

## Identification of quantitative trait loci associated with sheath rot resistance (*Sarocladium oryzae*) and panicle exertion in rice (*Oryza sativa* L.)

Sheath rot, caused by *Sarocladium oryzae* (Sawada) Gams & Hawksw, has gained the status of a major disease in rice<sup>1,2</sup> and yield loss varies from 9.6 to 85%, depending on the weather conditions during the crop growth-period<sup>3,4</sup>. In the upland rice, it can cause as high as 1.7% loss in grain yield with every one per cent increase in disease incidence<sup>5,6</sup>. This disease was first reported in rice from Taiwan by Sawada<sup>6</sup> and was subsequently reported from different rice-growing ecosystems of the world<sup>7-10</sup>. Agnihothrudu<sup>11</sup> recorded this disease in India for the first time and later several workers reported this from different parts of the country<sup>12-17</sup>.

The pathogen mainly infects the uppermost leaf sheath and retards translocation of nutrients from the foliage to the panicle<sup>18</sup>. The young panicles either remain within the leaf sheath or emerge partially. The emerged panicles produce chaffy grains/partially-filled grains and affect the whole grain recovery during milling and severely affect physico-chemical characteristics of rice grains<sup>12,19,20</sup>. Relationship between sheath rot incidence and other biotic stresses such as rice tungro virus and stem borer incidence has been studied by some workers<sup>13,18</sup> and higher incidence of sheath rot has been reported under stressed conditions.

Fungicides and disease-free seeds have been used extensively for the management of this disease, including non-target pesticides; however none of these efforts have been successful in curtailing the disease. Development of resistance varieties plays a pivotal role in the management of the disease. Conventional genetic studies have provided little information on the inheritance of genes controlling the resistance. With the availability of saturated molecular maps in rice, and rice being a fast-emerging model plant for molecular studies, identifying the resistance genes using DNA extraction, microsatellite, RAPD work, etc. markers during host-pathogen interactions has received wide attention<sup>21-23</sup>. Genetically, stable mapping populations facilitate tagging the resistance loci with molecular markers for possible use in marker-assisted selection.

Hence, an attempt was made to tag sheath rot resistance and PE to molecular markers. It is an attempt on identification and mapping sheath rot resistance loci in rice.

The plant material comprised of 188 F<sub>8</sub> recombinant inbred lines (RILs), a mapping population derived from a dwarf, susceptible *indica* cultivar, CO39 and West African tall, *japonica* cultivar, Moroberekan. The molecular data of mapping population consisted of 127 RFLP markers, comprising genomic and cDNA clones (kindly provided by Plant Pathology Division, IRRI, Philippines).

The 188 RILs along with their parents were evaluated for field resistance to

sheath rot, during the wet season of 1997, in completely randomized block design at Main Research Station, Hebbal, Bangalore and Agricultural Research Station, VC Farm, Mandya, University of Agricultural Sciences, Bangalore, India. All the genotypes and parents were raised in the nursery and 21-day-old seedlings were transplanted in the main field. The cultural practices required for good crop growth, both in the nursery and in the main field, were followed. HR12 and CO39, which showed hundred per cent infection in our earlier disease-screening trials, were used as susceptible checks. At the time of screening, the check varieties showed

**Table 1.** QTLs associated with field resistance to sheath rot in CO39/Moroberekan RIL population in rice (threshold LOD  $\geq$  2.00)

Chromosome number	Flanking markers	LOD	Per cent variation	QTL effect
1	RG612-RG140 (RZ744)*	3.83	10.5	8.67
	RZ276-CDO920 (RG400/RG331)*	2.72	8	7.31
2	RG109-RG236 (RG188)*	6.13	18.8	11.82
	RG102-RG73	3.19	8.6	7.61
4	RG498-RG864	2.29	7.4	8.23
5	RG360-RG573	2.46	8.4	9.32
6	(RG64)*	3.52	12.5	9.63
	RG192-RG172			
7	RZ272-RG511	2.25	8.1	9.62
8	RG20-RG333	2.75	8.8	7.16

\*Markers also associated with leaf blast resistance (Wang *et al.*<sup>25</sup>).

**Table 2.** QTLs associated with panicle exertion in CO39/Moroberekan RIL population in rice

Chromosome number	Marker interval	LOD	Per cent variation	QTL effect
1	RG612-RG811	3.71	15.5	-1.8772
	RZ276-RG162	3.15	11	-1.6332
	RG197-RG400	9.6	30.1	-2.284
2	RG324-RG322	2.75	8.4	-1.2557
3	RG450-RG227	2.19	6.7	-1.7977
	RG96-RG910	3.49	11.6	-1.7598
4	RG788-RG684	3.84	12.1	-1.5235
6	RZ192-RG172	4.55	17.5	-1.7086
7	RZ272-RG511	2.83	21.4	-2.498
	RG4-RG517	2.352	10	-1.504
8	RG20-RG333	2.7	10.6	-1.2441
9	RG553-RG206	5.43	13.7	-2.114
11	RG98-RG118	2.59	7.4	-1.3524
12	RG181-RG323	2.08	5.6	-1.0125

more than ninety per cent sheath rot infection. Fifteen days prior to the harvest, RILs, parents and check varieties were scored for the disease.

Ten plants were selected randomly from each line per replication and scored for sheath rot according to standard evaluation for rice (IRRI, 1988). Per cent incidence for all replications was averaged for each genotype and the data were used for QTL mapping.

The panicle emergence (PE) values were measured in centimetres, as the distance from the flag collar to the node of the panicle. Depending on whether the panicle base was above, equal or below the flag leaf collar, the exertion was computed as positive, zero or negative, respectively.

The data on both sheath rot and PE and the RFLP database with 127 RFLP markers across 12 chromosomes (kindly provided by Genome Mapping Laboratory, Plant Pathology Division, IRRI, Philippines) were subjected to linkage analysis using MAPMAKER QTL VII. b (ref. 22), to identify putative loci controlling the two traits. The analysis was conducted using threshold LOD score of  $\geq 2.00$ .

Mapping population of CO39/Moroberekan, an *indica* and *japonica* cross, was screened for sheath rot and panicle exertion in two different rice-growing ecosystems. CO39, an *indica* variety recorded maximum disease incidence (97%) and Moroberekan, a *japonica* variety, recorded lowest incidence ( $< 0.5\%$ ). The mean disease incidence for the entire population was 27.13%. The phenotypic data of the population with their parents were analysed using 127 RFLP markers for both sheath rot and PE. The correlation for the two traits observed was 1, indicating the close association between the two traits.

A total of nine QTLs for sheath rot on seven different chromosomes 1, 2, 4, 5, 6, 7 and 8 were identified (Table 1). Out of these, three QTLs flanked by RG109-RG236, RG612-RG140 and RG192-RG172 recorded the LOD value of  $> 3.5$ . The QTL flanked by RG109-RG236 recorded 18.8% variation. Similarly, fourteen QTLs were identified for PE on ten different chromosomes (Table 2). Five QTLs recorded the LOD value of 3.5 and above. Maximum LOD value of 9.6 was recorded by the QTLs flanked by RG197-RG400 on chromosome 1. Four QTLs flanked by RG197-RG400, RZ272-RG511, RZ192-RG172 and RG612-

RG811 recorded a per cent variation of 30.1, 21.4, 17.5 and 15.5, respectively.

Seven common QTLs were mapped on five chromosomes, namely 1, 4, 6, 7 and 8 (Figure 1). Three QTLs flanked by RG612-RG140, RG811-CDO920 and RG109-RG236 were common to both the traits on chromosome 1. Two QTLs flanked by RG102-RG73 (chromosome 2) and RG573-RG360 (chromosome 5) identified for sheath rot were not associated with PE.

Sheath rot, once considered as a minor disease in rice, has now made its presence in almost all rice-growing ecosystems of the world. It has become a serious menace to rice cultivation, causing devastating damage. Genetic information on this disease has been limited and been confined only to physiology of the host, host-pathogen genetics and control through fungicides<sup>19</sup>. Chen and Thseng<sup>23</sup> observed that the number of infected panicles as well as brown spots and sterile grains during sheath-rot infection were higher in *indica* than *japonica* rice. Earlier reports also indicated that *japonicas* were highly resistant to blast compared to *indicas*. Varieties with a short stature were more prone to sheath rot than tall plants, emphasizing the fact that shorter varieties

have mostly originated from the *indica* group. In this study, CO39/Moroberekan population which was extensively used for mapping of blast-resistant major and minor genes<sup>25</sup>, was used to identify the QTL for sheath rot resistance. The parents of this RIL population exhibited significant variation for the disease. No disease was recorded on Moroberekan (0%), a *japonica* variety, while CO39, *indica* variety recorded maximum incidence (97%) of the disease. The mean disease incidence of the population that consisted of 188 lines was 27.13% (data not shown).

Earlier phenotypic studies on rice indicated the close association of sheath rot with PE. To discern whether the same kind of association existed at the molecular level, the phenotypic observations of both the traits for the entire population along with their parents were analysed using 127 RFLP markers. A total of nine QTLs were identified on seven linkage groups of rice associated with sheath rot and fourteen QTLs for PE. In this study, the QTLs identified for sheath rot, flanked by RG612-RG140, RZ276-CDO920 markers on chromosome 1, have also been reported to confer resistance to blast disease<sup>25</sup>. QTL on chromosome 4 between RG498 and RG864 marker interval, QTL

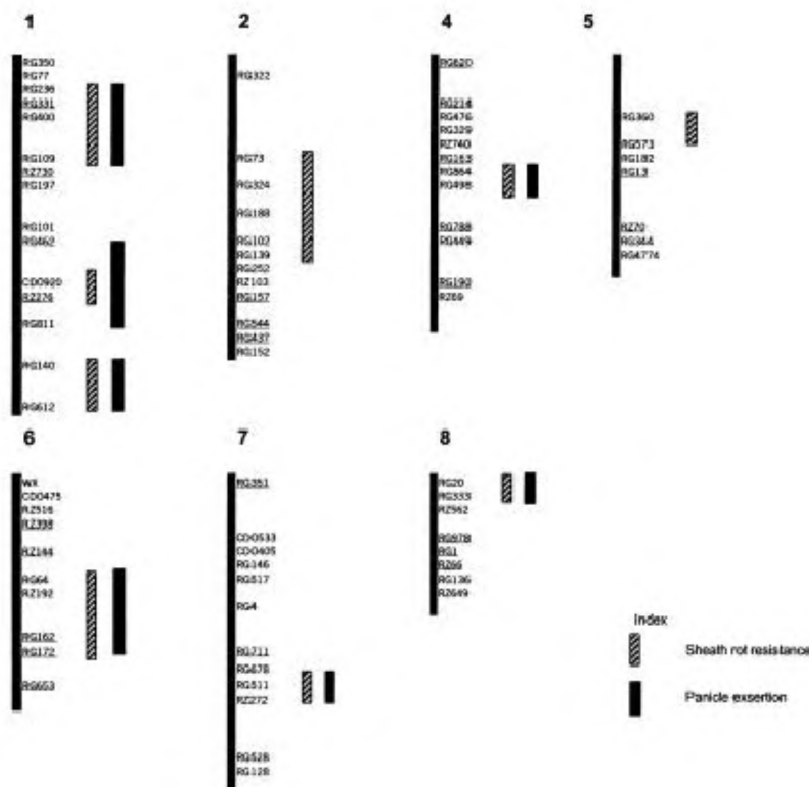


Figure 1. Rice chromosome map showing the location of QTL associated with sheath rot and panicle exertion in CO39/Moroberekan RIL mapping population.

flanked by RG192-RG172 markers, on chromosome 6 and RG20-RG333 on chromosome 8, have been reported to contain major genes for blast, viz. *Pi-5(t)*, *Pi-2 (Piz)* and *Pi-ZG*, as reported by Wang *et al.*<sup>25</sup>, Yu *et al.*<sup>26</sup> and Zhu *et al.*<sup>27</sup>, respectively. The QTL flanked by RG109-RG236 on chromosome 1 revealed a maximum LOD of 6.13, with 11.82% phenotypic variation.

The two major and eleven minor QTLs were located for PE on ten different linkage groups. The LOD score ranged between 2.08 and 9.6 with phenotypic variation of 5.6 to 30.1%. The QTL on chromosome 1 flanked by RG197-RG400, recorded the highest LOD value (9.60) with 30.1% phenotypic variation. Courtois *et al.*<sup>24</sup> reported a major QTL on chromosome 1 for PE in IR64/Azucena doubled, haploid mapping population. The phenotypic variation observed at some of the QTL loci indicates that some of the loci have major effect (18.8% phenotypic variation) on sheath rot resistance. Nine QTLs accounted for approximately 75% of the phenotypic variation.

Trait correlation may arise either due to pleiotropic effect of single gene or due to tight linkage of genes affecting different traits. Most workers have reported that highly correlated phenotypic traits share similar QTL regions on chromosomes with common marker intervals<sup>28-33</sup>. In the present study, a total of fourteen QTLs were found to be associated with PE, of which seven QTLs overlapped with those for sheath rot. Such an association for sheath blight with plant height and heading date in rice has been reported by several other workers<sup>34-36</sup>. Of all the QTLs identified for sheath rot, the loci flanked by the markers RG102-RG73 on chromosome 2 and RG360-RG573 on chromosome 5, were not associated with panicle exertion.

Most of the QTLs identified for sheath rot resistance overlapped with blast QTL<sup>29</sup>. This may be due to generalized resistance or because of pleiotropy or tight linkage of resistance genes. Such a situation would help in selection of genotypes for two characters simultaneously, using markers at early stage of the development. The correlation between PE and sheath rot indicates overlapping of QTL on common chromosomal regions. Further, the mark-

ers linked to QTL for sheath rot resistance can be used in marker-assisted selection after fine mapping of the resistance loci.

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