

Genome-wide association mapping for identification of sheath blight resistance loci from wild rice *Oryza rufipogon*

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Abstract

Sheath blight (ShB) caused by *Rhizoctonia solani* is one of the serious constraints that hamper global rice production. It causes severe yield loss of up to 69% under favorable environmental conditions. The application of chemical fungicides remains the primary approach for the management of this disease. But, overuse of chemical fungicides causes potential health and environmental risk. The use of host plant resistance is a very effective, economic, and sustainable strategy to control sheath blight disease in rice. In this report, we have evaluated 405 accessions of *Oryza rufipogon* for four ShB-related traits i.e. lesion height (LH), plant height (PH), relative lesion length (RLH), and disease score (DS) during the years 2015, 2016, and 2017. A total of 44,109 high-quality SNP markers on 301 accessions were employed to identify significant marker-trait associations (MTAs) using a genome-wide association study (GWAS). The GWAS analysis revealed a total of 22 significant SNPs associated with the ShB-related traits distributed on all the rice chromosomes except 10 and 12. Among them, eleven were associated with RLH, seven with PH, and one each with LH and DS. The SNPs on chromosomes 3 and 9 were associated with multiple traits. Furthermore, seven ShB resistance loci were also found to be co-localized with previously reported ShB resistance genes/QTLs. These findings provide valuable information to identify key SNPs associated with ShB resistance. Significantly associated SNPs could be used for introgression of ShB resistance traits into rice cultivars using marker-assisted selection (MAS).

Introduction

Rice sheath blight (ShB) disease, caused by a soil-borne, necrotrophic fungus *Rhizoctonia solani* Kühn (*R. solani*, teleomorph: *Thanatephorus cucumeris*), is one of the most destructive diseases of rice (*Oryza sativa* L.) worldwide, with an estimated yield loss up to 69% (Lee et al. 1983; Rush and Lee, 1992; Sivalingam et al. 2006). In India, it causes approximately 20% yield loss (Ghosh et al. 2016; Yuan et al. 2019). The widespread adaptation of high-yielding semi-dwarf cultivars combined with the application of high levels of nitrogenous fertilizers has led to increased severity of this pathogen (Savary et al. 2000). The disease initially occurs in the leaf sheath and spreads upwards, creating lesions in the leaf and leaf sheath (Goad et al. 2020). The disease is highly destructive when it reaches the panicle and reduces the grain quality by infecting the grains.

Application of fungicides and cultural practices remain the primary method to manage this disease (Chin and Bhandhufalck, 1990; Damicone et al. 1993). However, the use of chemical fungicides is costly, ineffective, and potentially harmful to the environment, and human health (Groth et al. 1990). Furthermore, overuse of these chemical fungicides has resulted in the resurgence of new resistant pathotypes. Breeding resistant varieties are believed to be one of the most economical, safe, and effective strategies to manage this disease. So far, no germplasm with complete resistance to sheath blight is known in *O. sativa* and its wild relatives (Chen et al. 2000; Eizenga et al. 2002; Liu et al. 2006; Zhang et al. 2006; Kamboj et al. 2016). However, significant variation in resistance to sheath blight has been observed among rice cultivars in many reports (Jia et al. 2007). To date, only a few varieties and lines have been reported as promising resistance sources that exhibit stable resistance to sheath blight, such as Tetep (Channamallikarjuna et al. 2010), Teting (Li et al. 1995; Pinson et al. 2005), and Jasmine 85 (Liu et al. 2009; Zou et al. 2000), etc. Thus, exploring new resistant germplasm has become an international effort (Xie et al. 1992; Zuo et al. 2009; Jia et al. 2007).

Rice sheath blight resistance loci are reported to be quantitative implying to be controlled by many quantitative traits loci (QTL) or polygenes (Li et al. 1995; Jia et al. 2007). More than 110 QTLs for sheath blight have been detected on all the 12 chromosomes of rice using different bi-parental mapping populations including F₂, backcross, doubled haploid, recombinant inbred lines, etc. (Li et al. 2021; Molla et al. 2020; Zhang et al. 2019). Among them, the majority of the QTLs exhibit minor effects on the trait. Only a few loci such as *qShB9-2* (Liu et al. 2009) and *qSBR11-1* (Channamallikarjuna et al. 2010) have been reported as major QTLs. In addition, *qSB-9TQ* (Zuo et al. 2014) and *qSB-11LE* (Zuo et al. 2013) have been fine mapped but none of the ShB resistance QTLs have been cloned yet. Despite the rapid progress in the mapping

of ShB resistance QTLs, very few QTLs are being exploited in rice breeding programs. Traditional QTL mapping is very time-consuming and requires a large bi-parental population segregating for the trait of interest. Moreover, limited recombination in the bi-parental population reduces the resolution of QTL analysis. In contrast, genome-wide association study (GWAS) allows the identification of genes at higher resolution as GWAS captures ancient recombination by employing diverse and natural populations. In rice, very few reports have utilized the GWAS approach for the identification of sheath blight resistance genes by employing cultivated varieties or lines (Chen et al. 2019; Jia et al. 2012; Sun et al. 2014; Zhang et al. 2019).

Wild relatives of rice are valuable sources of important agronomic traits as well as genes for abiotic and biotic stress tolerance (Brar et al. 1991; Khush et al. 1990). So far, wild species of rice have not been widely utilized for the identification and mapping of ShB resistance QTLs despite their potential to carry unique resistance alleles. Earlier studies have identified a few wild relatives of rice such as *O. minuta* acc. IRGC101089 and *O. rufipogon* acc. IRGC100907 to be potential sources of resistance to ShB (Amante et al. 1990). Few accessions of *O. nivara* were also shown to be resistant to sheath blight (Eizenga et al. 2009; Prasad and Eizenga, 2008). Out of them, two moderately resistant accessions of *O. nivara* (IRGC100898 and IRGC104705) were used to map sheath blight and blast resistance genes (Eizenga et al. 2013). Recently, Aggarwal et al (2019) reported novel sources of sheath blight resistance in *O. nivara*. More recently, mapping the population derived from weedy rice was employed to map new genes conferring resistance to sheath blight disease (Goad et al. 2020).

In this study, 405 accessions of *O. rufipogon* were evaluated for sheath blight resistance for three years. Among them, 301 accessions were employed for GWAS analysis for the identification of significant genomic regions associated with sheath blight resistance. SNP markers linked to resistance loci would serve as potential genomic resources for developing sheath blight-resistant elite cultivars with broadened genetic bases.

Materials And Methods

O. rufipogon diversity panel

The diversity panel consisting of 405 accessions of *O. rufipogon* was originally procured from International Rice Research Institute (IRRI, Manila, Philippines) and National Rice Research Institute (NRRI), Cuttack, India. The detailed information on *O. rufipogon* accessions is listed in Supplementary Table 1. The diversity panel was screened for sheath blight resistance under field conditions during the years 2015, 2016, and 2017. Seeds of each accession along with susceptible check PR114 were sown in the nursery and 30-days old seedlings were transplanted in the experimental field according to randomized block design (RBD) with three replications. The plant material was raised as per the package of practices (POP) for rice recommended by Punjab Agricultural University (PAU), Ludhiana, India. Each row (30cm x 60cm spacing) is comprised of five plants. Of these, three central plants were inoculated with highly pathogenic local Ludhiana isolate to screen the wild accessions.

Evaluation of sheath blight resistance

The fungal strain was isolated from the susceptible cultivar PR121 showing sheath blight symptoms. The infected leaf sheaths were sliced into 1 cm pieces and washed in running tap water followed by surface sterilization with 0.1 percent aqueous solution of mercuric chloride for one minute in a Petri plate and washed thrice with autoclaved distilled water to remove remnants of the chemical. The surface-sterilized sheath pieces were plated into the sterilized Petri-plates containing potato dextrose agar medium (PDA) followed by their incubation at $26 \pm 2^\circ\text{C}$ for 5-7 under a BOD incubator. The pathogen was mass multiplied on maize meal-sand (1:3) medium supplemented with 20g sucrose. The one-week-old culture of *R. solani* was transferred to a maize meal-sand medium and incubated at $27 \pm 2^\circ\text{C}$. The inoculum was ready to use for inoculation within 10 days (Lore et al. 2012). For disease evaluation, five grams of inoculum were placed

at the center of the hill of each plant. Disease assessment was made 21 days after inoculation (DAI) under field conditions. The lesion height (LH) was recorded from the base of the plant to the tip of the topmost lesions on the stem. Plant height (PH) was also measured from the bottom of the plant to the top leaf. Relative lesion height (RLH) was calculated using the following formula given by Sharma et al (1990).

$$\text{RLH (\%)} = \frac{\text{Lesion height}}{\text{Plant height}} \times 100$$

Disease score (DS) was measured based on the Standard Evaluation System (SES) for rice sheath using a scale of 0-9 (IRRI, 2014). The accessions showing resistance responses were further evaluated during the years 2016 and 2017.

Statistical Analysis

Data were analysed using the general linear model procedure (PROC GLM) in the SAS system (SAS, Cary, NC). Correlation coefficients for sheath blight and its component traits recorded across different seasons were determined by the correlation procedure (PROC CORR) in SAS. The least significant difference (LSD) values were calculated using PROC GLM. The cluster is reported as an unweighted pair group method using arithmetic means (UPGMA) based on the Mahalanobis distance algorithm using PAST 3.0 software (Hammer et al. 2001).

Genome-wide association mapping

The genome-wide association mapping was performed in a panel of 301 accessions using Genomic Association and Prediction Integrated Tool version 3.0 (GAPIT 3.0) program (Lipka et al. 2012; Wang and Zhang, 2019). A total of 44,109 SNPs comprised the working SNP set for the association study. The working set of SNP markers from 301 accessions together with pooled phenotypic data was used for GWAS analysis (Malik, 2019). The fixed and Random Model Circulating Unification (FarmCPU) model was used to determine the associations between each SNP and traits related to ShB resistance (LH, PH, RLH, and DS). Principal components (PC) and FaSTLMM algorithm-based kinship computed from SNP data describing the genetic structure of the panel were iteratively added to the FarmCPU model to avoid false positives.

Prediction of putative candidate gene

The SNP markers associated with ShB-related traits were used to predict putative candidate genes using the MSU Rice Genome Annotation Project database (RGAP) V.7 (<https://rice.plantbiology.msu.edu/>). The physical positions of associated SNPs were searched in RGAP for the prediction of putative candidate genes. In addition to that, 10 kb flanking regions both upstream and downstream of the significant markers, designated as LD regions for the present study, were investigated for the presence of loci in RGAP. This was followed by *in-silico* functional annotation of the loci to establish their relationship with ShB resistance.

Results

Evaluation of wild accessions of *Oryza rufipogon* and statistical analysis

A total of 405 accessions of *O. rufipogon* with susceptible check PR114 were screened under natural field conditions. Significant variations were observed for three ShB-related traits during three years of screening. Based on the standard evaluation system of rice, 22 accessions (5.43%) were moderately resistant (MR), 297 accessions (73.33%) were moderately susceptible (MS), and 86 accessions (21.23%) were susceptible (Fig. 1). The PH ranged from 53 cm to 200

cm, LH ranged from 18 cm to 103 cm, relative lesion height (RLH) ranged from 13.15–93.18% and DS ranged from 1 to 9.

The distance-based hierarchical clustering was performed using the Mahalanobis method based on RLH and DS. All the accessions were clustered into 4 groups (namely G1, G2, G3, and G4). Group G4 comprised accessions that showed the MR reaction with the minimum values of LH, RLH, and DS i.e., 37.52cm, 27.38%, and 3 respectively. This was followed by the G2 group which showed MS reaction with moderate LH (49.03 cm), RLH (35.86%), and DS (5.01), respectively. Group G1 had the highest values of LH, RLH and DS i.e. 65.10 cm, 70.12%, and 8.23 respectively (Table 1). Further, cluster analysis performed on twenty-two moderately resistant accessions which exhibit consistent MR response over 2 seasons of screening showed clear grouping of accessions in 4 groups (G1, G2, G3, and G4). The first group G1 had susceptible check PR114 that exhibits the highest LH (62.67cm), RLH (62.40%), and DS (7.0) respectively. The group G2 also had one accession which showed MR with LH (36.83 cm), RLH (29.51), and DS (3.0) respectively. Group G4 had 3 accessions with the susceptible level of disease reaction. However, group G3 had 19 accessions that showed MR reaction with the lowest values of LH (31.44cm), RLH (25.26%), and DS (3.0) respectively (Fig. 2).

Table 1
Clustered grouping of 405 accessions of *O. rufipogon* during 2015

Groups	Acc. No.	PH	Range of PH	LH	Range of LH	Mean RLH	Range of RLH	DS	Range of DS
G1	25	93.38	56-123.33	65.10	39.67–86.67	70.12	59.42–86.90	8.23	5–9
G2	296	137.65	68-193.33	49.03	22.33–73.67	35.86	30.26–51.47	5.01	5–7
G3	62	113.21	65.33–158	55.75	31.33–90.67	49.58	36.16–57.40	6.94	5–7
G4	22	138.41	101.33-179.33	37.52	28.67-52	27.38	22.14–29.69	3	3–3

Effect of *Oryza rufipogon* genotypes on disease variables

Based on evaluation during the year 2015, 22 moderately resistant accessions of *O. rufipogon* were further evaluated during the years 2016 and 2017. All the genotypes had a significant ($P < 0.001$) effect on all the disease variables. The experiment interaction had a significant ($P < 0.001$) effect on all the disease variables except LH ($P > 0.12$). The *O. rufipogon* genotype x experiment interaction had a significant ($P < 0.001$) effect on all ShB components traits except RLH ($P = 0.37$) and disease score ($P = 0.21$) (Table 2). Of these, 19 accessions namely CR100004, CR100472B, CR100469, CR100465, CR100462A, CR100461B, CR100438, CR100436, CR100309, CR100036, CR100018A, IRGC104404D, IRGC104404A, IRGC103404, IRGC80762, IRGC80660, IRGC80610, IRGC80600, IRGC805629 were found moderately resistant, 3 accessions namely CR100002, CR100006A, IRGC106512 were moderately susceptible during the year 2016 (Table 3). No accession showed a completely resistant reaction. PH ranged from 82 cm to 189 cm, LH ranged from 19 cm to 66 cm, RLH ranged from 12.96–66%, and DS ranged from 1 to 9 for both years (Table 3).

Table 2
Analysis of variance for effects of *O. rufipogon* genotypes on different disease variables of rice sheath blight

Disease variables	Effect	Df	F ratio	P > F*	LSD
Plant Height	Exp	1	105.45	< .0001	7.89
	Genotypes	22	9.05	< .0001	25.81
	Exp x Genotypes	22	6.38	< .0001	33.17
Lesion Height	Exp	1	2.43	0.1226	NS
	Genotypes	22	16.62	< .0001	7.67
	Exp x Genotypes	22	3.55	< .0001	12.39
Relative Lesion Height	Exp	1	24.48	0.0003	2.85
	Genotypes	22	27.88	< .0001	4.85
	Exp x Genotypes	22	1.09	0.3727	NS
Disease score	Exp	1	22.37	< .0001	0.43
	Genotypes	22	11.87	< .0001	1.01
	Exp x Genotypes	22	1.26	0.2187	NS
*Significant at 5% level of confidence, NS = Non significant					

Table 3
Evaluation of promising *O. rufipogon* accessions for sheath blight resistance during 2016 and 2017

S.No.	Acc. No.	Year 2016				Year 2017			
		PH	LH	RLH	DS	PH	LH	RLH	DS
1	IRGC80562	133.33	29.00	22.08	3	110.67	24.67	24.4	3
2	IRGC80600	137.33	34.3	24.97	3	97.67	23.67	24.19	3
3	IRGC80610	126.33	30.33	24.91	3	116.33	28.67	24.63	3
4	IRGC80660	119.0	26.33	23.26	3	96.33	24.33	25.21	3
5	IRGC80762	105.33	26.00	24.79	3	111.67	25.33	22.68	3
6	IRGC103404	138.67	29.67	22.49	3	106.17	30.33	28.5	3
7	IRGC104404A	181	39.67	22.1	3	120.67	28.67	23.73	3
8	IRGC104404D	145.33	36.00	24.77	3	94.33	23.67	25.09	3
9	CR100018A	124.67	31.33	25.31	3	108.67	30.67	28.21	3
10	CR100036	135.67	34.33	25.25	3	95	28	29.44	3
11	CR100309	128.67	32.00	25.05	3	163.33	43.67	26.77	3
12	CR100436	147.33	34.33	22.86	3	86.33	25.67	29.72	3
13	CR100438	154.67	34.67	22.71	3	102.33	25	24.43	3
14	CR100461B	167	40.67	24.07	3	109.67	30.67	27.95	3
15	CR100462A	134	34.33	25.69	3	113.67	30.67	26.75	3
16	CR100465	141	35.00	24.70	3	99.33	27.67	27.84	3
17	CR100469	175.33	41.67	23.85	3	156	46	29.47	3
18	CR100472B	151	37.17	24.96	3	104.33	27.67	26.53	3
19	CR100002	160	45.67	29.15	3	152.67	59.33	38.84	5
20	CR100004	111.67	32.33	29.06	3	137.67	41.33	29.95	3
21	CR100006A	144.33	42.33	29.34	3	141.67	51.67	36.36	5
22	IRGC106512	116	33.67	29.46	3	121.67	45.33	37.52	5
23	PR114	99.67	60	60.15	7	101	65.33	64.71	7
Standard Error		3.04	1.12	1.03	0.15	2.58	1.44	1.01	0.15
LSD(C.D) 5%		3.18	5.67	3.25	1.10	9.80	6.33	4.22	1.18

Disease Variables And Their Correlation With Genotypes

The different disease variables measured were significantly ($P < 0.05$) correlated. The RLH had a positive correlation with LH (0.76) and DS (0.89). The LH also showed a positive correlation of 0.68 with the disease score. There was a negative correlation between PH and DS with a correlation coefficient of -0.24 (Table 4).

Table 4
Pearson correlation coefficients between the different disease variables of rice sheath blight among *O. rufipogon* genotypes

Disease variables	Plant Height(cm)	Lesion Height(cm)	Relative Lesion Height (%)	Disease score
Plant Height(cm)	1	0.41	-0.24	-0.24
Lesion Height(cm)		1	0.76	0.68
Relative Lesion Height (%)			1	0.89
Disease score				1

Genome-wide Association Mapping

GWAS performed on a set of 44,109 SNPs identified 22 strong associations for the four traits LH, PH, RLH, and DS (Table 5). The SNP density has been presented chromosome-wise in Fig. 3. The issue of false positives was controlled by modelling kinship and 2 PCs as identified by the BIC criterion. P-value based on the minimum Bayes Factor calculated to be 4.21838E-05 was used as a threshold for determining the significance level in the current study. Significant associations were obtained on all the chromosomes except the 10th and 12th as evident from Manhattan plots (Fig. 4). Only two genomic regions on chromosomes 3 and 9 were strongly associated with multiple traits, PH and RLH. Besides these regions, 7 and 11 MTAs were obtained for PH and RLH, respectively, in addition to one strong association obtained each for LH and DS. It has also been observed that 81.8% of the genomic regions significantly associated with RLH were also associated with PH at $p < 0.05$. Similarly, SNPs significantly related to PH were also associated with DS and LH. The genomic regions strongly associated with LH showed association with DS and vice-versa at $p\text{-value} < 0.05$, indicating these two traits to be correlated. The association trends seen at the genomic level were also observed at the phenotypic level as evident from significant positive values of the Pearson correlation coefficient observed between the genotypically-associated traits as evident in Table 4.

Table 5
GWAS analysis identified significant marker-trait associations in a panel of *O. rufipogon* accessions

Significant SNPs	Traits	Chromosome	Physical position (Mb)	Locus Id	Encoded protein	Reported gene/QTL
S1_2885944	LH , DS	1	2.87– 2.89	LOC_Os01g06020	Aspartyl tRNA synthetase	
S3_15,307,919	PH , RLH	3	15.29– 15.31	LOC_Os03g26800	LTPL51 - Protease inhibitor/seed storage/LTP family protein	
				LOC_Os03g26820	LTPL52 - Protease inhibitor/seed storage/LTP family protein precursor	
				LOC_Os03g26840	OsFBX93 - F-box domain containing protein	
S6_4,029,679	PH	6	4.01– 4.03	LOC_Os06g08300	FAD dependent oxidoreductase domain containing protein	
				LOC_Os06g08310	Plasma membrane ATPase	
				LOC_Os06g08320	60S ribosomal protein L39	
S3_8,444,174	PH , DS	3	8.43– 8.45	LOC_Os03g15420	Dynamin family protein	
				LOC_Os03g15430	Ser/Thr protein phosphatase family protein	
S7_7,314,458	PH , DS	7	7.30– 7.32	LOC_Os07g12770	Transmembrane amino acid transporter protein	<i>qSB-7</i> (Zou JH et al 2000)
				LOC_Os07g12780	Cyclin, putative, expressed	
S5_26,958,980	PH , RLH	5	26.94– 26.96	LOC_Os05g46530	Invertase/pectin methylesterase inhibitor	<i>qSB-5</i> (Han YP 2002)
				LOC_Os05g46550	Adaptor complexes medium subunit family domain containing protein	
				LOC_Os05g46560	RAN GTPase-activating protein 1	
				LOC_Os05g46570	WD domain, G-beta repeat domain containing protein	
*The traits marked in bold indicate primary traits (significant at p-value < 4.21838E-05) and remaining are secondary traits (p-value < 0.05). The italicized and underlined loci harbor significant SNPs.						

Significant SNPs	Traits	Chromosome	Physical position (Mb)	Locus Id	Encoded protein	Reported gene/QTL
				LOC_Os05g46580	Polyprenyl synthetase	
S9_21,632,022	PH, RLH	9	21.62–21.64	LOC_Os09g37520	DUF630/DUF632 domains containing protein	
S3_16736722	PH	3	16.72–16.74	LOC_Os03g29389		
S4_35068903	PH	4	35.05–35.07	LOC_Os04g58940		
				LOC_Os04g58960	Regulator of chromosome condensation	
S2_29,199,388	PH, RLH	2	29.18–29.20	LOC_Os02g47744	MYB family transcription factor	<i>qSB-2</i> (Pinson et al 2005)
				LOC_Os02g47760	AAA-type ATPase family protein	
				LOC_Os02g47770	ZF-HD protein dimerisation region containing protein	
S7_3,920,076	RLH, LH	7	39.10–39.30	LOC_Os07g07790	LTPL75 - Protease inhibitor/seed storage/LTP family protein precursor	
				LOC_Os07g07800	PHA synthase	
S4_25,149,326	RLH, LH	4	25.13–25.15	LOC_Os04g42480	Receptor-like protein kinase At3g46290 precursor	
				LOC_Os04g42490	DIE2/ALG10 family, putative, expressed	
				LOC_Os04g42500	Trafficking protein particle complex subunit, putative, expressed	
S7_20,921,579	RLH, PH	7	20.91–20.93	LOC_Os07g034900	Aspartic proteinase nepenthesin precursor, putative, expressed	<i>qshb7.2</i> (Yadav et al 2015)
				LOC_Os07g034910	Aspartic proteinase nepenthesin precursor, putative, expressed	
				LOC_Os07g034920	Aspartic proteinase nepenthesin precursor, putative, expressed	
S5_29,097,966	RLH	5	29.08–29.10	LOC_Os05g50750	AAA family ATPase, putative, expressed	

*The traits marked in bold indicate primary traits (significant at p-value < 4.21838E-05) and remaining are secondary traits (p-value < 0.05). The italicized and underlined loci harbor significant SNPs.

Significant SNPs	Traits	Chromosome	Physical position (Mb)	Locus Id	Encoded protein	Reported gene/QTL
S2_25,483,277	RLH, PH	2	25.47–25.49	LOC_Os02g42370	Receptor-like protein kinase 2 precursor, putative, expressed	<i>qSB-2</i> (Pinson et al 2005)
				LOC_Os02g42380	e2f-associated phosphoprotein, putative, expressed	
S8_5,347,068	RLH, PH	8	53.37–53.57	LOC_Os08g09210	Phosphoribosylamine–glycine ligase, putative, expressed	<i>qshb8.1</i> (Yadav et al 2015)
				LOC_Os08g09220	OsFBX262 - F-box domain containing protein, expressed	
				LOC_Os08g09230	Starch synthase III	
S2_11,343,884	RLH, LH	2	11.33–11.35	LOC_Os02g19440	Sirohydrochlorin ferrochelatase	<i>qSBR-2</i> (Kunihiro Y 2002)
S3_15,836,747	RLH, PH	3	15.82–15.84	LOC_Os03g27590	OsSCP18 - Putative Serine Carboxypeptidase homologue	
				LOC_Os03g27610	Patatin, putative, expressed	
S2_7,170,057	RLH, PH	2	71.60–71.80	LOC_Os02g13420	Leucine-rich repeat receptor protein kinase	
				LOC_Os02g13430	Receptor-like protein kinase 5	
				LOC_Os02g13450	MEGL7 - Maternally expressed gene	
S6_28,836,919	RLH	6	28.82–28.84	LOC_Os06g47600	Thaumatococcus family domain containing protein	
				LOC_Os06g47620	Peptidase	
				LOC_Os06g47630	Expressed protein	
S11_19,435,536	RLH, PH	11	19.42–19.44	LOC_Os11g32880	DEAD-box ATP-dependent RNA helicase	<i>qSBR-11</i> (Kunihiro Y 2002)
				LOC_Os11g32900	Retinoblastoma-related protein-like	
S6_9975427	DS, LH	6	9.96–9.98	LOC_Os06g17220	UDP-glycosyltransferase	
*The traits marked in bold indicate primary traits (significant at p-value < 4.21838E-05) and remaining are secondary traits (p-value < 0.05). The italicized and underlined loci harbor significant SNPs.						

The present study revealed only a single genomic region on chromosome 1 to be strongly associated with LH which altered the trait over its mean value by 18%. However, for RLH, 11 genomic regions on chromosomes 2, 3, 4, 5, 6, 7, 8, and

11 showed significant association with effects ranging from 3.12–8.66% over the mean value of the trait. Most of these regions also showed an association with LH and PH at a p-value < 0.05.

Of the 9 SNPs significantly associated with PH, two SNPs with the highest effect on chromosomes 3 and 6 altered the trait by a value of 9% over the mean value. The MTA that altered the trait RLH maximally was found on chromosome 2. A single genomic region on chromosome 6 was found to be strongly associated with DS and altered the trait over its mean value by 15.84%.

Candidate Genes Prediction

Candidate genes were predicted by searching the physical position of significant SNP markers in a 20-Kb window in the rice genome annotation project database. A list of predicted candidate genes is given in Table 5. Of the 22 significant MTAs, 13 were genic SNPs. The functional annotation revealed their roles as protease inhibitors, ATPases, cyclins, F-box domain-containing proteins, patatins. Apart from these, functional annotation of 36 more loci in the LD region was also done. Moreover, 6 strongly associated SNPs were found to be in the LD region/ overlapped previously reported 5 genes/QTLs associated with ShB resistance, providing analytical proof of our study.

Discussion

Variations in *O. rufipogon* for ShB resistance

Developing sheath blight-resistant rice cultivars is likely the most effective, economic, and environment-friendly approach for managing sheath blight disease compared to the overall usage of fungicides (Molla et al. 2020). Several germplasm resources showing sheath blight resistance have been reported (Aggarwal et al. 2019; Bashyal et al. 2017; Eizenga et al. 2002; Pavani et al. 2020; Prasad and Eizenga, 2008; Srinivasachary et al. 2011). But being a quantitative trait, sheath blight resistance is controlled by multiple minor genes, thus, highly influenced by environmental conditions which limit the accuracy of phenotypic data. However, most of these studies have been conducted in cultivated germplasm. A very few reports have been published that have shown the evaluation of wild species against sheath blight (Aggarwal et al. 2019; Bashyal et al. 2017; Ram et al. 2008). So far, wild species of rice have not been extensively exploited in the breeding program for developing sheath blight-resistant cultivars. In the present study, 405 accessions of *O. rufipogon* showed a variable reaction to sheath blight indicating the existence of genetic variability among them. Based on disease reaction over three years of screening, 19 accessions showing moderately resistant reactions have been identified. These accessions can be utilized for the genetic enhancement of elite cultivars.

ShB resistance loci

GWAS identified fifteen genomic locations associated with sheath blight resistance in *O. rufipogon* accessions. These genomic regions were distributed across all the rice chromosomes except 10 and 12. Besides these regions, 7 significant MTAs on chromosomes 2, 3, 4, 5, 6, and 7 were obtained for PH. In the present study, the utilization of dense marker coverage allowed the identification of highly significant SNP markers associated with the ShB resistance at a higher resolution that could accelerate the marker-assisted breeding of ShB resistance rice cultivars. The majority of the loci identified in this study are co-localized with the previously mapped ShB resistance QTLs which authenticates our finding. In the present report, out of 22 genomic regions associated with the ShB traits, seven regions overlapped with previously reported genes/QTLs for ShB namely, *qSB2* (Pinson et al. 2005), *qSBR-2* (Kunihiro Y, 2002), *qSB-5* (Han Y P, 2002), *qSB-7* (Zou J H et al. 2000), *qshb7.2* (Yadav et al. 2015), *qshb8.1* is at 5.1 Mb (Yadav et al. 2015) and *qSBR11* (Kunihiro Y, 2002). This information indicates the reliability of GWAS methods for the detection of significant SNPs.

Apart from the correlation of the level of infection with the resistance response of germplasm, the biggest complication is its correlation with plant growth, particularly plant height (PH) and heading date (HD) (Channamallikarjuna et al. 2010; Zou et al. 2000). The standard evaluation system for disease scoring is based on a 0-9 scale which measures lesion length above the waterline (Zeng et al. 2015). According to this system, greater PH is correlated with higher resistance. Many reports showed that plant height is frequently correlated with ShB resistance (Channamallikarjuna et al. 2010; Eizenga et al. 2013; Kunihiro et al. 2002; Li Z et al. 1995; Wen Z H et al. 2015; Zou et al. 2000), and loci for plant height and ShB resistance often co-localized at the same region (Channamallikarjuna et al. 2010; Li Z et al. 1995; Wen Z H et al. 2015; Zou et al. 2000). Similarly, our study also determined seven regions significantly associated with RLH to be also associated with PH. Moreover, two SNPs on chromosomes 3 and 9 showed strong associations with both PH and RLH.

Functional annotation of candidate genes

In this report, GWAS was conducted to identify the significant association of SNP markers to sheath blight resistance in the accessions of wild rice *O. rufipogon*. Physical scanning of 20 Kb regions around the GWAS identified 22 significant SNP markers identified 69 predicted open reading frames (ORFs). Among them, 46 ORFs encode proteins with a known functional domain while the remaining encodes either a hypothetical or an expressed protein with unknown functions. Out of these, 13 were genic SNPs. The majority of the functional domain-containing genes are involved in the rice immune response against sheath blight and other fungal and bacterial pathogens. The functional annotation of candidate genomic regions helped establish the roles of genic and LD-region loci in conferring ShB resistance. Functional annotation of loci in the LD region of significant SNPs followed by a detailed literature survey helped establish the role of genomic regions in providing ShB resistance. The role of aspartyl tRNA synthetases in conferring disease resistance has been demonstrated by Luna et al (2014). Similarly, the role of ATPases linked to S6_4,029,679 in conferring defense response has been functionally analyzed in response to blast fungus *Magnaporthe oryzae* by Liu et al (2020). Likewise, the role of Dynamin-related proteins in conferring immunity against bacterial and fungal pathogens has been reported by Tang et al (2006); Smith et al (2006); Smith et al (2014) and Chaparro-Garcia et al (2015). Durian et al (2016) have highlighted the role of protein phosphatases in defense response against biotic stresses. The role of cyclins in detection and adaptation to stress by altering their level and altering the cell cycle in plants has been documented in several studies (Qi and Zhang, 2020). Likewise, Liu et al. (2018) established the defense role of pectin methylesterase-inhibiting proteins in response to a fungal infection caused by *Verticillium dahliae* in cotton where the enzyme interacts with Pectin Methylesterases to regulate the expression of specific fungal polygalacturonase which enhanced resistance. Similarly, the role of adaptor protein complexes in conferring resistance to bacterial blight and rice blast has been determined by Qiao et al (2010). Tameling et al (2007) have determined Ran GTPase-Activating proteins to physically associate with NB-LRR resistance proteins to confer resistance to Potato virus X in potato. Apart from this, SNP S4_25,149,326 on chromosome 4 was found to be closely linked to LOC_Os04g42480 which encodes a receptor-like protein kinase. Earlier, fine-mapping of sheath blight resistance locus *qSB11-LE* revealed a receptor-like protein kinase in the QTL region on chromosome 11 (Zuo et al. 2013). Other than chromosome 4, we have also determined the receptor-like protein kinase genes on chromosome 2 affecting relative lesion height closely linked to the SNPs S2_7,170,057 and S2_25,483,277. Previously, the sheath blight resistance QTL, *qSB-2*, was identified on chromosome 2 which overlapped these two SNPs (Pinson et al. 2005). Recently, comparative transcriptome analysis showed up-regulation of receptor-like protein kinase with other pathogenesis-related genes when challenged with *R. solani* (Yuan et al. 2018). For successful pathogenesis, host-pathogen interaction is very crucial for producing disease symptoms under a favorable environment. Several small molecular weight proteins act as receptors to mediate the interaction of pathogen effectors on the cell surface. The lipid transfers proteins (LTP) belong to a large family of pathogenesis-related 14 (PR-14) proteins (Finkina et al. 2016). The functional characterization of LTP genes showed that up-regulation of these proteins confers the resistance response to the major fungal and bacterial pathogens in plants (Ge et al. 2003; Kim et al. 2006; Maldonado et al. 2002). The inducible expression of several LTP protease inhibitors was also seen during pollen development in the

male-sterile plants (Khlaïmongkhon et al. 2021; Zhang et al. 2010). In the present report, identification of LTPL51, LTPL52, and LTPL75 linked to S3_15,307,919 and S7_3,920,076 on chromosomes 3 and 7 suggested the possible role of lipid-mediated long-distance signalling of a systemic immune response upon *R. solani* infection. Several studies suggested that LTP genes are co-expressed with other transcription factors. To date, numerous transcription factors have been reported that are critical for elicitation of resistance response upon pathogen infection. Among them, WRKY30 and WRKY80 are known to enhance the sheath blight resistance by up-regulation of the jasmonic acid biosynthesis pathway (Peng et al. 2012; Peng et al. 2016). Another locus in the LD region of S3_15,307,919 encoded F-box proteins which have been reported by Li et al (2019) to provide resistance to banded leaf and sheath blight in maize, making this genomic region a strong candidate for conferring SHB resistance. Both the genomic regions encoding LTP proteins showed association with RLH. LOC_Os06g47600 in LD region of significant SNP on chromosome 6, encoded thaumatin-like proteins which belong to an important class of pathogenesis-related protein family (PR-5). The antifungal role of thaumatin-like proteins is well documented in rice against sheath blight disease by overexpression studies (Datta et al. 1999; Kalpana et al. 2006; Velazhahan et al. 1998; Xue et al. 2016). Thaumatin-like proteins associated with the S6_28,836,919 in the GWAS analysis could be a novel gene resource that could be deployed in the breeding elite ShB resistant cultivars. Another significant association obtained on chromosome 6, S6_9975427, harbored in locus encoding UDP-glycosyltransferases whose over-expression has been reported to confer root tolerance to the mycotoxin and spike resistance to the fungus *Fusarium graminearum* by Pasquet et al 2016.

The function of retinoblastoma-related genes in conferring powdery mildew resistance in grapes has been documented by Wen Z et al (2012). Li et al (2008) have demonstrated the role of an OsBIRH1 coding for DEAD-box RNA helicase protein in blast resistance in rice and by over-expression studies in Arabidopsis confirmed its role in both abiotic and biotic stresses. However, identification of loci regions coding for MYB transcription factors, Sirohydrochlorin ferrochelatase could not establish a relationship with defense response, indicating that regulation of sheath blight resistance in *O. rufipogon* might have a diverse mechanism which is poorly understood and might require further investigation.

The breeding implication of GWAS for developing ShB resistance cultivars

The development of rice cultivars resistant to ShB is challenging due to the non-availability of promising donors and effective resistance genes, a wide range of host compatibility, and dynamic variability in resistance pathotypes (Molla et al. 2020). So far, no source of competing resistance has been identified in wild and cultivated rice. This report promises identification of a total of 22 accessions of *O. rufipogon* which showed consistent resistant response across three years of screening to *R. solani*. These accessions could be used as potential donors for breeding elite ShB resistant cultivars. Sheath blight resistance in rice is mostly quantitative and controlled by multiple genes. However, due to poor mapping resolution and the use of few SSR markers, most of the loci were mapped to large genomic regions that limit the suitability of QTLs in gene pyramiding through marker-assisted breeding. Fine-mapping of ShB resistance loci allowed the pyramiding of multiple genes in the same genetic background. Numerous studies showed pyramiding of ShB resistance QTLs for developing resistance cultivars (Chen et al. 2014; Ramalingam et al. 2020; Wang et al. 2012; Zuo et al. 2014; Zuo et al. 2011). Presently, the use of dense SNP markers using GWAS allows identification of the resistance locus at higher resolution. In this report, a total of 22 significant associations harboring 79 genes in LD regions contributed to moderate resistance in *O. rufipogon* accessions using GWAS. The significant SNPs could be converted into PCR-based markers or KASP markers for introgression of resistance gene into cultivated rice through MAS.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

KN, KS, JSL- Conceived the idea, proofread the manuscript; SA, JSL- Screening of the germplasm and phenotypic data analysis; PM- Performed GWAS and analysed the results; RK, KN- Handling and maintenance of the germplasm; PM, SA, KK, KN- Writing the manuscript. All the authors read the manuscript carefully and approved it.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Supplementary Tables

Supplementary Table 1 is not available with this version

Figures

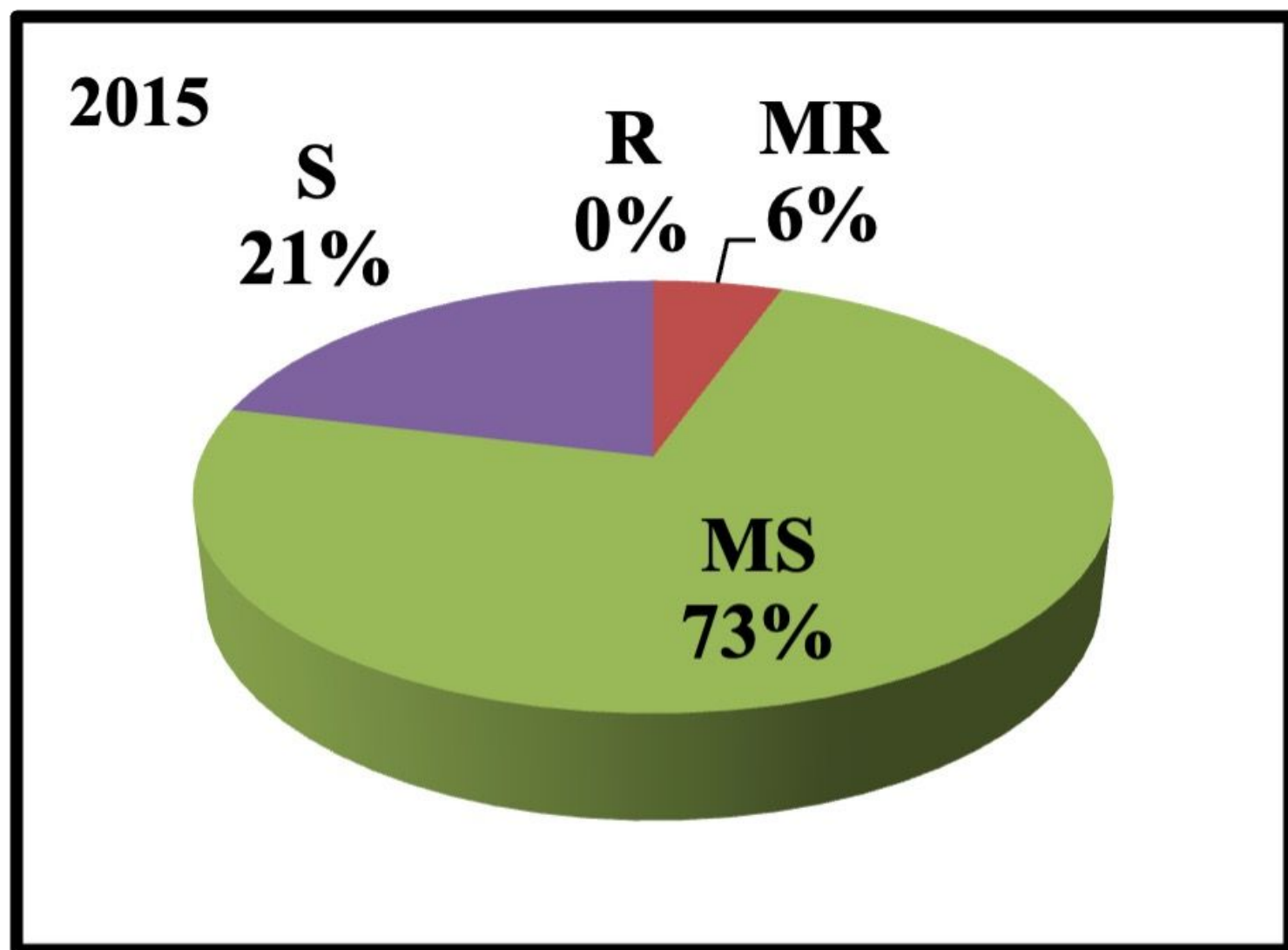


Figure 1

Pie chart describing the proportion of disease response in *Oryza rufipogon* panel during 2015

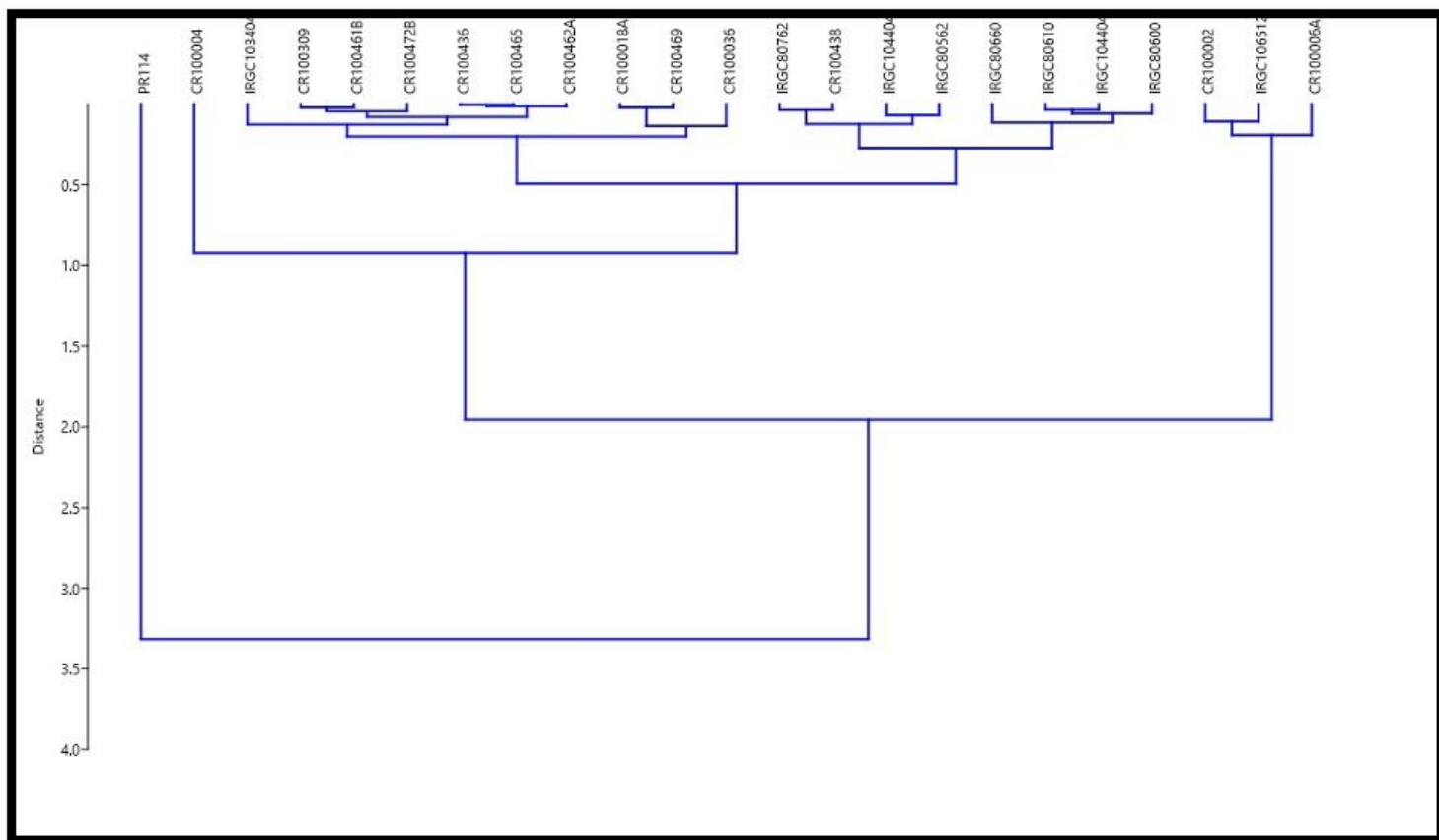


Figure 2

Cluster representation among *Oryza rufipogon* accessions during 2016 and 2017

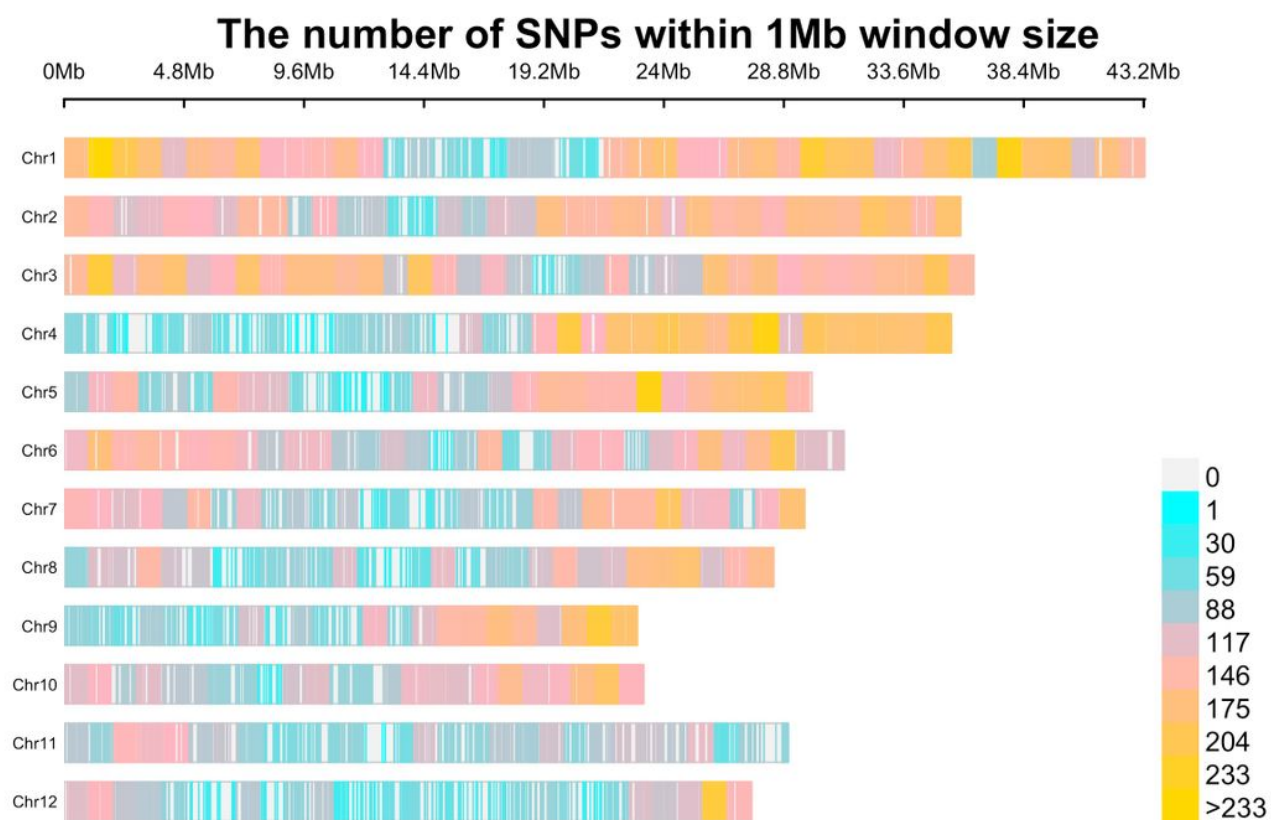


Figure 3

Number of SNPs within 1Mb window for all the 12 chromosomes

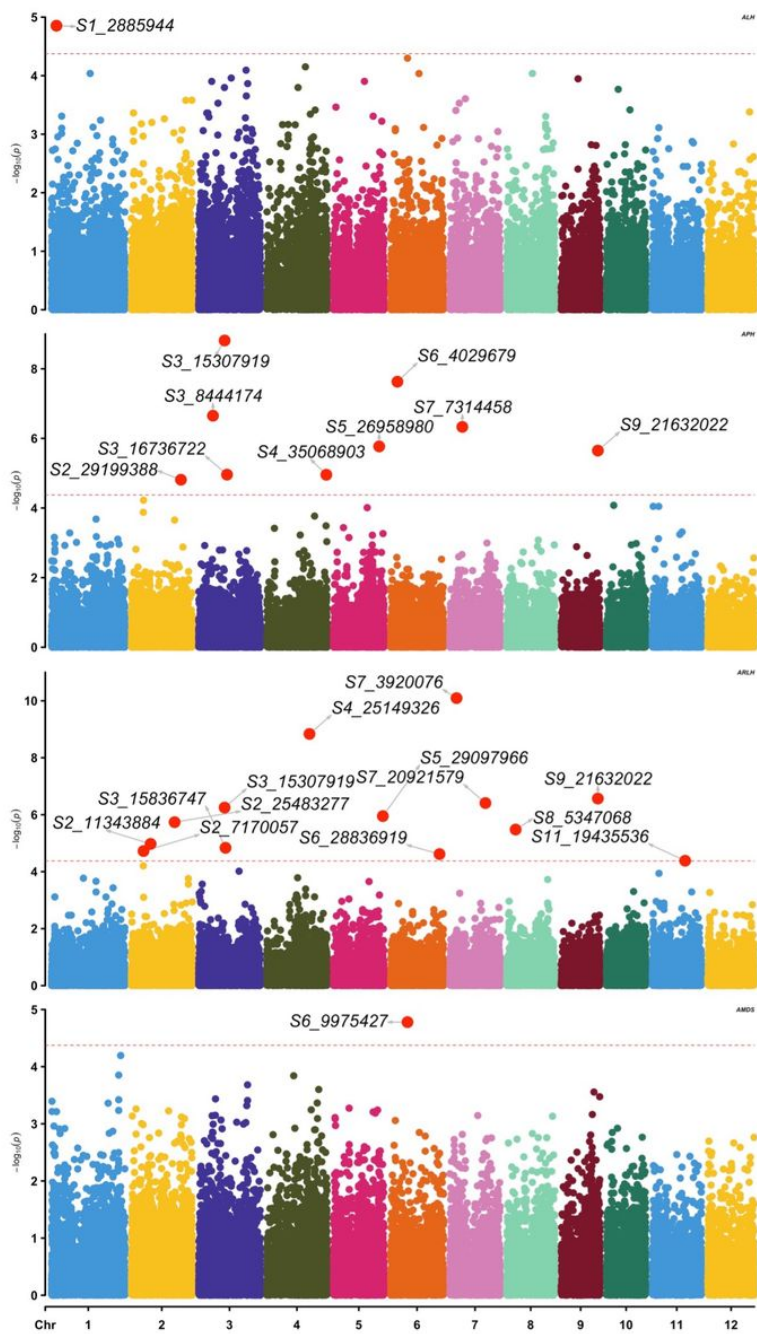


Figure 4

Manhattan plots depicting significant marker-trait associations for various traits LH, PH, RLH, and DS from top to bottom where red dotted line marks p-value threshold of 4.21838×10^{-5}