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Parental polymorphism survey using genome-wide SSR markers for brown planthopper resistance in rice

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Abstract
For introgressing Brown planthopper (BPH) resistance into a rice variety using marker assisted selection, study of parental polymorphism between the donor and recipient parents is a pre-requisite step. Unless the parents are polymorphic for the BPH reaction, further selection of plants carrying the traits may not be possible. Thus, the present investigation was conducted to analyze the polymorphism of two BPH resistant donors (M229, 10-3) and a recipient (Telangana Sona) parent using SSR markers. 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, while 93 were polymorphic between 10-3 and Telangana Sona by producing distinct reproducible amplification patterns. The highest percentage of polymorphism was observed on chromosome 12 (30.59%) for Telangana Sona and M229, while chromosome 1 (30.61%) recorded the highest polymorphism percentage for Telangana Sona and 10-3 indicating that these chromosomes are useful in studying the variation between the parents. Accordingly, the maximum number of polymorphic markers were obtained on chromosome 6 for the parents of both the crosses. The identified polymorphic markers across the 12 chromosomes will be useful in subsequent linkage map construction along with mapping of QTLs associated with BPH resistance.

Keywords: rice, brown planthopper, parental polymorphism, marker-assisted breeding, SSR markers

Introduction
Rice (*Oryza sativa* L.) is one of the world's most widely grown and consumed cereals playing a fundamental role in food security and socio-economic development. Over 3.5 billion people depend on rice as a major source of food, obtaining more than 20% of the dietary calorie intake (https://ricepedia.org/rice-as-food/the-global-staple-rice-consumers) [5]. But production of the crop is severely hampered by biotic stresses which are escalating rapidly at an alarming rate in recent times due to the erratic climatic conditions. Of the biotic stresses, Brown Planthopper (BPH), *Nilaparvata lugens* (Stål) is considered to be the most destructive biological constraint, often causing severe yield loss due to its monophagous nature and migration ability (Normile, 2008) [12]. The pest usually feeds on vascular sap sucking the phloem from leaf sheath leading to hopper burn symptoms in addition to the transmission of viral diseases such as ragged stunt virus and grassy stunt virus (Khush and Brar, 1991) [7]. Development of resistant cultivars by understanding the mechanisms of host plant resistance is considered to be the most economic and environmentally friendly strategy for controlling this pest. This resistance can be deployed into rice cultivars using molecular markers (Khush, 2001) [8] that have been a boon by providing realistic information about resistant cultivars through the creation of high-resolution genetic maps among the important crop traits (Moose and Mumm, 2008 [10]; Alsaleh *et al*., 2015 [1]). Thus, selection of plants using these markers has become an important tool in plant breeding in recent years for detecting valuable traits such as insect resistance in individuals or populations (Miah *et al.*, 2013 [9]; Balta *et al.*, 2014 [2]). Among all the molecular markers, microsatellites (SSRs) have been utilized extensively (Miah *et al.*, 2013) [9] since they have better advantages over other markers as they can be amplified by Polymerase chain reaction, available in abundance, distributed throughout the genome, highly polymorphic and co-dominant. These markers have also been proven very useful in improving breeding efficiency through precise transfer of target genomic regions. Therefore, systematic introgression of the resistance genes into elite rice cultivars by marker-assisted selection (MAS) with SSRs can be an important strategy for developing stable resistance for BPH (Jairin *et al.*, 2005 [6]; Choudhary *et al*., 2008 [3]; Singh and Singh, 2015) [13].

Screening of markers for parental polymorphism among rice cultivars for BPH resistance forms the basis for tagging of the genes along with fine mapping. A clear polymorphism between the donor and recurrent parents is essential in undertaking gene/Quantitative trait loci (QTL) introgression programmes. Thus, the present study was undertaken to detect polymorphism between two BPH donors and a recipient parent using genome-wide SSR markers. The polymorphic markers identified in the present study will be further utilized in the identification of QTLs responsible for BPH resistance.

Materials and Methods

In order to map QTL's for BPH resistance, initially parental polymorphic survey using 494 SSR markers equally distributed across all the 12 chromosomes was conducted to assess the extent of diversity between the parents.

1. Parent material

The experimental material for the investigation comprised of two BPH resistant donors (M229 and 10-3) and a BPH susceptible variety Telangana Sona. The donor parent M229 is a MAGIC (Multi-parent Advanced Generation Inter Cross) line, while, 10-3 is an Assam landrace that were procured from the Institute of Biotechnology, PJTSAU (Professor Jayashankar Telangana State Agricultural University), Hyderabad. On the other hand, Telangana Sona (RNR 15048) is a popular high yielding, short duration, slender grain variety developed by PJTSAU, Hyderabad.

2. DNA Isolation

Genomic DNA of the three rice parents was extracted by CTAB method suggested by Murray and Thompson, 1980 [11]. 2-3 cm leaf pieces from 20-25 days old seedlings were taken, cut into small pieces and were grinded in a mortar using 350 µl of extraction buffer (100 mM Tris HCl, pH 8.0; 20 mM EDTA; 1.4 mM NaCl). Another 350 µl of the extraction buffer was added to the well containing the homogenized leaf sample. The samples were kept in hot water bath at 65°C for 1 hour and the mixture was and centrifuged at 13000 rpm for 15 minutes. Then the supernatant was collected and equal volume of Chloroform: Isoamyl alcohol (24:1) was added after which the contents were mixed well for about 10 minutes by inversion and again centrifuged at 13000 rpm for about 15 minutes. After the centrifugation, the supernatant was aliquoted from the micro centrifuge tube without disturbing the intermediate layer into a fresh 1.5 ml micro centrifuge tube. Then to the clear supernatant, 5-10 µl of RNase (10 mg/ml) was added and incubated for about 45-60 minutes at room temperature. The supernatant was drained gently without disturbing the DNA pellet and about 200 µl of 70% ethanol was added to the pellet after which the contents were centrifuged again. Finally, the pellet was left for overnight air drying at room temperature and depending on the size of the pellet, about 50-100 µl of 1X TE buffer was added for dissolving the pellet. The purity of DNA was measured by Nanodrop.

3. Polymerase chain reaction (PCR) and Agarose gel electrophoresis

PCR was carried out in a total volume of 10µL containing 2µl of template DNA, random markers (0.5µl each), 4.0µl Takara PCR master mix and 3.0µl sterile distilled water. The PCR mix was centrifuged at 1000 rpm for 1 minute and loaded in a 96 wells thermal cycler of PCR (Eppendorf). The program consists of initial denaturation at 94°C for 5 min which was further followed by 35 cycles of denaturation at 94°C for 0.45 min, annealing temperature of 56°C for 0.45 min and final extension of 72°C for 1 min. The amplified PCR products were resolved on 3% agarose gel by adding 9 g of agarose (Seakem LE) to 300 ml 1X TAE buffer in a flask of 1000 ml capacity. The gel was run at a constant voltage of 90V for about 1 hour 30 minutes. The DNA fragments was then visualized under UV-transilluminator and documented using a documentation system (GELSTAN) which was stored for further scoring and permanent records.

4. Percentage of polymorphism

The percentage of polymorphism between the parents was calculated using the formula:

$$= \frac{\text{Markers showing polymorphism}}{\text{The total number of markers used}} \text{ x } 100$$

Results and Discussion

Polymorphic survey between the BPH resistant donors (M229, 10-3) and susceptible recipient (Telangana Sona) parents is essential for the construction of linkage map and effective identification of QTLs linked with BPH resistance. Thus, in order to identify the QTLs, a total of 494 SSR markers spanning across all the 12 rice chromosomes were used for genome-wide parental polymorphism survey.

Polymorphic survey between Telangana Sona and M229

Out of the 494 markers, 87 were found to be polymorphic between the parents Telangana Sona and M229 exhibiting 17.1% polymorphism. The List of SSR markers identified polymorphic are provided in Table 1, Fig. 2, 3.

Table 1: List of SSR markers polymorphic between Telangana Sona and M229

S. No	CHR.	Marker	S. No	CHR.	Marker	S. No	CHR.	Marker
1	1	RM12292	33	4	RM127	65	9	RM251
2	1	RM11069	34	4	RM335	66	10	RM228
3	1	RM3598	35	4	RM518	67	10	RM25404
4	1	RM151	36	4	RM6997	68	10	RM25661
5	1	RM129	37	5	RM259	69	11	RM25969
6	1	RM302	38	5	RM151	70	11	RM254
7	1	RM11135	39	5	RM3598	71	11	RM224
8	1	RM493	40	6	RM20615	72	11	RM4112
9	1	RM1282	41	6	RM275	73	11	RM202
10	1	RM11522	42	6	RM133	74	11	RM206
11	1	RM10033	43	6	RM190	75	12	RM28089
12	1	RM3148	44	6	RM111	76	12	RM1261
13	1	RM212	45	6	RM589	77	12	RM28067

14	2	RM13584	46	6	RM469	78	12	RM28157
15	2	RM13823	47	6	RM8072	79	12	RM28096
16	2	RM13131	48	6	RM586	80	12	RM28781
17	2	RM341	49	6	RM19291	81	12	RM28120
18	2	RM423	50	6	RM588	82	12	RM2529
19	2	RM109	51	6	RM508	83	12	RM17
20	2	RM240	52	6	RM19311	84	12	RM9
21	2	RM250	53	6	RM204	85	12	RM19
22	3	RM15580	54	7	RM21881	86	12	RM7102
23	3	RM14270	55	7	RM20818	87	12	RM3331
24	3	RM231	56	7	RM6697			
25	3	RM85	57	7	RM336			
26	3	RM520	58	8	RM22250			
27	4	RM16415	59	8	RM22257			
28	4	RM307	60	8	RM264			
29	4	RM8213	61	8	RM281			
30	4	RM6365	62	8	RM404			
31	4	MS10	63	8	RM273			
32	4	RH078	64	9	RM105			

The frequency distribution of markers (Table 2, Fig. 1) depicted chromosome 6 to be having the highest number (14) of polymorphic markers, followed by chromosomes 1 and 12 with 13 polymorphic markers each. The lowest number (2) was identified on chromosome 9 followed by chromosomes 5 and 10 with three markers. The percentage of polymorphism ranged from 5.12% (Chromosome 9) to 30.59% (Chromosome 12) with an average of 16.94% indicating that genetic variability for Telangana Sona and M229 was more on the 12th chromosome and very less on 9th chromosome. Similar polymorphism studies using 454 SSR primer pairs mapped on all the 12 chromosomes were used for testing polymorphism between parents APMS-6B and BF-16B was reported by Shruthi *et al.* (2016) [14].

Table 2: Chromosome wise percentage of SSR markers showing polymorphism between parents Telangana Sona and M229

Chromosome no	Total Number 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	49	13	41	26.531
2	41	8	36	19.512
3	38	5	28	13.158
4	39	10	36	25.641
5	30	3	16	10.000
6	79	14	75	17.722
7	35	4	29	11.429
8	37	6	35	16.216
9	39	2	36	5.128
10	29	3	23	10.345
11	36	6	23	16.667
12	42	13	29	30.952
	494	87		

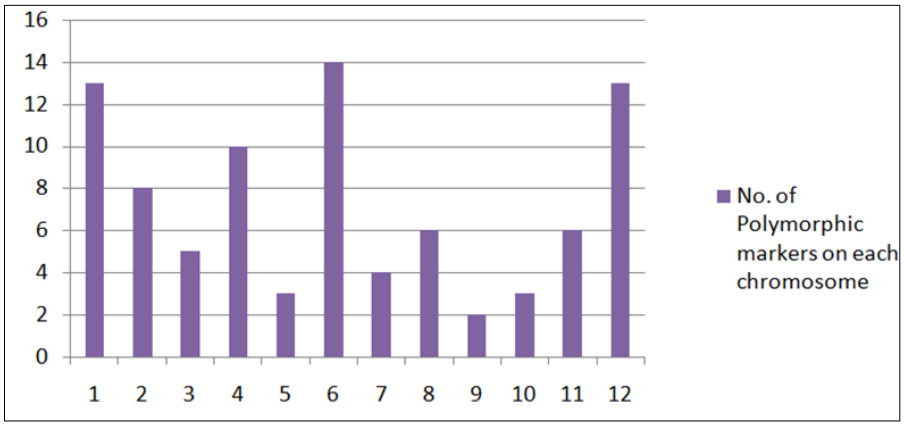

Fig 1: Histogram depicting the number of polymorphic markers on each chromosome between parents Telangana Sona and M229

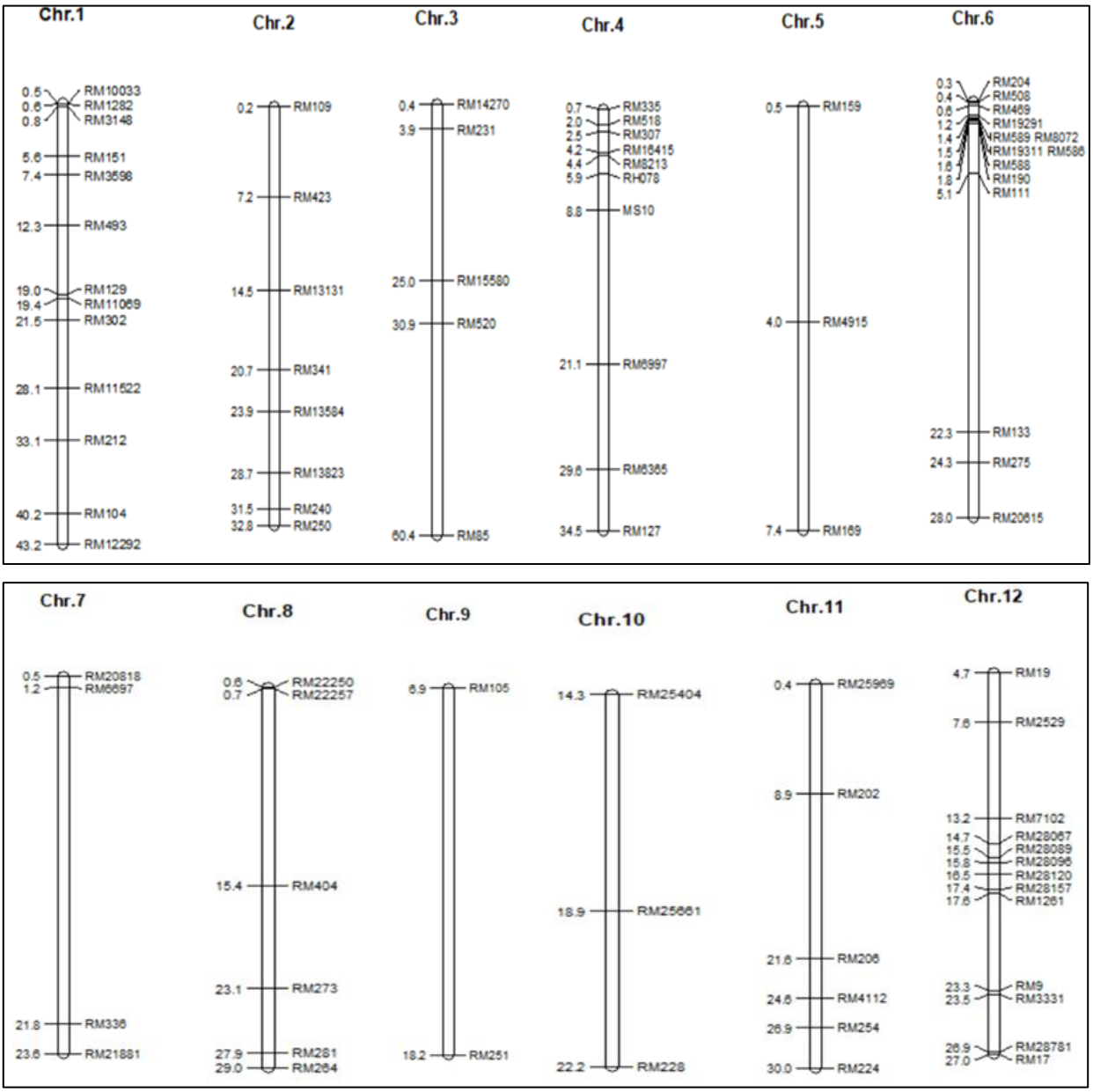

Fig 2: Chromosome wise distribution of polymorphic SSR markers between Telangana Sona and M229

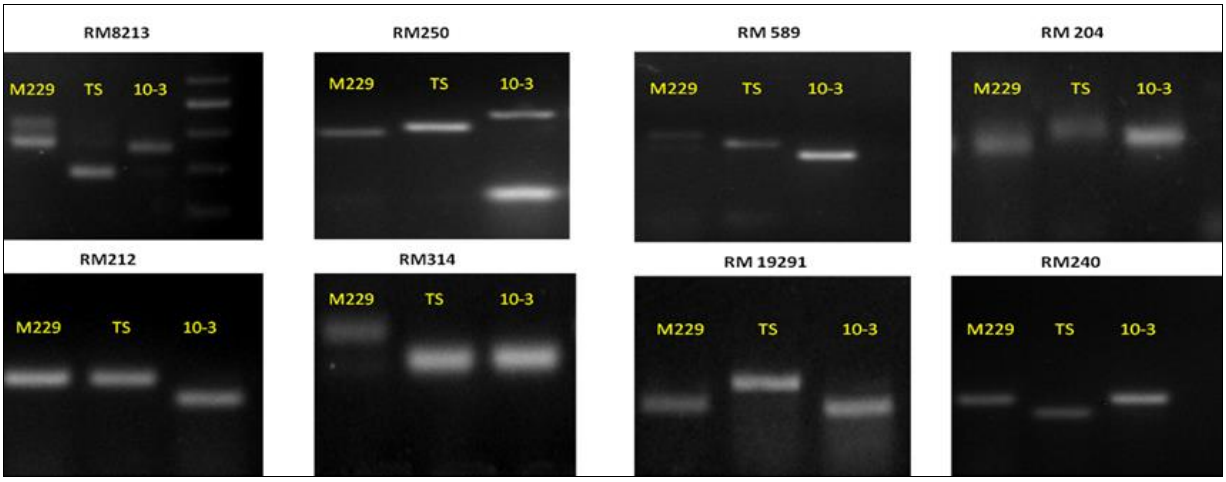

Fig 3: Polymorphism between the parents M229, Telangana Sona (TS) and 10-3 using SSR markers

Polymorphic survey between Telangana Sona and 10-3

For the parents RNR15048 and 10-3, the polymorphism survey indicated a clear distinction across all the chromosomes (Fig. 3). The same 494 SSR markers mapped on all the 12 chromosomes were used which detected a total of 93 polymorphic markers (Table 3, Fig. 5). The percentage of polymorphism ranged from 8.57% (Chromosome 7) to 30.61% (Chromosome 1) with an average of 17.97%. Similar

results for polymorphism percentage in rice were reported by Yerva *et al.* (2018) [15] and Dixit *et al.* (2018) [4] with 4-24% and 7-34% polymorphism respectively between the parents used in their study.

Table 3: List of SSR markers polymorphic between Telangana Sona and 10-3

S. No	CHR.	Marker	S. No	CHR.	Marker	S. No	CHR.	Marker
1	1	RM582	33	4	RM16373	65	7	RM21649
2	1	RM490	34	4	RM335	66	8	RM149
3	1	RM10009	35	4	RM17345	67	8	RM264
4	1	RM11135	36	4	RM16649	68	8	RM6925
5	1	RM10167	37	4	RM307	69	8	RM6699
6	1	RM3375	38	4	RM6365	70	8	RM264
7	1	RM428	39	4	RM127	71	9	RM242
8	1	RM9	40	4	RM261	72	9	9-18.1
9	1	RM5	41	4	RM8213	73	9	9-2.4
10	1	RM259	42	5	RM18939	74	9	RM24542
11	1	RM151	43	5	RM169	75	9	RM3808
12	1	RM3598	44	5	RM4915	76	9	RM105
13	1	RM3602	45	6	RM435	77	9	RM24199
14	1	RM5310	46	6	RM588	78	10	RM258
15	1	RM488	47	6	RM589	79	10	RM5271
16	2	RM13263	48	6	RM19410	80	10	RM25404
17	2	RM250	49	6	RM584	81	10	RM25661
18	2	RM6318	50	6	RM253	82	11	RM287
19	2	RM263	51	6	RM19902	83	11	RM26972
20	2	RM240	52	6	RM19660	84	11	RM26868
21	3	RM15630	53	6	RM19680	85	11	RM26352
22	3	RM16221	54	6	RM527	86	11	RM26829
23	3	RM15580	55	6	RM508	87	11	RM26972
24	3	RM231	56	6	RM7213	88	11	RM27096
25	3	RM15580	57	6	RM19291	89	11	RM206
26	3	RM14391	58	6	RM204	90	12	RM28424
27	3	RM251	59	6	RM314	91	12	RM558
28	3	RM16	60	6	RM8072	92	12	RM28157
29	3	RM218	61	6	RM469	93	12	RM247
30	3	RM273	62	6	RM8101			
31	3	RM347	63	7	RM21842			
32	4	RM16601	64	7	RM336			

15 markers on chromosome 1, five markers on chromosomes 2 and 8, 11 markers on chromosome 3, ten markers on chromosome 4, three markers on chromosomes 5 and 7, 18 markers on chromosome 6, seven markers on chromosome 9, four markers on chromosomes 10 and 12 and eight markers on chromosome 11 were found to be polymorphic between the parents (Table 4, Fig. 4). These results indicate that the genetic variability for Telangana Sona and 10-3 was more on the chromosome 1 and less on the chromosomes 5 and 7.

Table 4: Chromosome wise percentage of SSR markers showing polymorphism between parents Telangana Sona and 10-3

Chromosome no	Total Number 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	49	15	34	30.612
2	41	5	36	12.195
3	38	11	27	28.947
4	39	10	29	25.641
5	30	3	27	10
6	79	18	61	22.785
7	35	3	32	8.571
8	37	5	32	13.514
9	39	7	32	17.949
10	29	4	25	13.793
11	36	8	28	22.222
12	42	4	38	9.524
	494	93		

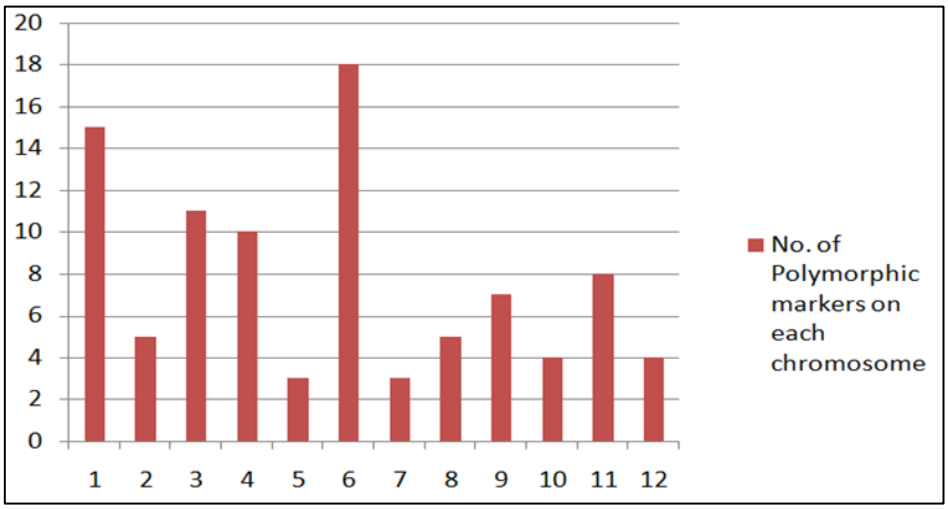

Fig 4: Histogram depicting the number of polymorphic markers on each chromosome between parents Telangana Sona and 10-3

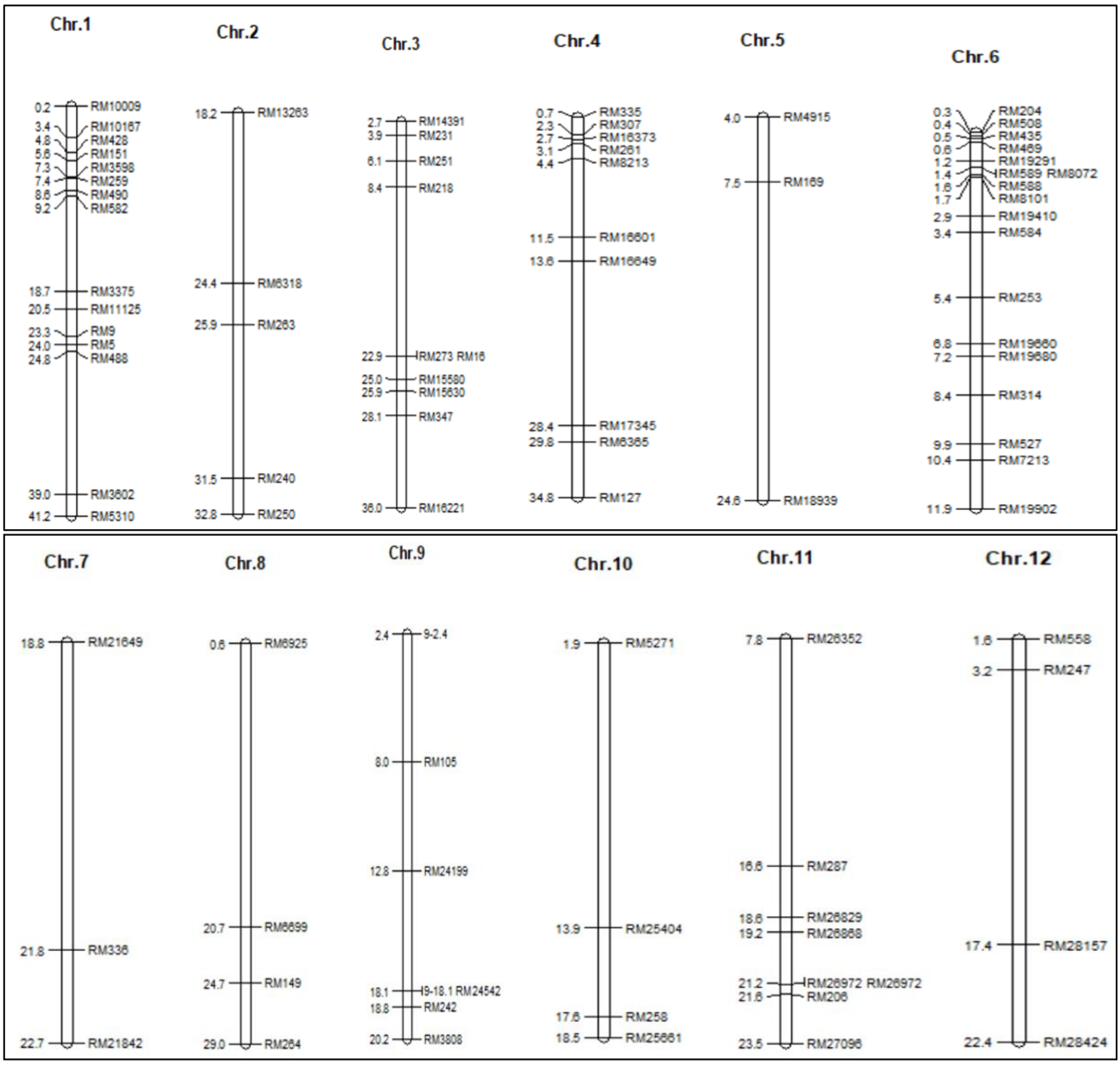

Fig 5: Chromosome wise distribution of polymorphic SSR markers between Telangana Sona and 10-3

Conclusion

In the present study, large number of parental polymorphic SSR markers (494) covering the entire genome, particularly focusing on the target chromosomes related with BPH resistance (i.e., Chromosomes 1, 4, 6, 12) were used. For Telangana Sona and M229, 87 markers were found to be

polymorphic exhibiting 17.1% polymorphism, while 93 were polymorphic between Telangana Sona and 10-3 with a polymorphism of 17.9%. Chromosome 6 detected the maximum number of polymorphic markers for all the three parents. Markers RM589, RM19291, RM231 and RM11135 were found as robust since their usage was high in many other studies related to BPH resistance. The polymorphic markers recognized will be helpful in mapping of Quantitative trait loci, ultimately identifying the genes responsible for understanding of genetic mechanisms of the variation underlying BPH resistance.

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