



RESEARCH ARTICLE

Identification of Novel QTLs for BPH Tolerance in Rice Using Resistant Donor BM 71

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Abstract

Rice is the most widely grown crop in the world, feeding half of the world's population. Brown plant hopper (BPH) is a considerable risk to rice fields carrying 20-90% yield losses. Hopper burn can be effectively managed by the recognition and use of BPH genes. Marker based genetic analysis of 136 RIL collected from a high yielding susceptible variety, MTU 3626 and BM 71, a BPH donor developed at RARS, identified 3 minor novel QTLs viz; *qmbph 2.1*, *qmbph 4.1* and *qmbph12.1* on chromosomes 2, 4 and 12 and two other QTLs on chromosome 5 and 7, namely *qmbph 5.1* and *qmbph 7.1*. The Phenotyping of RIL's revealed that ten RIL's (2711 – 31, 2711 – 37, 2711 – 50, 2711 – 69, 2711 – 84, 2711 – 88, 2711 – 94, 2711 – 100, 2711 – 168 and 2711 – 191) recorded yields comparable to checks, Swarna and Pushyami along with BPH score similar to donor. The BPH resistance lines recognised will be further evaluated, and the confirmed lines can be employed in rice breeding programs.

Keywords

RILs; BPH; SSR; QTLs

Introduction

Rice (*Oryza sativa* L.) is the major cereal food crops cultivated worldwide and also treated as "Global Grain". It belongs to Gramineae with a genome size of 430MB. Globally cultivated in 162.06 million ha with a produce of 500 million metric tonnes and average yield of 5.0 t/ ha (1). India has been the largest producer after China. In India, rice is being cultivated approximately in an area of 43.78 million hectares with a production of 118.43 million tones and productivity of about 2705kg/ha of milled rice (2).

Among the 65 species of plant hoppers found feeding on rice, brown plant hopper (BPH) *Nilaparvata lugen* (Stal) (Homoptera: Delphacidae) is devastating pest in all tropical rice growing areas in South Asia that causes damage to rice crop by sucking the cell sap from nutritionally rich phloem and occasionally from the xylem of plants. Infield conditions, an infestation of BPH spreads circularly and is technically called as "hopper burn". In rice unprecedented yield losses in Asian countries and several parts of India are due to out breaks of BPH in 1972, 1973 and 1974 (3). Resistant varieties development is an effective economical and eco-friendly method to manage brown plant hopper, in consequence it is requisite to identify brown plant hopper resistant genes and introgress them into rice cultivars through molecular tools (4, 5). To eradicate the BPH, many pest management approaches are available, including chemical control and developing field practises, but large usage of chemicals is unsafe for natural biodiversity and human health.

Till date, 37 BPH resistance genes were expressed from distinct resistance sources (6-8). Out of these, 20 genes were fine mapped and seven genes were cloned and characterised (BPH14, BPH17, BPH18, BPH26, BPH29, BPH9, BPH32 and BPH6 (9-17). Although remarkable attainments have been made so far by the identification and transfer of BPH-tolerant genes into popular varieties, quick transformation of virulent BPH populations is of primary concern (18). Hence, it is most important to identify novel genes or genetic variations from diverse genotypes to build resistance against virulent BPH populations. To address these problems, the current work was focussed on recognition of BPH genes in RIL population generated using BM 71 in the genetic background of productive variety MTU3626.

Materials and Methods

Plant material

Development of RIL population

MTU 3626, obtained from IR8/MTU3, is a long, bold grain of medium duration with high yield potential developed at RARS. Maruteru, which was used as a female parent, and BM 71, a resistant BPH donor, were used as male parents. Crosses were initiated between MTU 3626 and BM 71. The confirmed true F₁ plants were self-pollinated to produce F₂ seeds. The F₂ progeny were advanced via the single seed descent (SSD) method to the F₅ generation.

Phenotyping of RILs population for BPH screening

Mapping population of 136 RILs obtained from MTU 3626/ BM 71 was used for identification of BPH related QTL's. The 136 RILs, including parents, MTU 3626 and BM 71 and susceptible check TN1 were screened using standard seed box technique (SSBT) under laboratory conditions as well as under field condition as Maruteru is hotspot for BPH. Argo-morphological data was taken up on five plants per each RIL. Phenotyping data was noted for two seasons for five plants from each RIL along with parents and checks along with yield related traits (Table 2).

BPH population rearing and bioassay for resistance

Rearing of BPH was done by collecting adults of BPH and growing them on susceptible check (TN1). Seedlings of 2-3 leaf stage was infested with second and third instar

nymphs @10-12 nymphs/per seedling. Once the standard susceptible check started wilting (90%) data was documented as seedlings survival rate and finally expressed as per the SES score (standard evaluation system) (19). High BPH score with 9, indicates susceptibility and the lowest score 1, indicates the resistance of the genotype (Supplementary Table 2).

For Phenotyping of mapping population, seedlings of F₅ population (MTU 3626/ BM71) were planted in trays in 2 rows of 10 hills each. Five rows of test variety were planted alternating with 1 row of BM71 resistant check and susceptible check TN1. In addition, outskirts rows of the field were transplanted with TN1 seedlings to provide as bombardment rows of infestation to test seedlings. A complete resistance score (0-1) was given for progenies expressing 91-100% seedling survival, resistance score (2-3) was given for 70-90% seedling survival, scores of 4-7 was given for progenies with 11-75% survival and scores of 8-9 were given for progenies with complete susceptible and 0-10 % survival.

Genomic DNA extraction and genotyping of the RIL population

Extraction of genomic DNA was done from leaves of F₅ generation 142 RILs using CTAB method. DNA quantification was checked with spectrophotometer. PCR reaction of 15µl containing 50µg of DNA, 1x PCR buffer containing 10Mm Tris-HCL (pH 8.3), 50mm KCl, 1.5Mm MgCl₂, 0.01% (v/v) gelatine, 0.2Mm dNTPs, 5p mol of primer and 1U of Taq polymerase (Bangalore Genie, India). The PCR condition was set to 94°C of initial denaturation for 5 min, 35 cycles of denaturation at 94°C for 30s, Annealing for 30s at 55°C±20°C, extension at 72°C for 1 min and final extension at 72°C for 5 min. The PCR product were fractioned in a 3% agarose gel (Bangalore, Genei) in electrophoresis unit (Bio Rad, USA), stained with 0.5µg/ml ethidium bromide, visualised under UV light and was documented. A total of 315 SSR markers were used to study parental polymorphism between susceptible parent MTU 3626 and resistant parent BM 71 across 12 chromosomes. Out of 315 SSR markers studied, only 77 markers were identified as polymorphic and also used for QTL mapping. Fragments which were distinct and clear, amplification were considered for scoring with 100bp ladder.

Table 2. Mean performance of RILs for yield and yield attributing traits

Sl.No.	RIL name	DFF	Plant height (cm)	Ear bearing tillers per plant	Panicle length (cm)	SES score for BPH		Grain yield per plant
						In field conditions	In lab conditions	
1	2711-31	99.00	100.60	8.00	29.00	3.70	3.52	17.90
2	2711-37	93.00	119.00	8.00	28.10	3.90	4.20	20.36
3	2711-50	99.00	133.10	8.00	24.90	2.75	2.96	16.65
4	2711-69	97.00	142.80	7.00	29.50	4.95	3.23	16.05
5	2711-84	97.00	142.40	8.00	28.00	1.90	2.30	16.65
6	2711-88	98.00	138.10	8.00	29.90	2.65	4.95	19.85
7	2711-94	93.00	123.20	8.00	26.00	4.65	4.12	16.60
8	2711-100	96.00	147.90	8.00	29.95	2.40	2.50	17.65
9	2711-168	93.00	140.20	8.00	30.75	3.20	3.85	16.38
10	2711-191	99.00	112.00	8.00	32.10	2.40	1.45	16.15
11	MTU 7029	119.00	106.00	8.00	25.40	-	-	16.00
12	MTU 1075	113.00	114.70	9.00	26.70	-	-	17.80

Linkage map studies and QTL identification

QTL mapping was based on the expectation that linkage disequilibrium between the marker and a chromosomal region effecting the trait under study. Such linkage disequilibrium inferred depends on likelihood ratio (LR). In this study, QTL Ici Mapping Version 4.1 software was used to identify QTLs by using interval mapping (QIM) and Composite Interval Mapping (QIC). Composite Interval Mapping uses two flanking markers to construct an interval to search for QTL. A LOD score was calculated at each walking step in the intervals. When a peak exceeds the threshold value, it was observed that QTL has been found at that location (Supplementary Table 4 & Supplementary Fig. 1).

Results

Phenotypic evaluation of RIL population

Evaluation of RIL population was done by both SSBT and field screening. Screening results under standard seed box technique showed 44 RILs had a score of 1-3, 62 RILs with 3-5 score, 30 RILs with 5-7 and 6 RILs showed susceptible score 7-9, where as in field condition 67 RILs showed 1-3 score, 34 RILs showed score of 3-5, 21 RILs with score of 7-9 scores (Figure 1&2). BPH score for tolerant lines was presented in Table No. 1. Furthermore, ten RILs, 2711 – 31, 2711 – 37, 2711 – 50, 2711 – 69, 2711 – 84, 2711 – 88, 2711 – 94, 2711 – 100, 2711 – 168 and 2711 – 191 indicates BPH resistance with score less than 5.0 in both the screening methods and higher grain yield than the yield checks, MTU 7029 (Swarna) and MTU 1075 (Pushyami) (Table 1; Fig. 1 & 2).



Layout for standard seed box method



Hopper burn in seed box

Fig. 1. Seedling screening of RILs against BPH tolerance in lab conditions at RARS, Maruteru

Table 1. BPH tolerant lines with 1-3 score in both SSBT and field score given

SL.NO	BPH TOLERANT RIL	SSBT SCORE	FIELD SCORE	SL.NO	BPH TOLERANT RIL	SSBT SCORE	FIELD SCORE
1	2711-2	2.90	1.50	15	2711-84	1.90	1.90
2	2711-19	3.60	3.40	16	2711-89	2.95	2.70
3	2711-24	3.65	2.20	17	2711-104	1.29	2.70
4	2711-25	1.52	2.40	18	2711-105	2.96	1.10
5	2711-27	3.21	2.45	19	2711-106	1.92	1.23
6	2711-28	2.56	1.90	20	2711-108	2.56	1.30
7	2711-32	2.92	1.60	21	2711-113	1.86	1.34
8	2711-50	2.75	2.75	22	2711-120	2.96	1.2
9	2711-56	1.33	1.33	23	2711-122	2.85	1.12
10	2711-57	1.80	1.80	24	2711-123	1.00	1.02
11	2711-58	1.08	1.08	25	2711-129	1.00	1.4
12	2711-59	2.30	2.30	26	2711-133	2.98	1.5
13	2711-75	1.10	1.10	27	2711-175	2.95	1.70
14	2711-78	1.14	1.14	28	2711-188	1.22	1.06



Layout for field screening



BPH Burning of RILs

Fig. 2. Field screening of RILs against brown plant hopper in natural field conditions, during Kharif, 2018

Table 3. Analysis of variance (mean sum of squares) for yield and yield contributing in RILs and checks during Kharif, 2018

Source of variation	Degrees of freedom	Days to 50% flowering	Plant height (cm)	No of ear bearing tillers	Panicle length (cm)	Grain yield per plant (g)
Replications	1	32.00	5.95	9.28	3.42	8.35
Blocks with in replication	22	9.91	59.92	1.46	4.04	2.06
Treatments	143	39.77**	413.08**	2.83**	10.67**	12.21**
Intra block error	121	4.16	46.10	0.85	3.85	1.26
Total	287	22.44	229.87	1.91	7.26	6.80

*Significant at 5% level; **Significant at 1% level

2).

Statistical analysis on trait performance of RILs

The ANNOVA indicated presence of notable distinctness among the 142 RILs derived from MTU3626/BM71 together with two checks MTU 1075 (Pushyami) and MTU 7029 (Swarna) for five characters (DFF, PH, EBT, PL, GYP) at 1% level of significance indicating considerable difference in the RILs population (Table 3).

Genetic parameters of yield and yield contributing characters in RILs

Phenotype Coefficient Variation (PCV) and Genotype

Coefficient Variation (GCV) estimation will provide information regarding non-heritable and heritable portion of differences in the material under study. High broad-sense heritability (H^2) was noticed for all traits with a range of 31.93-89.68 in the RIL population. Low PCV (6.51) and GCV (4.36) were reported for days to 50% flowering indicating less variability, for plant height a moderate PCV (15.57) and low GCV (10.18), for ear bearing tillers/plant moderate values of PCV (19.36) and GCV (11.44), for panicle length moderate PCV (11.47) and low GCV (6.49), for grain yield/plant both high PCV (26.63) and GCV (25.21) indicating genetic deviation among the RILs studied (Table

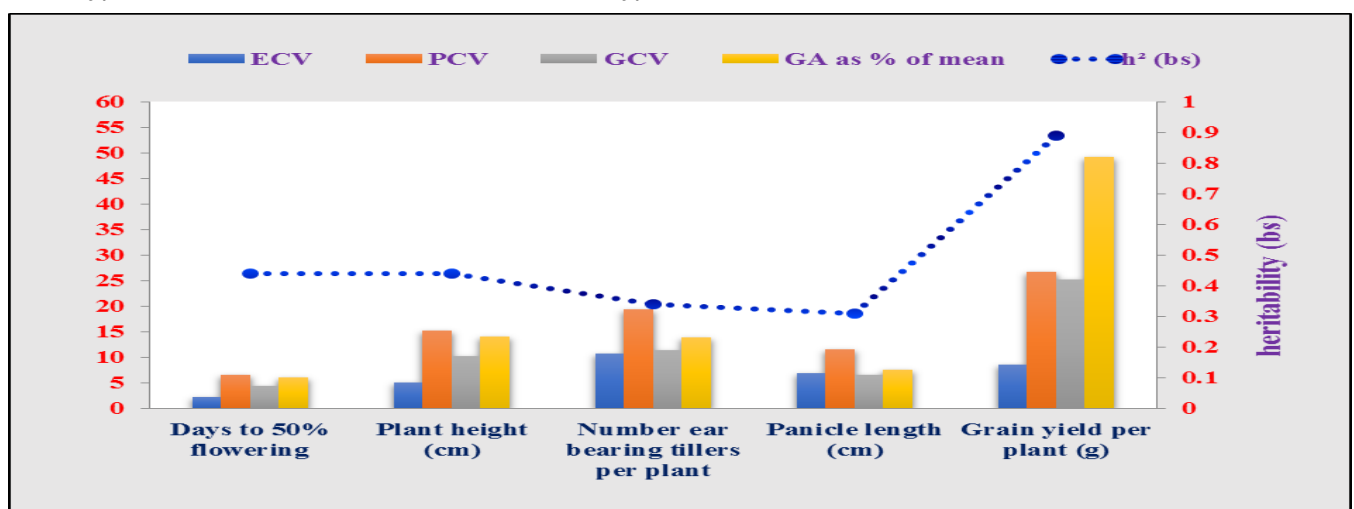


Fig. 4. Variability parameters for five characters studied in 142 RILs and two check

Table 4. Estimates of genetic variability parameters for evaluation of yield and yield contributing characters in RILs during Kharif, 2018

Character	Mean	Range		PCV	GCV	H2	GA	GA as % of mean
		Minimum	Maximum					
Days to 50% flowering (DFF)	96.00	91.00	107.00	6.51	4.36	44.77	5.83	6.03
Plant height (cm) (PH)	133.05	100.60	172.00	15.27	10.18	44.42	18.62	14.00
No of ear bearing tillers per plant (EBT)	8.70	7.00	14.30	19.36	11.44	34.90	1.22	13.94
Panicle length (PL)	28.49	12.00	32.15	11.47	6.49	31.93	2.16	7.56
Grain yield per plant (GYP)	13.13	4.05	20.36	26.63	25.21	89.68	6.47	49.25

4; Fig. 4).

For days to 50% flowering moderate heritability (44.77%) coupled with low GAM (6.03), for plant height both heritability (44.42%) and GAM (14.00), for ear bearing tillers/plant heritability (34.90%) and GAM (13.94), were moderate revealing the existence of both additive and non-additive gene actions, for panicle length moderate heritability (31.93%) and low GAM (7.56), for grain yield/plant high heritability (89.68%) and high GAM (49.25) indicated presence of additive gene action.

Correlation studies for yield attributing traits and BPH

Correlation studies are important in any crop improvement programme. Days to 50% flowering had significant positive association with ear bearing tiller and grain yield plant and plant height had significant association with panicle length. Ear bearing tillers had significant association with grain yield indicating RIL's with more tillers gives higher grain yield per plant, whereas there was a negative correlation for grain yield with BPH

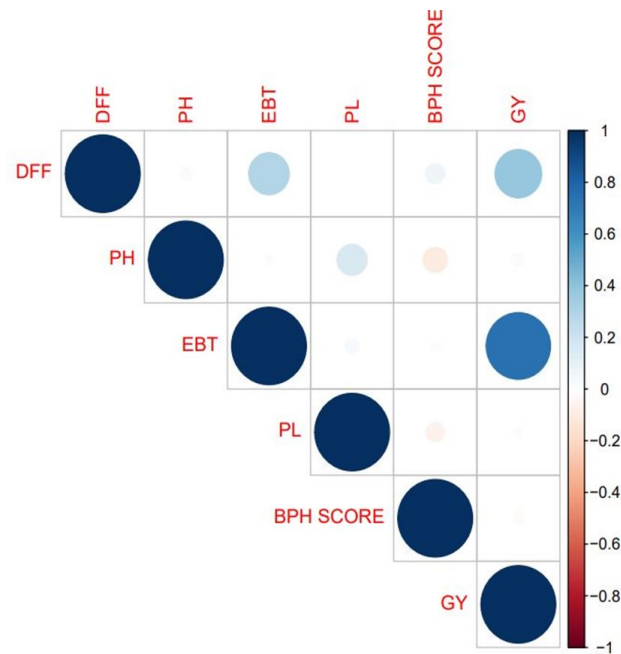


Fig. 3. Phenotypic correlation analysis for yield attributing traits and BPH

Table 5. Phenotypic correlation coefficients of yield and yield attributing traits in rice

	DFF	PH	EBT	PL	BPH	GY
DFF	1	0.026 4	0.295* *	0.0019	0.0653 55	0.3887 *
PH		1	0.015 23	0.161* *	- 0.1064 4	0.0243 6
EBT			1	0.0351 48	0.0168 91	0.7412 **
PL				1	- 0.0608 8	0.0103 88
BPH					1	- 0.0238 5
GY						1

*Significant at $\alpha=0.05$; **significant at $\alpha=0.01$; ***significant at $\alpha=0.001$
DFF: Days to 50% flowering; PH: Plant height; EBT: Ear bearing tillers;
PL: Plant length; BPH: Brown plant hopper; GY: Grain yield

(Table 5 & Fig. 3).

QTL mapping

Parental polymorphism

Parental polymorphism was studied between MTU 3626 and resistant parent BM71 using 315 SSR markers spanning on all the 12 chromosomes.

Parental polymorphism studies were done using 315 SSR markers spanning on all the twelve chromosomes between susceptible parent MTU 3626 and resistant parent BM 71. Among 315 SSR markers screened, 77 SSR markers showed polymorphism. The percent of polymorphism was 24.44%. Highest polymorphism was observed on chromosome 3 with eleven markers, while the least polymorphism was on chromosome 8 and 9 with four markers each (Supplementary Table 1, 3; Fig. 5).

Linkage map construction

Construction of linkage map was done using data on 77 polymorphic SSR markers for 136 RILs with Kosambi mapping function by QTL Ici Mapping Version 4.1 based on their positions in cM. For identification of QTL, CIM uses two flanking markers to construct an interval to search for QTL. A LOD score was calculated at each walking step in the intervals (Supplementary Fig. 1). QTL was identified when the peak exceeds the threshold value at that location. The linkage map covered 3433.74 cM involving all chromosomes with an average marker interval of 44.59 cM (Supplementary Table 4 & Supplementary Fig. 2).

Identification of novel QTLs for BPH resistance

In the current study, by composite interval mapping three QTLs were identified one on each on chromosome 2, 4 and 12. Apart from this using interval mapping 2 more QTLs were identified one on chromosome 5 and chromosome 7.

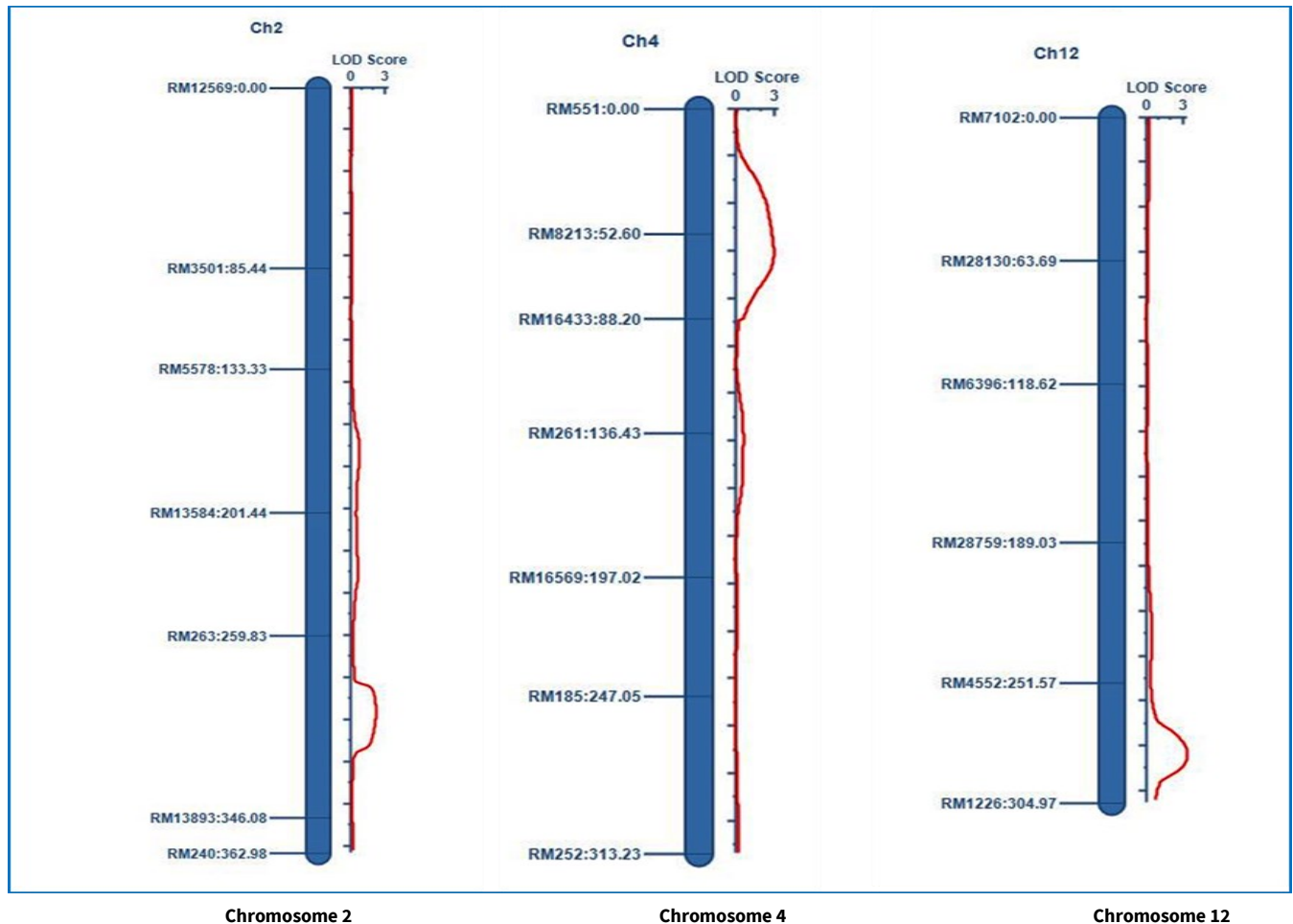
A minor QTL *qmbph 2.1* on chromosome 2 was flanked by the left marker RM263, positioned at distance of 25.86 cM and right marker RM13893, positioned at 29.72 cM with phenotypic variance of 5.96% and LOD score of 2.17 and the QTL positive additive effect at an interval of 3.86 cM.

The QTL *qmbph4.1* on chromosome 4 was flanked by the left marker RM8213 (4.44cM) and right marker RM16433 (4.62 cM) with phenotypic variance of 1.70% and LOD value of 2.91 and the QTL contributed by BM 71, position at an interval of 0.18cM. While, *qmbph12.1* on chromosome 12 flanked by left marker RM4552, positioned at distance of 26.94cM and right marker RM1226, positioned at a distance of 27.37cM with phenotypic variance of 5.76% and LOD score of 3.31 and the QTL contributed by MTU 3626, with an interval 0.43cM (Table 6; Fig. 6).

A minor QTL, using interval mapping *qmbph5.1* on chromosome 5 flanked by left marker RM163 (19.18 cM) and right marker RM3160 (19.98 cM) with phenotypic variance of 4.40% and LOD score of 2.91 and the QTL contributed by BM 71, with position at distance of 0.80cM interval and *qmbph7.1* on chromosome 7 flanked by left marker RM500 (15.91cM) and right marker RM336 (21.87 cM) with phenotypic variance of 4.42% and LOD score of

Table 6. QTLs identified for tolerant to BPH in the RIL population derived from cross MTU 3626 and BM 71 by composite interval mapping

Trait	QTL	Flanking markers		chr	Start position	End position	LOD peak		PVE (%)		Add	
		LB	RB				QIC	QIM	QIC	QIM	QIC	QIM
BPH	<i>Qmbph 2.1</i>	RM263	RM13893	2	25.86	29.72	2.17	4.21	5.96	4.46	1.73	1.98
BPH	<i>Qmbph 4.1</i>	RM8213	RM16433	4	4.44	4.62	2.91	2.91	1.70	1.03	0.89	0.89
BPH	<i>Qmbph 12.1</i>	RM4552	RM1226	12	26.94	27.37	3.31	5.95	5.76	4.42	1.70	1.97
BPH	<i>Qmbph 5.1</i>	RM163	RM3160	5	19.18	19.98	-	2.91	-	4.40	-	1.93
BPH	<i>Qmbph 7.1</i>	RM500	RM336	7	15.91	21.87	-	2.81	-	4.42	-	1.97

**Fig. 6.** Linkage map and LOD curve for chromosomes 2, 4 and 12 using composite interval mapping

2.81 and the QTL contributed by BM 71, with position at distance of 5.96cM interval. Further, the RILs (2711-31, 2711-37, 2711-50, 2711-69, 2711-84, 2711-88, 2711-94, 2711-100, 2711-168 and 2711-191) were at par with the high yielding checks, Swarna and Pushyami coupled with resistance to BPH score similar to BM 71, the resistant RILs promoted for evaluation in yield trials (Table 1).

Discussion

BPH is the major pest of the rice crop causing severe infection hopper burn, resulting in death of rice plants (20). Only few resistant donors available for BPH resistance till date. Identification of QTLs from resistance donors is essential, which help in breeding programmes. The BPH resistance source was from BM 71 which shows resistance to BPH in both field screening and laboratory screening

from many years. MTU 3626 is susceptible to BPH but commercially cultivated rice variety in Andhra Pradesh. Hence, identification of QTLs for BPH tolerance using BM71 in the genetic background of MTU3626 will help in subsequent breeding program.

Phenotyping of 142 RILs for BPH tolerance was done by using both by standard seed box technique (seedling screening) and field screening. Further, 10 RILs showed resistant to BPH with score of less than 5.0 in both the screening methods and highest grain yield than both the checks MTU 7029 (Swarna) and MTU 1075 (Pushyami).

Correlation analysis were studied to find out association between characters for yield related traits and BPH score. Ear bearing tillers had significant correlation with grain yield suggesting RIL's with more tillers gives higher grain yield per plant. Results presented by (21) for grain yield/plant at both phenotypic and genotypic levels

was similar. Whereas, there was a negative association between grain yield and BPH, indicating that when there is a high incidence of BPH, there is damage to the entire crop and a low yield.

SSR markers used to recognise the targeted regions coupled with trait of interest in genome of rice. Polymorphism percent ranged from 7% to 90% (22) was reported in different studies with a donor of *O. rufipogon* and three recurrent parents of *indica* (23) conducted polymorphism survey with 200 SSR markers between Swarna and PTB 33 and only 21 SSR markers considered as polymorphic. The identified QTL *qmbph2.1* on 2nd chromosome was far away from BPH 13(t) gene identified by (24) in a study involving resistant parent *Oryzaeichingeri* and susceptible parent 02428. Two QTLs associated on chromosome 2 earlier viz., *Qbph3* (25) and *BPH 13(t)*. *qmbph4.1* was in the domain of *BPH 20(t)* reported by (26) in a study on IR71033-121-15 and Junambyeo F₂ population. Identified QTL *qmbph12.1* was away from the QTL reported by (27) in a study involving introgression lines between IR31917-45-3-2 and *O. Australianises* (Acc. No. 100882).

Interestingly, about half of the resistance genes for BPH were identified and mapped to chromosomes 4 and 12 using SSRs. Eight genes, *Bph3* (28), *Bph6* (29), *Bph12* (30), *Bph15* (31), *Bph16* (32), *Bph17* (33), *Bph20* (26) and *Bph27* (34) were linked to chromosome 4 while remaining genes, *Bph1* (35), *Bph2* (36), *Bph9* (37), *Bph10* (38), *Bph18* (27), *Bph19* (39), *Bph21* (40) and *Bph26* (10) were represent on chromosome 12, indicates these two chromosomes harbour hot-spots for resistance to BPH.

Conclusion

The current study identified five minor QTLs located on chromosomes 2, 4, 5, 7 and 12 by mapping studies. The developed RILs showed not only resistance to BPH score similar to BM 71, but also gave higher yield, character of parent MTU 3626 on par with yield checks Swarna and Pushyami. These QTLs have to be fine mapped by using a greater number of polymorphic markers in the identified regions. The resistant RILs may be promoted for evaluation in yield trials. To overcome susceptibility to pests and yield barriers novel OTLs possibly introgressed into distinctive genetic backgrounds to understand complex interactions among QTLs so as to ensure food security and deeper for trait development.

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Authors contributions

SKG carried out experiment, Phenotyping, genotyping and QTL analysis as the part of M.Sc. thesis work and preparation of manuscript. BNVS RK participated in design of the study supervised the work and revised the manuscript. BVL had given guidance in preparation of the manuscript. MNK supervised in BPH screening of plant material. PVR participated in manuscript corrections and suggestions. VS in manuscript revision and finalization. TS supported to carry out the presented work.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

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