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Allelic Variation of Sheath Blight QTLs among Genotypes Promising for Sheath Blight Tolerance

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Abstract

The fungus, *Rhizoctonia solani* causing sheath blight disease in rice is omnipresent and no durable resistance source is available in the rice germplasm for this pathogen. However, moderate resistance has been reported in some of the rice genotypes and more than fifty QTLs for sheath blight tolerance have been identified in the past. The present investigation validates seven QTLs initially from two moderately resistant genotypes 'Tetep' and 'Teqing' in a set of rice lines, *viz*., Gumdhan, Ngonolasha, Wazuhophek, Phougak, RP 2068-18-3-5 and 10-3, which have been recently reported to be novel sources of moderate resistance to sheath blight. Allelic variation for a QTL marker was not observed between the source genotype and the selected susceptible genotypes and both the source genotype and susceptible genotypes showed similar allelic position at the QTL loci.

Keywords: Rice, Sheath blight, QTLs, Allelic variation

Introduction

Rice sheath blight (ShB), caused by the soil-borne fungal pathogen *Rhizoctonia solani* Ku¨hn, is one of the three major diseases of rice that greatly reduces yield and grain quality worldwide (Savary *et al* 2006). The pathogen *R. solani* is a semi-saprophytic fungus with wide host range. Even though research has been focused on identification of sources of resistance, till date, no major source of resistance has been identified (Susmita Dey *et al* 2016). Thus the major problem in the development of ShB-resistant rice varieties is the lack of donors having high degree of resistance to the pathogen.

A few rice varieties, *viz*., Teqing, Tetep, Tadukan, Jasmine 85 and WSS5 were frequently used in the genetic analysis of ShB resistance (Pan *et al* 1999a, 1999b; Zuo *et al* 2000; Pinson *et al* 2005; Liu *et al* 2006; Channamallikarjuna *et al* 2010; Shiobara *et al* 2013, Zeng *et al* 2014; Yadav *et al* 2015). In these studies, over 50 QTLs associated with resistance have been report and also these studies have concluded that resistance to ShB is a complex, quantitative trait, governed by polygenes and in some rice varieties it is controlled by few major genes and several minor genes. However, neither the identified QTLs have been utilized in development of sheath blight resistant cultivars nor their breeding value has been assessed so far. Moreover, Jasmine 85 which was earlier reported as tolerant and even though QTLs have been dentified from the variety, showed high level of susceptibility in one of our recent studies (Susmita Dey *et al* 2016). QTL analysis can provide genetic information about individual components of a complex trait. As earlier reports indicate that sheath blight resistance in rice is governed by several minor genes or QTLs each with small effect, pyramiding of such QTLs is expected to result in considerably increased resistance to ShB in the pyramided cultivars. In our earlier work, we have identified four land races (Phougak, Gumdhan, Wazuhophek, Ngonolasha) and two elite breeding lines (RP 2068-18-3-5 and 10-3) with moderate resistance to sheath blight. These results were based on four years (2012-2015) of stringent screening both under field and glasshouse conditions coupled with characterization of agro-morphological traits (Dey *et al* 2016). The present investigation is undertaken to assess the allelic variation of reported ShB QTLs in these moderately resistant genotypes.

Material and Methods

A total of 11 genotypes including two moderately resistant checks (Tetep and Teqing), six tolerant to ShB as identified by Dey *et al.*, 2016 (RP-2068-18-3-5, 10-3, Wazuhophek, Ngonolasha, Gumdhan and Phougak) and three susceptible checks (IR 50, Swarna and BPT 5204) were screened for the eight reported QTLs of which six were from Tetep and two from Teqing (Table 1).

Table 1. Details about QTL used in the present investigation

Sl. No.	QTL	Chr	Marker interval	PV (%)	Reference
1	*qSBR 1-1*	1	RM1232 - RM 306	15.01	Channamallikarjuna *et al.*, 2010
2	*qSBR 3-1*	3	RM 251- RM 338	9.96	
3	*qSBR 7-1*	7	RM 3691-RM 336	10.02	
4	*qSBR 7-1*	7	RM 5481- RM 3691	26.05	
5	*qSBR 11-2*	11	RM 3428 – RM 209	7.81	
6	*qSBR 11-3*	11	RM 536 - RM 202	21.59	
7	*Qsbr 2a*	2	RM 29-RM 341	7.81	Loan *et al.*, 2004
8	*Qsbr3*	3	RM 156-RM16	9.30	

Results

Out of 15 SSRs reported to be linked to the eight QTLs analyzed in this study, six were monomorphic, while nine were polymorphic with PIC values ranging from 0.3696 to 0.6044 (Table 2).

Table 2. Allelic variation and PIC Values for 15 SSR loci identified among 15 genotypes

Sl. No.	Chr	SSR	No. of alleles	PIC
1	1	RM 1232	3	0.3696
2	1	RM 306	3	0.5644
3	2	RM 29	1	-
4	2	RM 341	3	0.5333
5	3	RM 338	1	-
6	3	RM 156	1	-
7	3	RM 251	3	0.4756
8	3	RM16	3	0.4178
9	7	RM 5481	1	-
10	7	RM 336	3	0.44
11	7	RM 3691	3	0.5244
12	11	RM 209	1	-
13	11	RM 536	1	-
14	11	RM 3428	3	0.6
15	11	RM 202	3	0.6044

qSBR1-1

The left flanking marker RM 1232 was polymorphic with three alleles. Tetep type allele was shown by all the tolerant genotypes except RP 2068-18-3-5. The right flanking marker RM 306 had shown polymorphism with three alleles ranging from147-182 bp, while the Tetep specific type allele was a 175 bp allele. The allele similar to that of Tetep was shown by another moderately resistance check- Teqing and five genotypes *viz*., 10-3, Ngonolasha, Gumdhan, RP-2068-18-3-5 and Phougak. One genotype, Wazuhophek had allele size of 182 bp similar to that of susceptible check IR50 and BPT 5204. There was no amplification for this marker in one susceptible check Swarna. Both the flanking markers showed amplification of the Tetep specific allele among one or two susceptible checks.

qSBR3-1

The left flanking marker RM 251 was polymorphic, amplifying three alleles ranging from 127-179 bp with PIC value of 0.4756. Tetep type type allele was 165 bp and it was present in four genotypes *viz*., 10-3, Ngonolasha, Gumdhan, RP-2068-18-3-5, and Phougak. However, the same type of allele was also present in the susceptible check Swarna. The other moderately resistance check Teqing and one susceptible check BPT 5204 had second type of allele at 179 bp. Third type allele (127 bp) was shown by one susceptible check, IR 50. Tolerant genotype Wazuho phek was found to have heterozygous alleles of which one was similar to Teqing type and other similar to IR 50 type. Though RM 251 was polymorphic with three alleles, the alleles could not be differentiated in terms of resistance and susceptibility as the same type of allele was present in both moderately resistant genotypes and susceptible genotypes. On the other hand, the right flanking marker RM 338 was found to be monomorphic in all the genotypes.

qSBR7-1

The left flanking marker RM 3691 displayed polymorphism, amplifying three alleles ranging from 135-180 bp, of which, the 165 bp is the Tetep specific allele. A similar allele was also amplified by the tolerance genotyped RP-2068-18-3-5 and the susceptible check BPT. The other moderately resistant check Teqing, two susceptible checks (IR50 and Swarna) and four moderately resistant genotypes *viz.*, 10-3, Wazuho phek, Gumdhan and Phougak amplified an allele of size 180 bp. Only, Ngonolasha, another moderately suscceptible variety, amplified a different allele (135 bp) as compared to genotypes. The right flanking marker RM 336 was also found to be polymorphic, amplifying three alleles ranging from173-225 bp, of which 218 bp was

amplified in Tetep. Similar allele was amplified by the susceptible check BPT 5204 and the moderately resistant genotype, Ngonolasha. The other moderately resistance check Teqing, two susceptible checks (IR50 and Swarna) and four genotypes *viz.*, 10-3, Gumdhan, RP-2068-18-3-5 and Phougak amplified an alleles of size 173 bp. Wazuho phek amplified an allele of size 225 bp with repect to RM 336. Though the flanking markers of the QTL *qSBR7-1* showed polymorphism with three alleles, the alleles cannot be differentiated in terms of resistance and susceptibility as the same type of allele was observed present in both moderately resistant genotypes and susceptible genotypes.

SBR7-1

The left flanking marker for the QTL was monomorphic among the rice lines analyzed amplifying a 166 bp fragment. The right flanking marker RM 3691 displayed polymorphism with three alleles ranging from135-180 bp. Among them, the 165 bp is Tetep specific. The same allele was also present in the moderate resistant genotype RP-2068-18-3-5 and also in the susceptible check BPT 5204. The other moderately resistance check Teqing, two susceptible checks (IR50 and Swarna) and four moderately resistant genotypes *viz.*, 10-3, Wazuhophek, Gumdhan and Phougak amplified an allele if size 180 bp. Ngonolasha, another moderately resistant genotype was found to amplify a different allele (135 bp.

qSBR11-2

The left flanking marker RM 3428 showed polymorphism, amplifying three alleles ranging from 230-305 bp, of which 255 bp is specific for Tetep. A similar allele was amplified by two susceptible checks (IR 50 and BPT 5204) and two moderately tolerant rice lines, *viz.*, 10-3 and RP-2068-18-3-5. The other moderately resistance checks- Jasmine 85 amplified an alleles of size 230 bp. The susceptible check Swarna and seven promising moderately tolerant genotypes *viz.*, SM-801, Ngonolasha, Wazuho phek, Gumdhan, BG-380-2, Phougak and Thangmoi amplified an allele of size 305 bp. Though the left flanking marker, RM 3428 was polymorphic with three alleles, the alleles cannot be differentiated in terms of resistance and susceptibility as the same type of allele was present in both moderately resistant genotypes and susceptible genotypes. On the other hand, the right flanking marker, RM209 was observed to be monomorphic amplifying a 133 bp fragment.

qSBR11-3

The left flanking marker RM536 was observed to be monomorphic amplifying a 110 bp fragment. The right flanking marker RM 202 was polymorphic with three allelic positions ranging from 194-252 bp, of which the 194 bp was Tetep specific allele. Similar allele was amplified by moderately resistance check Teqing and two moderately resistant genotypes viz., Wazuhophek and Phougak. All the three susceptible checks along with 10-3 amplified an allele of size 243bp. Only one land race Ngonolasha displayed allelic variation at this locus amplifying a 252 bp fragment, while Gumdhan was found to have heterozygous alleles of which one was similar to the Tetep type and other similar to Ngonolasha type allele.

QSbr2a

The left flanking marker RM 29 was monomorphic amplifying a fragement of size 196 bp. The right flanking marker RM 341 displayed polymorphism, amplifying three alleles ranging from 139-212 bp. Among them, a 187 bp was specific for Teqing. A similar allele was amplified by the two suseptible checks (Swarna and BPT 5204), the moderately resistant genotype RP-2068-18-3-5 and Tetep. Two promosing genotypes *viz*., 10-3 and Wazuhophek amplified the second type allele at 139 bp. Susceptible check IR50 and two genotypes *viz.*, Ngonolasha and Phougak amplified the third type of allele of size 212 bp. Only Gumdhan was found to be heterozygous with three alleles at139 bp, 187 bp and 212 bp.

QSbr3

The left flanking marker for the QTL, RM156, displayed monomorphism. The right flanking RM16 was polymorphic with three alleles ranging 181-266 bp, of which 187 bp is of Teqing type. A similar allele was amplified in the moderately resistance check Tetep and six promising genotypes *viz*., 10-3, Ngonolasha, Wazuho phek, Gumdhan, RP-2068-18-3-5 and Phougak. Three susceptible checks (IR50, Swarna and BPT5204) amplified the third type allele of 266 bp. Allelic variation with respect to the marker, RM16 was observed to clearly distinguish moderately resistant genotypes and susceptible genotypes.

Discussion

The present investigation validated few of the reported QTLs from Tetep and Teqing for their association with tolerance to sheath blight. None of the QTLs except *QSbr 3* from Teqing showed allelic difference among tolerant genotype (from which it was reported) and susceptible genotype. For all the QTLs except *QSbr 3* (QTL from Teqing), either or both the flanking markers were amplified similar type of alleles both in Tetep/Teqing and one or two susceptible checks. Thus, the allele responsible for tolerance to sheath blight in novel sources *viz*., Gumdhan, Wazuhophek, Ngonolasha, Phougak, RP 2068-18-3-5 and 10-3 may or may not be a different one from that of the Tetep or Teqing.

Mostly, the ShB resistance phenotyping methods are based only on relative lesion height (SES scale 2002) that do not take into account a comprehensive phenotyping of the component traits based on agro-morphological traits (Susmita Dey *et al* 2016). Hence, it is often reported

that there is no consistency in disease reaction among genotypes and genotype reported as resistant in one season shows susceptible reaction in the next season. Furthermore, Zheng *et al* (2015) after surveying the phenotypes of different lines/individuals in mapping populations stated that majority of the reported QTLs are co-localized with plant height associated QTLs and are irrelevant for physiological/genetic ShB resistance. As there is no consistency in disease reaction and no evidence on practical utility of reported Shb-QTLs, it can be inferred that traits used so far to evaluate ShB resistance are quite inadequate. To gain deeper insights and to come out with substantial knowledge on ShB resistance/tolerance, comprehensive phenotyping for several associated traits can be considered imperative.

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