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Parental polymorphic survey for white backed plant hopper resistance in rice (*Oryza sativa* L.) using SSR markers

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Abstract
This study aims to find the polymorphic simple sequence repeat markers for white backed plant hopper insect resistance among the recipient parent (Narendra Dhan 359) and donor parent (MO1) of rice. A total of 856 random microsatellite markers are evenly distributed across the 12 chromosomes were used for the current polymorphic survey. Among these, 126 markers were found polymorphic by distinct banding patterns. The highest percentage of polymorphism was observed on chromosome 5 (28%), while chromosome 9 (4%) recorded the lowest polymorphism. The identified polymorphic markers across the 12 chromosomes will be harness in further linkage map construction and mapping of QTLs associated with WBPH resistance.

Keywords: White backed plant hopper, resistance, polymorphism and QTLs

Introduction
Rice (*Oryza sativa* L.) is the staple food crop for more than half of the world's population. India ranks 1st in area (47m ha) and second in production (132 mt) globally (USDA/ FAO, 2023) [5]. The productivity of rice is being affected by a number of biotic and abiotic stresses. Among various biotic stresses affecting rice crop insect pests occupy a major position. The warm and humid environment prevalent in rice growing ecosystems is conducive for the proliferation of insect pests. Losses caused by the insect pests are the main constraint in achieving high yield of rice. Both nymphs and adults of white backed plant hopper (WBPH) suck the phloem sap and under severe infestation complete drying and death of crop occurs, popularly called as hopper-burn. Host plant resistance is the most effective breeding strategy to manage with WBPH.

White-backed plant hopper have emerged as serious pests in many rice growing states round the year. During the *Kharif*, 2008 there was a severe hoppers in Punjab and Haryana in about 120, 000 ha. These have been a regular pest in the East & West Godavari districts in Andhra Pradesh, Bellary and Sindanur areas in Karnataka and in Burdwan district of West Bengal during *kharif*, 2009-2012 (Prakash *et al*., 2014) [3].

With this background the present experiment is carried to understand the genetic basis of resistance and location of WBPH resistance genes/QTLs present in an universal donor namely MO1 the standard resistant check variety used in WBPH screening programmes using SSR markers. Once identified, the genes/QTLs may further be fine mapped and validated in future for utilization in marker assisted selection of WBPH resistance in breeding programs.

Materials and Methods
The present experiment was conducted at Indian Institute of Rice Research (Molecular breeding laboratory at Crop Improvement Division), Rajendranagar, Hyderabad. In order to map QTLs for white backed plant hopper resistance trait, parents were screened by using SSR markers to establish parental polymorphism survey among them. Resistant parent (MO1) and susceptible parent (Narendra Dhan 359) were screened for polymorphism survey by SSR primers. About 856 SSR primers distributed equidistantly over the 12 rice chromosomes are utilized to conduct current study.

Leaf sample collection and DNA isolation

Total genomic DNA of 25 days old seedlings was extracted using 2% Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thomson, 1980) [2] as described below. About 100g leaves from nursery were collected in zip lock plastic covers. These samples were then stored in -20ºc deep freezer. Frozen leaf tissues about 100mg were ground to a fine powder with liquid nitrogen using mortar and pestle and finely grinded powder was transferred to 2.0 ml centrifuge tubes. 800µl of CTAB buffer was added and incubated for 30 minutes at 65 ºC in water bath. During incubation, the contents were occasionally mixed two to three times by inverting the tubes gently. 800µl of chloroform: iso-amylalcohol (24:1) was added to the tubes and then centrifuged at 10,000 rpm for 15 minutes at room temperature. The upper aqueous phase was transferred to fresh tubes with the help of micropipette. Care was taken to avoid debris inclusion. 700ul of chilled iso-propanol was added and mixed well by inverting the tubes. The samples were refrigerated at -20 ºC for overnight and then centrifuged at 10,000 rpm for 15 minutes to deposit pellet and the supernatant was discarded. The DNA pellet was washed with 200µl of 70% ethanol and centrifuged at 10,000 rpm for 5 minutes. Ethanol was discarded and the pellet was air-dried and finally the purified DNA pellet was dissolved in 100 µl of 1x TE buffer. It was kept overnight at room temperature. The DNA samples were stored at -20 ºC for long term stability. The extracted genomic DNA quantity and quality was analyzed by nano drop.

Polymerase Chain Reaction (PCR)

A set of 856 SSR markers covering all the 12 chromosomes of rice were used for parental polymorphism survey in the present study. The polymerase chain reaction (PCR) was carried out in a total volume of 10 µl containing 2 µl of template DNA, random (forward and reverse) primers (0.5µl each), 4.0µl Takara PCR master mix and 3.0 µl sterile distilled water. It was placed in a PCR thermal cycler with 96 wells. The protocol begins with a 5 minutes initial denaturation at 94 ºC and continues with 35 cycles of 30 seconds at 94 ºC for denaturation, 30 seconds at 55 ºC for primer annealing, 1 minute at 72 ºC for extension, and 7 minutes at 72 ºC for final extension.

Agarose gel electrophoresis and gel documentation

The PCR products were analysed by electrophoresis using a 3.0% agarose gel using a gel Electrophoresis Unit. About 12.0 g of agarose and 400 ml of 1X TAE buffer added and place into a microwave oven to melt agarose. Gel-casting tray and combs were wiped with ethanol. After the agarose had cooled to room temperature, two microlitres of ethidium bromide added to the melted agarose solution and the mixture was poured onto a gel cast tray, solidification takes about 20 to 30 minutes. The gel was transferred to the electrophoresis unit containing 1X TAE buffer at 120volts. The DNA fragments were visualized under UV transilluminator and documented using gel documentation system.

Statistical Analysis

The percentage of polymorphism was calculated by using the formula

$$\text{Number of polymorphic markers} = \frac{\text{Number of polymorphic markers}}{\text{Total number of markers}} \times 100$$

To visualise the marker data, GGT 2.0 programme was used. Visualization of the distribution of polymorphic markers along the length of the chromosome according to their physical positions (Mb) was obtained by GGT, as shown in Figure.1.

Results and Discussion

Polymorphic survey carried out between WBPH resistant donor parents (MO1) and the susceptible parent (NDR 359). Total 856 SSR markers were selected equidistantly to cover the entire genome, out of 856 markers 126 markers were found to be polymorphic between the parents exhibiting 17.1% polymorphism. Total number of markers on each chromosome and percentage of polymorphism on each chromosome between parents are provided in the Table 1. The List of identified polymorphic SSR markers are provided in Table 2. The frequency distribution of markers on each chromosome depicted in Figure2. Chromosome 5 to be having the highest number (15) of polymorphic markers, followed by chromosomes 3 (14) and chromosome 1, 6, 7 shown 13 polymorphic markers each. The lowest number (5) was identified on chromosome 9, followed by chromosomes 8 and 12 shown (7) markers on each chromosome. The percentage of polymorphism ranged from 4% (Chromosome 9) to 28% (Chromosome 5) with an average of 15.4%. Highest genetic variability observed on chromosome 5 whereas lowest observed on chromosome 9. Similar polymorphic study reported by using a set of 514 SSR primers covered all the 12 chromosomes were used for testing polymorphism between parents TN1 and Sinnasivappu and of these, 128 markers were found polymorphic between the parents was reported by Ramesh *et al.* (2014) [4]. Similar finding reported by Ishwarya Lakshmi *et al.* (2021) [1] by using a total of 494 random microsatellite markers evenly distributed across 12 chromosomes were used for the polymorphic survey, among these 87 markers were found polymorphic between M229 and Telangana Sona parents. The identified polymorphic markers will be further utilized in construction of linkage map, mapping of quantitative trait loci, identifying the genes responsible for understanding of genetic mechanisms of the variation underlying WBPH resistance.

Table 1: Chromosome wise percentage of SSR markers showing polymorphism between parents NDR 359 and MO1

Chromosome number	Total No. of markers screened for each chromosome	No. of Polymorphic markers on each chromosome	No. of monomorphic markers on each chromosome	Percentage of polymorphism on each chromosome
1	81	13	68	16
2	84	12	72	14
3	81	14	67	17
4	72	10	62	14
5	53	15	38	28
6	68	13	55	19
7	79	13	66	16
8	66	7	59	11
9	114	5	109	4
10	48	9	39	19
11	52	8	44	15
12	58	7	51	12
	856	126	730	

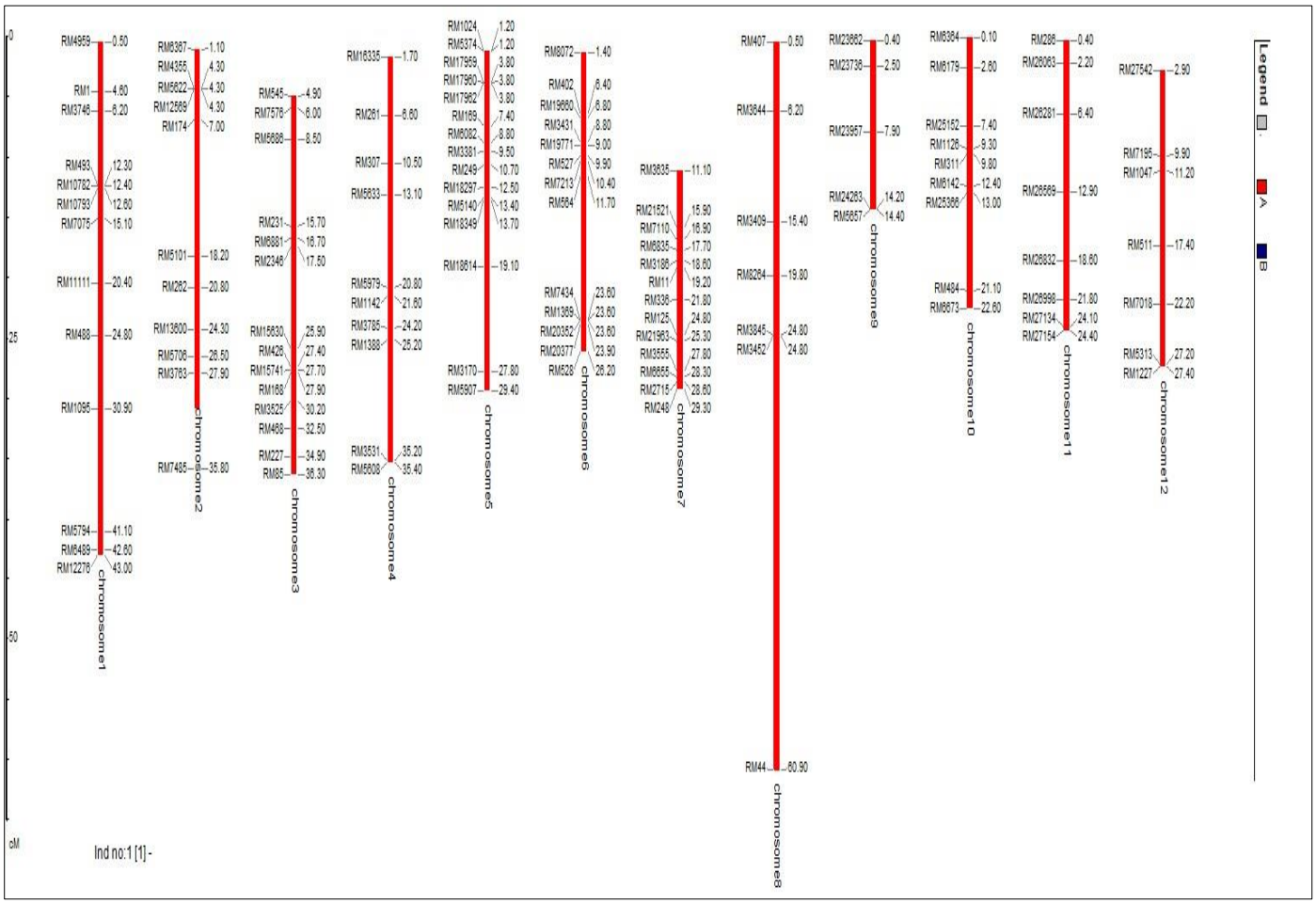

Fig 1: Distribution of 126 polymorphic SSR markers on 12 chromosomes of rice (image by GGT 2.0)

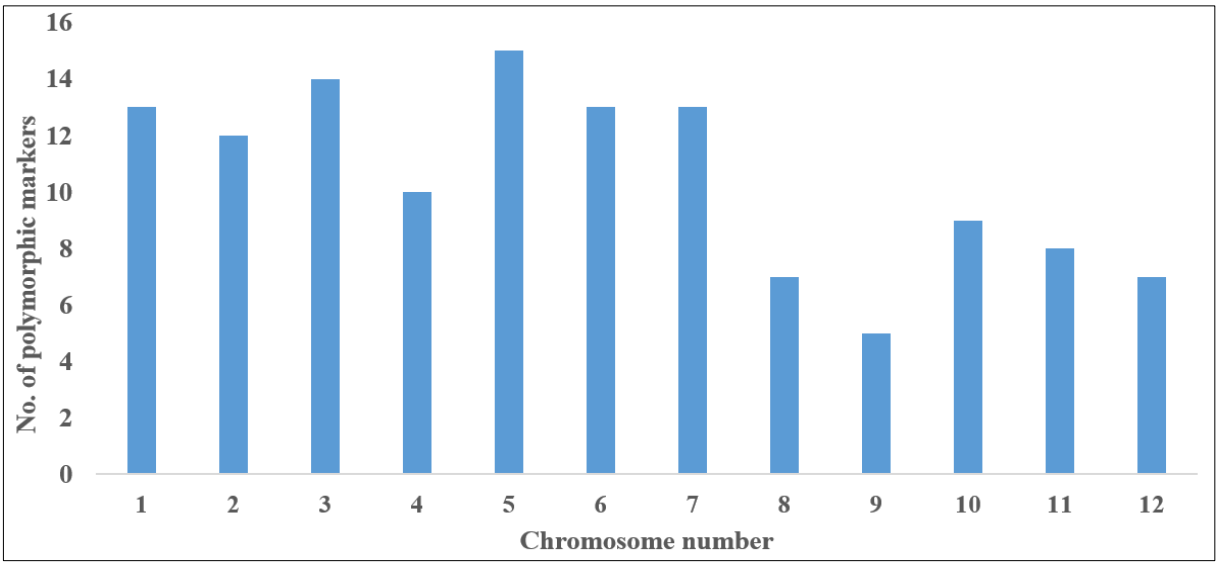

Fig 2: Histogram depicting the number of polymorphic markers on each chromosome between parents NDR 359 and MO1

Table 2: List of polymorphic markers between NDR 359 and MO1

S. No.	Ch. No.	Marker	S. No.	Ch. No.	Marker	S. No.	Ch. No.	Marker	S. No.	Ch. No.	Marker
1	1	RM1	33	3	RM426	65	6	RM528	97	8	RM3452
2	1	RM11111	34	3	RM15741	66	6	RM8072	98	9	RM23957
3	1	RM493	35	3	RM168	67	6	RM7213	99	9	RM23662
4	1	RM1095	36	3	RM227	68	6	RM564	100	9	RM24263
5	1	RM6489	37	3	RM15630	69	6	RM402	101	9	RM5657
6	1	RM5794	38	3	RM5686	70	6	RM7434	102	9	RM23736
7	1	RM12276	39	3	RM545	71	6	RM1369	103	10	RM1126
8	1	RM10782	40	4	RM3785	72	6	RM3431	104	10	RM6179
9	1	RM7075	41	4	RM3531	73	6	RM20352	105	10	RM6142
10	1	RM3746	42	4	RM1142	74	6	RM527	106	10	RM311
11	1	RM4959	43	4	RM5608	75	6	RM19660	107	10	RM6364
12	1	RM10793	44	4	RM5979	76	6	RM19771	108	10	RM484
13	1	RM488	45	4	RM1388	77	6	RM20377	109	10	RM25152
14	2	RM3763	46	4	RM307	78	7	RM3555	110	10	RM6673
15	2	RM4355	47	4	RM16335	79	7	RM336	111	10	RM25366
16	2	RM6	48	4	RM261	80	7	RM21521	112	11	RM286
17	2	RM5622	49	4	RM5633	81	7	RM3635	113	11	RM26063
18	2	RM6367	50	5	RM3170	82	7	RM3186	114	11	RM26998
19	2	RM5101	51	5	RM18297	83	7	RM248	115	11	RM27154
20	2	RM5706	52	5	RM6082	84	7	RM11	116	11	RM26281
21	2	RM262	53	5	RM169	85	7	RM6655	117	11	RM26569
22	2	RM7485	54	5	RM5140	86	7	RM21963	118	11	RM26832
23	2	RM12569	55	5	RM1024	87	7	RM6835	119	11	RM27134
24	2	RM174	56	5	RM5374	88	7	RM7110	120	12	RM1227
25	2	RM13600	57	5	RM17959	89	7	RM2715	121	12	RM5313
26	3	RM231	58	5	RM17960	90	7	RM125	122	12	RM27542
27	3	RM468	59	5	RM17962	91	8	RM44	123	12	RM1047
28	3	RM85	60	5	RM18349	92	8	RM3845	124	12	RM7018
29	3	RM3525	61	5	RM18614	93	8	RM3644	125	12	RM7195
30	3	RM2346	62	5	RM3381	94	8	RM3409	126	12	RM511
31	3	RM6881	63	5	RM249	95	8	RM407			
32	3	RM7576	64	5	RM5907	96	8	RM8264			

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