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Full Length Research Paper

DNA marker characterization for allele mining of blast and bacterial leaf blight resistant genes and evaluation for grain yield

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Landraces of rice were evaluated for blast and bacterial leaf blight (BLB) resistance, via tightly linked SSR markers and by phenotyping for flowering time, maturity and grain yield. Correlation between flowering maturity and grain yield was carried out using 162 local landraces and traditional rice cultivars. Days for 50% flowering ranged from 59 to 157 days, maturity from 93 to 192 days and seed yield per plant ranged from 0.24 to 33.3 g. Strong association was observed between flowering time and maturity time. Marker RG64 linked to *Pi*-2, a major dominant blast resistance gene on Chromosome 6 and marker *pTA*248 on Chromosome 11 linked to *Xa*21, a resistant gene to bacterial leaf blight were used to detect the presence of resistant alleles. Three different types of bands of 1 kb carrying *Xa*21 resistant allele and two susceptible alleles of 700 and 750 bp were amplified using *pTA*248. 14 rice genotypes were resistant for BLB, 46 genotypes showed susceptible banding pattern, and 87 genotypes were in heterozygous condition for resistance. 28 genotypes carried resistant alleles for both blast and bacterial leaf from among them Gowri Sanna, Ponni, Antharsali and Doddabyranellu were popularly preferred by the farmers. These can serve as donor lines for transferring of both resistances simultaneously.

Key words: Landraces, blast, bacterial leaf blight, grain yield, DNA markers.

INTRODUCTION

Rice is an important food crop of South and Southeast Asia (Mutert and Fairhurst, 2002). Narrow genetic diversity in the existing rice (*Oryza sativa* L.) varieties is problematic in breeding for adaptation to biotic and abiotic stresses. Therefore, it is necessary to explore the genetic diversity in rice germplasm for use in broadening the genetic variation in future rice breeding (Vanaja et al., 2010). Recent studies demonstrated that genetic linkage maps constructed with various DNA markers and different mapping populations such as F2, RIL, BC etc are very useful for the analysis and detection of QTLs (Sabouri et al., 2011). Donors could prove invaluable in the hybridization programme to improve rice quality and disease resistance (Chauhan et al., 2000). Local landraces can serve as donors of allele for future breeding programme. Rice is grown in landraces are reservoirs of many traits such as high yield, taste, color, grain nutritional value, grain quality, resistant to diseases and pests (Quaye et al., 2009). These local wide ranges of temperature and soil conditions. Before the advent of hybrids and improved varieties, rice was grown locally by farmers. There exists more than one hundred thousand genotypes of land races of rice and they carry various alleles of economic importance. Slowly, the use of local rice varieties has reduced significantly as the newly bred semi-dwarf varieties which are high yielding and fertilizer responsive have taken over to meet the food demand of the growing population. The genetic base of the modern varieties has decreased plant yield potential and could succumb to various diseases and pests. In this context, the germplasm accessions can provide useful genes to combat the situation.

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Rice blast disease caused by the fungus *Magnaporthae grisea (*Ou, 1985*)* and bacterial blight (BB) caused by *Xanthomonas oryzae* pv. o*ryzae* (*Xoo*) are the two most serious diseases of rice. Blast disease can lead to 20 to40% loss in yield and reducing grain quality (Le Huu et al., 2007) and 30 to 50% is due to bacterial leaf blight (Reddy et al., 1979).

Genetic mapping has resulted in much useful information on important major genes such as those for disease resistance and morphological traits. This information is particularly helpful in clarifying the allelism of gene conferring similar phenotypes. Linked markers are already being used in marker-assisted selection (MAS) programs for developing improved rice cultivars (Hittalmani et al., 2000; Sanchez et al., 2000).

Major genes for blast resistance have been detected. In recent years MAS has been employed for transferring Pita (Rybka et al. 1997) Pil, Pig (DU et al. 2007) Piz (Conaway – Bormans et al. 2003), Pi35 (Nguyen et al. 2006) and Pi37 (Chen et al. 2005) to new varieties. As well as, new sources of resistance against *Xoo* were identified*. O. nivara, O. longistminata,* and *O. punctata* are recommended for utilizing in rice breeding program. (Afzal et al., 2011). Thimmegowda et al (2011) deter-mined the differential virulence of isolates of *Xoo* to different rice cultivars. Ghazanfar,Usman et al (2009) screened rice germplasm for sources of resistance against rice blast disease. They said that the prevalence of the resistance against rice blast pathogen is more common in the course as compared to the fine germplasm lines of rice.

Genes conferring resistance in several rice species have been mapped with linked DNA markers, facilitating marker - assisted selection (MAS) for disease resistance in the crop. The efficiency of identifying blast-resistance gene(s) depended on the genotype and gene-linked markers used (Girija Rani and Adilakshmi, 2011). *Pi*-2 and *Xa*21 are the two major genes conferring resistance to many strains of blast and bacterial leaf blight (BLB) pathogens. Survey of the landraces for the presence of *Pi*-2 and *Xa*21 genes will facilitate the utilization of resistant genotypes in future breeding program. The limitations of conventional plant breeding methods have been eased by molecular tools and techniques. The blast and bacterial blight associated markers used in this study have been proved to be effective in the selection for resistance. Marker aided selection for selection of resistant genes help to screen large number of genotypes in short time. It can be practiced using tightly linked markers devoid of the disease also. In this study, the two markers selected are routinely used to select resistant alleles in the germplasm line and in the variety development and tracked by banding pattern.

The objective of the present study was to evaluate grain yields and to identify the local land races carrying resistant alleles for blast and bacterial leaf blight as shown by tightly linked markers for the two major rice disease resistance genes *Pi*-2 and *Xa21* in landraces of rice and evaluate flowering and maturity duration in land races (Shivapriya and Hitttalmani, 2006).

MATERIALS AND METHODS

A total of 162 genotypes comprising of landraces (Table 1) of rice were grown along with IR64, IR36, IR72, Azucena and Moroberekan as check varieties (Table 1).Phenotypic observations were recorded on days for 50% flowering, days to maturity, seed yield per plant (g) and screened for the presence of *Pi*-2 and *Xa21* disease resistance genes with linked markers (Table 3). Two tightly linked markers for blast (RG64) and bacterial leaf blight (*pTA*248) of rice which had chromosome 6 and chromosome 11 were used to detect the resistance gene in the local genotypes. These two markers are well established and are routinely used in the marker aided selection for blast and blight in rice. Hence phenotypic screening for the disease was not essential.

An average from five plants each was calculated. The range, which is minimum and maximum value for each traits observed is presented in Table 1a). Simple correlation was estimated as per the formula suggested by Aljibouri et al. (1985). Simple correlation coefficients among 50% flowering time, Days to maturity and seed yield per plant in local and traditional landraces are presented in Table 2.

DNA isolation and PCR amplification

Healthy leaves from 25 days old seedlings, grown in greenhouse were collected and DNA was extracted using the hexadecyl trimethyl ammonium bromide (CTAB) method (Cao and Oard, 1997). The DNA was quantified at 260 nm wavelength using a UV spectrophotometer. PCR-based markers, RG64 (SAP) reported by Hittalmani et al. (1995) and *pTA*248 (STS) reported by Ronald et al. (1992) were used to detect and confirm the presence of *Pi-2* and *Xa21* genes in the landraces (Table 3), respectively.

The PCR reaction mixture contained 40 ng of template DNA, 0.2 mM of each primer pair (Sigma Aldrich), 1 mM dNTP's, 10X PCR buffer (1X is 10 mM Tris HCl pH 8.8 at 25°C, 1.5 mM KCl and 0.1% triton X- 100), 1 unit of *taq* polymerase (Bangalore Genei) and deionized water to get a total reaction volume of 20 µl. The PCR profile for RG64 SAP marker using *Hae*III restriction enzyme was used (Hittalmani et al., 1994). An initial denaturation of genomic DNA at 94°C for 3 min followed by 30 cycles of PCR amplification under the following profile: denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, primer extension at 72°C for 2 min and a final extension for 5 min at 72°C was done. The PCR amplification profile for pTA248 STS marker was similar to that of RG64 except that the annealing temperature was 56°C instead of 60°C (Huang et al., 1997).

Restriction digestion of PCR products

The PCR amplified products of *pTA*248 showed polymorphism among the landraces but the products amplified by RG64 primers were monomorphic, hence restriction digestion of the PCR products was carried out by *Hae* III enzyme (Hittalmani et al., 1995). The success of PCR amplification by RG64 primer pair was first confirmed by running 5 µl of the amplified product on 1.4% agarose gel. Restriction enzyme digestion was then carried out directly in the PCR tube without separating the PCR product from the oil overlay. The enzyme mix of 5 µl [2.5 µl of 10x restriction enzyme buffer, 0.5 µl of restriction enzyme (10 u/µl) and 2 µl of sterile distilled water] was added to make up the final volume to 20 µl. After a brief spin and 4 h of incubation at 37°C, the digested products were run on a 1.4% agarose gel to resolve digested PCR fragments.

Table 1. List of rice landraces used for screening *Pi-2* (blast) and Xa21 (bacterial leaf blight) resistance genes.

S/N	Name of genotype	50% flowering (Days)	Days to maturity	Seed yield per plant (g)	S/N	Name of genotype	50% flowering (Days)	Days to maturity	Seed yield per plant (g)
1	Abhilash	140	177	2.3	41	Dodda Byranellu	114	151	8.88
2	Adinya	80	117	1.64	42	Doddi	119	155	6.72
3	Ajaya	100	134	3.73	43	Dodiga	132	169	4.53
4	Alugidda	135	171	3.06	44	Gouri Sanna	118	157	6.36
5	Alur Sana	136	176	5.25	45	Gowri	135	169	1.46
6	Ambemori	120	155	12.55	46	Gowri Sanna-1	124	164	7.8
7	Amruth	126	162	3.2	47	Gumsali	119	153	4.78
8	Amrutham	59	93	2.5	48	Hakkalsali	101	138	21.85
9	Andrewsali	131	164	10.79	49	Halaga	124	146	1.42
10	Annada	139	178	5.18	50	Halubblu	141	173	5.51
11	Antarsali	100	138	13.11	51	Halugidda Sele	142	182	2
12	Antharsali-1	104	137	4.76	52	Heera	83	119	4.38
13	Aruna	135	174	1.91	53	Hemavathi	122	170	1.15
14	Asha	132	160	7.04	54	Hilly Paddy	99	132	4.09
15	Azucena	118	154	17.78	55	Himdhan	117	151	11.75
16	Beeriga	104	139	33.3	56	Intan	130	171	3.65
17	Belgodsele	146	192	4.23	57	IR36	114	149	4.32
18	Bhadur	136	180	1.35	58	IR64	124	157	6.81
19	Bhagya Jyothi	123	159	6.96	59	IR72	119	149	5.18
20	Bharani	123	176	8.1	60	Jaddu Batta	127	169	20.88
21	Bidar Local-2	128	166	4.43	61	Jalamagna	150	182	4.14
22	Bidar Local-3	128	152	1.89	62	Jaya	114	180	3.09
23	Bili Dodiga	107	153	12.55	63	Jaya Madhuri	112	152	2.09
24	Biliakki	130	164	4.5	64	Jeerasali	113	148	8.16
25	Bilihasadi	141	173	3.67	65	Jeerige Sanna	116	148	10
26	Biliya	139	160	2.67	66	Johamini	141	176	8.14
27	Birsagohra	66	100	2	67	Jolaga	117	152	5.43
28	Bitiga	102	138	12.25	68	Jyothi	105	136	4.00
29	Boliya	144	178	3.68	69	Kaggali Kirwana	130	163	1.53
30	Budda	102	137	10.29	70	Kagu	117	154	3.33
31	Budda-1	102	135	12.92	71	Kalinga	136	186	1.09
32	Byranellu	139	150	11.75	72	Kanakam	145	187	4.64
33	Champakali	97	134	9	73	Kanakasali	143	183	0.24
34	Champakali-1	100	132	2.32	74	Kanihosadi	124	159	12.72
35	Chamundi	125	163	5.29	75	Kannanur Local	128	150	1.75
36	Chitaga	117	122	1.84	76	Karibatta	110	154	6.26
37	Chitiga-1	100	132	3.3	77	Karidoddi	102	133	1.78
38	Dambarsali	83	116	4.5	78	Karikaldiddya	101	133	8.72
39	Dodda Abhilash	141	182	1.94	79	Karimurdiga-'A'	99	135	6.09
40	Dodda Batta	141	182	3.82	80	Karna	144	186	2.78

RESULTS AND DISCUSSION

Character association analysis (Table 2) between flowering time, days to maturity, seed yield per plant revealed strong positive association of 50% flowering with days to maturity (0.92) and negative association with seed yield per plant (-0.27) and also negative association between days to maturity with seed yield per plant (-0.28). Distribution pattern of 162 local landraces are shown in Figures 5, 6 and 7. Frequency distribution was skewed for seed yield

Table 1. Contd.

S/N	Name of genotype	50% flowering (Days)	Days to maturity	Seed yield per plant (g)	S/N	Name of genotype	50% flowering (days)	Days to maturity	Seed yield per plant (g)
81	Karthik	135	174	4.73	122	Puri-Chipiga	119	151	15.21
82	Katchet	153	185	4.36	123	Purmuri	101	132	19.16
83	Kemphasadi	139	172	1.45	124	Purnendu	138	181	6.08
84	Kempumaras	118	152	13.49	125	Rajamudi	124	178	1.29
85	Kesari	123	160	2.19	126	Rajkamal	137	183	3.05
86	Khaima	118	151	2.93	127	Ramya	123	161	10.1
87	Khaima-1	108	142	6.86	128	Rare	108	171	6.75
88	Kirwana	131	165	4.98	129	Ratan Sagar	134	158	2.58
89	Madras Sanna	148	182	2.59	130	Rathnachudi	127	165	5.23
90	Madras-Sanna-1	118	153	11.25	131	Ratna Chuda	139	178	2.48
91	Mahsuri Prakash	128	165	2.53	132	Sabitha	129	161	4.09
92	Makam	128	158	4	133	Sagar Seln-2	105	135	4.06
93	Mallige	123	163	2.37	134	Salavahan	139	184	4.27
94	Mandya Vijaya	131	169	5.75	135	Sampige Batta	138	181	8.33
95	Manila	157	192	2.83	136	Sampigedala	117	149	8.17
96	Masale Putta Batta	157	187	3.91	137	Sampigedala	142	180	2.62
97	Mattalaga	139	170	10.19	138	Samsali	105	138	6.37
98	Mingola	129	183	0.69	139	Sannavallya	123	163	5.58
99	Morian-18	118	149	4.73	140	Scented Rice Purple	119	168	1.87
100	Moroberekan	119	154	28.42	141	Shakthi	125	160	10.07
101	Mullugeddu	115	143	10.72	142	Shankar Poonam	137	169	1.41
102	Mundoni	147	178	2.2	143	Siddasale	136	182	3.64
103	Murakata Bhatta	109	142	4.33	144	Sona Mahsuri	145	189	5.47
104	Nandantarasali	104	139	6.13	145	Suraj	99	135	5.16
105	Nati Batta	124	158	3.94	146	Surath Red	134	179	1.71
106	Navalsali	108	143	3.28	147	Thirthalli Sel	126	161	4.52
107	Neergoli-2	123	161	7.63	148	Udarsali	107	142	3.08
108	Neerugoliga	123	163	8.14	149	Uduru Mallige	130	164	5.75
109	Neeruguli-1	122	157	5.52	150	Udurusali	109	143	12.29
110	Nereguli	125	157	9	151	Uma	103	138	20.91
111	Onnmaridinellu	123	161	4.8	152	Utkal Prabha	125	158	5.91
112	Padma	136	175	7.67	153	Valle Farm Bhatta	130	158	4.55
113	Padma Rekha-1	142	182	4.92	154	Vallya-1	142	180	1.67
114	Padmarekha	138	179	1.26	155	Valya	107	153	5.25
115	Pankaj	137	172	3.85	156	Vanangala	102	137	18.5
116	Pavizam	125	162	4.59	157	Vani	134	169	2.47
117	P-Doddi	130	166	2.44	158	Wari Sanna	119	164	3.63
118	Ponni	131	169	3.89	159	Warner-1	102	137	5.38
119	Pooja	132	172	2.2	160	Warris	107	140	6.38
120	Pothana	130	169	2.97	161	White-ponni	131	169	1.73
121	Pranava	131	172	5.44	162	Yedkuni	118	148	7.64

per plant and normal distribution was observed for 50% flowering, and days to maturity.

RG64 SAP marker linked to *Pi*-2 was used to detect the presence of resistant marker allele for the gene of interest in rice landraces. The amplified products revealed monomorphic banding pattern of 1155 bp band. Further digestion of the amplified PCR products of genotypes with *Hae*III enzyme revealed polymorphic bands. The results are shown in Figure 3. Four different alleles of RG64 marker locus tightly linked to *Pi*-2 gene were observed as shown in Figure 1) among the genotypes. Of 162 local rice accessions screened with RG64 marker, 28 genotypes

Table 1a. Mean and range for the three traits 50% flowering, days to maturity, and seed yield per plant in local landraces.

Character	50% flowering (days)	Days to maturity	Seed yields / plant(g)
Mean	122.27	158.09	5.94
Range	59-157	93-192	0.24-33.3

Table 2. Correlation coefficients between 50% flowering, days to maturity, seed yield per plant in rice local landraces.

Character	50% flowering (days)	Days to maturity	Seed yields / plant (g)
50% flowering (days)	1	0.92	-0.27
Days to maturity	0.92	1	-0.28
Seed yields / plant (g)	-0.27	-0.28	1

Marked correlation are significant at p<0 .05000; N=162.

Figure 1. Banding patterns and respective scores of RG64-SAP marker Associated with *Pi2* blast resistance gene. Type 1, *Pi*2 resistant type allele; type 2, heterozygote alleles; type 3, CO39 type susceptible allele; type 4, IR64 type susceptible allele; type 5, null allele

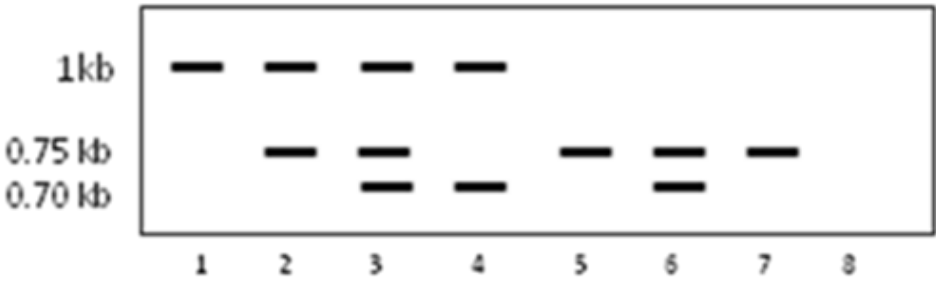

Figure 2. Banding patterns and respective scores of pTA 248 STS marker Associated with *Xa*21 gene. Type 1, resistant type allele; type 2, heterozygote allele; type 3, heterozygote allele; type 4, heterozygote allele; type 5, susceptible allele; type 6, heterozygote allele; type 7, susceptible allele; type 8, null allele

had *Pi*-2 resistant banding pattern (type 1 allele) (Figure 1), 44 showed the banding pattern of CO39 susceptible check (type 3 allele) (Figure 1), 27 genotypes showed type 2 (heterozygote) alleles and 32 genotypes showed type 4 (IR64 type) susceptible allele.

In the remaining genotypes, the marker locus failed to

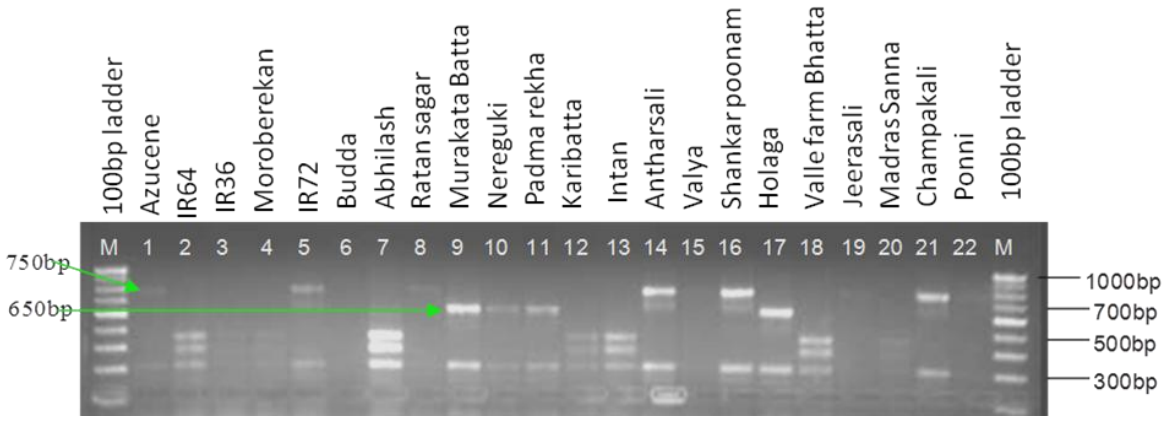

Figure 3. Local and landraces of rice genotyped with RG64-SAP marker linked to *Pi*-2 blast resistance gene on chromosome 6.

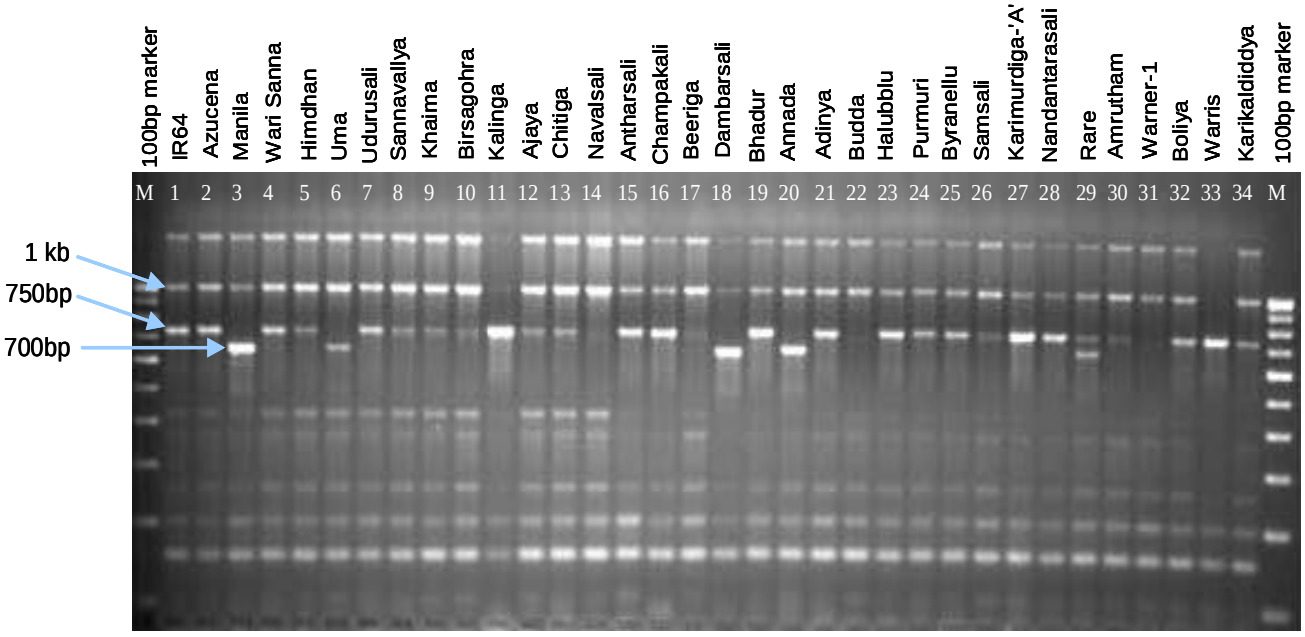

Figure 4. Local and landraces of rice genotyped with *pTA*248-STS marker linked to *Xa*21 bacterial blight resistance gene on chromosome 11.

Table 3. Details of RG64 and pTA248 primer sequence and their chromosomal location.

Gene	Trait/Chromosome/Distance	Marker	Sequence	Reference
Pi-2 (*Pi*-z[5])	*Blast/6/0.06cM*	RG64-F	5'GTTGTTTCAGCTCTCCAATGCCTGTTC 3'	Hittalmani et al.,
		RG64-R	5' CTGCAGTGCAATGTACGGCCAGG 3'	1995
*Xa*21	*Bacterial blight/11/0-1cM*	pTA-248 F	5' AGA CGC GGA AGG GTG GTT CCC GGA 3'	Ronald et al.,
		pTA-248 R	5' AGA CCG GTA ATC GAA AGA TGA AA 3'	1992

amplify or produce scorable amplicons. There was a wide range of variation in 50% flowering, maturity and grain yield among the genotypes. Since land races are sensitive to photo period and temperature condition, it was observed that there was a wide difference between flowering time to maturity which is typically observed by some local genotypes (Shivapriya and Hitttalmani, 2006). Singh et al., (2001) used molecular markers to introduce

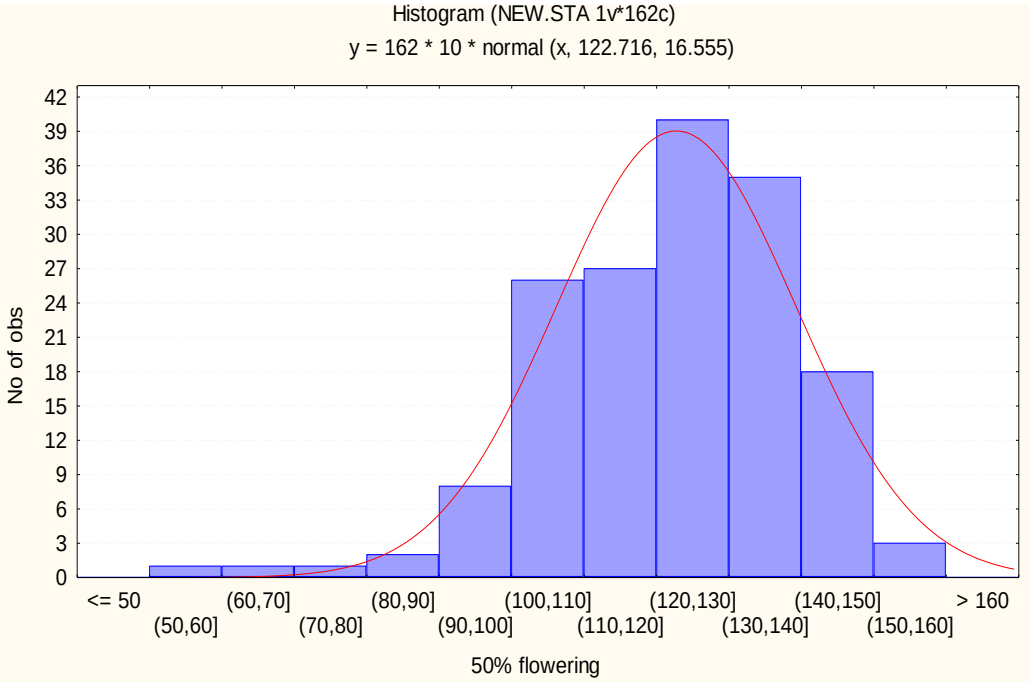

Figure 5. Frequency distribution for 50% flowering.

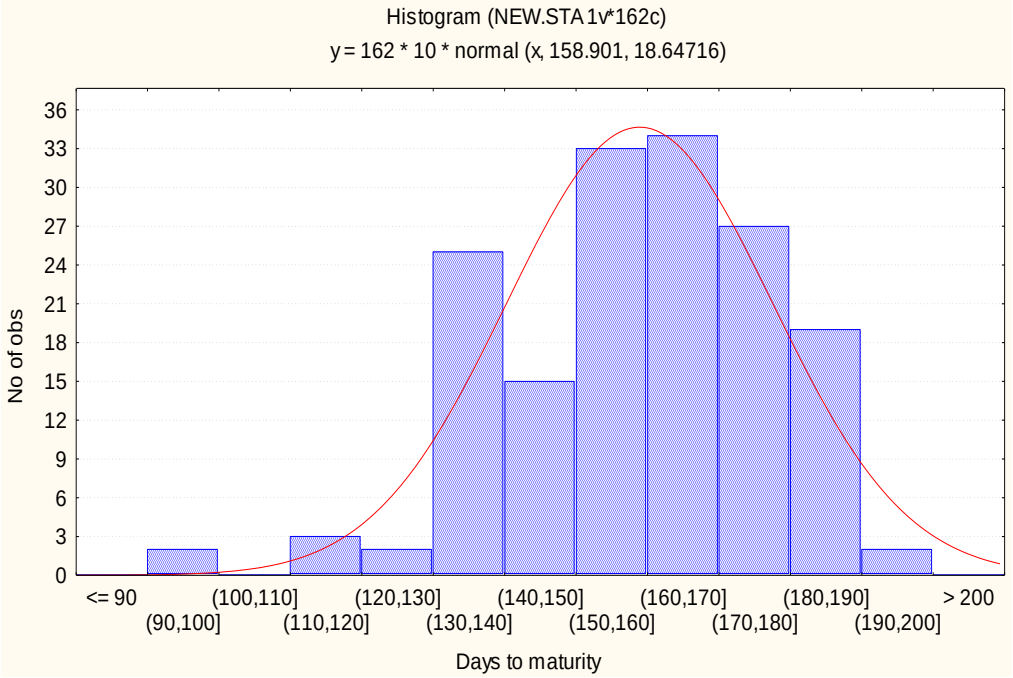

Figure 6. Frequency distribution for Days to maturity.

xa5, *xa13* and *Xa21* into PR106, a BB susceptible line widely grown in the Punjab. A three-gene combination appeared to be the most effective, with *Xa21* contributing the largest component of resistance. *pTA*248 linked to *Xa*21 (bacterial leaf blight) amplified three different sized bands of 1 kb carrying *Xa*21 resistant allele and bands of either 700 bp or 750 bp carrying susceptible alleles as shown in Figure 4. Different allelic combinations and their respective scores are as shown in Figures 1 and 2. Results of screening and type of alleles of each genotype are shown

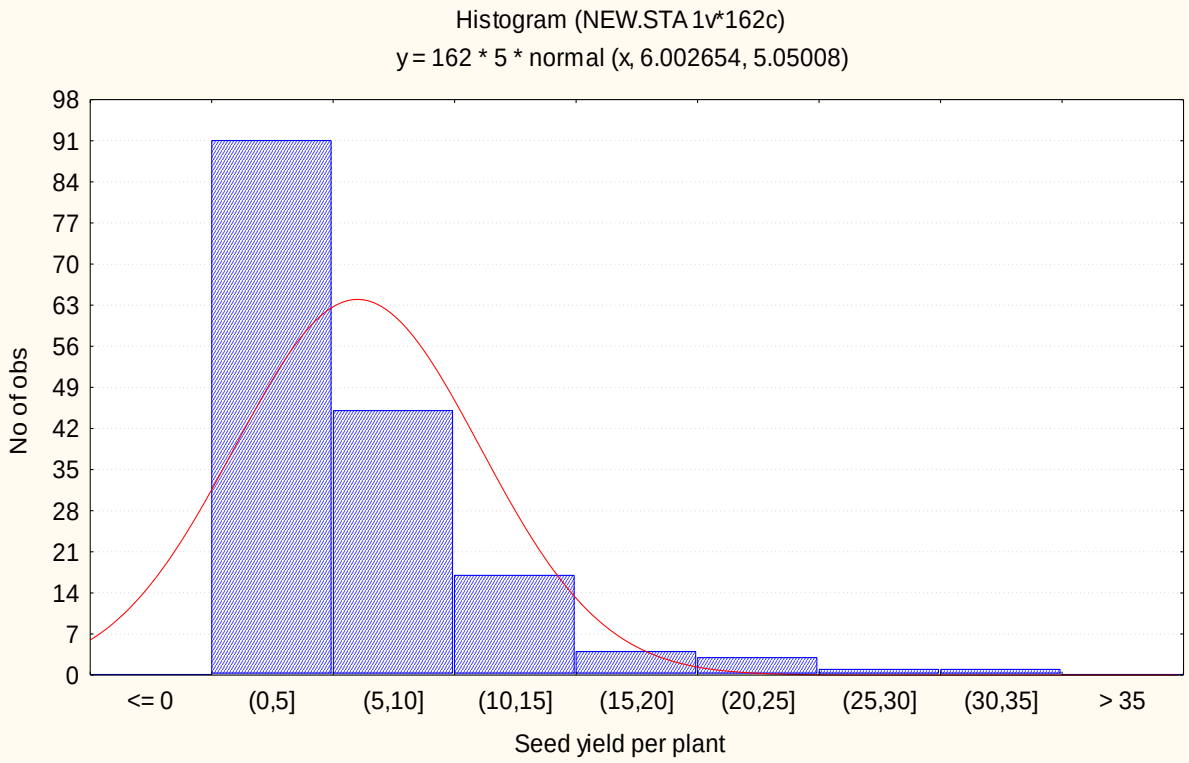

Figure 7. Frequency distribution for seed yield per plant.

Table 4. List of Resistant genotypes for Blast, and bacterial leaf Blight disease as identified by linked markers.

Disease	Genotype
Blast linked to *Pi-2* gene resistant genotypes	Azucena, Ratan sagar, Ponni, Himdhan, Champakali-1, Bhadur, Purmuri, Hilly Paddy, Gowri, Asha, Gowri Sanna-1, Ramya, Madras-Sanna-1, Karthik, White ponni, Vani, Kanakam, Makam, Morian-18, Sona Mahsuri, Thirthalli Sel, Yedkuni, Surath Red, Nati Batta, Kanihosadi, Uduru Mallige, Jaddu Batta, Mandya Vijaya, IR72, Antarsali, Shankar Poonam, Champakali, Pothana, Bidar Local 3, Sampigedala, Chitaga, Bidar Local-2, odda Byranellu, Gouri Sanna, Wari Sanna, Nandantarasali, Rare, Warner-1, Warris, Jyothi, Bitiga, Suraj, Bhagya Jyothi, Utkal Prabha, Kempumaras, Purnendu, Hemavathi, Kagu, Sampige Batta, Kirwana.
Bacterial leaf Blight linked to *Xa*21 gene resistant genotypes	Moroberekan, Ponni, Hakkalsali, Navalsali, Beeriga, Budda-1, Warner-1, Gowri, Gowri Sanna-1, Rajamudi, Kanakam, Shakthi, Sona Mahsuri, DoddaBatta, IR64, Azucena, IR36, IR72, Budda, Abhilash, Ratan Sagar, Murakata Bhatta, Nereguli, Padmarekha, Karibatta, Antarsali, Valya, Shankar Poonam, Halaga, Valle Farm Bhatta, Jeerasali, Sampigedala, Sagar Seln-2, Pothana, Udarsali, Champakali, Chitaga, Khaima, Kannanur Local, Dodda Byranellu, Gouri Sanna, Khaima-1, Himdhan, Udurusali, Sannavallya, Wari Sanna, Birsagohra, Ajaya, Chitiga -1, Antharsali-1, Champakali-1, Bhadur, Adinya, Halubblu, Purmuri, Byranellu, Samsali, Karimurdiga-'A', Nandantarasali, Amrutham, Boliya, Karikaldiddya, Karidoddi, Bitiga, Bili Dodiga, Suraj, Hilly Paddy, Vanangala, Dodda Abhilash, Bhagya Jyothi, Amruth, Bharani, Utkal Prabha, Ramya, Madras-Sanna-1, Mullugeddu, Kesari, Karna, Morian-18, Jolaga, Onnmaridinellu, Pranava
Blast and Bacterial leaf Blight resistant genotypes	Azucena, Ratan sagar, Ponni, Himdhan, Champakali-1, Bhadur, Purmuri, Hilly Paddy, Gowri, , Gowri Sanna-1, Ramya, Madras-Sanna-1, Kanakam, Morian-18, IR72, Antarsali, Shankar Poonam, Champakali, Pothana, Sampigedal, Chitaga, Dodda Byranellu, Gouri Sanna, Wari Sanna, Nandantarasali, Warner-1, Jyothi, Bitiga, Bhagya Jyothi, Utkal Prabha,

in Table 4. Out of 162 landraces screened with *pTA*248 marker for the presence of *Xa*21 gene, 14 genotypes showed resistant banding pattern (Type 1 allele) (Figure 2), 46 genotypes showed susceptible banding pattern (either type 5 or type 7 allele) (Figure 2), 16 genotypes did not amplify any bands whereas 87 genotypes were in heterozygous condition (scored as either 2, 3, 4 or 6) (Figure 2). Heterozygosity was also reported by Davierwala et al. (2001). The absence of band(s)/marker allele(s) in both cases (RG64 and *pTA*248) could be due to nucleotide-

sequence variation/alteration resulting from insertion, deletion or substitution in one or both the primer binding sites which results in non-amplification (Huang et al., 1997; Davierwala et al., 2000; Shivapriya, 2000; Ni et al., 2002; Saito et al., 2004). Ramalingam et al., (2001) performed similar type of molecular survey for the presence of bacterial blight resistance genes *xa5, xa13* and *Xa21* in Chinese rice germplasm. They surveyed 56 germplasm, 23 were reported to carry allele 3, 30 had allele 2 and no allele was reported in one genotype. Naveed et al., (2010) generated molecular information regarding presence and absence of resistant gene for *xa*5 in their germplasm, which can facilitate rice breeder to incorporate the resistant genes from the known source to their cultivated basmati varieties through marker assisted selection. Four genotypes *viz*., Ponni, Gowri Sanna, Sona Mahsuri, Gowri and Kanakam were identified to carry resistant alleles at both *Pi*-2 and *Xa*21 loci for blast and bacterial blight resistance respectively in homozygous condition (Table 4). In the present study, we identified *Pi*2 using tightly linked marker RG64 and *Xa*21 using linked marker *pTA*248 in local landraces of rice and these two markers can be used to screen diversegenotypes. The study indicates that, the land races and local rices are a good source of resistant alleles for both diseases and also useful for developing varieties with varying duration and be used in breeding programs.

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