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Study of Genetic Diversity of Near Isogenic Lines of Rice Using RAPD Markers

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Bacterial leaf blight is the one of the major diseases of rice caused by *Xanthomonas oryzae* pv. *oryzae* thought the world. The yield loss due to bacterial leaf blight disease occurs up to 20-50%. This disease can be most effectively and economically controlled by using bacterial leaf blight resistant pyramided lines. The present investigation was carried out to study the molecular characterization of 30 rice genotypes for BLB resistance using 29 RAPD (Randomly amplified polymorphic DNA) markers. The analysis of 29 RAPD primers generated total 5118 scorable bands with 308 loci, among them 273 loci were found polymorphic, showing 86.68% polymorphism. The PIC values ranged from 0.72 (OPC 11) to 0.94 (OPP-06) with an average of 0.86. Some primers such as, OPA 05, OPA 09, OPA 12, OPAB 09, OPAE 03, OPB 17, OPH 04, OPP 03, OPP 17 and ES 19 showed 100% polymorphism, the size of amplified fragments varies from 95 bp (OPP 01) to 5713 bp (OPP 06). The range of Jaccard's similarity coefficient value was from 0.35 (between IET 20667 and IET 21067) to 0.82 (between IET 20667 and IET 20669). Overall results suggested the genetic diversity between some of the pyramided lines with their respective recurrent parents and it would be useful for successful genetic diversity analysis.

Keywords: Rice, Bacterial Leaf Blight (BLB), Near Isogenic Lines (NILs), Disease resistance genes, RAPD (Randomly Amplified Polymorphic DNA).

Rice is agronomically and nutritionally, one of the world's most important staple food crops, with approximately half of the world's population dependent on it. China ranks first for rice production and India is the second largest rice producing country in the world. In India, higher rice producing states include: West Bengal, Andhra Pradesh, Uttar Pradesh, Punjab, Orissa, Tamil Nadu, Chhattisgarh, Bihar, Karnataka and Haryana. The rice production was recorded 112 million tonnes in 2013-14 in India (FAO, Agricultural Outlook and Situation Analysis Reports, United Nations, 2013-14). In rice, monocot seeds develop by following self-pollination. There are n=12 basic chromosomes in rice. Due to its relatively smaller genome size (430 Mb), rice is consider as a model crop for genome study (Causse et al., 1994; Kurata et al., 1994).

In rice more than 70 diseases caused by fungi, bacteria, viruses and nematodes are prevalent (*Oryza sativa*). The most devastating of them are the ones caused by *Magnaporthe grisea* (rice blast), *Xanthomonas oryzae* pv. *oryzae* (bacterial leaf blight, BLB) and *Rhizoctonia solani* (sheath blight) (Jamil et al., 2012). BLB caused by the vascular pathogen *Xanthomonas oryzae* pv. *oryzae (Xoo)* is one of the most serious diseases leading

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to crop failure in rice growing countries including Korea, Taiwan, Philippines, Indonesia, Thailand, India and China. *Xanthomonas* is a large genus of gram-negative and yellow-pigmented bacteria. *Xoo* enters rice leaf typically through the hydathodes at the leaf margin, multiplies in the intercellular spaces of the underlying epithelial tissue, and moves to the xylem vessels to cause systemic infection, which is known as kresek (Noda and Kaku., 1999; Ou., 1985).

BLB causes severe yield losses of up to 20-50% (Srinivasan and Gnanamanickam, 2005; Jamil et al., 2012), depending on the stage of the crop, cultivar susceptibility and the environmental conditions. Several chemicals have been identified, but they are impractical to use. The improvement of rice varieties for resistance to the diseases that are prevalent and destructive is necessary for the sustainability of rice grain yields (Pinta et al., 2013). Breeding and deployment of resistant cultivars carrying major resistance (R) genes has been the most effective approach to control BLB disease. Most R genes to BLB have been introgressed into the background of the susceptible indica cultivar to develop a set of near isogenic lines (NILs), and some have been pyramided, either through classical breeding and marker-assisted selection or through genetic engineering, to develop new plant types and NILs (Sanchez et al., 2000; Singh et al., 2001; Narayanan et al., 2002). To date, at least 38 BLB resistance genes conferring host resistance against various strains of *Xoo* have been identified and six genes have cloned (Gautam et al., 2014; Pradhan et al., 2015). Pyramided lines have shown higher levels and wider spectra of resistance to BLB than the parental NILs with single R genes (Huang et al., 1997; Adhikari et al., 1999).

Molecular markers offer great scope for improving the efficiency of conventional plant breeding by selecting markers linked to the trait rather than the trait itself. The development of PCR based RAPD markers, in which random fragment of DNA are amplified from DNA samples using short, arbitrary primers, led to the tagging of several resistance genes with RAPD markers. The number of different amplification products for each primer depends upon the primer sequence, the genome sequence and the genome size (Williams et al., 1991).

In this investigation, RAPD marker which is universal and mostly used for diversity study, has been used to quickly identify genetic similarities and dissimilarities among near-isogenic lines, their recurrent parents and susceptible check cultivars.

MATERIALS AND METHODS

In this study, the seeds of 30 rice genotypes (Table-1) have used. There were 19 NILs, six recurrent parents and five susceptible check cultivars used which were obtained from the Main Rice Research Station, Anand Agricultural University, Nawagam, Gujarat, India.

Plant material and DNA extraction

The seeds of 30 rice genotypes (Table-1) were grown in pots, 15 days old seedlings were collected and used for isolating genomic DNA.

DNA was extracted using a modified CTAB method (Zidani et al., 2005). All of the required reagents were prepared as per Sambrook et al., (1989). Fresh leaf tissues (0.3 g) was ground in liquid nitrogen and taken into a 2 ml microcentrifuge tube. The ground sample was extracted with 0.8 ml of CTAB extraction buffer. [To prepare 10 ml of CTAB extraction buffer, 1 M Tris HCL (1ml), 0.5 M EDTA (1ml), 5 M NaCl (2.8ml) were mixed with 4 ml of distilled water. Then, 4% CTAB (0.4g) and 1% PVP (0.1g) were added to this mixture. Dissolved PVP and CTAB properly and heated this mixture at 60^0C (about 20-30 minute). Two percentage of β-mercaptoethanol (200µl) was added, just before use]. Sample was mixed well with extraction buffer by inversion. The sample was incubated for one hour at 65^0C in water bath (allowed it to cool down). Equal amount of 800 µl of chloroform: isoamylalcohol (24:1) was added to centrifuge tube and mixed by inversion. Centrifugation was carried out it for 20 minutes at 10,000 rpm at 4°C. Supernatant was transferred into a new tube and further extracted with chloroform: isoamyl alcohol (24:1), and the DNA was precipitated with 80% ethanol. The pellet was air dried and resuspended in 100µl of Tris–EDTA (TE) buffer. To estimate the quantity and quality (in terms of protein and RNA contamination) of isolated genomic DNA, spectrophotometry was performed. One microliter of DNA sample was loaded on to the well of Nanodrop instrument. The

concentration of DNA and absorbance at 260 nm and 280 nm were measured. To check the DNA quality of isolated genomic DNA, electrophoresis was done using 0.8 % agarose gel prepared in 1X TBE buffer.

RAPD amplification

Amplification of RAPD fragments was performed according to Williams et al., (1991) using general decamer arbitrary Operon primers (Operon technologies Inc, USA; SIGMA-D, USA) (Table-2). Amplification were performed in a 25 µl reaction volume containing 1.5 µl DNA (50 ng/ml), 2.5µl of PCR buffer (10 x) with 15 mM $MgCl_2$, Bangalore Genei, India, 1 µl of Primer (10 p moles/ml) (MWG), 0.5µl of dNTPs (2.5 mM) Bangalore Genei, India, 0.5µl of *Taq* DNA polymerase (3U/ml) and 19 µl of sterile distilled water. Amplification was performed in a programmed thermocycler with initial denaturation at 94^0C for 5min, 35 cycles of denaturation at 94^0C for 1min, primer annealing at 38^0C for 1min, extension at 72^0C for 1min, and final extension at 72^0C for 5 min. Amplified products were electrophoresed on 1.5% agarose in 1*x*TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system (Bio-Rad, Hercules, California).

Analysis of RAPD

Scoring of clear and distinct bands amplified by RAPD primers were carried out for the presence and absence of the corresponding band in the 30 rice genotypes. The scores '1' for presence of band and '0' indicates absence of band. The NTSYSpc (Numerical Taxonomy System Applied Biostatistics, Setauket, New York) version 2.02 software was used for data entry and their subsequent analysis (Rohlf, 1994).

RESULTS

Randomly Amplified Polymorphic DNA (RAPD) assay

Total 80 RAPD primers were screened for the study of molecular characterization of bacterial leaf blight resistant near isogenic lines of rice along with recurrent parents and susceptible check cultivars; out of which, 29 primers were polymorphic. These 29 primers were used for RAPD analysis of 30 rice genotypes as discussed in Table-2.

Study of genetic diversity in rice genotypes using RAPD markers

Out of 29 used primers, the individual descriptions of few primers have given below:

OPA 04 amplified genotype specific band of 520 bp, which was observed in IET 20671 (NIL of Swarna) and another specific band of 406 bp, was also found in IET 19046 (NIL of Samba Masuri). These fragments can be developed into SCAR marker, if they are reproducible.

The banding pattern given by OPA 12 is shown in figure-2. The primer OPA 12 scored 138 scorable bands. The size of the bands ranged from 277 to 1892 bp. In all, 12 loci were generated

Table 1. Rice genotypes used for molecular analysis

S.No.	Genotypes
Bacterial leaf blight resistant near isogenic lines	
1	IR 64 (Recurrent parent)
2	IET 20667 (NIL of IR 64) *(Xa4, xa5, xa13, Xa21)*
3	IET 20668 (NIL of IR 64) *(Xa4, xa5, xa13, Xa21)*
4	IET 20669 (NIL of IR 64) *(Xa4, xa5, xa13, Xa21)*
5	SWARNA (Recurrent parent)
6	IET 20670 (NIL of Swarna) (*xa5, xa13, Xa21*)
7	IET 20671 (NIL of Swarna) (*xa5, xa13, Xa21*)
8	IET20672 (NIL of Swarna) (*xa5, xa13, Xa21*)
9	LALAT (Recurrent parent)
10	IET 21063 (NIL of Lalat) (*xa5, xa13, Xa21*)
11	IET 21064 (NIL of Lalat) (*xa5, xa13, Xa21*)
12	IET 21065 (NIL of Lalat) (*xa5, xa13, Xa21*)
13	IET 21066 (NIL of Lalat) (*xa5, xa13, Xa21*)
14	IET 21068 (NIL of Lalat) (*xa5, xa13, Xa21*)
15	TAPASWINI (Recurrent parent)
16	IET 21067 (NIL of Tapaswini) (*xa5, xa13, Xa21*)
17	IET 21069 (NIL of Tapaswini) (*xa5, xa13, Xa21*)
18	IET 21070 (NIL of Tapaswini) (*xa5, xa13, Xa21*)
19	IET 21071 (NIL of Tapaswini) (*xa5, xa13, Xa21*)
20	IET 21072 (NIL of Tapaswini) (*xa5, xa13, Xa21*)
21	IRBB 60 (NIL of IR 24) *(Xa4, xa5, xa13, Xa21)*
22	GR-11(Bacterial leaf blight susceptible rice genotypes)
23	SAMBA MASURI(Recurrent parent)
24	IET 19046 (NIL of Samba Masuri) (*xa5, xa13, Xa21*)
25	PUSA BASMATI-1(Recurrent parent)
26	IET 18990 (NIL of Pusa Basmati) (*xa13, Xa21*)
Bacterial leaf blight susceptible rice genotypes	
27	GR 4
28	JAYA
29	GR 3
30	TN-1

and all of them were polymorphic, giving 100% polymorphism with PIC value of 0.88.

Rao et al., (2003) reported that OPA 12 amplified *xa5* gene specific band of 1467 bp, which was observed in all three near isogenic lines of IR 64 (IET 20667, IET 20668 and IET 20669). If this fragment is reproducible, then there will be a chance to convert it into a *xa5* gene specific SCAR marker.

ES 15

The banding pattern amplified by ES 15 is shown in fig. 3. The primer ES 15 registered 251scorable bands. The size of the bands ranged from 132 to 2072 bp. In all, 12 loci were observed, out of which 11 were polymorphic, having 91.67% polymorphism with PIC value of 0.90.

Pooled RAPD analysis

Pooled RAPD analysis of all 29 primers generated total 5118 scorable bands with 308 loci, among them 273 loci were found polymorphic, showing 86.68% polymorphism. The PIC values ranged from 0.72 (OPC 11) to 0.94 (OPP-06) (Table 2) with an average of 0.86. On an average, per primer 11 loci were generated. The RAPD primers tested in present investigation produced fragments of different size. The minimum sized

Table 2. List of RAPD primers, range of molecular weight amplified by primers, total number of bands and loci, number of polymorphic loci and % polymorphism, Polymorphism Information Content (PIC) values obtained by analysing 30 genotypes of rice

S. No.	Primer Name	Range of molecular weight (bp)	Total No. of bands	Total No. of Loci	No of polymorphic loci	Percent Polymorphism (%)	PIC value
1	OPA-03	245-4702	277	13	10	76.92	0.91
2	OPA-04	210-4939	263	11	7	63.64	0.90
3	OPA-05	165-1283	129	9	9	100	0.86
4	OPA-09	247-3771	100	7	7	100	0.82
5	OPA-12	227-1892	138	12	12	100	0.88
6	OPA-15	168-2055	157	11	10	90.91	0.88
7	OPAB-09	194-1837	215	12	12	100	0.90
8	OPAC-05	212-1164	138	9	7	77.78	0.84
9	OPAE-03	201-5226	150	9	9	100	0.86
10	OPB-17	319-1130	193	9	9	100	0.88
11	OPC-11	442-1602	88	4	2	50	0.72
12	OPH-03	182-1411	160	8	6	75	0.84
13	OPH-04	217-768	100	7	7	100	0.84
14	OPK-03	278-1439	109	5	4	80	0.78
15	OPK-07	107-1047	138	9	7	77.78	0.84
16	OPL-11	310-1313	115	9	8	88.89	0.83
17	OPM-02	617-5193	216	15	14	93.33	0.91
18	OPP-01	95-930	136	8	7	87.5	0.84
19	OPP-03	155-1732	183	18	18	100	0.93
20	OPP-06	138-5713	335	18	16	88.89	0.94
21	OPP-17	135-1685	200	15	15	100	0.92
22	S-34	140-2932	155	11	9	81.82	0.87
23	S-70	177-2287	133	10	9	90	0.87
24	ES-13	134-1838	201	13	11	84.62	0.89
25	ES-14	172-1359	254	16	14	87.5	0.92
26	ES-15	132-2072	251	12	11	91.67	0.90
27	ES-17	179-3210	378	16	14	87.5	0.93
28	ES-19	281-1614	78	7	7	100	0.78
29	ES-22	410-1001	128	5	2	40	0.79
TOTAL	5118	308	273	2513.75	25.07		
AVERAGE		176	10.62	9.41	86.68	0.86	

Table 3. Jaccard's similarity coefficient of 30 rice genotypes based on RAPD data analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1																													
2	0.74	1																												
3	0.66	0.77	1																											
4	0.67	0.82	0.72	1																										
5	0.62	0.73	0.74	0.73	1																									
6	0.62	0.7	0.64	0.69	0.63	1																								
7	0.66	0.71	0.73	0.68	0.67	0.63	1																							
8	0.54	0.63	0.64	0.56	0.64	0.6	0.6	1																						
9	0.64	0.75	0.72	0.72	0.71	0.68	0.73	0.7	1																					
10	0.64	0.78	0.68	0.78	0.72	0.69	0.68	0.63	0.75	1																				
11	0.58	0.7	0.69	0.68	0.73	0.64	0.67	0.64	0.7	0.7	1																			
12	0.53	0.64	0.62	0.64	0.66	0.59	0.59	0.59	0.65	0.64	0.69	1																		
13	0.61	0.71	0.71	0.69	0.73	0.71	0.67	0.7	0.72	0.71	0.75	0.66	1																	
14	0.59	0.69	0.65	0.7	0.77	0.61	0.67	0.61	0.67	0.68	0.71	0.66	0.73	1																
15	0.59	0.66	0.67	0.65	0.7	0.58	0.64	0.58	0.68	0.7	0.72	0.76	0.67	0.69	1															
16	0.38	0.35	0.37	0.36	0.37	0.38	0.4	0.37	0.39	0.4	0.39	0.42	0.42	0.4	0.4	1														
17	0.44	0.45	0.44	0.45	0.42	0.43	0.49	0.42	0.48	0.47	0.43	0.42	0.43	0.46	0.44	0.65	1													
18	0.38	0.42	0.44	0.44	0.46	0.39	0.43	0.41	0.45	0.46	0.44	0.43	0.45	0.46	0.45	0.6	0.63	1												
19	0.43	0.46	0.47	0.48	0.47	0.46	0.53	0.42	0.51	0.5	0.45	0.43	0.48	0.5	0.46	0.64	0.74	0.64	1											
20	0.41	0.39	0.4	0.39	0.4	0.44	0.44	0.38	0.43	0.44	0.39	0.4	0.44	0.45	0.38	0.61	0.58	0.62	0.67	1										
21	0.44	0.47	0.48	0.48	0.5	0.45	0.53	0.43	0.51	0.52	0.5	0.46	0.49	0.52	0.5	0.51	0.62	0.64	0.69	0.65	1									
22	0.41	0.46	0.48	0.47	0.48	0.41	0.48	0.44	0.48	0.49	0.48	0.45	0.48	0.49	0.48	0.52	0.59	0.65	0.63	0.62	0.81	1								
23	0.46	0.45	0.46	0.44	0.47	0.45	0.47	0.42	0.49	0.48	0.47	0.42	0.49	0.5	0.46	0.59	0.61	0.57	0.71	0.63	0.68	0.73	1							
24	0.41	0.48	0.48	0.49	0.5	0.42	0.48	0.41	0.49	0.51	0.49	0.44	0.5	0.48	0.48	0.5	0.6	0.64	0.67	0.6	0.75	0.78	0.71	1						
25	0.41	0.47	0.5	0.46	0.48	0.44	0.49	0.45	0.5	0.48	0.49	0.46	0.48	0.47	0.47	0.54	0.64	0.61	0.66	0.6	0.71	0.76	0.67	0.78	1					
26	0.38	0.41	0.41	0.42	0.44	0.37	0.42	0.39	0.43	0.45	0.4	0.41	0.42	0.43	0.43	0.49	0.52	0.6	0.59	0.54	0.64	0.6	0.6	0.67	0.63	1				
27	0.44	0.47	0.48	0.49	0.47	0.41	0.5	0.41	0.48	0.49	0.46	0.45	0.45	0.5	0.48	0.52	0.58	0.57	0.67	0.54	0.67	0.67	0.65	0.67	0.71	0.68	1			
28	0.44	0.48	0.51	0.48	0.48	0.42	0.49	0.44	0.49	0.49	0.48	0.45	0.47	0.49	0.47	0.58	0.65	0.57	0.67	0.57	0.67	0.69	0.67	0.73	0.77	0.67	0.74	1		
29	0.44	0.47	0.51	0.48	0.48	0.42	0.47	0.42	0.47	0.48	0.45	0.44	0.44	0.48	0.44	0.53	0.62	0.58	0.68	0.57	0.66	0.69	0.67	0.68	0.71	0.65	0.73	0.75	1	
30	0.38	0.4	0.41	0.4	0.41	0.39	0.4	0.43	0.43	0.42	0.42	0.43	0.43	0.43	0.41	0.54	0.54	0.54	0.57	0.51	0.53	0.59	0.59	0.56	0.58	0.56	0.59	0.59	0.6	1

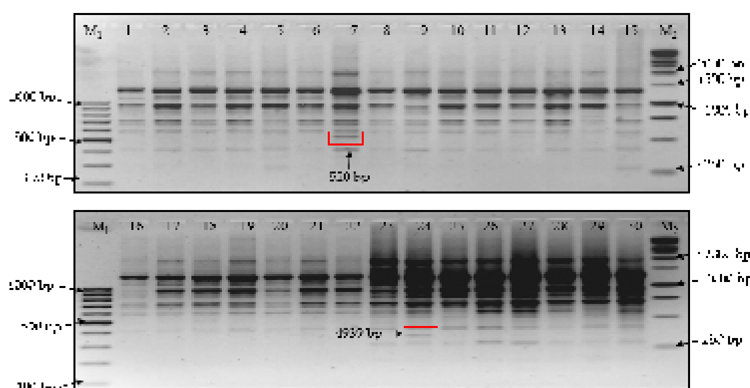
fragment (95 bp) was amplified by primer OPP 01, whereas maximum sized fragment (5713 bp) was amplified by primer OPP 06. The highest (100%) polymorphism were exhibited by primers, OPA 05, OPA 09, OPA 12, OPAB 09, OPAE 03, OPB 17, OPH 04, OPP 03, OPP 17 and ES 19 while the lowest polymorphism (40%) was observed with primer ES 22. Since primers OPA 05, OPA 09, OPA 12, OPAB 09, OPAE 03, OPB 17, OPH 04, OPP 03, OPP 17 and ES 19 showed 100% polymorphism, they will be very useful for the study of genetic diversity among rice genotypes.

Primer ES 17 generated maximum scorable bands 378 and the maximum loci 18 were generated by primer OPP 03 and OPP 06, whereas primer ES 19 generated minimum scorable bands 78. Primer OPC 11 amplified minimum four loci out of which, only two were found polymorphic.

The Jaccard's similarity coefficient value ranged from 0.35 (between IET 20667 and IET 21067) to 0.82 (between IET 20667 and IET 20669) (Table 3). Out of three NILs of IR 64, IET 20667 showed maximum genetic similarity (0.74) with recurrent parent IR 64; while for Swarna, all three lines exhibited moderate degree of genetic similarity ranging from 0.63 to 0.67. IET 21063 (NIL of Lalat) recorded maximum similarity (0.75) with its recurrent parent Lalat. All the NILs of Tapaswini showed low values of similarity coefficient (d" 5.0). IET 19046 (NIL of Samba Masuri) and IET 18990 (NIL of Pusa Basmati-1) registered 0.71 and 0.63 similarity coefficient, respectively, with their recurrent parents.

IET 21067 (NIL of Tapaswini) showed least similarity values with all other NILs. Similarly, IRBB 60 also exhibited lower values. These NILs along with NILs of IR 64 can be used as parents, to bypass marker assisted selection. Since, these NILs have three common R genes viz., *xa5, xa13* and *Xa21.* There will be no segregation between NILs. Hence, they can be used as potential parents in breeding programmes.

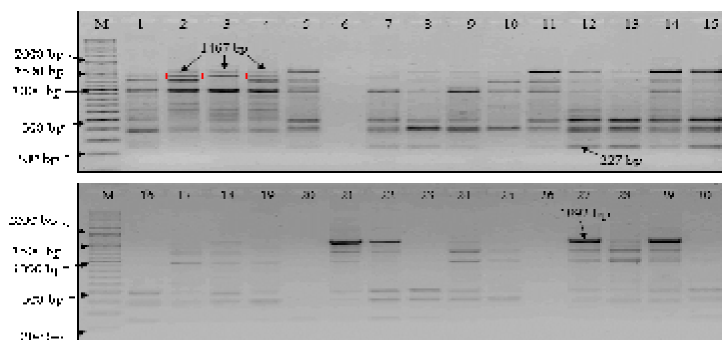
Clustering pattern of dendrogram (Fig. 4) produced by pooled RAPD data showed two major clusters A and B. Cluster A included recurrent parents IR 64, Swarna, Lalat and their respective NILs and Tapaswini and its near isogenic line (IET 20672). Cluster B comprised recurrent parents



- Genotype specific band; M_1 = 100 bp DNA ladder; M_2 = 1 kb DNA ladder

1) IR-64
2) IET-20667
3) IET-20668
4) IET-20669
5) SWARNA
6) IET-20670
7) IET-20671
8) IET-20672
9) LALAT
10) IET-21063
11) IET-21064
12) IET-21065
13) IET-21066
14) IET-21068
15) TAPASWINI
16) IET-21067
17) IET-21069
18) IET-21070
19) IET-21071
20) IET-21072
21) IRBB-60
22) GR-11
23) SAMBA MASURI
24) IET-19046
25) PUSA BASMATI
26) IET-18990
27) GR-4
28) JAYA
29) GR-3
30) TN-1

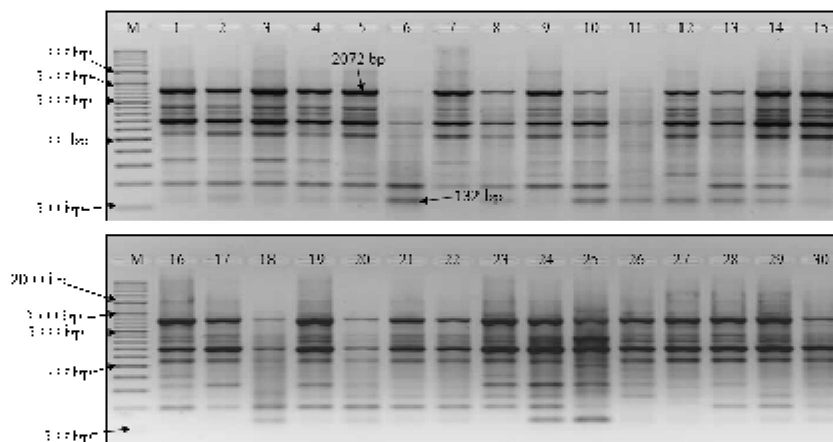
Fig. 1. RAPD profile of OPA 04



- Genotype specific band; M= 100 + 500 bp DNA ladder

1) IR-64
2) IET-20667
3) IET-20668
4) IET-20669
5) SWARNA
6) IET-20670
7) IET-20671
8) IET-20672
9) LALAT
10) IET-21063
11) IET-21064
12) IET-21065
13) IET-21066
14) IET-21068
15) TAPASWINI
16) IET-21067
17) IET-21069
18) IET-21070
19) IET-21071
20) IET-21072
21) IRBB-60
22) GR-11
23) SAMBA MASURI
24) IET-19046
25) PUSA BASMATI
26) IET-18990
27) GR-4
28) JAYA
29) GR-3
30) TN-1

Fig. 2. RAPD profile of OPA 12



(M= 100 + 500 bp DNA ladder)

1) IR-64
2) IET-20667
3) IET-20668
4) IET-20669
5) SWARNA
6) IET-20670
7) IET-20671
8) IET-20672
9) LALAT
10) IET-21063
11) IET-21064
12) IET-21065
13) IET-21066
14) IET-21068
15) TAPASWINI
16) IET-21067
17) IET-21069
18) IET-21070
19) IET-21071
20) IET-21072
21) IRBB-60
22) GR-11
23) SAMBA MASURI
24) IET-19046
25) PUSA BASMATI
26) IET-18990
27) GR-4
28) JAYA
29) GR-3
30) TN-1

Fig. 3. RAPD profile of ES 15

Samba Masuri, Pusa Basmati-1 and their respective NILs, NILs of Tapaswini and susceptible check cultivars. Cluster A was divided into two sub clusters A1 and A2. Sub-cluster A1 was further divided into A1a and A1b. Solitary genotype IR 64 formed group A1a, whereas Lalat, Swarna and their NILs, Tapaswini and NILs of IR 64 were in group A1b. Group A1b showed the maximum genetic proximity between NILs of Lalat to its recurrent parent and NILs of Swarna to its recurrent parent. Group A1a and A2b suggested that all the three NILs of IR 64 were genetically different from their recurrent parent IR 64. Sub-cluster A2 consists of single genotype IET 20672 (NIL of Swarna).

Cluster B was divided into two sub clusters B1 and B2. Sub-cluster B1 was further divided into group B1a and B1b. NILs of Tapaswini were in group B1a, whereas group B1b comprised of Samba Masuri, Pusa Basmati-1 and their near isogenic lines, IRBB 60, GR 11, GR 3, GR 4 and Jaya. Sub-cluster B2 consisted of TN 1.

Grouping of genotypes evaluated by principal coordinate analysis (PCA), which was performed by extracting Eigen value and Eigen vectors from a correlation matrix generated using a standardized data matrix. PCA revealed similar groupings as one obtained with UPGMA clustering which shown in 2D (two dimensional) plot (Fig. 5) and 3D (three dimensional) plot (Fig. 6).

DISCUSSION

Bacterial leaf blight (BLB) caused by the vascular pathogen *Xanthomonas oryzae pv. oryzae (Xoo)* is one of the most serious diseases leading to crop failure in rice growing countries. Exploitation of host plant resistance is considered the most effective, economical and environmentally safe measure for controlling BLB (Jamil et al., 2012). Breeding Marker assisted selection (MAS) for pyramiding important genes along with rapid background recovery of the recurrent parent, while maintaining the exquisite quality characteristics of rice, could be an effective approach for rice improvement (Suh et al. 2011; Ye., 2010).

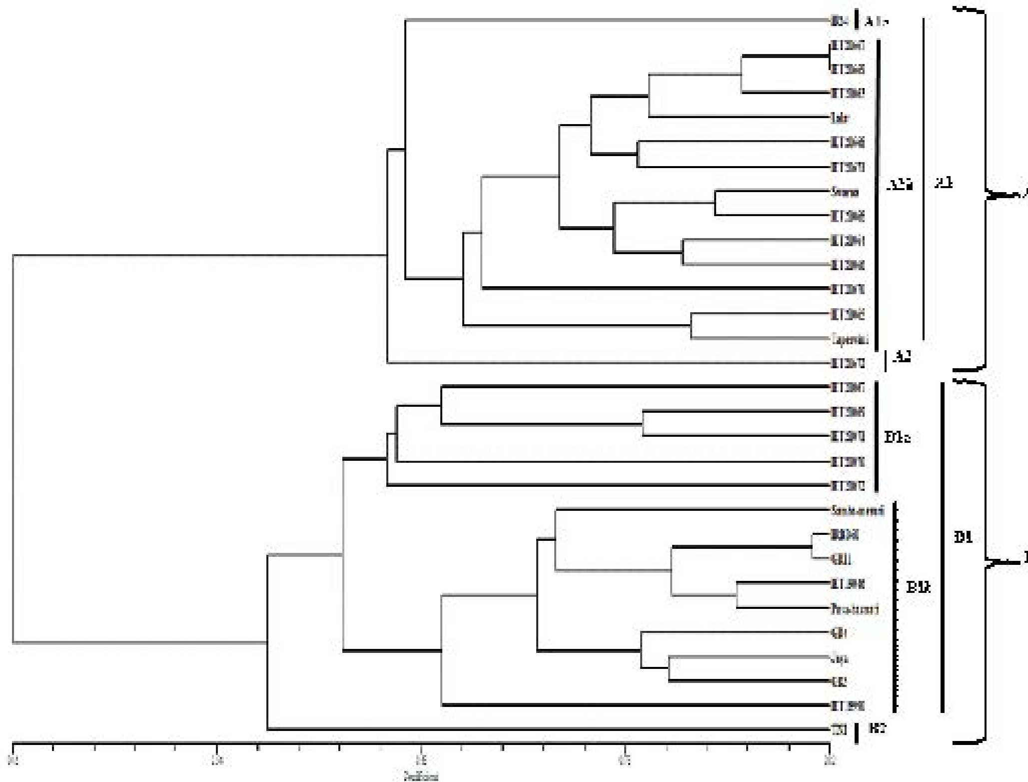


Fig. 4. Dendrogram showing clustering of 30 rice genotypes constructed using UPGMA based on Jacquard's similarity coefficient obtained from RAPD analysis

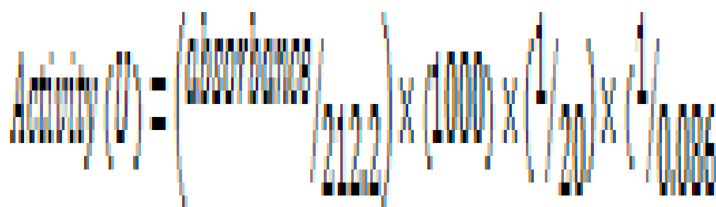


Fig. 5. Two-dimensional (2D) plot of genotypes obtained principal coordinate analysis of RAPD data

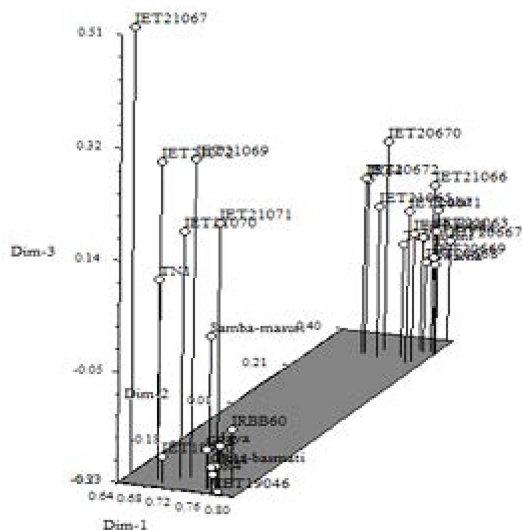


Fig. 6. Three-dimensional (3D) plot of genotypes obtained using principal coordinate analysis of RAPD data

In present study, RAPD molecular markers used to study the genetic diversity among near isogenic lines and their checks. Some of the RAPD markers show higher polymorphism such as OPA 05, OPA 09, OPA 12, OPAB 09, OPAE 03, OPB 17, OPH 04, OPP 03, OPP 17 and ES 19, were more informative and conclusive to unravel the molecular variability among near isogenic lines. RAPD primers OPA 12 and OPA 4 showed BLB resistance gene specific bands (Rao *et al*., 2003), which can be convert into more useful co-dominant SCAR markers, but these fragments were not reproducible may be due to mechanical error.

The Jaccard's similarity coefficient values ranged from 0.35 (between IET 20667 and IET 21067) to 0.82 (between IET 20667 and IET 20669). NIL IET 20667 showed maximum genetic proximity to its recurrent parent IR 64. NILs of Swarna exhibited moderate degree of genetic similarity to their recurrent parent. IET 21063 (NIL of Lalat) showed maximum genetic similarity to its recurrent parent Lalat. NILs IET 19046 and IET 18990 exhibited genetic proximity to their recurrent parents Samba Masuri and Pusa Basmati-1, respectively; whereas, NILs of Tapaswini showed low degree of genetic similarity to their recurrent parent. The Jaccard's similarity coefficient values suggested the genetic distance among the NILs and their recurrent parents and which line is more similar to its recurrent parent and recovered the maximum genetic background from recurrent parent.

Clustering pattern of dendrogram generated by pooled RAPD data showed that, all five NILs of Lalat exhibited genetic proximity to their recurrent parent; Solitary NILs IET 19046 and IET 18990 exhibited more genetic similarity to their recurrent parents Samba Masuri and Pusa Basmati-1, respectively. All three NILs of IR 64 were genetically dissimilar from their recurrent parent. IET 20672 (NIL of Swarna) genetically differed from its recurrent parent and all five NILs of Tapaswini were also genetically different from their recurrent parent. The genetic diversity

between NILs and their recurrent parents suggested the less percentages of recovery of the parental genome. The more diverse lines such as NILs of Tapaswini, NILs of IR 64 would be select as a recurrent parent aimed to develop resistant lines against BLB disease in breeding programs.

CONCLUSIONS

Host-plant resistance is a cost-effective and environmentally safe approach to reduce yield loss caused by BLB disease of rice. Several BLB resistance genes identified to date are either race specific or express susceptibility to the emerging races of the pathogen. The RAPD markers have successfully detected genetic diversity among bacterial leaf blight resistant near isogenic lines of rice. The study of Jaccard's similarity coefficient values and clustering patterns of dendrogram based on RAPD amplifications data suggested the relative genetic difference between near isogenic lines and their respective recurrent parents. Genetically, the most diverse near isogenic lines can be used as potential parents for breeding programmes aimed at development of resistant lines.

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