QTLs meet genomics: targeting superior disease QTL alleles in the rice genome

Extensive genetic mapping data coupled with the complete sequence of the rice genome has enabled us to combine classical genetics and comparative genomics to target superior disease quantitative trait loci (dQTLs) associated with resistance to multiple diseases in cereals. We have previously identified and mapped candidate genes (CGs) conferring quantitative resistance to rice blast concomitant with selection for good agronomic traits in an advanced backcross populations of Vandana x Moroberekan. Using gene-based primers as well as simple sequence repeat markers, we identified oxalate oxidase, oxalate oxidase-like protein, thaumatin, and peroxidase significantly correlated with blast resistance in 108 introgression lines (84 selected for blast resistance and 24 for drought tolerance). We have also characterized the chromosomal regions containing dQTLs by *in silico* and expression approaches. Information from this study as well as gene-based molecular markers developed were used to identify candidate genes associated with resistance to rice blast in an advance backcross population of Way Rarem (WR) x Oryzica Llanos 5 (OL5).

Scanning the genome of Vandana x Moroberekan and Way Rarem x Oryzica Llanos 5 using gene-based markers and SSRs

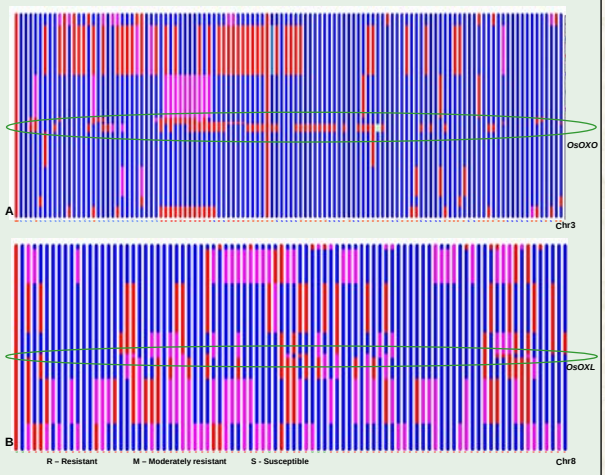
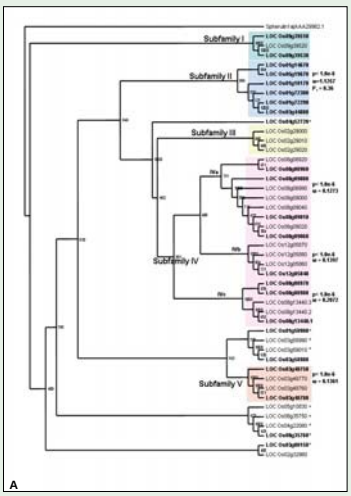
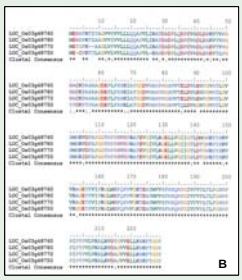


Figure 1. A) A genome scan using CG-based markers, SSRs and SSRs co-localizing with CGs identified introgression of Moroberekan alleles for oxalate oxidase, oxalate oxidase-like proteins, thaumatin and peroxidase in resistant lines. Oxalate oxidase (*OXO*) in chromosome 3 was significantly associated with resistance in multiple locations. B) Molecular markers developed and identified from V x M study were used to identify introgression of OL5 CG alleles into advance backcross populations of WR x OL5. Two-gene analysis showed interaction of a eukaryotic aspartyl protease (EAP)*Thaumatin_Chr6, oxalate oxidase-like (*OsOXL*) protein*PR10 (probenazole-induced protein) and PR1*PR10 as significantly associated with resistance to seedling blast in Sukabumi, Indonesia. The physical location of *OsOXO* (Chr3) and *OsOXL* (Chr8) revealed that multiple members are present in each loci.

Characterizing dQTL chromosome regions: focus on oxalate oxidase and oxalate oxidase-like proteins





Locus	Full length cDNA	# of mapped ESTs	# of mapped peptides
LOC_Os03g48750	AK103716	1	1
LOC_Os03g48760	n/a	0	0
LOC_Os03g48770	n/a	0	0
LOC_Os03g48780	AK059872	15	15

n/a: not available

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Figure 2. Genome organization and phylogenetic analysis of germin-like proteins in rice. Oxalate oxidase and oxalate oxidase-like proteins are members of the germin-like protein (GLP) family. A) Analysis of the rice genome revealed 45 GLPs divided into five subfamilies. Sequences in Subfamily I to V contain members that are 50% or more identical in the nucleotide level. Sequences marked with asterisk cannot be assigned to a particular subfamily based on nucleotide identity. Genes with expression evidence (http://www.tigr.org/tdb/e2k1/osa1/locus_expression_evidence.shtml) are boldfaced. The values to the right of the subfamilies are the results of positive selection analyses, where p is the probability value, ω is the dN/dS value under M8 of codeml, and P_1 is the inferred proportion of positively selected sites. Subfamily IV (12 Chr8 *OsOXL* genes) and Subfamily V (four Chr3 *OsOXO* genes) have been associated with quantitative resistance to blast. B) Multiple alignment of the four tandemly duplicated Chr3 *OsOXO* genes reveal >90% similarity at the gene coding region. C) EST and full-length clones reported for rice *OsOXO* genes

Expression analysis of oxalate oxidase genes in selected Vandana x Moroberekan advance backcross lines

Line	Reaction to seedling blast		Oxalalate oxidase gene			
	India	Philippines	LOC_Os03g48750	LOC_Os03g48760	LOC_Os03g48770	LOC_Os03g48780
			0 6 12 24 48	0 6 12 24 48	0 6 12 24 48	0 6 12 24 48
IR78221-19-6-7-B	2	1				
IR78221-19-6-56-B	3	1				
IR78221-19-6-99-B	3	1				
IR78221-19-3-196-B	8.25	4				
Moroberekan	1.5	0.3				
Vandana	8.5	7				

Figure 3. Expression analysis by RT-PCR of *OsOXO* genes after inoculation of *Magnaporthe grisea* isolate PO6-6 selected advanced backcross Vandana x Moroberekan lines. Lines were selected on the basis of their reaction to field blast in India and the Philippines, detection of the Moroberekan *OsOXO* allele in the progenies and similarity of morphological and agronomic traits with Vandana, with the exception of IR78221-19-3-196-B which has the *OsOXO* allele from Vandana. Leaf samples were harvested at 0, 6, 12, 24 and 48 hours after inoculation.

Validating gene function through mutational analysis

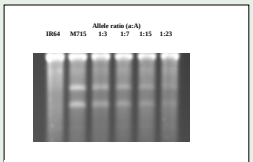

Figure 4A. Agarose TILLING. M715 carries a heterozygous mutation (A/a) at the locus encoding oxalate oxidase-like protein on chromosome 8 (LOC_Os08g09010). Heteroduplex DNA formed by the heterozygote is detectable in 1a:15A, suggesting that a single heterozygous mutant, common in the M_2 population, can be readily detected from DNA pool of 8 M_2 plants

Figure 4. Candidate gene mutation in IR64 mutants were identified by SNP-based PCR approaches. Mutants with SNPs in candidate genes were phenotyped for their reaction to rice blast.

Mutant	Gene	Mutation	Amino acid change
M3E715	LOC_Os08g09010	G to A	Arg to Lys
M3E93	LOC_Os03g48750	C to T	Pro to Leu
M3E97	LOC_Os08g09000	C to T	Ser to Phe
M3E183	LOC_Os08g9040	C to T	Arg to Val
M3E543	LOC_Os08g9040	C to T	Pro to Leu

B

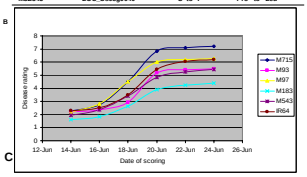

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Figure 4B and 4C. Five EMS-mutagenized IR64 lines were identified with SNPs in OXO (Chr3) and OXL (OChr8) genes. Phenotypic evaluation of five mutants with missense mutations in oxalate oxidase and oxalate oxidase like-protein. The mutant lines were inoculated with blast isolate PO-6-6 in the greenhouse, and disease scored over a 14 day period.

Leveraging information from rice dQTLs to cereal genomes

- In addition to molecular, phenotypic and in silico approaches to dissect dQTLs in rice, we used the information from this study to identify putative orthologous sequences of CGs in other cereal genomes. Maize ESTs were identified for all CGs except PR10.
- Degenerate primers for each CGs were designed for SNP-based PCR. These will be used to mine maize germplasm for functional dQTLs for maize diseases.
- Gene-based molecular markers developed from this study can be used for dQTL analysis of other rice mapping populations
- Genetic materials are available for further studies on characterizing dQTLs in rice and other cereals

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