through phenotyping were used along with the control Akshaya for gene profiling studies. All the amplicons of mutant lines were not similar to that of putative controls. The results of gene profiling studies revealed that these resistant mutant lines do not possess the tested 8 major blast resistant genes (Fig.5).

With the polymorphic survey, it was observed that the mutation might have occurred in the chromosome 2 and 4. But, the gene profiling results indicated that the resistance is not due to the P*i b* gene which was reported from chromosome 2 and also the polymorphic markers *viz.,* RM 266 and RM208 which were reported to be linked with P*i b* gene, so the resistance could be due to the loci which was not earlier reported on chromosome 2. To identify the gene/genes responsible for the resistant reaction in these mutant lines the resistant lines have to be crossed with the control/ any other susceptible variety and the genetics of resistance can be further studied in the F2 generation to unravel the effect of mutation in conferring the resistance.

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Table 1. Major blast resistant gene specific markers

S.No.	Blast resistant gene	Chromosome No.	Positive check	Primer
A	*Pi1*	11	C101LAC	RM 224
B	*Pi9*	6	*O.minuta*,SP48	RM 7103
C	*Pi54*	11	Tetep	RM 206
D	*Pi2*	6	C101A51	RM 56595
E	*Pib*	2	SP 51	RM 166
F	*Pita*	12	Tadukan	RM7 102
G	*Pi33*	8	Bala, IR 64	RM 72
H	*Pita2*	12	IR 64	RM 7102

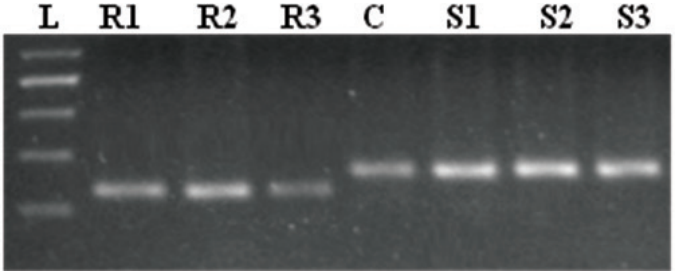
+ Positive check R1-T9 R2- T17 R3-T19

-Negative check C- T25 (Akshaya) S1- T14 S2- T16 S3 –T22

Table 2. Screening of mutant lines of Akshaya for blast resistance at IIRR, Rajendranagar and at Agriculture College, Bapatla

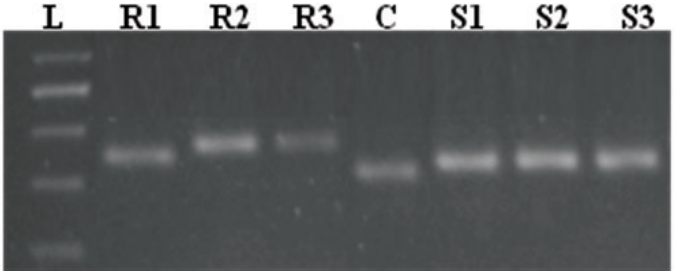
S.No	Treatment	IIRR, Rajendranagar		Bapatla	
		Score	Reaction	Score	Reaction
1	T1 (10kr+0.1%EMS)	8	HS	8	HS
2	T2 (10kr+0.2%EMS)	8	HS	8	HS
3	T3(10kr+0.25%EMS)	4	MR	3	R
4	T4 (10kr+0.3%EMS)	8	HS	7	S
5	T5 (10kr)	7	S	6	MS
6	T6 (0.1% EMS)	6	MS	6	MS
7	T7 (20kr+ 0.1%EMS)	8	HS	8	HS
8	T8 (20kr+ 0.2%EMS)	4	MR	3	R
9	T9 (20kr+ 0.25%EMS)	1	HR	1	HR
10	T10 (20kr+ 0.3%EMS)	4	MR	3	R
11	T11 (20kr)	8	HS	7	S
12	T12 (0.2%EMS)	8	HS	7	S
13	T13 (30kr+0.1%EMS)	5	MR	4	MR
14	T14 (30kr+0.2%EMS)	9	HS	9	HS
15	T15 (30kr+0.25%EMS)	9	HS	9	HS
16	T16 (30kr+0.3%EMS)	9	HS	8	HS
17	T17 (30kr)	1	HR	0	HR
18	T18 (0.1%EMS)	7	S	6	MS
19	T19 (40kr+ 0.1% EMS)	1	HR	1	HR
20	T20 (40kr+ 0.2% EMS)	3	R	3	R
21	T21 (40kr+ 0.25% EMS)	3	R	3	R
22	T22 (40kr+ 0.3% EMS)	9	HS	8	HS
23	T23 (40kr)	5	MR	4	MR
24	T24 (0.3% EMS)	4	MR	3	R
25	T25 (Control variety Akshaya)-	5	MR	5	MR

R: Resistant; MR: Moderately resistant; HR: Highly resistant; S: Susceptible; MS: Moderately susceptible; HS: Highly susceptible



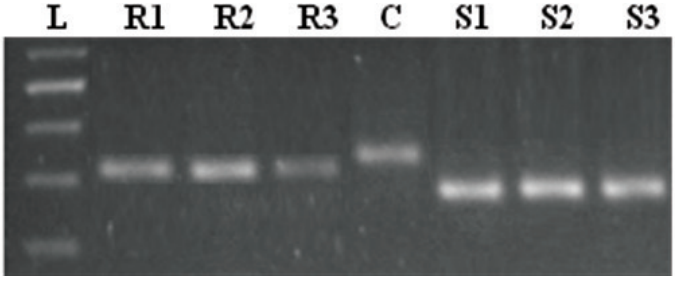
Chromosome 2 RM 266

Fig 1. Banding pattern of PCR amplified product of microsatellite marker RM266 on chromosome 2



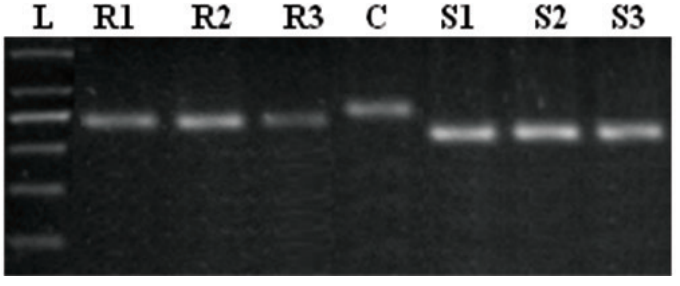
Chromosome 4 RM280

Fig. 2. Banding pattern of PCR amplified product of microsatellite marker RM280 on chromosome 4



Chromosome 2 RM 208

Fig. 3. Banding pattern of PCR amplified product of microsatellite marker RM208 on chromosome



Chromosome 4 RM 567

Fig. 4. Banding pattern of PCR amplified product of microsatellite marker RM567 on chromosome 4

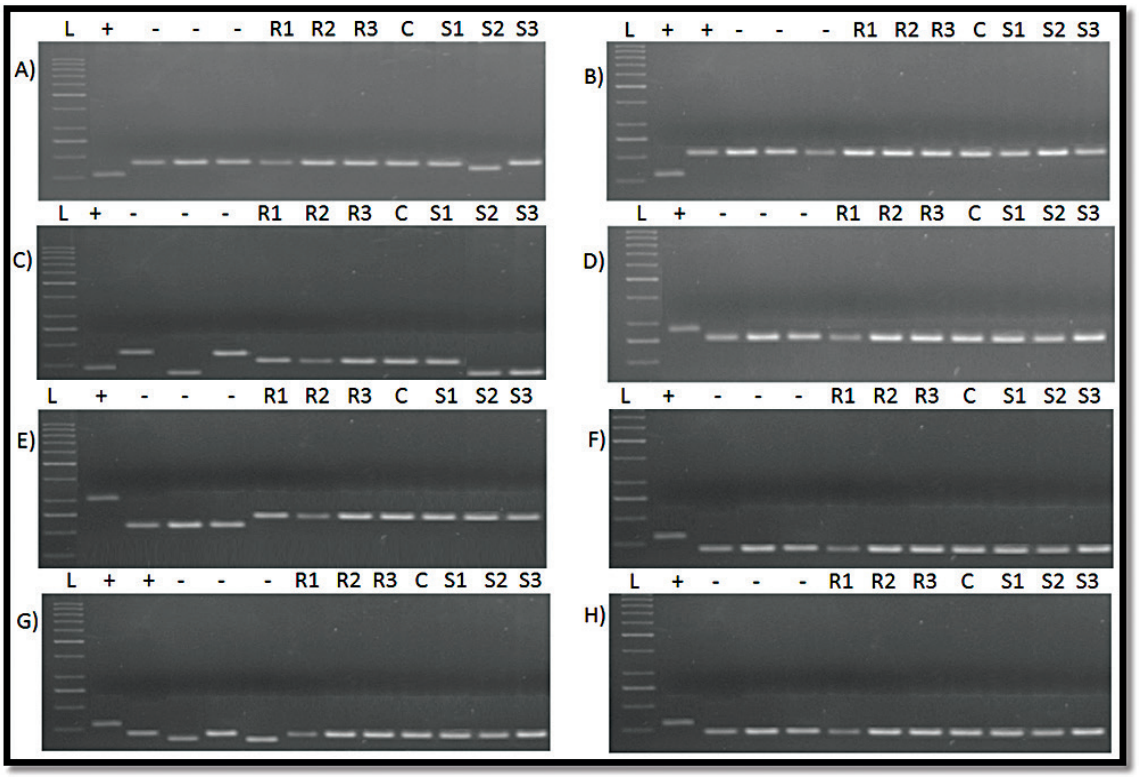


Fig. 5. Gene profiling with the primers specific to blast resistance genes *Pi1, Pi9,Pi54, Pi2,Pib, Pita, Pi33 and Pita2* on chromosomes 6,11, 2, 8 and 12 of rice