Plant Archives Vol. 24, No. 1, 2024 pp. 536-542 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.1.072

SSR MARKER BASED GENETIC EVALUATION FOR LEAF BLAST RESISTANCE GENES IN KALANAMAK RICE

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(Date of Receiving-01-12-2023; Date of Acceptance-06-02-2024)

ABSTRACT

In this investigation, we carried out phenotyping for Blast resistance in Rice for two consecutive seasons of *Kharif* (2016 and 2017) at Crop Research Centre, Govind Ballabh Pant University of Agriculture & Technology (G.B.P.U.A.T.), Pantnagar, Uttarakhand, India. The phenotyping was followed by genotyping at Plant Molecular Biology laboratory, G.B.P.U.A.T., Pantnagar. Based on genotyping KARL 1was found to harbor ten resistant genes, KARL 3, KARL4, KARL 8 and KARL 9 had nine resistant genes ,KARL 6, KARL7 and KARL 10 had eight resistant genes, KARL 11 and KARL 5 had six resistant genes, KARL 2 had five resistant genes and PSD 17 had four resistant genes from super cluster and dendrogram analysis KARL 1, 2, 3, 4, 5, 6, 7 and 8 showed 59% similarity with KARL 9, 10 and 11, as well as with PSD 17. The highest Polymorphic information content (PIC) value (0.97) was observed for marker RM 21 having 140-600 bp amplicon and lowest PIC value (0.77) was observed for marker RM 168 having 100-110 bp amplicon. The genotypes showing high correspondence between genotypic and phenotypic data can be used in crossing programme aimed at improving the resistance in otherwise superior varieties and simultaneously, validity for true hybridity and selection of resistant genotypes in segregating population can be done using the associated markers in context.

Key words : Kalanamak rice, Leaf blast, Genotyping, Dendrogram, Polymorphic information content.

Introduction

Rice (*Oryza sativa* L.) is the staple food for over 50% of the world's population. Globally, it is cultivated over an estimated area of 160.1 million hectares producing about 483.8 million tons of grain (USDA, 2017), bestowing 21% of dietary energy and 15% of global protein requirement. Owing to its extensive cultivation, its genetic yield potential is severely affected by rice blast. The rice blast disease, caused by fungus, *Magnaporthe oryzae*, is considered as the most threatening disease of rice because of its worldwide distribution and extensive crop devastation under favorable conditions (Talboot and Foster, 2001). Various cultivars, which are released as a resistant cultivar become susceptible to new races of pathogen in a few years of cultivation because of high genetic instability (Huang *et al*., 2014) of blast fungus. The major problem in resistance breeding for Blast resistance is ephemeral resistance because of fast evolving nature of this fungus, which aids to overcome the major gene resistance easily. Thus, there is an urgent need to explore for newer resistance source and subsequently, their phenotypic evaluation followed by genotypic evaluation to validate the resistance source. Thus, to improve the duration of varietal resistance we need to pyramid different resistance gene in a single genotype (Pinta *et al*., 2013). This approach demands for identification of the new resistance source (Barman

et al., 2004), which needs to be validated over location through phenotyping supported by genotyping in a different environment especially at hot spots. Validation of genes at different locations also help to select for the different gene combination for different locations involving gene deployment technique. Such combining of genes having overlapping resistance from diverse sources is an effective approach to increase the durability of resistance (Ahn *et al*., 1982). Therefore, to gain durable resistance through desired gene combination after their validation requires characterization of reported resistance gene (Shikari *et al*., 2013). To confer durable resistance to *M. oryzae*, partial and field resistance strategies are recognized as a better alternative to combat the pathogen under natural field condition (Hittalmani *et al*., 2000 and Liu *et al*., 2005). Molecular markers have high potential for improving the efficiency of conventional breeding by carrying out selection both directly on blast resistant phenotype and indirectly based on linked molecular markers to blast resistant gene. The conventional and molecular approaches together have sped up the resistance breeding programme. Keeping this in view, we carried out the present study with objectives of SSR marker based evaluation of 11 advanced recombinant lines of Kalanamak rice and Pant Sugandh Dhan 17 regarding major genes for blast resistance.

Materials and Methods

Experimental material

Eleven Kalanamak Advanced Recombinant Lines (KARL) of rice along with the check variety Pant Sugandh Dhan 17 were evaluated for leaf blast resistance in this study. These lines were procured from DRR, Hyderabad and the Pant Sugandh Dhan 17 was procured from G.B.P.U.A.T., Pantnagar, India.

Phenotyping for Blast Resistance

The phenotypic evaluation was done by visual scoring (7 and 14 days after infection) of the entire row and comparing the infection level with standard SES scale for leaf blast resistance. For visual scoring, 10 plants per genotype were selected and their infection level for leaf blast was scored based on SES scale (Chen *et al*., 1996).

Genotyping for Blast Resistance

The genotypes were scored with SSR markers, synthesized by Genei Pvt. Ltd., Bangalore which is listed in Table 1. Genotyping involves isolating genomic DNA from the plant leaves of fifteen-day-old seedlings using CTAB method (Doyle and Doyle, 1990). The isolated DNA was quantified using UV spectrophotometer and purified by RNase treatment. DNA quality was checked by electrophoresis in 0.8% agarose gel. The PCR amplification was carried out and the banding pattern of SSR markers was analysed to establish a relationship between phenotypic and genotypic observation. Similarity analysis was done with the NTSYS-pc ver. 2.02 software (Rohlf, 1998) and dendrogram was produced according to the Unweighted Pair-Group Mean Arithmetic method (UPGMA) using NTSYS-pc software.

Statistical analysis

This involves scoring of band, comparative study of phenotypic and genotypic data for blast resistance and analysis of genotypic data for degree of genetic association among the genotypes. For scoring of bands, only intense bands were scored based on presence (designated as '1') or absence (designated as '0') of the amplified product for a particular DNA fragment, bands that were faint or having a smeared background were avoided. Only the specific amplified PCR products that showed consistency in the successive amplifications were selected to minimize the possibility of false scoring of markers. Band scoring is followed by comparative study of both phenotypic data and genotypic data for blast resistance, done by tabulating them and deriving relationship between them for presence or absence of character. This was compared with the result from phenotypic data for host response. If positive association occurs between the two data, then it can be documented as presence of blast resistance gene in that genotype. The extent of genetic relationship among the included material in the present study was revealed by Jaccard's coefficient of similarity. Cluster analysis was used to represent the degree of genetic association among the genotypes using the algorithm of UPGMA, by feeding similarity matrix as input data. Dendrogram is used for graphical representation of genetic relationship among the genotypes.

Results and Discussion

Genotyping

KARL 1, KARL 3, KARL 4 and KARL 8 gave positive bands with respect to all the primers used in the study. KARL 2 gave positive bands for only 4 primers namely, 3, 5, 6 and 7. KARL 5 gave positive bands with 6 primers *i.e.*, 1, 2, 4, 6, 7 and 8. KARL 6, KARL 9 and KARL 10 gave positive bands with all the primers except primer no.10 and primer no. 7, respectively. KARL 7 gave positive bands with all the primers except primer 5 and 10. KARL 11 gave positive bands with primer no. 1, 2, 4, 5, 6, 8 and 10. PSD 17 gave positive bands with primer no. 2,3, 4, 6, 8, 9 and 10. In the investigation of different genotypes showed a specific banding pattern

Table 1 : General information on Primer used for genotyping.

S. no.	Primer Name	Primer linked with Blast Resistant gene	Expected amplicon size (bp)	References	Size range of allele observed (bp)	Total no. of alleles observed	Forward Primer	Reverse Primer	PIC Value
1	RM21	Pi 38	157	Gowda *et al.* (2005)	140-600	3	ACAGTATTCCG TAGGCACGG	GCTCCATGAGG GTGGTAGAG	0.97
2	RM44	Pi 33	140	Berruyer *et al.* (2003)	100-600	4	ACGGGCAATCCG AACAACC	TCGGGAAAAC CTACCCTACC	0.91
3	RM168	Pi66(t)	116	Liang *et al.* (2015)	100-110	1	TGCTGCTTGCCTG CTTCCTTT	GAAACGAATC AATCCACGGC	0.77
4	RM224	Pi-1	157	Fuentes *et al.* (2007)	180-200	1	ATCGATCGATCTT CACGAGG	TGCTATAA AAGGCATTCGGG	0.79
5	RM226	Piz-t	157	Yan *et al.* (2017)	170-190	1	AGCTAAGGTCTG GGAGAAACC	AAGTAGGATGG GGCACAAGCTC	0.93
6	RM527	Piz-5	233	Fjellostorm *et al.* (2006)	100-250	1	GGCTCGATCTAG AAAATCCG	TTGCACAGGT TGCGATAGAG	0.95
7	RM1233	Pik –S	175	Fjellostorm *et al.* (2004)	130-220	1	GTGTAAATCAT GGGCACGTG	AGATTGGCTCC TGAAGAAGG	0.91
8	RM6836	Pi-z	178	Fjellostorm *et al.* (2004)	120-250	2	GTTCTCGAGCT CCACGTAGG	CTCCACCTCCA TCTCCGTC	0.82
9	RM8226	Pi-z	233	Fjellostorm *et al.* (2004)	210-260	1	TTAGGATACGGC TTCTAGGC	CGTAATTGTTG CATATGGTG	0.88
10	RM413	Pi 26	118	Masuduzzaman *et al.* (2016)	110-130	1	GGCGATTCTT GGATGAAGAG	TCCCCACCA ATCTTGTCTTC	0.89

with different primers that shows presence of multiple gene conferring blast resistance.

Correlation between genotyping and phenotyping

The genotypic and phenotypic data for blast resistance is shown in Table 2. KARL 3 to KARL7 and KARL 9 to KARL 11 produced a similar disease reaction, and all were resistant. These lines showed amplification for at least 6 primers linked to different leaf blast resistance that confers resistance. KARL 1 and KARL 2 were Phenotypically moderately resistant. KARL 1 had amplification for all the 10 primers and still it was not resistant that is indicative of some markers being dissociated from the genes for which they are reported to be linked. In KARL 2, only five markers showed amplification. This shows that out of ten markers showing amplification in KARL 1, the markers absent in KARL2 are even farther from the linked genes and hence showed anomaly between the number of markers amplified and the disease reaction. KARL 8 and PSD 17 were Phenotypically susceptible showing amplification for nine and four markers. The five markers in excess (RM21, RM 226, RM1233, RM8226, RM413) in the former may have been dissociated with the reported gene due to recombination.

Marker based genetic association among the genotypes

The extent of genetic relationship among the studied material in the present study was determined through dendrogram using Jaccard's coefficient of similarity. Establishing accurate genetic similarity and dissimilarity between individuals is

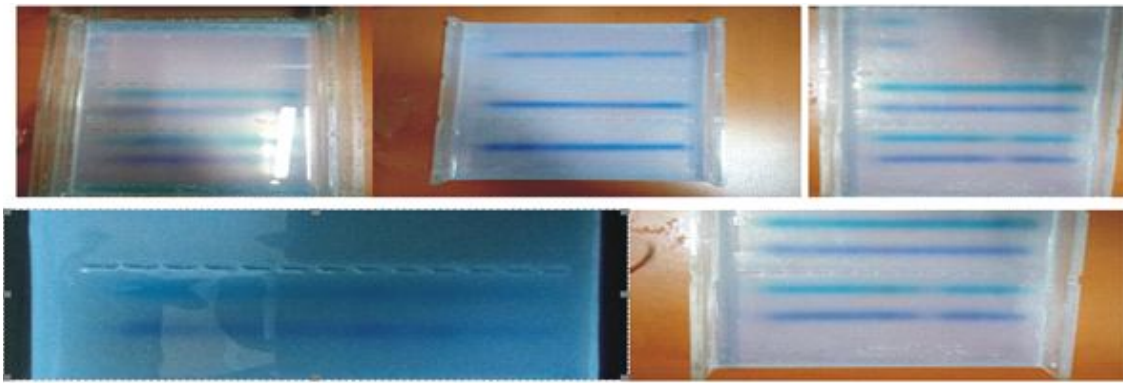

Fig. 1 : Images of raw gel showing banding patterns of different markers.

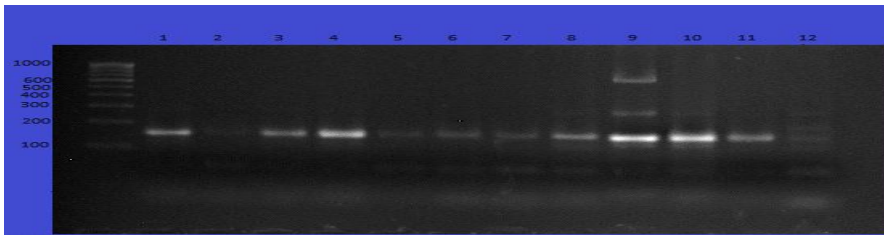

Fig. 2 : Marker banding pattern of KARL for SSR marker RM 21 linked with gene Pi 38.

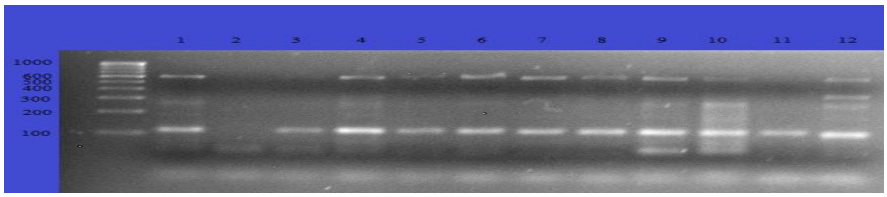

Fig. 3 : Marker banding pattern of KARL for SSR marker RM44 linked with gene Pi 33.

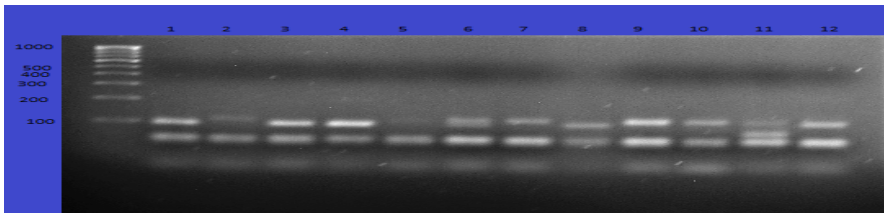

Fig. 4 : Marker banding pattern of KARL for SSR marker RM 168 linked with gene Pi 66(t).

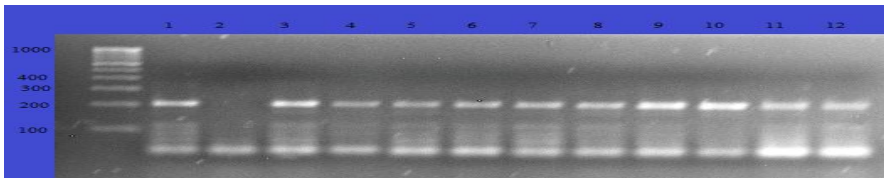

Fig. 5 : Marker banding pattern of KARL for SSR marker RM 224 linked with gene Pi 1.

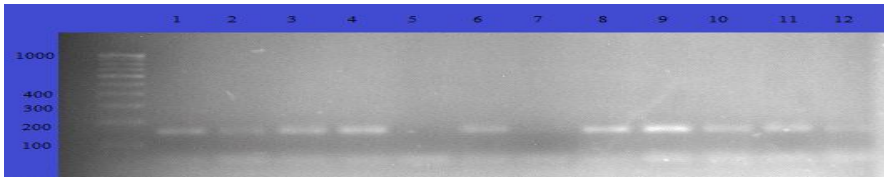

Fig. 6 : Marker banding pattern of KARL for SSR marker RM 226 linked with gene Piz-t.

an essential and decisive point for clustering and analyzing inter- and intra-population diversity (Dalirsefat *et al.*, 2009). Genetic similarity (or dissimilarity) matrix constructed from all potential pair-wise combinations of individuals based on binary combination (1 and 0) for presence and absence of band respectively, was used to characterize population structure based on relative affinities of each individual to all other individuals tested. Cluster analysis was used to represent genetic association among the genotypes. The dendrogram of genotypes for blast resistance gene is shown in Fig. 12. All the 12 genotypes were grouped in to 2 super cluster and 4 clusters. From super cluster and dendrogram analysis, KARL 1 to KARL 8 showed 59% similarity with KARL 9 to KARL11 and with PSD 17. In the cluster1 (KARL 1, 3 and 4) showed 66% similarity with cluster 2 (KARL 2, 5, 6, 7 and 8). In the cluster 1, KARL 1 showed 68% similarity with KARL 3 and 4. KARL 3 and 4 shared 85% similarity between themselves. In the cluster 2, KARL 2 showed 71% similarity with KARL 5, 6, 7 and 8. KARL 8 showed 75% similarity with KARL 5, 6 and 7. KARL 7 showed 78% similarity with KARL 5 and 6 while KARL 5 and 6 are almost similar in genetic constitution for blast resistance. In the cluster 3, KARL 9, 10 and 11 showed 68% similarity with cluster 4 (PSD 17). KARL 9 and 10 showed 77% similarity

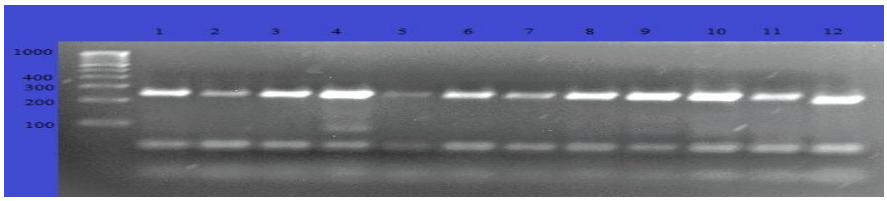

Fig. 7 : Marker banding pattern of KARL for SSR marker RM 527 linked with gene Piz-5.

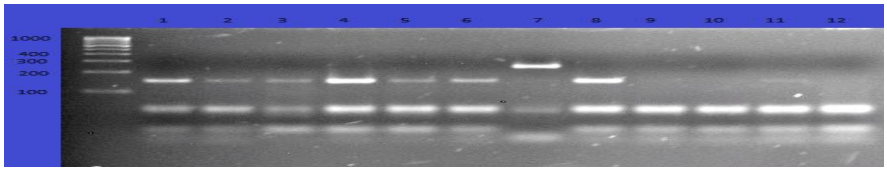

Fig. 8 : Marker banding pattern of KARL for SSR marker RM 1233 linked with gene Pi k-s.

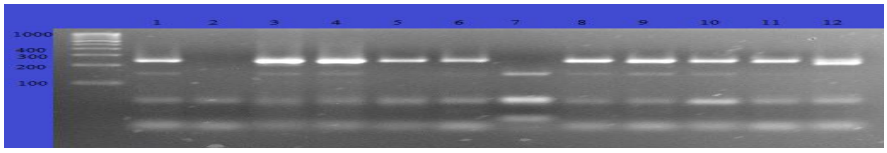

Fig. 9 : Marker banding pattern of KARL for SSR marker RM 6836 linked with gene Pi-z.

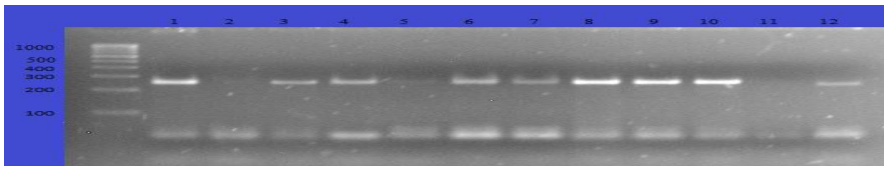

Fig. 10 : Marker banding pattern of KARL for SSR marker RM 8226 linked with gene Pi-z.

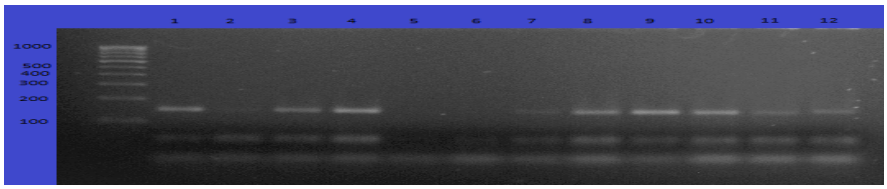

Fig. 11 : Marker banding pattern of KARL for SSR marker RM 413 linked with gene Pi 26.

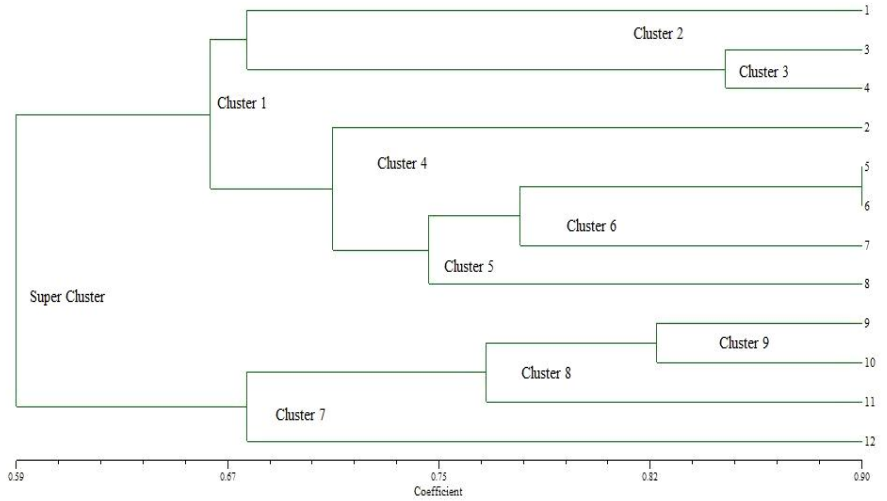

Fig. 12 : Dendrogram of studied genotypes with respect to gene for leaf blast resistance.

with PSD 17. In the cluster 3, KARL 9 and 10 showed 83% similarity. From above information, it is evident that the various genotypes included in the study were moderately diverse source for blast resistance gene hence, could be effectively utilized in durable resistance breeding programme against the continuous evolving blast pathogen. Thus, they prove to be a good genetic resource to cope with the frequent breakdown of field resistance against leaf blast.

Polumorphic information content (PIC) of SSR markers

Some basic information on primers used in this investigation regarding specific blast resistance gene, position of these genes on respective chromosomes, expected amplicon size, level of polymorphism and PIC value of primers are presented in Table 1. Study of polymorphism in plants, which are rich in genetic variability, can play an efficient role in building genetic bank and further breeding. The PIC value will be almost zero if there is no allelic variation and it can reach a max of 1.0 if a genotype has only new allele, which is a rare phenomenon. In the present study ten blast gene-specific primers are used to screen experimental population to establish if his is as a genetically diverse source for blast resistance. PIC value is a good measure to access the genetic diversity of a gene linked with specific primers in a genetic stock. Higher PIC value suggests for multiple allelism and the allele have an equal frequency in the population. The highest PIC value (0.97) was observed for RM 21 with three alleles and 140-600 bp amplicon. All the three allele were polymorphic, which indicate for 100% polymorphism. Lowest PIC value (0.77) was observed for RM 168 with one allele which was 100% polymorphic and have 100-110 bp amplicon. A PIC value more than 0.5 is considered to be highly polymorphic

Table 2 : Comparative study of molecular characterization and field screening for blast resistance.

S. no.	Genotypes	Pi 38 RM21	Pi 33 RM44	Pi66(t) RM168	Pi 1 RM224	Piz-t RM226	Piz-5 RM527	Pik –S RM1233	Pi-z RM6836	Pi-z RM8226	Pi 26 RM413	No. of genes	Resistance reaction in the field at hotspot				
													HR	R	MR	MS	S
1	KARL1	+	+	+	+	+	+	+	+	+	+	10			+		
2	KARL2	+	-	+	-	+	+	+	-	-	-	5			+		
3	KARL3	+	+	-	+	+	+	+	+	+	+	9		+			
4	KARL4	+	+	-	+	+	+	+	+	+	+	9		+			
5	KARL5	+	+	-	+	-	+	+	+	-	-	6		+			
6	KARL6	+	+	+	+	-	+	+	+	+	-	8		+			
7	KARL7	+	+	+	+	-	+	+	+	+	-	8		+			
8	KARL8	+	+	-	+	+	+	+	+	+	+	9					+
9	KARL9	+	+	+	+	+	+	-	+	+	+	9		+			
10	KARL10	+	+	-	+	+	+	-	+	+	+	8		+			
11	KARL11	+	+	-	+	+	+	-	+	-	-	6		+			
12	PSD 17	-	+	-	+	-	+	-	+	-	-	4					+

and informative. In the present study the average PIC value was 0.88 which suggest that the primer studied in this investigation were highly polymorphic and informative, which aids significantly in screening and incorporation of blast resistance gene in any breeding programmes to improve the cultivar for last resistance.

Conclusion

Based on genotyping KARL 1 was found to harbor ten resistant genes, KARL 3, KARL4, KARL 8 and KARL 9 had nine resistant genes, KARL 6, KARL7 and KARL 10 had eight resistant genes, KARL 11 and KARL 5 had six resistant genes, KARL 2 had five resistant genes and PSD 17 had four resistant genes. From super cluster and dendrogram analysis KARL 1, 2, 3, 4, 5, 6, 7 and 8 showed 59% similarity with KARL 9, 10 and 11, as well as with PSD 17. The highest Polymorphic information content (PIC) value (0.97) was observed for marker RM 21 having 140-600 bp amplicon and lowest PIC value (0.77) was observed for marker RM 168 having 100-110 bp amplicon. The genotypes showing high correspondence between genotypic and phenotypic data can be used in crossing programme aimed at improving the resistance.

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