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Genetic studies and identification of QTLs for sheath rot disease resistance in rice (*Oryza sativa* L.)

C. Mahadevaiah[1,3], Shailaja Hittalmani[1,*], G. Uday[1] and M. K. Prasanna Kumar[2]

[1]Marker Assisted Selection Laboratory, Dept. of Genetics and Plant Breeding; [2]Dept. of Plant Pathology, University of Agricultural Sciences, G.K.V.K., Bangalore 560 065; [3]Current address: Division of Crop Improvement, ICAR-Sugarcane Breeding Institute, Coimbatore

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Abstract

Rice genotypes BPT5204 and HP14, their direct and reciprocal F_1s (DF_1&RF_1), F_2 populations (DF_2&RF_2) and recombinant inbred lines (RILs) were evaluated to study the genetics of field resistance to sheath rot disease (ShR). Observed frequencies of resistant and susceptible plants in DF_2 and RILs showed 27R:37S ratio and 1R:7S ratio indicating involvement of three dominant genes with complementary gene action. Significant differences between two crosses were observed between DF_1&RF_1, and DF_2&RF_2 for panicle exertion and sheath rot attributing traits indicating the role of cytoplasm in manifestation of sheath rot resistance. Plants with anthocyanin pigmentation recorded significantly higher mean for panicle exertion and higher level of resistance to sheath rot disease in both F_2s and RILs. Single marker analysis indicated association of anthocyanin with sheath rot resistance making purple pigmentation as probable morpholigical marker for it. Composite interval marker analysis revealed q*ShR*-12 located on chromosome-12 and flanked by SSR markers RM7315 and RM28118 accounting 11.78% of cumulative phenotypic variation. This is the first report on genetics, role of cytoplasm, association of anthocyanin pigmentation and SSR markers with sheath rot disease resistance.

Key words: Rice, sheath rot disease, QTLs, complementary genes, anthocyanin and morphological marker

Introduction

Sheath rot (ShR) disease caused by *Sarocladium oryzae* [(Sawada) W. Gams & D. Hawksw] emerged as one of the major disease affecting most of rice-growing ecosystems in the world (Bigirimana, 2015). Though there are no reports on extent of yield loss in India due to sheath rot disease in recent years, 3 to 85% yield loss has been reported depending upon the disease severity (Chen 1957; Amin et al. 1974; Chakravarthy and Biswas 1978). In severe cases, it causes complete chaffiness and suppression of panicle exertion (Raina and Singh, 1980). The pathogen produces secondary metabolites cerulenin and helvolic acid (Sakthivel and Gnanamanickam 1986; Tschen et al. 1997; Ghosh et al. 2002 and Nandakumar et al. 2007) and whole genome sequencing of *S. Oryzae* revealed the presence of genes involved in biosynthesis of cerulenin and helvolic acid (Hittalmani et al. 2016) causing the greyish brown necrotic lesion on flag leaf sheath. The pathogen is primarily seed borne and enters host through injuries or sucking pests such as mites, (*Leptocorisa acuta* Thumb) (Amin et al. 1974; Chakravarthy and Biswas, 1978; Milgrosa, 1987; Lakshmanan et al. 1992; Bigirimana, 2015). Disease symptoms which are invisible until the crop reaches booting stage, appear as minute brownish necrotic pricks on flag leaf sheath in booting stage, enlarges to a lesion in milky stage and lesion coalesce form dark brownish sheath covering significant portion of flag leaf sheath during maturity. Panicles enclosed with infected flag leaf sheath discoloures due to growing inoculum and chaffiness of grains causing loss of nutritional and economic value of produce (Gopalakrishnan et al. 2010).

Disease resistance is a complex phenomenon influenced by environment, host and pathogen factors (Agriose 2005). Genetics of disease resistance shall be dissected out by studying the segregating pattern in F_2 population (Padmavathi et al. 2005; Khumbar et al. 2013; Rajashekara et al. 2014) and recombinant inbred lines (Tullu et al. 1998; Graichen et al. 2010).

*Corresponding author's e-mail: shailajah_maslab@rediffmail.com
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Reciprocal cross difference reveals the role of cytoplasm in governing the disease resistance and dissected out by studying the direct and reciprocal cross F_2 populations (Rath and Padmanabhan 1972; Hooker 1974). Several alkaloids and phenolic compounds are reported to confer basal resistance to diseases in plants (Jones 2006). In rice, many secondary metabolites (proanthocyanidins, 3-deoxyanthocyanidins, flavonols, phenylpropanoids and naringenin) accumulated during anthocyanin biosynthesis were reported for insect pests and disease resistances (Reddy 1995; Poltrurak et al. 2017). Purple pigmentation in coleoptiles confer resistance to brown plant hoppers (Rao and Kalode 1987). Possible association of purple pigmentation with sheath rot disease resistance was examined in the present study. Parental lines (BPT5204 and HP14) were widely differing for anthocyanin pigmentation. Five different populations such as direct F_1 (DF_1), reciprocal F_1 (RF_1), direct F_2 (DF_2), reciprocal F_2 (RF_2) and recombinant inbred lines (RILs) and parental lines (BPT5204 and HP14) were evaluated under artificial disease screening to dissect out the genetics of ShR resistance, role of anthocyanin pigmentation on ShR resistance and to detect simple sequence repeat motifs (SSR) marker based QTLs associated with ShR resistance.

Material and methods

Plant material and experimental details

Plant material in the present study consisted of parental lines (BPT 5204, HP 14), DF_1, RF_1, DF_2, RF_2, F_{10} RILs and susceptible standard IR64. Parental lines *viz*., BPT5204 is a super-fine grain type variety, non-pigmented and highly susceptible to ShR whereas HP14 is a landrace, purple pigmented at basal sheath, leaf blade, culm, apiculi and highly resistant to ShR. To dissect out the role of cytoplasm in manifestation of ShR disease resistance, reciprocal crosses between parental lines (BPT5204 and HP14) were made during *kharif* season and F_1s were evaluated in *rabi* season, 2013. True DF_1 and RF_1 plants were identified based on polymorphic SSR marker (RM583) and morphological markers (basal sheath pigmentation in seedlings). F_{10} generation RILs derived from BPT5204 × HP14 developed by our group were used in the present study.

Previous studies on sheath rot disease resistance incidence and severity in aerobic condition was found on par with wetland rice (Mahadevaiah et al. 2016). Hence, the experiments were planted in aerobic condition and cultural operations were carried out as per the recommended package of practice (Gandhi et al. 2011). DF_2 (669 plants), RF_2 (579 plants), 10 plants of each DF_1 and RF_1, parental varieties (BPT5204 and HP14) were raised in the nursery, 21 days old seedlings were soaked in conidial solution for overnight and transplanted in field during late *kharif* season (October planting), 2014. RILs (276) were direct sown in randomized block design with two replications during *kharif* (June sowing) and late *kharif* (October sowing) seasons.

Artificial disease screening in field condition

Single spore isolate of *S. oryzae* (Gene bank ID: KT291723) sampled from GKVK campus, University of Agricultural Sciences, Bangalore was mass multiplied on potato dextrose agar, potato dextrose broth and rice kernels. Artificial disease screening (Mahadevaiah et al. 2015) was carried out using conidial suspension (10^5 conidia/ml). Seeds of RILs and parental lines, and 21 days seedlings of DF_2 and RF_2 were soaked in conidial solution (10^5 conidia/ml) overnight. Further inoculum augmented through cotton swab inoculation of DF_1, RF_1, DF_2, RF_2 and RILs on 62^{nd} days and, foliar application of conidial suspension (10^5 conidia/ml) using hand sprayer on 30^{th}, 40^{th}, 50^{th}, 60^{th} and 70^{th} days after sowing which coincides with vegetative stage to booting stage.

Genotyping of RILs

DNA isolated from leaf samples pooled from five plants collected from 25-30 days old seedlings of RILs were used for DNA isolation by using modified CTAB method (Cao and Oard, 1997) and DNA quantified using using 0.8 per cent agarose gel. Total of 360 SSR markers sampled from *Gramene* database (McCouch et al. 2002; www.gramene.org) and among them, 112 SSR markers showing parental polymorphism and uniformly distributed on all chromoses were used for genotyping of RILs. SSR marker were resolved using 2.5 per cent agarose gel and scored as 'A' for BPT5204 allele, and 'B' for HP14 allele. Missing bands were scored as '–'.

Data collection and descriptive statistics analysis

Sheath rot lesion length, proportion of diseased flag leaf sheath, simplified severity scores, panicle exertion and disease incidence scores were recorded (IRRI 2002; Mahadevaiah et al. 2017). Each plant of DF_1, RF_1, DF_2, RF_2 populations and RILs were scored for purple pigmentation for basal sheath (fully pigmented sheath, partially pigmented sheath and green or non-

pigmented sheath) and leaf blade (purple and green) during seedling stage and for culm colour (purple and green), apiculi (purple and pale green) and stigma (purple and pale green) during anthesis stage. Chi square (χ^2) statistics was used to test the goodness of fit between observed and expected frequency of resistance and susceptible plants in F_2 populations and RILs. Significance between fully pigmented, partially pigmented and green coloured sheaths was tested using one-way ANOVA. Significance of purple and green categories for anthocyanin pigmentation in leaf blade, culm, apiculi and stigma tip was tested using 't' test assuming unequal variances. Single marker analysis (SMA) was carried out by regressing anthocyanin pigmentation on ShR resistance using SPSS statistical software package (version 16; SPSS, Chicago, IL, USA).

Linkage map construction and QTL analysis

Genetic linkage map was constructed by using *GMendel* in *iMAS* server in RILs with threshold LOD of ≥3.0 and recombination value of 0.25 forming the 12 groups. The order of each loci in a group is calculated using three point test cross method and Haldane method used to estimate the genetic distance. Composite interval mapping (CIM) was performed using WinQTL Cartographer 2.5_011 version (Wang et al. 2012) with 1000 permutations.

Results

Reactions to sheath rot disease

Female parent BPT-5204 was highly susceptible to sheath rot disease as indicated by ShR incidence and severity scores, ShR lesion length, proportion of panicle exertion and disease indices (Table 1). Male parent (HP14) was devoid of infection in both seasons and immune to ShR with complete panicle exertion. Response of DF_1 and RF_1 were differed for their response to ShR in late *kharif* season. DF_1 took no infection whereas RF_1 infected on late formed tillers with lesion length of 6.00 cm, ShR incidence score of 1, severity score of 7 with partially exerted panicle and partially infected sheath. DF_1 was immune and RF_1 was highly resistant based on severity and lesion index. Similar trend was also observed in F_2 populations; mean ShR incidence (3.56) and severity scores (4.49) was significantly lower in DF_2 as compare that of mean ShR incidence (3.94) and severity scores (5.41) in RF_2 population. Mean panicle exertion (–2.88 cm) and proportion of panicle exertion (0.79) was significantly high in DF_2 as compared to mean panicle

Table 1. Response of parents, F_1s, F_2s and recombinant inbred lines for sheath rot disease attributing traits during *kharif* (K) and late *kharif* (LK) seasons, 2014

S.No.	Traits	Parents				F_1s		F_2 populations		RILs	
		BPT5204		HP14							
		K	LK	K	LK	DF_1	RF_1	DF_2	RF_2	K	LK
1	Plant height (cm)	37.96	37.96	107.28	78.23	78.00	39.00	59.80**	55.46**	89.68**	65.88**
2	Panicle exertion (cm)	–3.94	-8.56	5.25	3.88	–3.00	0.00	–2.88**	–4.31**	1.47**	–4.83**
3	Proportion of panicle exertion	0.69	0.38	1.26	1.20	0.83	1.00	0.79**	0.68**	1.07**	0.68**
4	Sheath rot lesion length (cm)	11.37	10.56	0.00	0.00	0.00	6.00	5.34ns	5.28ns	3.75**	7.55**
5	Proportion of diseased sheath	0.61	0.63	0.00	0.00	0.00	0.35	0.28ns	0.29ns	0.17**	0.39**
6	Sheath rot incidence scores (SES)	8.33	9.00	0.00	0.00	0.00	1.00	3.56**	3.94**	0.17**	4.17**
7	Simplified sheath rot severity scores	7.88	8.25	1.00	1.00	1.00	7.00	4.49**	5.41**	2.08**	5.53**
8	Severity index	758.33	825.00	0.00	0.00	0.00	7.00	269.00ns	261.00ns	159.00**	298.00**
9	Lesion index	1089.86	1056.25	0.00	0.00	0.00	6.00	422.00**	328.00**	292.00**	451.00**

*Significant @ 1 per cent, * *Significant @ 5 per cent
+Probability values for paired 't' test assuming unequal variance

exertion (-4.31 cm) and proportion of panicle exertion (0.68) in RF_2 (Table 1). Similarly, significantly higher mean for panicle exertion and lesser means for sheath rot disease attributing traits were observed in RILs between *kharif* and late *kharif* seasons.

Genetics of ShR disease

Out of 669 plants in DF_2, 289 plants were resistant (with no infection) and 380 plants were susceptible or took infection (Supplementary Table S1). *Chi* square test (χ^2) for three complementary gene model showed goodness of fit (p>0.5961) between observed and expected frequencies. Segregation in 27R:37S ratio confirmed the three dominant complementary genes governing the ShR resistance and further reconfirmed with genetic analysis in RILs. Out of 276 RILs, 32 RILs were resistant (with no infection) and 244 RILs were susceptible and segregating in 1:7 ratio (p>0.6491; S_1). In RF_2, 171 plants were resistant and 408 were susceptible, and did not fit to three complementary genes (p value for χ^2 statistics: 0.0001; S_2) or any of the known gene action (Supplementary Table S2). This could be attributed to cytoplasmic effect of HP14.

Association between anthocyanin pigmentation and sheath rot disease resistance in rice

Significant differences were observed for mean performances of fully pigmented, partially pigmented and green category of basal sheath, and among purple-green category of leaf blade, culm, apiculi and stigma in DF_2, RF_2 and RILs. Plants of fully purple pigmented sheath had high mean for panicle exertion as compared to plants of green sheathed in both F_2s and RILs. The incidence of ShR was significantly lower in plants of purple pigmented sheath, leaf blade, culm, apiculi and stigma as compared to green plants in DF_2, RF_2 and RILs.

Association of purple pigmentation with disease resistance using single marker analysis showed that purple pigmented sheath and leaf blade showed significant association with ShR resistance. Basal sheath and leaf blade pigmentation accounted 9.81% and 7.78% phenotypic variation for lesion length, 8.16% and 6.33% phenotypic variation for proportion of diseased sheath, 6.98% and 6.37% phenotypic variation for panicle exertion respectively (Table 2).

Linkage map construction

Linkage map construction using the *iMAS 2.0* (*GMendel*) with threshold LOD of 3.0 resulted in twelve linkage group covering distance of 1159 cM with an average of 10.35 cM between the markers. Rice linkage groups were identified from 1-12 as per the standard linkage map (McCouch et al. 1997; McCouch et al. 2002).

QTL analysis

Significant strong inverse correlation was observed for panicle exertion with ShR attributing traits such as sheath rot lesion length (–0.814), proportion of diseased sheath (–0.810), sheath rot disease incidence (SES) scores (–0.680), simplified severity scores (–0.699), severity (–0.660) and lesion (–0.640) indices (Mahadevaiah et al. 2017) indicating that incomplete partial exertion as indicator of susceptibility to ShR disease. Composite interval marker analysis in RILs using 112 SSR markers resulted in detection of two QTLs for panicle exertion and one QTL for ShR disease resistance (Fig. 1). QTL for panicle exertion (q*PE*-3) detected from *kharif*, 2014 was flanked by RM6712-RM168 with inter marker distance of 15.8 cM, explaining 8.58 *per cent* of phenotypic variation for panicle exertion and 11.81 *per cent* for proportion of panicle exertion. QTL 'q*PE*-5' detected for panicle exertion, flanked by RM592-RM249 accounting 5.9 per cent of phenotypic variation. QTL 'q*ShR*-12' detected for sheath rot lesion length and simplified severity scores located on chromosome-12 flanked by SSR marker RM7315 and RM-28118 with intermarker distance of 15.3 cM, explaining 5.51 and 6.27 *per cent* of phenotypic variation respectively. Genomic regions for sheath rot lesion length and sheath rot severity scores were contributed by male parent with additive effect of 1.97 and 3.31, respectively.

Discussion

Disease resistance is a complex trait governed by disease resistance genes, quantum of inoculum and influenced by environmental factors (Agriose, 2005). Our previous studies had showed that the incidence and severity of sheath rot disease under aerobic condition was on par with the wetland rice (Mahadevaiah et al. 2016). Late planting coupled with low temperature and higher humidity were favourable for ShR disease (Reddy et al. 1999; Reddy et al. 2001). ShR disease incidence was higher in dwarf cultivars due to incomplete panicle exertion (Srinivasachary et al. 2002). *Kharif* season generally favours ShR escape due to complete panicle exertion. In late *kharif* paddy (October planting), low temperature during December-January favours incomplete panicle exertion (Cruz et al. 2006; Jiang et al. 2011) and offers favourable

Table 2. Single marker analysis of anthocyanin pigmentation with sheath rot disease resistance attributing traits in F_2 & RIL population derived from BPT5204 and HP14 during late *kharif* seasons, 2014

Traits	Class	Population	Mean values of sheath rot disease resistance attributing traits							
			Plant height (cm)	Panicle exertion (cm)	Proportion of panicle exertion	ShR lesion length (cm)	Proportion of diseased sheath	ShR incidence (SES) scores	Simplified ShR severity scores	Grain yield/ plant (g)
Basal sheath pigmentation	Purple	DF_1	60.97	–1.30**	0.86*	3.40**	0.18**	2.17**	3.32**	6.85**
		DF_2	55.20*	–4.19**	0.68**	4.94**	0.29**	3.78**	5.13**	5.00*
		RILs	72.07*	–1.56**	0.86**	3.33**	0.82**	2.34**	3.57**	17.71
	Partial Purple	DF_1	60.44	–2.81**	0.81*	5.49**	0.29**	3.67**	4.49**	8.16**
		DF_2	56.60*	–3.72**	0.72**	4.70**	0.26**	3.55**	5.04**	5.58*
		RILs	63.92*	–5.05**	0.66**	8.20**	0.59**	4.53**	5.76**	17.92
	Green	DF_1	57.97	–3.87**	0.74*	6.07**	0.31**	4.10**	5.09**	8.14**
		DF_2	52.96*	–5.76**	0.59**	6.83**	0.38**	4.94**	6.44**	5.56*
		RILs	64.50*	–5.37**	0.65**	7.83**	0.60**	4.33**	5.75**	18.30
	R^2		3.06	6.98	4.9	9.81	8.16	4.46	5.33	0.05
Leaf blade pigmentation	Purple	DF_1	60.97	–1.29**	0.86	3.39**	0.18**	2.17**	3.32**	6.84**
		DF_2	55.20	–4.19	0.68	4.94	0.29	3.78	5.13	5.00**
		RILs	72.24	–1.50**	0.86**	3.59**	0.80**	3.83**	5.11**	3.35
	Green	DF_1	59.62	–3.16**	0.782	5.66**	0.29**	3.79**	4.68**	8.16**
		DF_2	55.50	–4.33	0.68	5.33	0.30	3.97	5.46	5.57**
		RILs	64.41	–5.20**	0.66**	7.85**	0.60**	4.43**	5.71**	3.40
	R^2		2.44	6.37	4.32	7.78	6.32	3.85	4	0.02
Culm, apiculi and stigma pigmentation	Purple	DF_1	60.58*	–2.49**	0.82**	5.04*	0.26*	3.34*	4.23**	7.90
		DF_2	56.34**	–3.80**	0.72**	4.74**	0.27**	3.58**	5.05**	5.47
		RILs	66.00	1.08**	1.05*	6.79*	0.35	3.74	5.05	3.29
	Green	DF_1	57.97*	–3.87**	0.74**	6.07*	0.31*	4.10*	5.09**	8.14
		DF_2	52.89**	–5.78**	0.59**	6.84**	0.38**	4.97**	6.45**	5.55
		RILs	64.76	–5.33**	0.65*	7.80*	0.40	4.51	5.77	3.47
	R^2		0	1.6	0.92	0.96	0.85	0.35	0.66	0.14

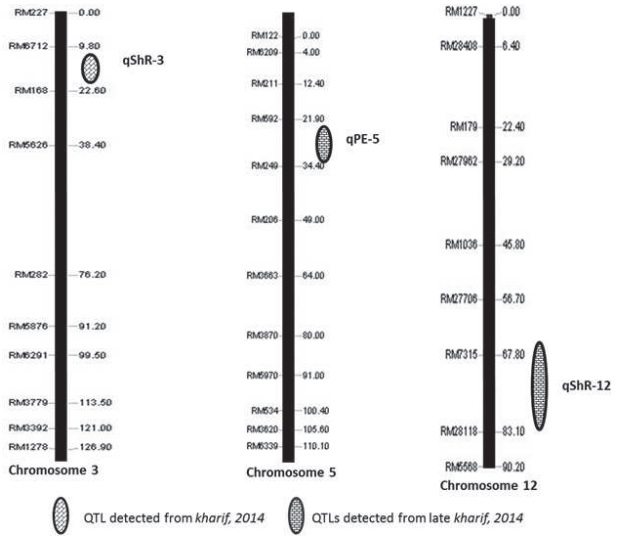

Fig. 1. Location of QTLs associated with sheath rot disease resistance attributing traits on rice chromosome map. qShR12 explained 5.51% and 6.27% of phenotypic variation for sheath rot lesion length and simplified ShR severity scores with LOD score of 3.58 and 3.36, respectively. QTL qPE5 accounted 5.9% of phenotypic variation for panicle exertion (LOD= 3.49). qPE3 accounted 8.58% and 10.12% of phenotypic variation for panicle exertion (LOD=3.58) and proportion of panicle exertion (LOD of 5.5)

ambience for ShR disease incidence. Hence, the two experiments in *kharif* and late *kharif* seasons were planted and phenotyped for ShR under artificial disease screening methods.

Parental diversity for one or more traits is prerequisite for QTL mapping (Collard et al. 2005). HP14 was resistant to ShR and took no infection, whereas BPT5204 was classified as highly susceptible to ShR based on estimates of severity (791) and lesion (1073.1) indices (Mahadevaiah et al. 2017). Segregation of DF_2 and RILs for sheath rot disease resistance attributing traits segregated in 27R:37S ratio in DF_2 and 1R:7S ratio in RILs confirmed that three dominant complementary genes governing the sheath rot disease resistance. The segregation pattern of RF_2 population did not fit to three complementary genes or any of the known gene action. Besides, significant reciprocal cross differences were observed between DF_1 and RF_1, DF_2 and RF_2, and RILs. DF_1 was resistant and RF_1 took infection in late emerged tertiary tillers. The significant reciprocal cross differences were attributed to the modifying effect or segregation distortions indicate the role of cytoplasm of HP-14. Similar reports on reciprocal cross differences or undesirable effects of cytoplasm were reported for leaf blade pigmentation in crosses involving Purpleputt (Reddy et al. 1995) and inhibitor genes (Kadam, 1974). Further studies from our group revealed polymorphic alleles for cytoplasmic genes as Nad, Cox1A, Cox3 and Nad1A indicating presence of cytoplasmic differences between BPT5204 and HP14 (Yallappa and Hittalmani, 2017).

Parental lines, both F_2s and RILs were differing for purple pigmentation at basal sheath, leaf blade, culm, apiculi and stigma. Purple pigmented plants possess significantly higher level of resistance to ShR due to accumulation secondary metabolites such as proanthocyanidins, 3-deoxyanthocyanidins, flavonols, phenylpropanoids, naringenin during anthocyanin biosynthesis and possesses antifungal properties (Reddy 1996; Poltrurak et al. 2017). Similar findings of purple pigmented plants conferring resistance to brown plant hopper was reported by Rao and Kalode (1987) and grey mold resistance in other crops (Poltrurak et al. 2017). Single marker analysis of purple pigmentation with sheath rot disease attributing traits had showed that purple basal sheath and leaf blade covered significant portion of phenotypic variation for lesion length, proportion of diseased sheath, panicle exertion. Therefore, anthocyanin pigmentation of basal sheath and leaf blade pigmentation does serves as morphological markers for sheath rot disease.

Two QTLs were detected for panicle exertion *viz*., *qPE-3* from *kharif* season located on linkage group 3 and 'q*PE*-5' from late *kharif* season, located on linkage group 5. Our studies had shown significant negative correlation between panicle exertion and ShR disease resistance attributing traits (Srinivasachary et al. 2002; Mahadevaiah et al. 2017; Mvuyekure et al. 2017). Sheath rot resistant 'q*ShR*-12' was detected from this study from late *kharif* season located on chromosome-12 flanked by SSR marker RM7315 and RM28118 explaining 5.51 and 6.27% of phenotypic variation for sheath rot lesion length and ShR severity respectively. The genomic regions comparing resistance were contributed by male parent with additive effect of 1.97 and 3.31, respectively. This is the first report of SSR marker based QTL for sheath rot disease resistance in rice. New locus and differing from the previous reports. Chromosome-12 of rice is enriched with disease resistance and defense genes (The Rice Chromosomes 11 and 12 Sequencing Consortia, 2005). The cursory examination of rice genome annotation database (http://rice.plantbiology.msu.edu/) for qShR12 showed the qShR12 co-localized with of many disease resistant and anthocyanin metabolic genes. The genomic size of qShR-12 was 14.21mb from 5801422 to 7311355 bp on chromosome 12 consisting of 1807 genes. Among them, 106 were disease resistant genes, 11 were abiotic stress related genes, 482 were retrotransposons and 298 were transposon. Anthocyanin metabolic genes such as Chalcone Synthase and O-Methyl Transferase were present in qShR12. Therefore, fine mapping of qShR-12 could able to dissect out the ShR resistant candidate genes. Besides, Mvuyekure et al. (2017) has identified the new resistant sources for sheath rot disease and governed by genes with reported additive effects. Therefore, further focused research on screening of germplasm and identification of resistant QTLs from different genetic backgrounds could able to dissect out the sheath rot disease resistance in rice.

Authors' contribution

Conceptualization of research (SH); Designing of the experiments (SH, CM, MK); Contribution of experimental materials (SH); Execution of field/lab experiments and data collection (CM, SH, GU, MK); Analysis of data and interpretation (CM, GV, SH); Preparation of manuscript (CM, SH).

Declaration

The authors declare no conflict of interest.

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Supplimentary Table S1. *Chi* square test for observed frequency and expected segregation of genes controlling sheath rot resistance in F_2 population and RIL's derived from BPT5204 x HP14 cross in rice

Traits	Parents/ generations	Response	Observed frequency	Expected frequency	Expected ratio	χ^2 statistics	Prob> χ^2
Sheath rot	BPT5204	Susceptible	10	-	-	-	-
incidence (SES)	HP14	Resistant	10	-	-	-	-
	F_1	Resistant	10	-	-	-	-
	F_2	Resistant	289	282.22	27:37	0.2809	0.5961
		Resistant	380	386.76			
	RILs	Resistant	32	34.5	1:7	0.2070	0.6491
		Susceptible	244	241.5			
Sheath rot	BPT5204	Susceptible	10	-	-	-	-
severity scores	HP14	Resistant	10	-	-	-	-
	F_1	Resistant	10	-	-	-	-
	F_2	Resistant	289	282.22	27:37	0.2809	0.5961
		Susceptible	380	386.76			
	RILs	Resistant	32	34.5	1:7	0.2070	0.6491
		Susceptible	244	241.5			
Sheath rot	BPT5204	Susceptible	10	-	-	-	-
lesion length	HP14	Resistant	10	-	-	-	-
(cm)	F_1	Resistant	10	-	-	-	-
	F_2	Resistant	289	282.22	27:37	0.2809	0.5961
		Susceptible	380	386.76			
	RILs	Resistant	32	34.5	1:7	0.2070	0.6491
		Susceptible	244	241.5			

Supplimentary Table S2. *Chi* square test for observed frequency and expected segregation of genes controlling sheath rot resistance in F_2 population derived from HP 14 x BPT5204 cross in rice

Traits	Parents/ generations	Response	Observed frequency	Expected frequency	Expected ratio	χ^2 statistics	Prob> χ^2
Sheath rot	BPT5204	Infected	10	.	.	-	-
incidence (SES)	HP14	Immune	10	-	-	-	-
	F_1	Infected	10	-	-	-	-
	F_2	Immune	171	244.26	27:37	38.010	0.0001
		Infected	408	334.73			
Sheath rot	BPT5204	Infected	10	-	-	-	-
severity scores	HP14	Immune	10	-	-	-	-
	F_1	Infected	10	-	-	-	-
	F_2	Immune	171	244.26	27:37	38.010	0.0001
		Infected	408	334.73			
Sheath rot	BPT5204	Infected	10	-	-	-	-
lesion length	HP14	Immune	10	-	-	-	-
(cm)	F_1	Infected	10	-	-	-	-
	F_2	Immune	171	244.26	27:37	38.010	0.0001
		Infected	408	334.73			