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RESEARCH ARTICLE

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Meta-QTL analysis identified stable quantitative trait loci (QTLs) and associated resistance gene analogues in rice

HED Shashiprabha[1], SP Abeysundara[1] and HACK Ariyarathna[2*]
[1] *Department of Statistics and Computer Science, Faculty of Science, University of Peradeniya, Peradeniya.*
[2] *Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya.*

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Abstract: Disease resistant rice varieties are mandatory for sustainable rice production to ensure the global food supply. This study aimed to identify meta-QTLs and associated with disease resistance and candidate R-genes. A consensus map was constructed by merging 9 QTL mapping studies, 115 QTLs and 943 markers. The consensus map was 1554.71 cM in length with a 1.65 cM-1 average marker density. Totally, 24 meta-QTLs were projected on the consensus chromosome maps except on chromosomes 5, 6, and 10. Larger effect meta-QTLs (R2 ≥ 25 to 56 %) were reported on chromosomes 1, 2, 3, 4, 8, and 11. Three or more meta-QTLs were predicted on 1, 2, 7, 8, and 9 chromosomes. More than 62 % of the meta-QTLs were contributed by 3 or more mapping studies. Furthermore, 6 of the putative meta-QTLs collocated with 11 meta-QTLs from previous studies, and 23 resistance loci that are used in existing resistance breeding programs indicating robust expression. Nine meta-QTLs were associated with blast (BL) resistance whereas 14 were associated with both BL and sheath blight (SHB) resistance. Candidate gene mining identified 79 disease resistance gene analogues (RGAs) of which 74 % were complete putative R genes. Nine of the meta-QTLs predicted clusters of RGAs. Out of these, expression of LOC_Os11g12340 was cross-validated using publicly available expression data in tissues infected with *M. oryzae,* indicating a functional role of this gene in the disease cycle. The outcome clearly exposed genomic abundance of R-genes and their potential functional redundancy. Further, these findings provide potential resources for rice breeding for disease resistance.

Keywords: BioMercator, Gramene, meta-QTLs, PRGdb, resistance genes, *Rice Genome Annotation Project.*

INTRODUCTION

Rice, the staple food for more than half of the global population provides 20 % of the daily human calorie intake (*http://rice.plantbiology.msu.edu/*), and is an integral component of global food and nutrition security. Rice is susceptible to over 70 pests and diseases that collectively cause 37 % yield reduction (*http://www.knowledgebank.irri.org/*). The impact is significant in food-deficit regions and food security hotspots in tropical Asia, where over 90 % of the total global rice is produced and consumed (Sharma *et al.*, 2012). Blast (BL), bacterial blight (BB), and sheath blight (SHB) are the most destructive diseases in rice worldwide (Gnanamanickam, 2009). BL, a fungal disease caused by *Magnaporthe oryzae,* infects all parts of the rice plant and can result in yield reductions of 10 - 60 % (Wang *et al.*, 2014). BB is due to infection by *Xanthomonas oryzae*. The disease causes yield losses up to 50 % and also affects rice grain quality (Liu *et al.*, 2014). Losses due to SHB, a disease caused by the soil-born fungus *Rhizoctonia solani*, can be as high as 10 to 30 % in severely infected fields (Banniza *et al.*, 2001; Sreenivasaprasad, 2004; Boukaew & Prasertsan, 2014; Liu *et al.*, 2014). Application of synthetic fungicides for the management of these diseases incurs both economic and environmental losses (Nalley *et al.*, 2016). Developing host resistance in elite cultivars provides cost-effective solutions to increase

* Corresponding author (chandimaariyarathna@pdn.ac.lk; https://orcid.org/0000-0002-1860-7754)

rice production and profits at minimal cost and damage to the environment. Disease-resistant varieties are vital components of sustainable rice cultivation to ensure global food supply and therefore genetic improvement for host resistance to BL, BB, and SHB is among the priority objectives of rice breeding programmes worldwide.

Genes and QTLs associated with the multilayers of innate defence mechanisms are potential resources for resistance/tolerance breeding of crops. Over 350 resistance loci that are associated with BL were mapped on rice (Miah *et al.*, 2013; Su *et al.*, 2015; Xiao *et al.*, 2017). Among these 27 allelic QTLs have been fully sequenced; *Pib, pb1, Pita, Pi9, Pi2, Pizt, Pid2, Pi33, Pii, Pi36, Pi37, Pikm, Pit, Pi5, Pid3, Pid3-A4, Pi54, Pish, Pik, Pikp, Pia, PiCO39, Pi25, Pi1, Pi21, P50* and *Pi650* (Miah *et al*, 2013; Liu *et al.*, 2014; Su *et al.*, 2015). Moreover, up to 200 QTLs that are associated with SHB resistance and 14 QTLs associated with BB are reported (Zarbafi & Ham, 2019). While some of these were discovered in isolated research, others were discovered in a variety of settings and experiments (Zarbafi & Ham, 2019). Rice breeding schemes, in which major effect resistance QTLs such as *Pib, Pita, Pia, Pi1, Pikh, Pi2* and *Pi4* , or minor effect resistance QTL alleles including *Pi-1, Pi2, Pi9, Pi20, Pi27, Pi39, Pi40* and *Pikh* were deployed in gene pyramids, were successful in producing race-specific or broad-spectrum resistance (Narayanan *et al.*, 2002; Ballini *et al.*, 2008; Gnanamanickam *et al.*, 2009; Djian- Caporalino *et al.*, 2014; Ellis *et al*., 2014; Ordon & Kühne, 2014; Jia *et al.*, 2019; Zarbafi & Ham, 2019). However, shifts in strains of the pathogen populations due to mutations result in frequent "resistance breakdown." Therefore, continuous searching and stacking of multiple loci or genes that are associated with disease resistance through strategic breeding schemes are important to sustain breeding activities for steady delivery of disease-resistant rice varieties.

Resistance genes (R-genes) interact with pathogen avirulence genes (*Avr*) and initiate signaling cascades to activate defence mechanisms. R-genes are vital elements of the host resistance mechanisms and are successfully used in cereal resistance breeding programmes (Miah *et al.*, 2013; Bouktila *et al.*, 2014; Zhang *et al.*, 2015). Conserved structural motifs and protein domains enable computational identification of R-genes by peptide sequence alignments and searching for structural similarity (Michelmore & Meyers, 1998). Thereby a large class of potential R-genes, known as Resistance Gene Analogs (RGAs), were predicted and mapped in rice (Miah *et al.*, 2013; Sekhwal *et al.*, 2015) and the orthologs of the rice RGAs were identified in wheat (Bouktila *et al.*, 2014), maize (Xiao *et al.*, 2007), rye (Dracatos *et al.*, 2010) and finger millet (Reddy *et al.*, 2011; Babu *et al.* 2014). The annotated rice genome and transcriptome data provide opportunities for the discovery of novel RGAs and these resources are increasingly investigated in search of RGAs as gene candidates for disease resistance.

Meta-analysis of QTLs integrates information from multiple QTL mapping studies into a single consensus map to enable contraction of QTL intervals and identification of robust and reliable QTLs. Thereby meta-QTLs identify consensus QTLs that are expressed across different environments, and genetic backgrounds and these stable QTLs are highly desired for their applicability in breeding (Goudemand *et al.*, 2013; Hamon *et al.*, 2013). Meta-QTL analysis identified genetic determinants of complex traits in a variety of crops, including yield, fiber quality, drought tolerance, and disease resistance in rice (Khowaja *et al.*, 2009), cotton (*Gossypium hirsutum* L.) (Said *et al.*, 2013), maize (*Zea mays* L.) (Semagn *et al.*, 2013), pea (*Pisum sativum* L.) (Hamon *et al.*, 2013), wheat (*Triticum aestivum* L.) (Griffiths *et al.*, 2009) and mustard (*Brassica juncea* L.) (Yadava *et al.*, 2012). This study aimed to predict meta-QTLs associated with BL, BB, and SHB resistance in rice. A consensus genetic map with high marker density was constructed to identify meta-QTLs with narrow genomic intervals. Candidate genes that collocate with the meta-QTL regions were predicted and cross-validated using genomic, and transcriptomic data available in the public domain. The findings of the study will aid in increacsing prepision of QTL mapping, the identification of candidate genes, and the generation of markers for effective marker-assisted selection (MAS) programs.

MATERIALS AND METHODS

QTL studies used for meta-QTL analysis

Rice QTLs associated with BL, BB, and SHB resistance were mined in the Gramene QTL database (*https://archive.gramene.org/qtl/*). In total, 9 mapping studies for disease resistance were selected from the Gramene QTL database (Table 1). The 95 % confidence interval (CI) of the QTLs predicted on backcross or F2 mapping population was estimated using the equation 95 % CI = $530/(NR^2)$ (Darvasi, 1997), whereas the equation 95 % CI = $163/(NR^2)$ was used for QTLs predicted on recombinant inbred line mapping population (Guo *et al.*, 2006). Here N is the population size and R^2 is the proportion of phenotypic variance explained by the QTLs. R (version 3.5.3) language was used for data web scrapping and preprocessing.

Table 1: Details of the nine rice QTL maps accessed through Gramene QTL database (*https://archive.gramene.org/qtl/*) and used in the meta-analysis of QTLs associated with resistance to blast, bacterial blight and sheath blight diseases in rice

Population	Population size	No.of markers on the map	Number of disease resistance QTLs mapped on each chromosome												Reference
			1	2	3	4	5	6	7	8	9	10	11	12	
Lemont × Teqing	284	244	-	2	2	2	-	2	1	-	2	-	-	2	(Tabien *et al*., 2002)
Jasmine × Lemont	128	76	-	2	1	-	-	-	1	-	2	-	2	-	(Zou *et al.*, 2000)
IR64 × Azucena	116	170	-	-	-	-	1	-	-	1	-	-	-	-	(Ramalingam *et al.*, 2003)
Lemont × Teqing	255	111	-	3	3	-	-	-	-	3	3	-	-	3	(Li *et al*., 1995)
Nipponbare × Owarihatamochi	146	110	-	-	-	2	-	-	-	-	1	-	-	1	(Fukuoka and Okuno, 2001)
Bala × Azucena	205	114	2	1	-	2	-	-	4	-	1	3	3	-	(Talukder *et al*., 2005)
IR64 × Azucena	105	322	-	-	-	-	-	1	-	1	-	1	-	3	(Sallaud *et al*., 2003)
Moroberekan × CO39	281	125	2	-	1	-	1	2	1	1	-	-	-	1	(Wang *et al*., 1994)
Zhenshan × Minghui	241	234	17	11	1	-	-	-	4	4	5	-	-	-	(Chen *et al*., 2003)

Construction of consensus map & QTL projection

A consensus map was constructed using BioMercator v4.2 (*http://moulon.inrae.fr/logiciels/biomercator/*) with default parameters. Nine selected rice mapping studies were projected on the map. The method implemented in BioMercator applies a classical weighted least square (WLS) strategy. Chromosomes that share less than two common markers between the mapping studies were excluded. The QTLs were projected on the consensus map using a simple scaling rule between the original QTL flanking marker interval and the corresponding one on the consensus chromosome. Projection of the initial QTLs on the high-density consensus map was based on LOD scores, phenotypic variation explained by each QTL, 95 % CIs, and QTL flanking marker positions. The 95 % CI of the QTLs that were projected on the consensus map were approximated using Gaussian distribution.

Meta-QTL analysis

The meta-analysis of QTLs was performed using the QTL clustering-based approach proposed by Veyrieras *et al.* (2007) using BioMercator v4.2. The distribution of QTLs is viewed as a Gaussian mixture model and parameter estimates were obtained using the Expectation-Maximization algorithm. The lowest Akaike-Information Criterion (AIC) was used to determine the best model for each chromosome. The accuracy of QTL model selection using AIC was studied by a simulation in the Veyrieras method (Veyrieras *et al*., 2007). The meta-QTLs thereby predicted were compared with meta-QTLs and QTLs associated with BL, SHB, and BB resistance, available in the public domain.

Identification of candidate genes

The nearest markers of 95 % CI of meta-QTLs were identified. Physical positions of the nearest markers were retrieved from the Gramene marker database (*https://archive.gramene.org/markers/*). The Rice Genome Annotation Project (*http://rice.plantbiology.msu.edu/*) was studied for the putative genes, the encoded proteins, and the functional annotation of the genes associated with the meta-QTLs, based on physical locations of the nearest markers.

The sequences of the putative R-genes were web scraped from Rice Genome Annotation Project and then used in BLAST search in the PRGdb (*http://prgdb.org/prgdb/*) to predict the domain types. The expression of the R-genes was studied in the susceptible and resistant varieties using expression libraries available from NCBI Sequence Read Archives: SRR074129, SRX032224, SRR074133, SRR074134, SRR074135, and SRR074136. Genes were reported 'expressed' when at least one sequence read mapped uniquely within an exon of the target gene. All steps of identification of candidate R-genes were carried out using R (version 3.5.3) language.

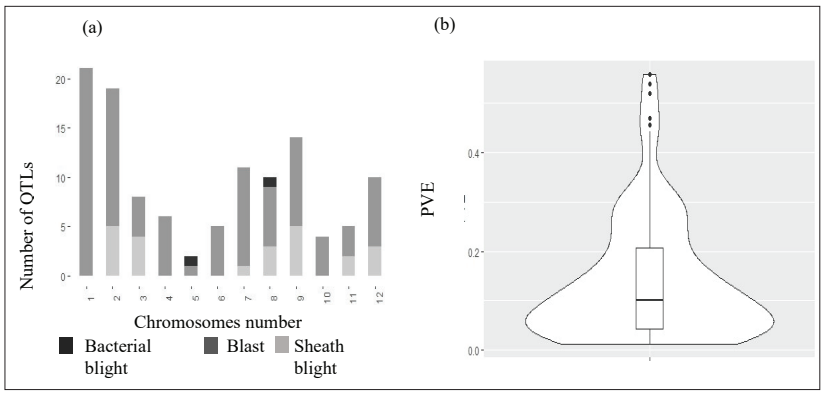


Figure 1: Overview of QTLs used in this study. (a) Total number of QTLs identified on the 12 rice chromosomes. (b) QTL density for phenotypic variance explained (PVE) by QTLs. Area of the graph demonstrate the QTL density

Table 2: Details of the consensus map

Chromosome	Number of markers	Map length (cM)	Marker density (Markers/cM)	Average distance between two adjacent markers (cM)
1	113	128.47	1.14	1.15
2	96	72.93	0.756	0.77
3	115	168.95	1.47	1.48
4	77	142.38	1.85	1.87
5	49	135.24	2.76	2.81
6	76	217.50	2.86	2.90
7	69	116.65	1.69	1.72
8	60	205.13	3.42	3.48
9	75	54.01	0.72	0.73
10	59	113.86	1.93	1.96
11	96	125.65	1.31	1.32
12	58	73.94	1.27	1.30
Total	943	1554.71		

RESULTS AND DISCUSSION

Overview of initial QTLs

Novel genetic resources are constantly required as inputs into the continuing process of crop improvement for disease resistance. The 9 maps predicted altogether 143 QTLs including 111, 26, and 6 QTLs that were associated with BL, SHB, and BB resistance, respectively (Figure 1-a). Out of the 143, a detailed analysis was available in the literature for 115 QTLs. The phenotypic variance explained by those 115 QTLs ranged from 1.2 % to 55.9 % (Figure 1-b). More than 80 % of the QTLs contributed to less than 30 % of the phenotypic variance of target traits indicating that the majority of the QTLs had minor to moderate effects on the phenotype (Figure 1-b). Furthermore, 95 % CI of the QTLs varied from 1.3 cM to 93.18 cM, indicating widely spread QTL intervals, thereby reducing plausibility of application of the mapping results in candidate gene and marker search.

Constructing a consensus map, QTL projection and meta-QTL analysis

A consensus map was generated by merging the 9 rice maps by BioMercator v4.2 using default parameters. The 115 QTLs along with 943 markers were projected on the

consensus map (Table 2). The total length of the consensus map was 1554.71 cM with 1.65 cM^{-1} average marker density. The average distance between adjacent markers across chromosomes ranged from 0.73 to 3.48 cM. A total of 24 meta-QTLs were projected on the consensus map on all except 5, 6, and 10 rice chromosomes (Table 3 and Figure 2). Out of the 24 meta-QTLs, 23 were clustered by two or more QTLs. More than 62 % of the meta-QTLs were common to more than 3 QTL mapping studies. Larger effect meta-QTLs (where $R^2 \geq 25$ to 56 %) were reported on chromosomes 1, 2, 3, 4, 8, and 11. Out of the 24 meta-QTLs, 22 showed 95 % CI estimates narrower than those of their clustered QTLs. Meta-QTL intervals varied from 0.18 cM to 12.09 cM. Meta-analysis resulted in a QTL length reduction of 1.07 % on the minimum and 91.61 % on the maximum, thereby attaining a more accurate prediction of chromosomal regions associated with phenotypic variability for the target traits.

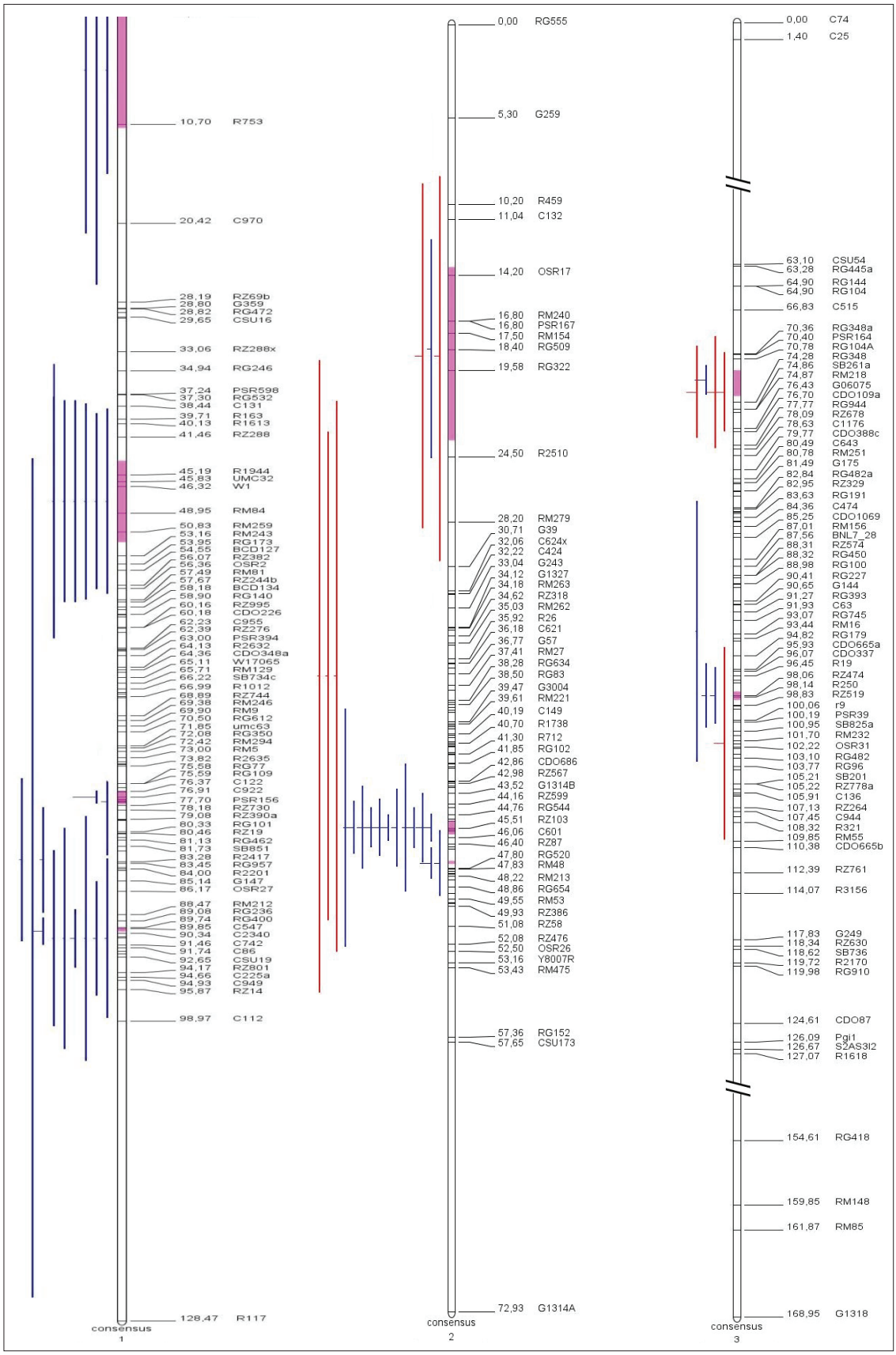
Table 3: Details of the predicted meta-QTLs

Meta-QTL	[1]No. of clustered QTLs[1]	[2]LOD max	[3]R^2 max	[4]Chrom	Model	AIC value	Meta-QTL position cM	Meta-QTL CI (95%) cM	Meta-QTL left markers	Meta-QTL right marker	Associated traits
MQTL011	1	2	3.3	1	4	166.87	5.35	11.39	C161	R753	BL
MQTL012	2	3.5	3.9				47.85	8.05	R1944	RM259	BL
MQTL013	3	22.5	52				77	1.29	C122	PSR156	BL
MQTL014	3	11.9	25				90.03	0.34	C547	C2340	BL
MQTL021	3	5.07	21.2	2	3	92.56	18.68	9.82	OSR17	R2510	BL, SHB
MQTL022	4	27.8	44.2				45.53	0.79	RZ531	C601	BL, SHB
MQTL023	3	13.8	31.7				47.46	0.18	RG520	RG520	BL, SHB
MQTL031	2	17.61	32.3	3	2	44.31	72.77	2.11	RG104A	RG348	BL, SHB
MQTL032	3	6.86	26.5				98.11	0.73	RZ474	R250	BL, SHB
MQTL041	3	19.3	45.7	4	1	39.11	120.9	1.78	RG190	G379	BL
MQTL071	2	6.02	22.2	7	3	86.82	51.09	6.04	G338	RG769	BL, SHB
MQTL072	3	6.02	22.2				65.64	12.09	CSU109	RZ471	BL
MQTL073	3	6.8	19				88.18	0.73	CDO418	R1245	BL
MQTL081	2	3.45	55.9	8	3	111.51	49.53	3.59	RM25	RM25	BL, SHB
MQTL082	3	10	29				59.43	2.29	RG333	RZ562	BL, SHB
MQTL083	2	6.3	22				72.19	7.13	C1121	C483	BL
MQTL091	4	4.2	12.2	9	3	102.71	24.84	6.56	RM105	RG463	BL, SHB
MQTL092	5	4.2	13				36.68	5.69	RZ422	RZ228	BL, SHB
MQTL093	3	4.08	13				46.49	3.65	RM215	C506	BL, SHB
MQTL111	1	4.4	14.5	11	2	41.18	0	4.91	R642	R642	BL
MQTL112	2	7.17	31.2				56.24	7.82	R2918	r2	BL, SHB
MQTL121	4	9.8	28	12	2	55.14	47.27	7.49	RM19	L102	BL, SHB
MQTL122	4	19.43	54				54.64	3.41	RG869	C443	BL, SHB

[1] Number of different initial QTL studies (detailed in Table 1) contributed to the meta-QTL

[2,3] Out of the clustered QTLs for a given meta-QTL, QTL that expressed highest LOD and/or R^2 were considered. The LOD and/or R^2 were extracted from the original references given in Table 1

[4] Chromosome where the meta-QTL is located


10,70 R753
20,42 C970
28,19 RZ69b
28,80 G359
28,82 RG472
29,65 CSU16
33,06 RZ288x
34,94 RG246
37,24 PSR598
37,30 RG532
38,44 C131
39,71 R163
40,13 R1613
41,46 RZ288
45,19 R1944
45,83 UMC32
46,32 W1
48,95 RM84
50,83 RM259
53,16 RM243
53,95 RG173
54,55 BCD127
56,07 RZ382
56,36 OSR2
57,49 RM81
57,67 RZ244b
58,18 BCD134
58,90 RG140
60,16 RZ995
60,18 CDO226
62,23 C955
62,39 RZ276
63,00 PSR394
64,13 R2632
64,36 CDO348a
65,11 W17065
65,71 RM129
66,22 SB734c
66,99 R1012
68,89 RZ744
69,38 RM246
69,90 RM9
70,50 RG612
71,85 umc63
72,08 RG350
72,42 RM294
73,00 RM5
73,82 R2635
75,58 RG77
75,59 RG109
76,37 G122
76,91 C922
77,70 PSR156
78,18 RZ730
79,08 RZ390a
80,33 RG101
80,46 RZ19
81,13 RG462
81,73 SB851
83,28 R2417
83,45 RG957
84,00 R2201
85,14 G147
86,17 OSR27
88,47 RM212
89,08 RG236
89,74 RG400
89,85 C547
90,34 C2340
91,46 C742
91,74 C86
92,65 CSU19
94,17 RZ801
94,66 C225a
94,93 C949
95,87 RZ14
98,97 C112
128,47 R117
consensus 1
0,00 RG555
5,30 G259
10,20 R459
11,04 C132
14,20 OSR17
16,80 RM240
16,80 PSR167
17,50 RM154
18,40 RG509
19,58 RG322
24,50 R2510
28,20 RM279
30,71 G39
32,06 C624x
32,22 C424
33,04 G243
34,12 G1327
34,18 RM263
34,62 RZ318
35,03 RM262
35,92 R26
36,18 C621
36,77 G57
37,41 RM27
38,28 RG634
38,50 RG83
39,47 G3004
39,61 RM221
40,19 C149
40,70 R1738
41,30 R712
41,85 RG102
42,86 CDO686
42,98 RZ567
43,52 G1314B
44,16 RZ599
44,76 RG544
45,51 RZ103
46,06 C601
46,40 RZ87
47,80 RG520
47,83 RM48
48,22 RM213
48,86 RG654
49,55 RM53
49,93 RZ386
51,08 RZ58
52,08 RZ476
52,50 OSR26
53,16 Y8007R
53,43 RM475
57,36 RG152
57,65 CSU173
72,93 G1314A
consensus 2
0,00 C74
1,40 C25
63,10 CSU54
63,28 RG445a
64,90 RG144
64,90 RG104
66,83 C515
70,36 RG348a
70,40 PSR164
70,78 RG104A
74,28 RG348
74,86 SB261a
74,87 RM218
76,43 G06075
76,70 CDO109a
77,77 RG944
78,09 RZ678
78,63 C1176
79,77 CDO388c
80,49 C643
80,78 RM251
81,49 G175
82,84 RG482a
82,95 RZ329
83,63 RG191
84,36 C474
85,25 CDO1069
87,01 RM156
87,56 BNL7_28
88,31 RZ574
88,32 RG450
88,98 RG100
90,41 RG227
90,65 G144
91,27 RG393
91,93 C63
93,07 RG745
93,44 RM16
94,82 RG179
95,93 CDO665a
96,07 CDO337
96,45 R19
98,06 RZ474
98,14 R250
98,83 RZ519
100,06 r9
100,19 PSR39
100,95 SB825a
101,70 RM232
102,22 OSR31
103,10 RG482
103,77 RG96
105,21 SB201
105,22 RZ778a
105,91 C136
107,13 RZ264
107,45 C944
108,32 R321
109,85 RM55
110,38 CDO665b
112,39 RZ761
114,07 R3156
117,83 G249
118,34 RZ630
118,62 SB736
119,72 R2170
119,98 RG910
124,61 CDO87
126,09 Pgi1
126,67 S2AS3I2
127,07 R1618
154,61 RG418
159,85 RM148
161,87 RM85
168,95 G1318
consensus 3

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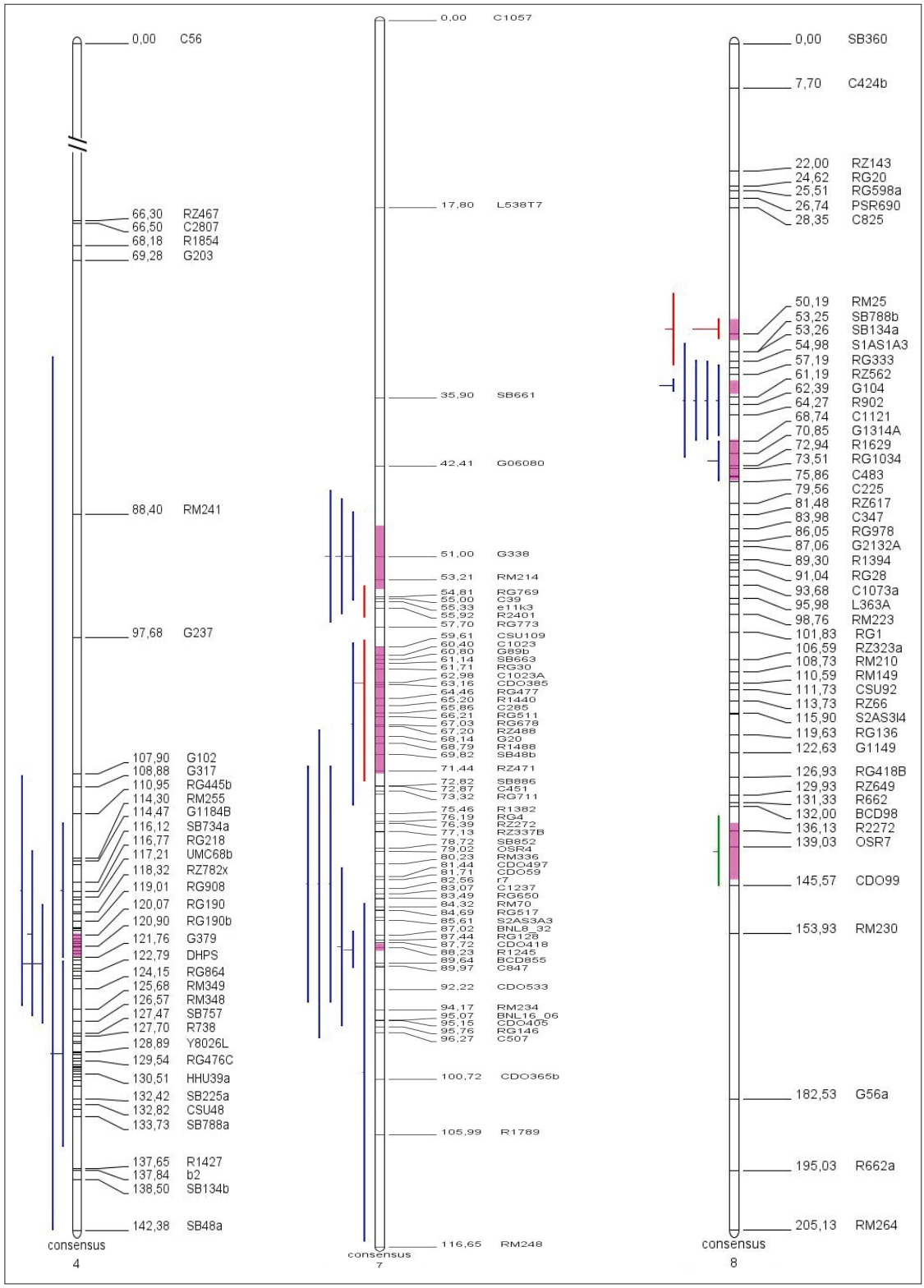

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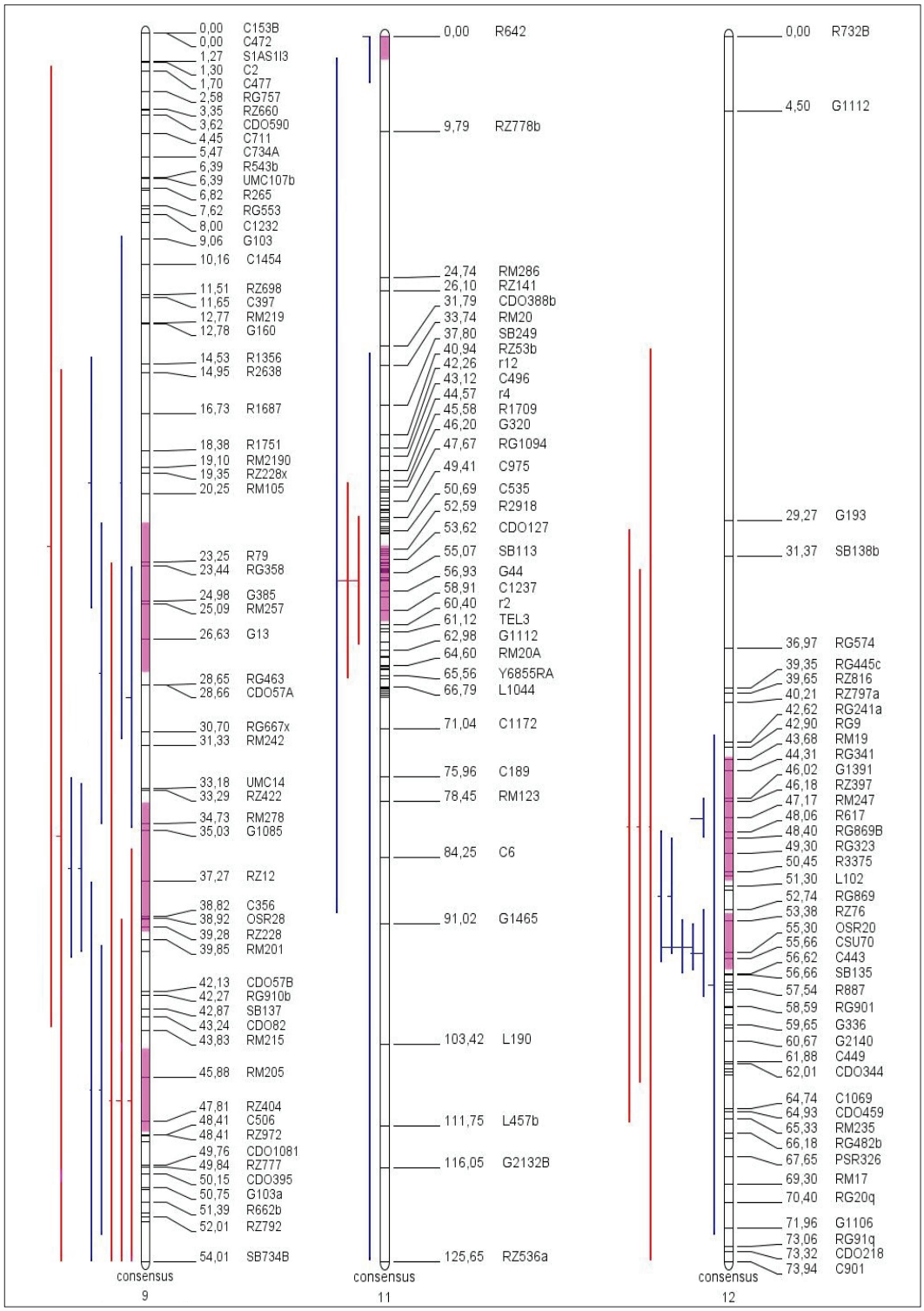


Figure 2: Meta-QTLs predicted on chromosome 1, 2, 3, 4, 7, 8, 9, 11, and 12. The chromosome number is given on the bottom of each map. Vertical lines to the left of each chromosome represent the 95 % Confidence Interval of clustered QTLs. QTLs associated with blast, bacterial blight and sheath blight are represented in blue, green and red lines. The meta-QTLs are represented by purple bars on the body of the chromosome.

Out of the 24 meta-QTLs, 9 were associated with BL resistance whereas 14 were associated with both BL and SHB resistance. This study did not identify meta-QTLs associated with BB resistance (Figure 2). Figure 3 is a graphical representation of physical positions (in Mbp) of the meta-QTLs predicted in this study, meta-QTLs identified in previous studies, and resistance QTLs that are successfully utilized in existing resistance breeding programmes. Interestingly, 6 of the meta-QTLs predicted herein collocate with 11 meta-QTLs from previous studies, and 23 resistance QTLs that are successfully utilized in existing resistance breeding programmes (Miah *et al*., 2013; Liu *et al*., 2014; Su *et al*., 2015) providing further confirmation of their stable expression and significant contribution in resistant phenotypes.

The QTLs associated with the predicted 24 meta-QTLs contributed phenotypic effects ranging from 1.2 % to 55.9 %. Major effect loci expressing dominant and clear resistant phenotypes are prone to "resistance break-down" due to rapid virulence formation within pathogen populations. On the other hand, quantitative resistance resulting from combinations of QTLs and QTLs specific to one or several isolates (Calenge *et al.*, 2004; Rocherieux *et al.* 2004; Ellis *et al.*, 2014; Wiesner-Hanks and Nelson, 2016) can provide partial resistance (Poland *et al.*, 2009; St. Clair, 2010). Stable expression of the meta-QTLs under differential strains and inoculum pressure of the same and /or different pathogens indicates broad-spectrum resistance and is highly desirable in developing durable resistance in crops. The 14 meta-QTLs that expressed resistant phenotypes for both BL and SHB presumably predicted Multiple Disease Resistance (MDR) loci or multiple disease susceptibility (MDS) loci, or loci associated with loss of baseline levels of resistance. Meta-QTLs predicted herein there by expressed a broad spectrum of disease resistance in rice and provide assorted targets for resistance breeding.

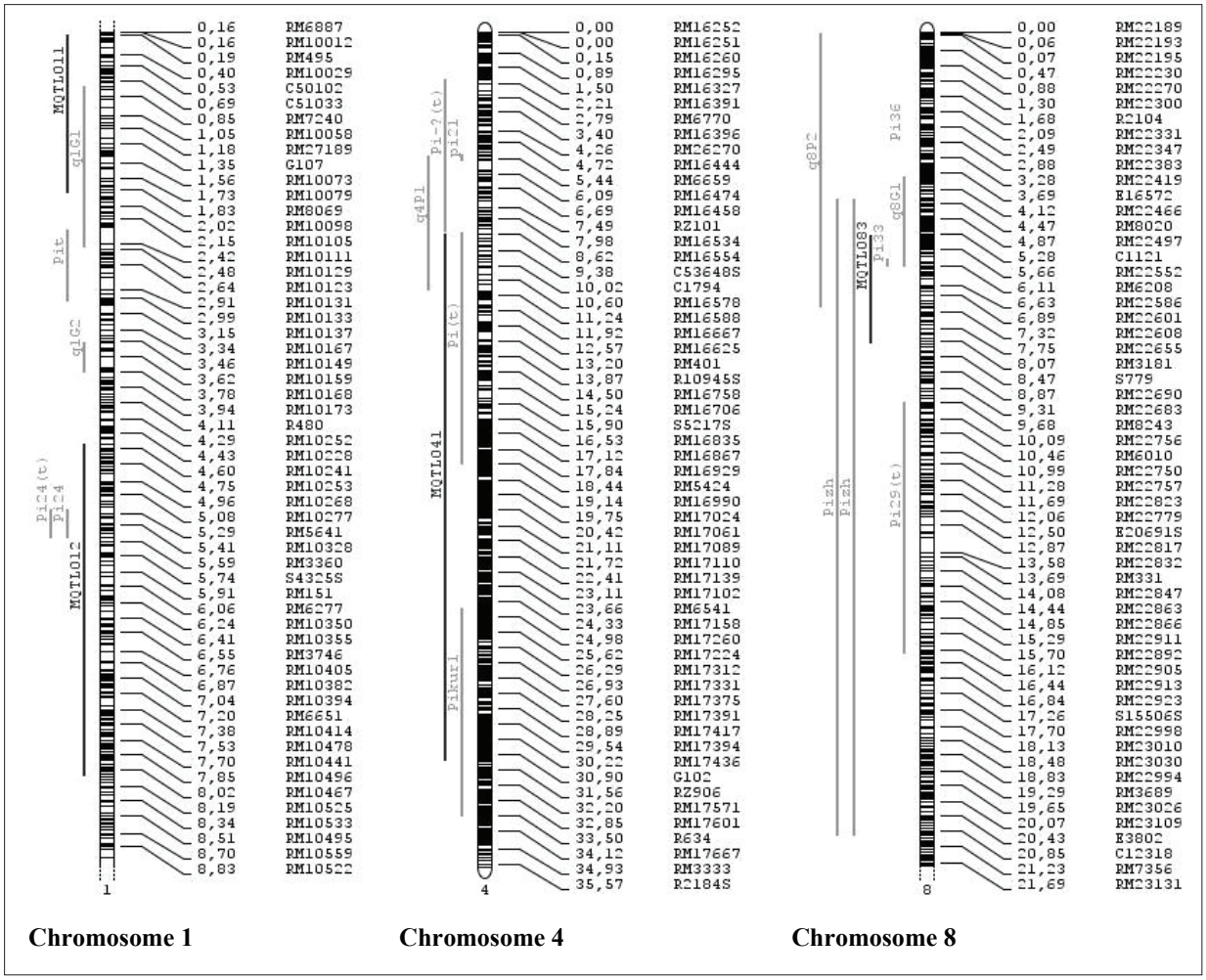
Identification of candidate genes

More than 3 meta-QTLs were predicted on the 1, 2, 7, 8, and 9 chromosomes where the meta-QTLs were tightly linked within 40 % to 50 % of the total length of the chromosome. Over 64 % of the QTLs associated with BL resistance that is reported in the literature were clustered on 6, 11, and 12 rice chromosomes (Ashkani *et al.*, 2016). These include, 15 loci including *Pi1, Pi7, Pi18, Pif, Pi34, Pi38, Pi44(t), PBR, Pilm2* and *Pik* on the long arm of the rice chromosome 11, and 17 including *Pita, Pita-2, Pitq6, Pi6(t), Pi12(t), Pi12(t), Pi19(t), Pi20(t), Pi21(t), Pi24(t), Pi31(t), Pi32(t), Pi39(t), Pi62(t), Pi157(t), IPi,* and *IPi3* loci on the centromere region of chromosome 12 (*http://www.ricedata.cn/gene/*) (Liu *et al.*, 2005; Tanweer, *et al.* 2015). These chromosome segments associated with clusters of QTLs are hotspots for identifying gene candidates and genetic/genomic resources for resistance breeding.

A total of 79 candidate Resistance gene analogues (RGAs) that collocate with the meta-QTLs were predicted (Table 4). Over 73 % of those were complete RGAs consisting of CC/NBS/LRR/TM domains. The rest were partial RGAs with NBS / TM (1.25 %), NBS/ LRR/ CC (5 %), or NBS/ LRR/ TM (20 %) domain types. Indeed, a large fraction of resistance QTLs in crops collocate with NBS-LRR genes, altered forms (*e.g.*, *Pi35*), or loss-of-function susceptibility alleles (*e.g.*, *Pi21*) of NBS-LRR genes (Poland *et al.*, 2009). For an example over 80 % out of 180 rice RGAs that were associated with BL resistance (*http://www.ricedata.cn/ontology*) were NBS-LRR candidates (Ballini *et al.*, 2008). Interestingly, 9 of the meta-QTLs collocated with clusters of RGAs. For example, meta-QTL, MQTL112 collocated with 45 RGAs. Tandem repeats of the NBS-LRR genes are a regular feature in plant genomes, whereby 50 % of the NBS-LRR genes in the rice genome are clustered tandem repeats (Yang *et al*., 2006). Some of these NBS-LRR clusters evolve under diversifying selection and contribute to functional redundancy of R-genes and enhance the potential to cope with rapidly evolving pathogens like *M. oryzae* (Zhong *et al.*, 2016). Interestingly, the variability of NBS-LRR gene sequences among paralogs within clusters can provide resistance against distantly related pathogen taxa, resulting in MDR loci (Stiekema *et al.*, 1999; Narusaka *et al.*, 2009). Based on transcriptome data available in the public domain (*http://rice.plantbiology.msu.edu/expression.shtml*), except one, none of the RGAs that collocate with the 24 meta-QTLs was expressed when rice is infected with *M. oryzae*. Out of the 79 RGAs, *RPR1h* (LOC_Os11g12340), a disease resistance gene in NB-LRR family is expressed in tissues of resistant Nipponbare genotypes, 12 hours after infection of *M. oryzae,* indicating a functional role of the gene in the disease cycle. The same gene is differentially expressed in resistant genotypes compared to susceptibility when infected with different fungal pathogens (Wamishe *et al*., 2018; Salvador-Guirao *et al*., 2019). This indicates complex and rigid regulation of the RGAs. Genomic abundance of R-genes that enable functional redundancy in the ability to recognize potential pathogens is widely studied in the rice-blast patho-system. For example, a given pathogen isolate can be recognized by approximately 8 % of the R-genes selected from a genome-wide NBS-LRR gene pool (Zhang *et al.*, 2015). Therefore, it is evident that the pool

of R-genes/RGAs assembles a robust surveillance system in rice for recognizing rapidly evolving pathogens like *M. oryzae* with a significant level of potential functional redundancy.

The 24 meta-QTLs including 6 previously known and 18 novel ones, the high resolution maps, and the RGAs made available herein are important additions to the existing genetic and genomic resources available for resistance breeding for BL and SHB. However, functional redundancy is a challenge when R-genes/RGAs are selectively targeted in resistance breeding programmes to improve host resistance. Because to be effective when integrated into a target host genetic background a given R-gene has to be (i) effective against the repertoire of *Avr* genes retained in the local pathogen pool, (ii) not already present or inherent in the host genetic background, and also (iii) functionally non-redundant in the host-pathogen system. Therefore, a systematic screening of the RGA pool for the above three criteria is a prerequisite for their effective use. Nonetheless the number of RGAs predicted herein that collocate with meta-QTLs identify putative R genes that significantly contribute to phenotypic expression of disease resistance in multiple locations and multiple genetic backgrounds and therefore are potential, stable genomic resources for breeding host resistance.



Chromosome 1 **Chromosome 4** **Chromosome 8**

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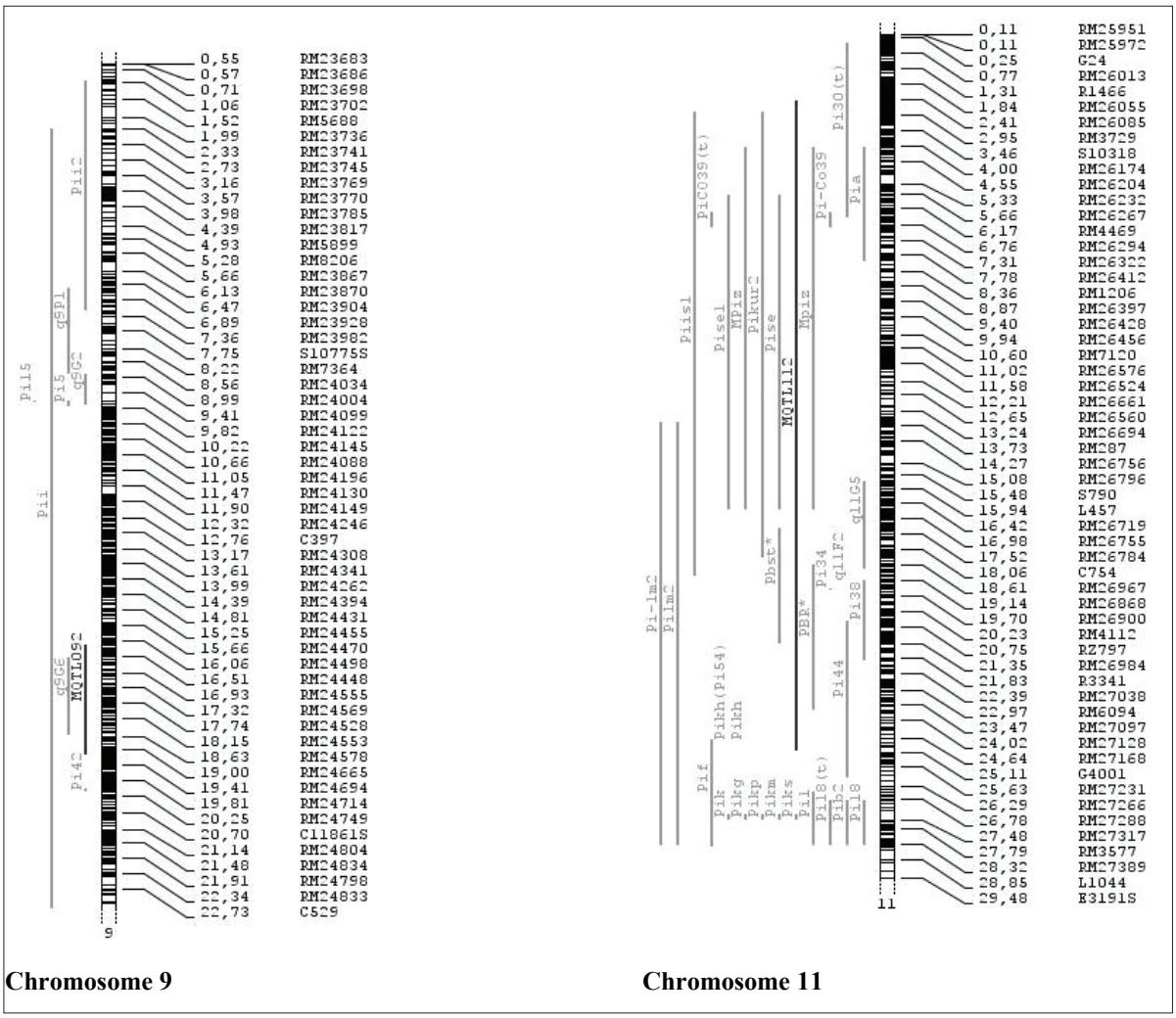

Figure 3: Collocation of meta-QTLs predicted herein with known meta-QTLs and resistance QTLs from previous studies. The dark vertical lines represent meta-QTLs predicted in this study. Light vertical lines represent meta-QTLs and QTLs from previous studies. Meta-QTLs reported in previous studies: *q1G2, q1G1, q4P1, q8P2, q9G2, q9P1* (Wang & Valent, 2009), *q8G1, q8G8, q9G6, q11G5, q11F2* (Ballini *et al*., 2008) and QTLs reported in previous studies: *Pi24, Pi-?(t), Pi24(t), Pi33, Pi29(t), Pi30(t)* (Sallaud *et al*., 2003), *Pi-Co39, PiCO39(t)* (Chauhan *et al*., 2002), *Pit, Pikp* (Hayashi *et al*., 2006), *pi21* (Fukuoka & Okuno, 2001), *Pi36* (Liu *et al*., 2005), *Pizh* (Causse *et al*., 1994; Tanksley, 1993), *Pi5* (Wang *et al*., 1994), *Pi-lm2* (Tabien *et al*., 2000), *Pikm* (Ashikawa *et al*., 2008), *Piks* (Fjellstrom *et al*., 2003), *Pi1* (Yu *et al*., 1991), *Pi18(t)* (Ahn *et al*., 2000), *Pise1, MPiz, Pikur1, Pikur2, Piis1, Pise, Pia* (GOTO, 1970), *Pikh* (Sharma *et al*., 2005), *Pii2* (Kinoshita and Kiyosawa, 1997), *Pi44* (Chen *et al*., 1999), *Pi38* (Gowda *et al*., 2006), *Pi34* (Zenbayashi *et al*., 2002), *Pik* (L. Wang *et al*., 2009), *Pif* (Toriyama, 1972), *Pikg* (Sharma *et al*., 2012), *Pib2* (Tabien *et al*., 1996) *PBR, Pbst* (Fujii *et al*., 1995), *Pi18* (Nagato, 1998), *Pii* (Ise, 1991), *Pi15* (Qing-Hua *et al*., 2003), *Pi42* (Ballini *et al*., 2008). Physical position of the markers are given in Mbp.

Table 4: Putative resistance genes that collocate with the meta-QTLs

Meta-QTL	Meta-QTL start (bp)	Meta-QTL stop (bp)	Gene ID	Gene start (bp)	Gene stop (bp)	Gene domain structure
MQTL014	41820536	43337474[1]	LOC_Os01g72390	41986078	41988856	NBS, CC, LRR, TM
			LOC_Os01g72680	42169412	42172204	NBS, CC, LRR
MQTL022	31163706	36444094	LOC_Os02g57305	35108829	35110107	CC, NBS, TM, LRR
MQTL041	8667054	31213886	LOC_Os04g21890	12396117	12398915	NBS, CC, TM, LRR
			LOC_Os04g30530	18247444	18250388	NBS, CC, LRR, TM
			LOC_Os04g30610	18279293	18281498	NBS, CC, LRR, TM
			LOC_Os04g30660	18318639	18322459	NBS, CC, LRR, TM
			LOC_Os04g30690	18340866	18344512	NBS, CC, LRR, TM
			LOC_Os04g39460	23506845	23509631	NBS, LRR, TM
			LOC_Os04g43340	25631835	25636842	CC, NBS, LRR, TM
			LOC_Os04g46300	27430842	27434629	NBS, CC, TM, LRR
			LOC_Os04g51172	30297864	30305606	NBS, TM
MQTL071	7157274	11393756[2]	LOC_Os07g17220	10142289	10145916	CC, NBS, LRR, TM
			LOC_Os07g17250	10173157	10176827	NBS, CC, LRR, TM
MQTL072	10115789[3]	19502523	LOC_Os07g17220	10142289	10145916	CC, NBS, LRR, TM
			LOC_Os07g17250	10173157	10176827	NBS, CC, LRR, TM
			LOC_Os07g29810	17524037	17528680	CC, NBS, TM, LRR
			LOC_Os07g29820	17529552	17535086	CC, NBS, TM, LRR
MQTL073	2350101[4]	24954614	LOC_Os07g08890	4618666	4622813	NBS, CC, TM, LRR
			LOC_Os07g09900	5263409	5267310	CC, NBS, TM, LRR
			LOC_Os07g09940	5292921	5293886	NBS, LRR, TM
			LOC_Os07g17220	10142289	10145916	CC, NBS, LRR, TM
			LOC_Os07g17250	10173157	10176827	NBS, CC, LRR, TM
			LOC_Os07g29810	17524037	17528680	CC, NBS, TM, LRR
			LOC_Os07g29820	17529552	17535086	CC, NBS, TM, LRR
			LOC_Os07g33690	20129648	20135321	CC, NBS, TM, LRR
			LOC_Os07g33720	20157460	20160904	CC, NBS, LRR, TM
			LOC_Os07g40810	24456130	24459910	NBS, LRR, TM
MQTL083	5326953	8156813	LOC_Os08g09430	5466374	5469304	NBS, CC, TM, LRR
			LOC_Os08g10260	5964410	5967847	NBS, CC, TM, LRR
			LOC_Os08g10430	6127418	6131390	NBS, CC, TM, LRR
			LOC_Os08g10440	6137622	6141505	CC, NBS, LRR, TM
			LOC_Os08g12740	7539941	7551989	NBS, LRR, TM
MQTL092	16201951	19168603	LOC_Os09g30220	18391869	18397839	NBS, CC, TM, LRR
			LOC_Os09g30230	18402208	18404264	NBS, CC, TM, LRR
MQTL112	2435497	25125119[5]	LOC_Os11g10550	5777015	5787839	CC, NBS, LRR, TM
			LOC_Os11g10570	5797007	5801720	NBS, TM, LRR
			LOC_Os11g10610	5820833	5826729	CC, NBS, LRR, TM
			LOC_Os11g10620	5828343	5828674	CC, NBS, LRR, TM
			LOC_Os11g10760	5907950	5911943	CC, NBS, LRR, TM
			LOC_Os11g10770	5911937	5919029	NBS, LRR, TM
			LOC_Os11g11550	6419845	6424350	NBS, LRR, TM
			LOC_Os11g11770	6532861	6535926	CC, NBS, LRR, TM
			LOC_Os11g11790	6541924	6546026	NBS, LRR, TM
			LOC_Os11g11810	6554514	6561687	NBS, CC, TM, LRR
			LOC_Os11g11950	6631958	6634662	CC, NBS, LRR, TM
			LOC_Os11g11960	6638262	6640922	CC, NBS, LRR, TM
			LOC_Os11g12000	6668507	6681159	CC, NBS, LRR, TM
			LOC_Os11g12020	6690417	6690681	CC, NBS, LRR, TM
			LOC_Os11g12040	6692025	6697566	CC, NBS, LRR, TM

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Meta-QTL	Meta-QTL start (bp)	Meta-QTL stop (bp)	Gene ID	Gene start (bp)	Gene stop (bp)	Gene domain structure
			LOC_Os11g12050	6703120	6709237	CC, NBS, LRR, TM
			LOC_Os11g12300	6872037	6873056	CC, NBS, LRR, TM
			LOC_Os11g12320	6875946	6877418	CC, NBS, LRR, TM
			LOC_Os11g12330	6878608	6884945	CC, NBS, LRR, TM
			LOC_Os11g12340	6888057	6896357	CC, NBS, LRR, TM
			LOC_Os11g12350	6896964	6898774	CC, NBS, LRR, TM
			LOC_Os11g13940	7712006	7717010	CC, NBS, TM, LRR
			LOC_Os11g15670	8883111	8885768	CC, NBS, TM, LRR
			LOC_Os11g16510	9136895	9137837	NBS, LRR, TM
			LOC_Os11g17110	9505035	9505904	NBS, TM, LRR
			LOC_Os11g17120	9508232	9509376	NBS, CC, TM, LRR
			LOC_Os11g29030	16807964	16809227	NBS, LRR, TM
			LOC_Os11g29050	16821579	16826538	NBS, LRR, TM
			LOC_Os11g29520	17126630	17132216	CC, NBS, TM, LRR
			LOC_Os11g29980	17425545	17429007	NBS, CC, LRR, TM
			LOC_Os11g29990	17434258	17438286	NBS, CC, LRR, TM
			LOC_Os11g30060	17484860	17489555	CC, NBS, TM, LRR
			LOC_Os11g30110	17508573	17509867	NBS, CC, LRR, TM
			LOC_Os11g30210	17561346	17565177	NBS, CC, LRR, TM
			LOC_Os11g35580	20859839	20862861	NBS, CC, TM, LRR
			LOC_Os11g36410	21468751	21472160	NBS, CC, LRR
			LOC_Os11g38480	22799288	22802116	NBS, CC, LRR
			LOC_Os11g38580	22862447	22867268	NBS, CC, LRR
			LOC_Os11g39160	23305866	23313424	NBS, LRR, TM
			LOC_Os11g39260	23377646	23378368	NBS, LRR, TM
			LOC_Os11g39280	23389785	23391708	NBS, CC, TM, LRR
			LOC_Os11g40780	24387280	24390444	CC, NBS, TM, LRR
			LOC_Os11g41170	24670967	24674338	NBS, TM, LRR
			LOC_Os11g41210	24712661	24719904	NBS, TM, LRR
			LOC_Os11g41540	24911501	24917050	NBS, CC, TM, LRR

[1] RG331; [2] C39; [3] C1023; [4] RG128; [5] G4001

[a, b, c, d, e] Second nearest marker was used due to the unavailability of physical position of the first nearest marker

Conflict of interest statement

The authors declare that they have no competing interests.

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