The Pharma Innovation Journal 2021; 10(6): 1224-1230

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(6): 1224-1230

www.thepharmajournal.com
Received: 14-04-2021
Accepted: 27-05-2021

Prakriti Meshram
Ph.D., Research Scholar, Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Sandeep Bhandarkar
Senior Scientist, Department of Genetics and Plant Breeding, IGKV, College of Agriculture and Research Station, Mahasamund, Chhattisgarh, India

Shubha Banerjee
Scientist, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Ritu R Saxena
Professor, Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Rameshwar Prasad Sah
Scientist, National Rice Research Institute, Cuttack, Odisha, India

Mangla Parikh
Assistance Professor, Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

DK Rana
Professor and Head, Department of Agricultural Entomology, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

SK Nair
Senior Scientist, Department of Genetics and Plant Breeding, IGKV, College of Agriculture and Research Station, Mahasamund, Chhattisgarh, India

Corresponding Author:
Prakriti Meshram
Ph.D., Research Scholar, Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Parental polymorphic survey for BPH resistant genes in rice (*Oryza sativa* L.)

Prakriti Meshram, Sandeep Bhandarkar, Shubha Banerjee, Ritu R Saxena, Rameshwar Prasad Sah, Mangla Parikh, DK Rana and SK Nair

Abstract
29 polymorphic simple sequence repeat (SSR) markers were analyzed in 34 F_3 progenies derived from the cross of CG Zn Rice I and IR64 rice cultivars to investigate the association with BPH resistance. Parental polymorphism survey was taken up between donor parent IR64 and recurrent parent CG Zn Rice I. The parent DNA was isolated to good purity using the chemical method of purification. The PCR reactions were carried out according to standard protocol for rice microsatellites. A total of 68 SSR markers spanning all the 12 chromosomes of rice genome were analyzed on the susceptible variety CG Zn Rice I and resistant variety IR64 for parental polymorphism. Out of 68 SSR markers, 29 were polymorphic showing overall 42.64% polymorphism. The maximum polymorphism of 100% was observed for chromosome 9 and minimum of 16.66% for chromosome 5. Markers RM5, RM237, RM6, RM489, OSR13, RM55, RM124, RM413, RM510, RM454, RM11, RM125, RM152, RM215, RM25, RM316, RM484, RM552, RM271, RM171, RM224, RM287, RM277, RM5479, RM313, RM1986, RM3331, RM28004 and RM7102 showed polymorphism.

Keywords: Rice, SSR, parental polymorphism, molecular markers, BPH

Introduction
The brown planthopper (BPH), *Nilaparvata lugens* Stål (Homoptera: Delphacidae), is a destructive and widespread insect pest throughout the rice areas in Asia. The BPH feeds mainly on the stems, and sucks assimilates from the phloem of rice plants. Feeding by a large number of BPH may result in drying of the leaves and wilting of the tillers, a condition called hopperburn. Developing resistant cultivars is generally considered the most effective and economical means for BPH control. Molecular markers have been utilized extensively for the preparation of saturated molecular maps. Their association with genes/QTLs controlling the traits of economic importance has also been utilized in some cases for indirect marker-assisted selection. A vast collection of SSRs has been developed in rice, which are very useful resource for mapping agronomically important genes. Polymorphism occurs whenever one genome is missing in one of the SSRs or has a deletion or insertion that modifies the distance between the repeats. Availability of polymorphic markers is a prerequisite for mapping of genomic regions influencing a trait.

Polymorphism in biology is a discontinuous genetic variation which results in several different forms or types of individuals occurring among the members of a single species. These variants /variations are controlled by multiple discrete alleles. A discontinuous genetic variation separates the individuals of a population into two or more distinctly distinct forms, while the individuals do not fall into rigid groups in continuous variation, but are graded between broad extremes. The best examples for polymorphism are the separation of higher organisms into male and female sexes and different blood groups and Rh factor among humans. Smooth graduation of height and skin colour among human beings is an example for continuous variation. Some polymorphisms do not have visible manifestations and require biochemical/molecular techniques to identify differences between the different forms of chromosomes, proteins or DNA.

Essentially, DNA markers reveal the genetic diversity within individuals which can be visualized by gel electrophoresis followed by staining with EtBr or detection with radioactive or colorimetric probes. They are especially useful when variations are discovered between individuals of the same or different species. These markers are called polymorphic markers, while markers are called monomorphic markers which do not differentiate between genotypes. Co-dominant or dominant can also be identified as polymorphic markers.

The basis of this explanation is whether such markers can differentiate between homozygous and heterozygous individuals. Co-dominant markers display variations in amplicon size while dominant markers can be classified either as present or as absent.

Material and Methods

Plant material and Insects

The experimental materials comprised of 32 F_3 population including 2 checks varieties IR64 and CG Zn Rice I. The experiment was laid out in randomized complete block design with three replications. The aims of defining and tracking functional resistance genes in Chhattisgarh, recognizing the inheritance mode of resistance to brown planthopper in population F_2 and F_3. Morphological characterization of 32 F_3 lines (16 resistant and 16 susceptible) of rice and two parents as checks. In the insect rearing, during the process of slowly moving the potted plants over the boxes, the dropped nymphs were visually estimated to drop approximately 8-10 nymphs onto each seedling. Thereafter, the boxes were returned to the cages individually. Observations were reported 7-10 days after insect release, when 90 percent of the plants were killed in the susceptible TN1 check-line. The entries were scored for damage based on the criteria used to score the damage of individual plants. When the TN1 seedlings in a box had become completely wilted due to planthopper feeding, the tests were terminated and the damage to all seedlings in a box was scored according to Horgan *et al.* 2015 [1] (Table 1), where higher scores indicated greater susceptibility to BPH.

Table 1: Evaluation standard for rice resistance to brown planthoppers based on seedling mortality (Adapted from Horgan *et al*. 2015) [1]

Score	Rice damage	Resistance level
0	No damage	Immune
1	Slight damage to a few plants within a row	Highly resistant
3	First and second leaves of each plant partially yellowing	Resistant
5	Pronounced yellowing or stunting of plants, or 10-25% of plants wilted within a row	Moderately resistant
7	More than 50% of plants wilted or dead and the remaining plants severely stunted or dying	Moderately susceptible
9	All plants wilted or dead	Susceptible

Evaluation of BPH resistance

IR64 is a medium duration, high yielding resistant to BPH which was used as male parent and CG Zn Rice I, Muskan and IET22290 susceptible for BPH were used as female parents in this study. CG Zn Rice I x IR64, Muskan x IR64 and IET22290 x IR64 were crossed and F_1 plants of cross was selfed to produce F_2 population and F_3 population was produced by selfing. A total of 2105 lines of F_2 population and 2105 lines of F_3 population were used for screening against brown planthopper.

Phenotypic data of scoring for BPH resistance was generated for 2105 F_2 lines including F_2 population from cross I (CG Zn Rice I x IR64) had 753 lines, cross II (Muskan x IR64) had 563 lines and cross III (IET22290 x IR64) had 789 lines of F_2 generation used for BPH scoring. In F_3 generation similar number of lines were used for BPH scoring. So total lines for screening in F_3 generation was 2105.

The parents IR64 showed highly resistance (BPH score of 1.33) to BPH whereas CG Zn Rice I showed complete susceptibility (BPH score of 9). Female parent Muskan showed resistance (BPH score of 6.7) to BPH and female IET22290 showed resistance (BPH score of 7.0) to BPH.

DNA extraction and PCR analysis

Molecular work was carried out in Plant Molecular Biology Laboratory (RRL), Department of Genetics and Plant Breeding, IGKV, Raipur (C.G.). Extracted total genomic DNA was done by CTAB method (Zheng *et al*., 1995) [6]. Genomic DNA was isolated using modified CTAB method to extract Rice DNA. Nucleic acid has maximum absorption of ultraviolet radiation, i.e., roughly 260 nm. The ratio between the readings at 260 nm and 280 nm (OD 260/OD 280) provides as estimate for the purity of nucleic acid. Pure DNA and RNA preparation have an average ratio of 1.8 and 2.0, respectively. When protein or phenol contamination occurs the ratio would be slightly lower than this amount (<1.8). A ratio greater than 2.0 reveals a high proportion of RNA in the sample DNA. A set of 68 microsatellite (SSR) markers (Table 2) was used to amplify PCR products using DNA samples of 34 lines in order to identify polymorphic markers for assessing the genetic diversity. 5% polyacrylamide gel (vertical) was used for better visualization and separation of PCR products, since PAGE have better resolution. Before creating the gel solution, glass plaques were prepared. Gels were put into electrophoresis unit CBS-SCIENTIFIC.

Table 2: List of microsatellite (SSR) markers used for molecular studies

S. No.	Primer	Chromosome No.	Sequence	Remarks
1.	RM5	1	TGCAACTTCTAGCTGCTCGA(F) GCATCCGATCTTGATGGG(R)	P
2.	RM283	1	GTCTACATGTACCCTTGTTGGG(F) CGGCATGAGAGTCTGTGATG(R)	M
3.	RM431	1	TCCTGCGAACTGAAGAGTTG(F) AGAGCAAAACCCTGGTTCAC(R)	M
4.	RM319	1	ATCAAGGTACCTAGACCACCAC(F) TCCTGGTGCAGCTATGTCTG(R)	M
5.	RM237	1	CAAATCCCGACTGCTGTCC(F) TGGGAAGAGAGCACTACAGC(R)	P
6.	RM312	1	GTATGCATATTTGATAAGAG(F) AAGTCACCGAGTTTACCTTC(R)	M
7.	RM259	1	TGGAGTTTGAGAGGAGGG(F) CTTGTTGCATGGTGCCATGT(R)	M
8.	RM1	1	GCGAAAACACAATGCAAAAA(F) GCGTTGGTTGGACCTGAC(R)	M
9.	RM341	2	CAAGAAACCTCAATCCGAGC(F) CTCCTCCCGATCCCAATC(R)	M
10.	RM6	2	GTCCCCTCCACCCAATTC(F) TCGTCTACTGTTGGCTGCAC(R)	P
11.	RM452	2	CTGATCGAGAGCGTTAAGGG(F) GGGATCAAACCACGTTTCTG(R)	M
12.	RM154	2	ACCCTCTCCGCCTCGCCTCCTC(F) CTCCTCCTCCTGCGACCGCTCC(R)	M
13.	RM514	3	AGATTGATCTCCCATTCCCC(F) CACGAGCATATTACTAGTGG(R)	M
14.	RM338	3	CACAGGAGCAGGAGAAGAGC(F) GGCAAACCGATCACTCAGTC(R)	M
15.	OSR13	3	CATTTGTGCGTCACGGAGTA(F) AGCCACAGCGCCCATCTCTC(R)	P
16.	RM55	3	CCGTCGCCGTAGTAGAGAAG(F) TCCCGGTTATTTTAAGGCG (R)	P
17.	RM489	3	ACTTGAGACGATCGGACACC(F) TCACCCATGGATGTTGTCAG(R)	P

18.	RM185	4	AGTTGTTGGGAGGGAGAAAGGCC(F) AGGAGGCGACGGCGATGTCCTC(R)	M
19.	RM124	4	ATCGTCTGCGTTGCGGCTGCTG(F) CATGGATCACCGAGCTCCCCCC(R)	P
20.	RM307	4	GTACTACCGACCTACCGTTCAC(F) CTGCTATGCATGAACTGCTC(R)	M
21.	RM185	4	AGTTGTTGGGAGGGAGAAAGGCC(F) AGGAGGCGACGGCGATGTCCTC(R)	M
22.	RM334	5	GTTCAGTGTTCAGTGCCACC(F) GACTTTGATCTTTGGTGGACG(R)	M
23.	RM178	5	TCGCGTGAAAGATAAGCGGCGC(F) GATCACCGTTCCCTCCGCCTGC(R)	M
24.	RM161	5	TGCAGATGAGAAGCGGCGCCTC(F) TGTGTCATCAGACGGCGCTCCG(R)	M
25.	RM413	5	GGCGATTCTTGGATGAAGAG(F) TCCCCACCAATCTTGTCTTC(R)	P
26.	RM507	5	CTTAAGCTCCAGCCGAAATG(F) CTCACCCTCATCATCGCC(R)	M
27.	RM421	5	AGCTCAGGTGAAACATCCAC(F) ATCCAGAATCCATTGACCCC(R)	M
28.	RM510	6	AACCGGATTAGTTTCTCGCC(F) TGAGGACGACGAGCAGATTC(R)	P
29.	RM3827	6	GGACGGATTGTAGGTAGGAC(F) CCTTTCTTCAATCTGCATTC(R)	M
30.	RM133	6	TTGGATTGTTTTGCTGGCTCGC(F) GGAACACGGGGTCGGAAGCGAC(R)	M
31.	RM454	6	CTCAAGCTTAGCTGCTGCTG(F) GTGATCAGTGCACCATAGCG(R)	P
32.	RM162	6	GCCAGCAAAACCAGGGATCCGG(F) CAAGGTCTTGTGCGGCTTGCGG(R)	M
33.	RM11	7	TCTCCTCTTCCCCCGATC(F) ATAGCGGGCGAGGCTTAG(R)	P
34.	RM455	7	AACAACCCACCACCTGTCTC(F) AGAAGGAAAAGGGCTCGATC(R)	M
35.	RM125	7	ATCAGCAGCCATGGCAGCGACC(F) AGGGGATCATGTGCCGAAGGCC(R)	P
36.	RM118	7	CCAATCGGAGCCACCGGAGAGC(F) CACATCCTCCAGCGACGCCGAG(R)	M
37.	RM25	8	GGAAAGAATGATCTTTTCATGG(F) CTACCATCAAAACCAATGTTC(R)	P
38.	RM408	8	CAACGAGCTAACTTCCGTCC(F) ACTGCTACTTGGGTAGCTGACC(R)	M
39.	RM152	8	GAAACCACCACACCTCACCG(F) CCGTAGACCTTCTTGAAGTAG(R)	P
40.	RM44	8	ACGGGCAATCCGAACAACC(F) TCGGGAAAACCTACCCTACC(R)	M
41.	RM284	8	ATCTCTGATACTCCATCCATCC(F) CCTGTACGTTGATCCGAAGC(R)	M
42.	RM433	8	TGCGCTGAACTAAACACAGC(F) AGACAAACCTGGCCATTCAC(R)	M
43.	RM447	8	CCCTTGTGCTGTCTCCTCTC(F) ACGGGCTTCTTCTCCTTCTC(R)	M
44.	RM215	8	CAAAATGGAGCAGCAAGAGC(F) TGAGCACCTCCTTCTCTGTAG(R)	P
45.	RM205	8	CTGGTTCTGTATGGGAGCAG (F) CTGGCCCTTCACGTTTCAGTG (R)	M
46.	RM316	9	CTAGTTGGGCATACGATGGC(F) ACGCTTATATGTTACGTCAAC(R)	P
47.	RM496	10	GACATGCGAACAACGACATC(F) GCTGCGGCGCTGTTATAC(R)	M
48.	RM171	10	AACGCGAGGACACGTACTTAC(F) ACGAGATACGTACGCCTTTG(R)	P
49.	RM484	10	TCTCCCTCCTCACCATTGTC(F) TGCTGCCCTCTCTCTCTCTC(R)	P
50.	RM552	10	CGCAGTTGTGGATTTCAGTG(F) TGCTCAACGTTTGACTGTCC(R)	P
51.	RM271	10	TCAGATCTACAATTCCATCC(F) TCGGTGAGACCTAGAGAGCC(R)	P
52.	RM287	11	TTCCCTGTTAAGAGAGAAATC(F) GTGTATTTGGTGAAAGCAAC(R)	P
53.	RM224	11	ATCGATCGATCTTCACGAGG(F) TGCTATAAAAGGCATTCGGG(R)	P
54.	RM536	11	TCTCTCCTCTTGTTTGGCTC(F) ACACACCAACACGACCACAC(R)	M
55.	RM19	12	CAAAAACAGAGCAGATGAC(F) CTCAAGATGGACGCCAAGA (R)	M
56.	RM7102	12	TTGAGAGCGTTTTTAGGATG(F) TCGGTTTACTTGGTTACTCG(R)	P
57.	RM3331	12	CCTCCTCCATGAGCTAATGC(F) AGGAGGAGCGGATTTCTCTC(R)	P
58.	RM6869	12	GAGCTCCTTGTAGTGACCCG(F) ATCAGCCTCGCCAGCTTC(R)	M
59.	RM1103	12	CAGCTGCTGCTACTACACCG(F) CTACTCCACGTCCATGCATG(R)	M
60.	RM7376	12	TCACCGTCACCTCTTAAGTC(F) GGTGGTTGTGTTCTGTTTGG(R)	M
61.	RM6947	12	ATTAAACGTCCACTGCTGGC(F) GCTAGGTTAGTGGTGCAGGG(R)	M
62.	RM19	12	CAAAAACAGAGCAGATGAC(F) CTCAAGATGGACGCCAAGA(R)	M
63.	RM5479	12	AACTCCTGATGCCTCCTAAG(F) TCCATAGAAACAATTTGTGC(R)	P
64.	RM313	12	TGCTACAAGTGTTCTTCAGGAC(F) GCTCACCTTTTGTGTTCCAC(R)	P
65.	RM6217	12	GCAGCAAGAGCAAGAAATCC(F) GTTCCTGCCGTACCAGCAG(R)	M
66.	RM1986	12	TAACGGAGGGAGTAGTTTTC(F) GAACCTACATATCGAGAGCA(R)	P
67.	RM28004	12	GGCTGCCTGCATGGATATATGG(F) ATTATTTCAAGGTCGGAGCCAAGG(R)	P
68.	RM277	12	CGGTCAATCATCACCTGAC(F) CAAGGCTTGCAAGGGAAG(R)	P

P = Polymorphic and M = Monomorphic

Microsatellite marker analysis

The SSR study employed a total of 34 rice genotypes (Table 3). The primary sequences and chromosome positions for priming pairs have been downloaded from the Rice Genome Microsatellite Markers genome database. The 68 primers were randomly picked representing all of the chromosomes or genomic regions. Forty primers exhibited monomorphic fragments and were therefore excluded from further analysis. PCR reaction was carried out using a Programmable Thermal Cycle. The reaction volume was 10 ml containing 1.00 ml of genomic DNA, 1 ml of 10 X PCR buffer, 1 ml of 1 mM dNTPs, 0.5 ml of each primer and 1 unit of Tag polymerase. For SSR PAGE and gene-specific PCR products the C.B.S. gel electrophoresis unit was used. The DNA bands were visualized using staining gel with 1 percent ethidium bromide solution and photographed using a GelDocXR (BioRad) device under UV light. For the determination of the molecular weight of the amplified materials, a 50 bp DNA phase up ladder was used.

Table 3: Reaction of 32 rice lines and 2 parents against BPH

S. No.	F3 population	BPH score	Reaction
1	P1 (IR64)	1.33	Highly Resistant
2	P2 (CG Zn Rice I)	9.00	Highly Susceptible
3	Line no. 1	1.00	Highly Resistant
4	Line no. 2	1.00	Highly Resistant
5	Line no. 3	1.00	Highly Resistant
6	Line no. 4	1.28	Highly Resistant
7	Line no. 5	1.25	Highly Resistant
8	Line no. 6	1.00	Highly Resistant
9	Line no. 7	1.31	Highly Resistant
10	Line no. 8	1.00	Highly Resistant
11	Line no. 9	1.26	Highly Resistant
12	Line no. 10	1.25	Highly Resistant
13	Line no. 11	1.00	Highly Resistant
14	Line no. 12	1.00	Highly Resistant
15	Line no. 13	1.26	Highly Resistant
16	Line no. 14	1.13	Highly Resistant
17	Line no. 15	1.07	Highly Resistant
18	Line no. 16	1.15	Highly Resistant
19	Line no. 17	9.00	Highly Susceptible
20	Line no. 18	9.00	Highly Susceptible
21	Line no. 19	9.00	Highly Susceptible
22	Line no. 20	9.00	Highly Susceptible
23	Line no. 21	9.00	Highly Susceptible
24	Line no. 22	9.00	Highly Susceptible
25	Line no. 23	9.00	Highly Susceptible
26	Line no. 24	9.00	Highly Susceptible
27	Line no. 25	9.00	Highly Susceptible
28	Line no. 26	9.00	Highly Susceptible
29	Line no. 27	9.00	Highly Susceptible
30	Line no. 28	9.00	Highly Susceptible
31	Line no. 29	9.00	Highly Susceptible
32	Line no. 30	9.00	Highly Susceptible
33	Line no. 31	9.00	Highly Susceptible
34	Line no. 32	9.00	Highly Susceptible

Scoring of marker and data analysis

All the genotypes were graded for the inclusion of the SSR bands and their absence. Gels staining of ethidium bromide typically exhibited many lines. Based on its electrophoretic mobility relative to molecular weight markers (50 increments) the size of the most intensively amplified band for each microsatellite marker has been calculated. Clearly defined unambiguous bands were graded visually with each primordial for their existence or absence. The score were obtained in the form of matrix with "1" and "0", which indicate the presence and absence of bands in each variety respectively.

Results and Discussion

Phenotypic data analysis

In total, 34 F_3 population, including two parents, were screened for BPH resistance based on the standard evaluation system of BPH damage to rice. Among the 32 lines of rice along with 2 parents IR64 (Resistant parent) and CG Zn Rice I (Susceptible parent) when screened against BPH, the damage score ranged from 1.0 to 9.0. Out of 34 lines of rice 17 lines *viz*., IR64 (Resistant parent), Line no. 1, Line no. 2, Line no. 3, Line no. 4, Line no. 5, Line no. 6, Line no. 7, Line no. 8, Line no. 9, Line no. 10, Line no. 11, Line no. 12, Line no. 13, Line no. 14, Line no. 15, Line no. 16 showed the average plant damage score of 1.33, 1.0, 1.0, 1.0, 1.28, 1.25, 1.0, 1.31, 1.0, 1.26, 1.25, 1.0, 1.0, 1.26, 1.13, 1.07 and 1.15, respectively i.e. highly resistant.

CG Zn Rice I (Susceptible parent), Line no. 17, Line no. 18, Line no. 19, Line no. 20, Line no. 21, Line no. 22, Line no. 23, Line no. 24, Line no. 25, Line no. 26, Line no. 27, Line no. 28, Line no. 29, Line no. 30, Line no. 31, Line no. 32 showed the average plant damage score of 9.0, 9.0, 9.0, 9.0, 9.0, 9.0, 9.0, 9.0, 9.0, 9.0, 9.0, 9.0, 9.0, 9.0, 9.0, 9.0 and 9.0 respectively i.e. highly susceptible. In 1991, Pophaly and Rana, (1993) screened 21 IR varieties at Raipur (M.P.) against BPH. Only, IR64 and IR62 were resistant and IR56, IR34 and IR36 were moderately resistant. Santhanalakshmi *et al*. (2010), evaluated the 106 F_3 families and their parents Swarna and PTB33 for resistance to Indian biotype brown planthopper using the standard IRRI seed box technique.

Molecular marker analysis

In the present study, parental polymorphism survey was taken up between donor parent IR64 and recurrent parent CG Zn Rice I. The parent DNA was isolated to good purity using the chemical method of purification. The PCR reactions were carried out according to standard protocol for rice microsatellites. A total of 68 SSR markers spanning all the 12 chromosomes of rice genome were analysed on the susceptible variety CG Zn Rice I and resistant variety IR64 for parental polymorphism. Of the 272 SSR markers, 191 SSR markers were chosen from SSR genetic maps by Orjuela *et al*. (2010), 43 SSR markers from Tenmykh *et al*. (2000) and 38 SSR markers from gramene. Out of 68 SSR markers, 29 were polymorphic showing overall 42.64% polymorphism. The maximum polymorphism of 100% was observed between parents for one marker studied located at chromosome 9 and minimum of 16.66% for six markers studied located at chromosome 5. Percent polymorphism obtained in the present

study (Table 4) is in agreement with other similar type of studies. Sun *et al*. (2005) [5] used 548 SSR markers to detect polymorphism between BPH resistant line (Rathu Heenati) and susceptible line (02428), of which 178 (32.5%) markers showed polymorphism. Sun *et al.* (2006) reported 34% polymorphism among BPH resistant (Karnataka) and susceptible (02428) line. Qiu *et al*. (2014) [4] surveyed 508 SSR on T12 and 93-11 varieties and reported 189 (37.2%) markers to be polymorphic. Qiu *et al*. (2012) [3] used 484 SSR markers, distributed on 12 rice chromosomes to survey B14 and 93-11 of which 185 (38.2%) of markers were polymorphic.

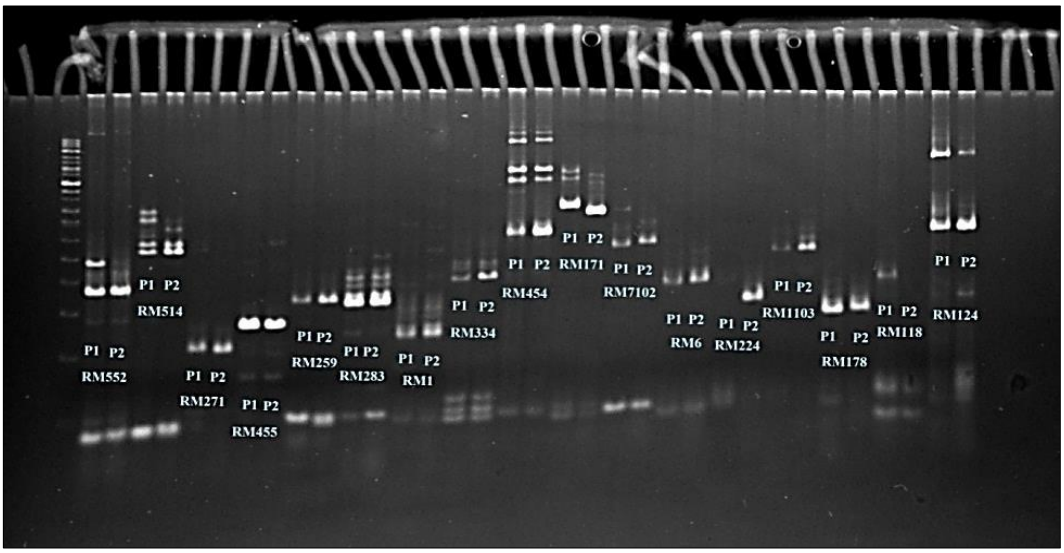

Fig 1: A representative figure of parental polymorphism survey using SSR markers: P1 (IR64) and P2 (CG Zn Rice I)

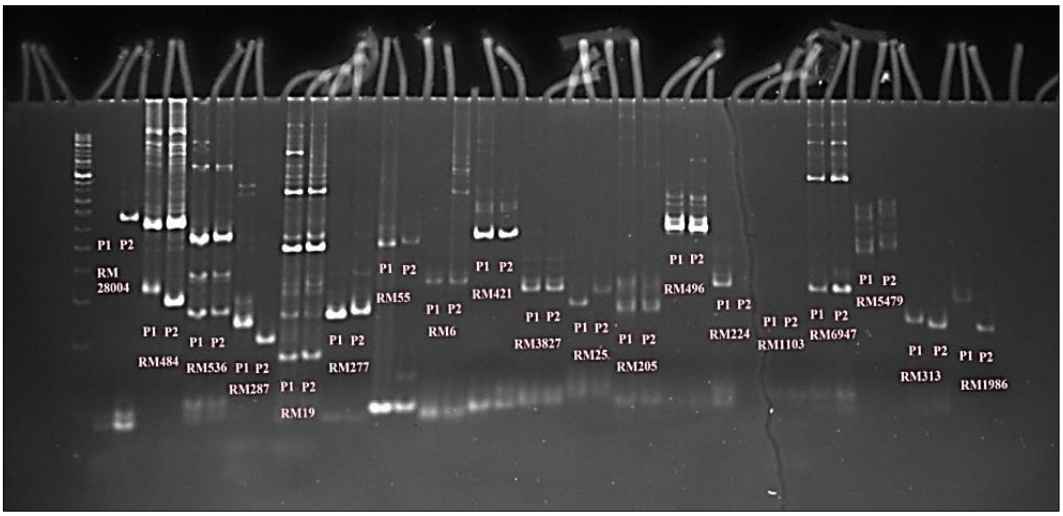

Fig 2: A representative figure of parental polymorphism survey using SSR markers: P1 (IR64) and P2 (CG Zn Rice I)

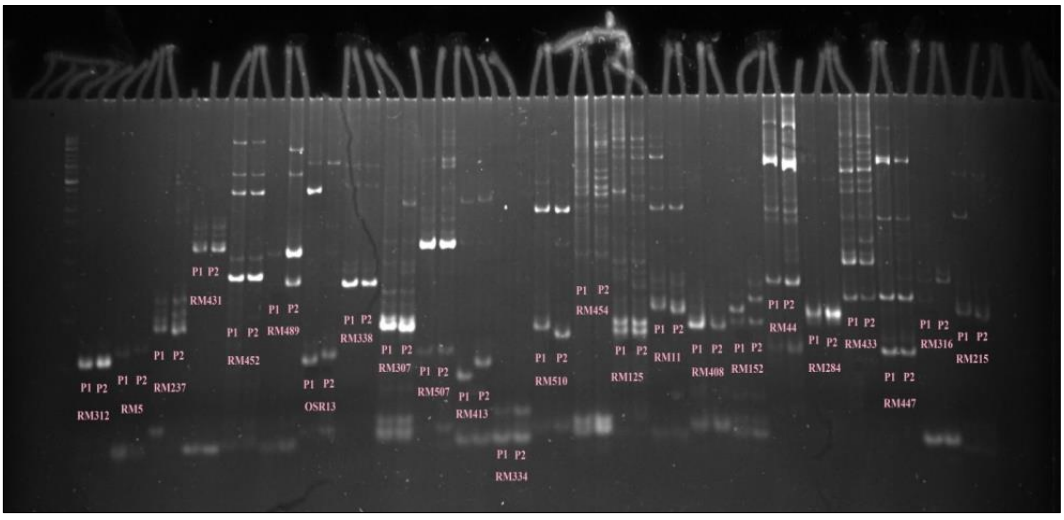

Fig 3: A representative figure of parental polymorphism survey using SSR markers: P1 (IR64) and P2 (CG Zn Rice I)

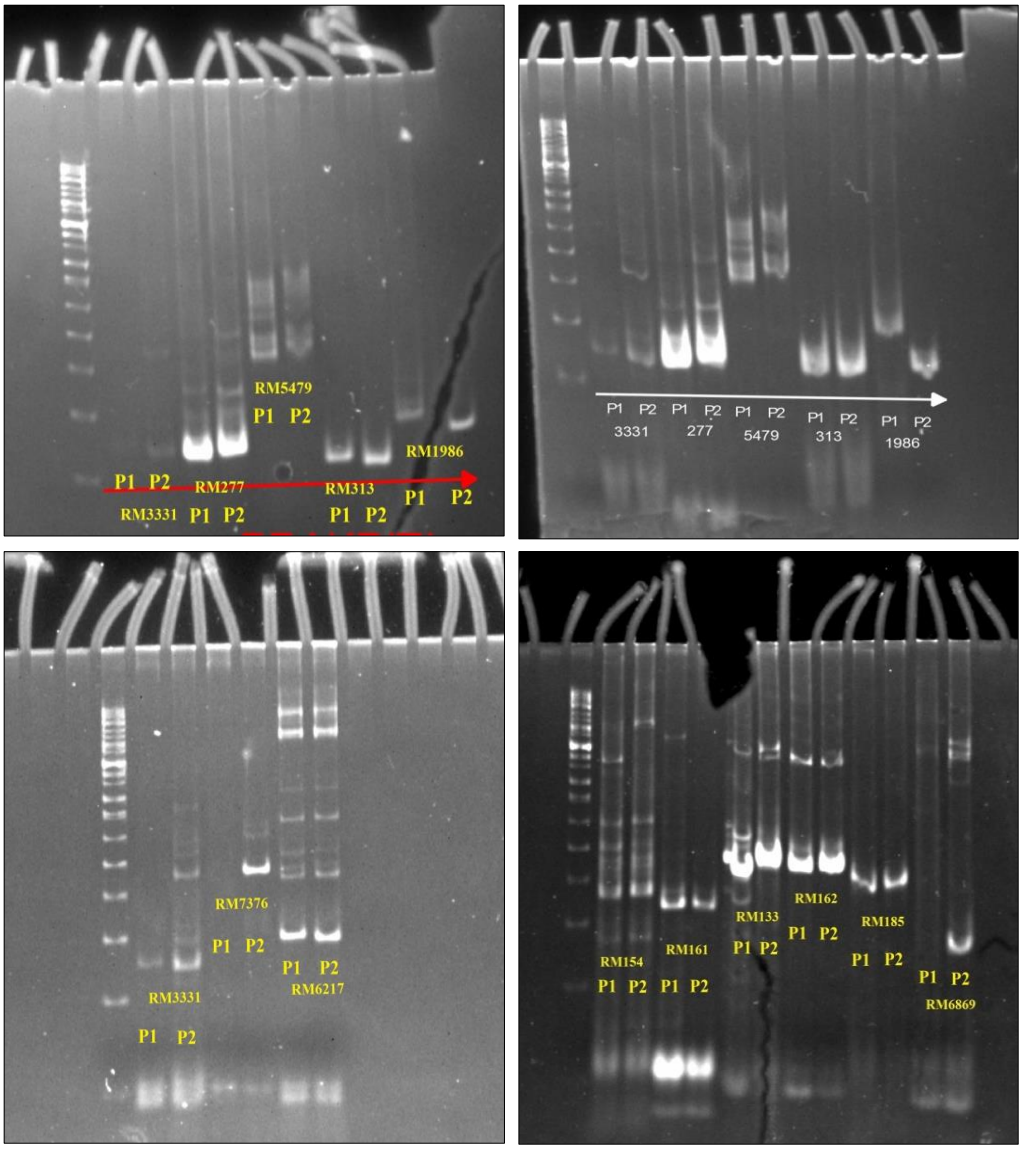

Fig 4: A representative figure of parental polymorphism survey using SSR markers: P1 (IR64) and P2 (CG Zn Rice I)

A representative gel picture showing polymorphism survey between parents is shown in Fig. 1, 2 and 3. Chromosome wise total no. of markers tested for polymorphism, number of polymorphic markers identified and percent polymorphism is given in Table 5. The number of markers per chromosome used for polymorphism survey ranged from 1 on chromosome 9 to 15 on chromosome 12. Number of polymorphic markers per chromosome ranged from 1 on chromosome 9 to 7 on chromosome 12. The reason for lower level of polymorphism might be due to similarity in the genetic background of the parents used (Marri *et al*., 2005) [2]. Polymorphic markers identified in the present study were used for selective genotyping of resistant and susceptible genotypes and identification of linked markers of BPH resistant gene (s). Markers RM5, RM237, RM6, RM489, OSR13, RM55, RM124, RM413, RM510, RM454, RM11, RM125, RM152, RM215, RM25, RM316, RM484, RM552, RM271, RM171, RM224, RM287, RM277, RM5479, RM313, RM1986, RM3331, RM28004 and RM7102 showed polymorphism table 4.

Table 4: List of polymorphic SSR markers

S. No.	Primer	Chromosome No.	Sequence
1	RM5	1	CAAATCCCGACTGCTGTCC(F) TGGGAAGAGAGCACTACAGC(R)
2	RM237	1	CAAATCCCGACTGCTGTCC(F) TGGGAAGAGAGCACTACAGC(R)
3	RM6	2	GTCCCCTCCACCCAATTC(F) TCGTCTACTGTTGGCTGCAC(R)
4	RM489	3	ACTTGAGACGATCGGACACC(F) TCACCCATGGATGTTGTCAG(R)
5	OSR 13	3	CATTTGTGCGTCACGGAGTA(F) AGCCACAGCGCCCATCTCTC(R)
6	RM55	3	CCGTCGCCGTAGTAGAGAAG(F) TCCCGGTTATTTTAAGGCG (R)
7	RM124	4	ATCGTCTGCGTTGCGGCTGCTG(F) CATGGATCACCGAGCTCCCCCC(R)
8	RM413	5	GGCGATTCTTGGATGAAGAG(F) TCCCCACCAATCTTGTCTTC(R)
9	RM510	6	AACCGGATTAGTTTCTCGCC(F) TGAGGACGACGAGCAGATTC(R)
10	RM454	6	CTCAAGCTTAGCTGCTGCTG(F) GTGATCAGTGCACCATAGCG(R)
11	RM11	7	TCTCCTCTTCCCCCGATC(F) ATAGCGGGCGAGGCTTAG(R)
12	RM125	7	ATCAGCAGCCATGGCAGCGACC(F) AGGGGATCATGTGCCGAAGGCC(R)

13	RM152	8	GAAACCACCACACCTCACCG(F) CCGTAGACCTTCTTGAAGTAG(R)
14	RM215	8	CAAAATGGAGCAGCAAGAGC(F) TGAGCACCTCCTTCTCTGTAG(R)
15	RM25	8	ATCAGCAGCCATGGCAGCGACC(F) AGGGGATCATGTGCCGAAGGCC(R)
16	RM316	9	CTAGTTGGGCATACGATGGC(F) ACGCTTATATGTTACGTCAAC(R)
17	RM484	10	TCTCCCTCCTCACCATTGTC(F) TGCTGCCCTCTCTCTCTCTC(R)
18	RM552	10	CGCAGTTGTGGATTTCAGTG(F) TGCTCAACGTTTGACTGTCC(R)
19	RM271	10	TCAGATCTACAATTCCATCC(F) TCGGTGAGACCTAGAGAGCC(R)
20	RM171	10	AACGCGAGGACACGTACTTAC(F) ACGAGATACGTACGCCTTTG(R)
21	RM224	11	ATCGATCGATCTTCACGAGG(F) TGCTATAAAAGGCATTCGGG(R)
22	RM287	11	TTCCCTGTTAAGAGAGAAATC(F) GTGTATTTGGTGAAAGCAAC(R)
23	RM277	12	CGGTCAATCATCACCTGAC(F) CAAGGCTTGCAAGGGAAG(R)
24	RM5479	12	AACTCCTGATGCCTCCTAAG(F) TCCATAGAAACAATTTGTGC(R)
25	RM313	12	TGCTACAAGTGTTCTTCAGGAC(F) GCTCACCTTTTGTGTTCCAC(R)
26	RM1986	12	TAACGGAGGGAGTAGTTTTC(F) GAACCTACATATCGAGAGCA(R)
27	RM3331	12	CCTCCTCCATGAGCTAATGC(F) AGGAGGAGCGGATTTCTCTC(R)
28	RM28004	12	GGCTGCCTGCATGGATATATGG(F) ATTATTTCAAGGTCGGAGCCAAGG(R)
29	RM7102	12	TTGAGAGCGTTTTTAGGATG(F) TCGGTTTACTTGGTTACTCG(R)

Table 5: Percent polymorphism observed between CG Zn rice I x IR64 per chromosome

Chromosome No.	Total no. of markers used	No. of polymorphic markers	Percent polymorphism
1	8	2	25%
2	4	1	25%
3	5	3	60%
4	4	1	25%
5	6	1	16.66%
6	5	2	40%
7	4	2	50%
8	9	3	33.33%
9	1	1	100%
10	5	4	80%
11	3	2	66.66%
12	14	7	50%

References

1. Horgan FG, Ramal AF, Bentur JS, Kumar R, Bhanu K, Sarao PS *et al*. Virulence of brown planthopper (*Nilaparvata lugens*) populations from South and South East Asia against resistant rice varieties. Crop Protection 2015;78:222-231.
2. Marri PR, Sarla N, Reddy LV, Siddiq EA. Identification and mapping of yield and yield related QTLs from an Indian accession of *Oryza rufipogon*. BMC genet 2005;6(1):33.
3. Qiu X, Gong R, Tan Y, Yu S. Mapping and characterization of the major quantitative trait locus qSS7 associated with increased length and decreased width of rice seeds. Theor. Appl. Genet 2012;125(8):1717-1726.
4. Qiu Y, Guo J, Jing S, Zhu L, He G. Fine mapping of the rice brown planthopper resistance gene *BPH7* and characterization of its resistance in the 93-11 background. Euphytica 2014;198(3):369-379.
5. Sun L, Su C, Wang C, Zai H, Wan J. Mapping of a major resistance gene to brown planthopper in the rice cultivar Rathu Heenati. Breed. Sci 2005;55: 391-396.
6. Zheng K, Huang N, Bennett J Khush GS. PCR-based marker assisted selection in rice breeding. IRRI Discussion Paper Series No. 12. International Rice Research Institute, Manila, the Philippines 1995.