

# In silico and molecular approaches for associating candidate defense genes with quantitative resistance to rice blast

A japonica cultivar, Moroberekan from Africa, known to exhibit durable resistance to rice blast in Asia was crossed to a popular upland cultivar, Vandana, following the advanced backcross QTL approach. This approach allows QTL analysis of the mapping population while simultaneously developing elite germplasm for commercial use ( Tanksley and Nelson 1996). To accumulate different mechanisms involved in quantitative resistance to blast, 15 BC<sub>3</sub>F<sub>3</sub> or BC<sub>3</sub>F<sub>4</sub> parental lines derived from Vandana/Moroberekan showing partial resistance and carrying positive candidate alleles were selected and crossed in all pairwise combinations. Seeds of 10 F<sub>2</sub> families were selected based on partial resistance of the BC<sub>3</sub>F<sub>3</sub> or BC<sub>3</sub>F<sub>4</sub> parental lines to seedling blast and neck blast, association of the parental lines with positive alleles, and phenotypic similarity to Vandana. Among the 10 F<sub>2</sub> families, 14 F<sub>2</sub> plants showed a good to moderate level of agronomic acceptability and high level of seedling blast resistance (0.75–3.2% DLA). Resistant lines from selected families were advanced to F<sub>3</sub> until F<sub>5</sub>. At F<sub>4</sub>, the top 10% of the lines (60 out of >600 lines) derived from the progenies of VM5/VM14, VM6/VM14, and VM82/VM14 have acceptable agronomic traits, and served as a basis for further selection and advancement to F<sub>5</sub>. The field performance of these advanced BC lines indicated that the major QTLs have been captured in the BC lines.



Fig. 1. Field-infected Vandana cultivar showing typical rice blast lesions (Cavinti, Philippines).

## Methodology

- Consensus candidate genes were identified from multiple mapping, linkage and microarray experiments
- Primers based on candidate gene sequence derived from the International Rice Genome Sequencing Program were designed in both 1kb upstream and gene-coding regions (<http://www.tigr.org/tdb/e2k1/osa/>)
- SSRs co-localizing with candidate genes and randomly selected SSRs (<http://www.gramene.org>) were also used to conduct a genome scan of selected F<sub>6</sub> Vandana x Moroberekan intermated lines
- Candidate gene analysis was correlated with field performance in terms of yield and reaction to seedling blast, panicle blast and neckblast across screening sites in India and the Philippines.
- For genes occurring in gene families such as the germin-like proteins (GLP), we derived phylogenetic trees using the retrieved sequences. We also checked for conserved promoter motifs and identified cis-elements in the 1,000-bp upstream regions using MEME (<http://meme.sdsc.edu/>) and PLACE (Plant Cis-Acting Elements, [www.dna.affrc.go.jp/PLACE/](http://www.dna.affrc.go.jp/PLACE/)).

## Candidate gene analysis of Vandana x Moroberekan progenies

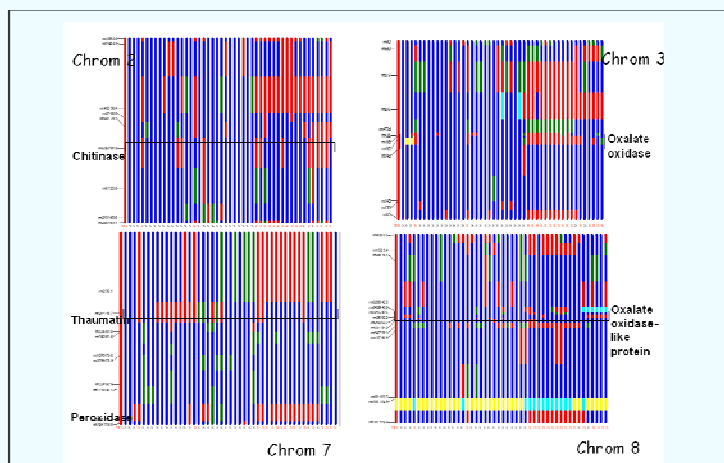


Figure 2. Genome scan of selected Vandana x Moroberekan progenies. Using 110 random SSRs polymorphic CG-associated markers in Vandana and Moroberekan, 108 lines were targeted for genome scanning of introgressed genomic regions from Moroberekan into Vandana. These lines included 84 lines which are resistant to blast based on multilocation testing and 24 drought-tolerant V x M lines. The genome scan was generated by Graphical Genotype or GGT software (van Berloo, 1999).

## Associating candidate genes with quantitative resistance to rice blast

Analysis	Yield (g), Almora India	Variable			
		Seedling Blast IRR1	Diseased leaf area IRR1	Panicle blast Almora India	Neckblast Cavinti Philippines
Single gene	Oxalate oxidase, peroxidase, PR1, PR10	Oxalate oxidase, Thaumatin_Chr7	Oxalate oxidase, Peroxidase	Oxalate oxidase, HSP90, Thaumatin_Chr7, Peroxidase, PR10	Oxalate oxidase, HSP90, Thaumatin_Chr7, Peroxidase
Two genes	None	Oxalate oxidase*Thaumatin_Chr7, HSP90*Thaumatin_Chr7, HSP90*peroxidase, Thaumatin_Chr7*Peroxidase, Thaumatin_Chr7*Oxalate oxidase-like	None	None	Chitinase*Thaumatin, Chitinase*Peroxidase, Chitinase*Oxalate oxidase-like, HSP90*Thaumatin_Chr7, HSP90_Peroxidase, Thaumatin*Oxalate oxidase-like, Peroxidase*Oxalate oxidase-like

Table 1. Significant candidate genes associated with quantitative resistance to rice blast using Analysis of Variance ( $\alpha = 0.05$ ). PR10 = probenazole induced protein; DHAP = deoxyphosphoheptanoate aldolase; HSP90 = heat shock protein 90

## In silico approach to characterizing candidate genes for quantitative resistance to blast – Oxalate oxidase in focus

For in silico approaches, we first focused on oxalate oxidase, a consensus candidate gene identified from multiple mapping, linkage, and microarray experiments as associated with resistance to fungal pathogens.

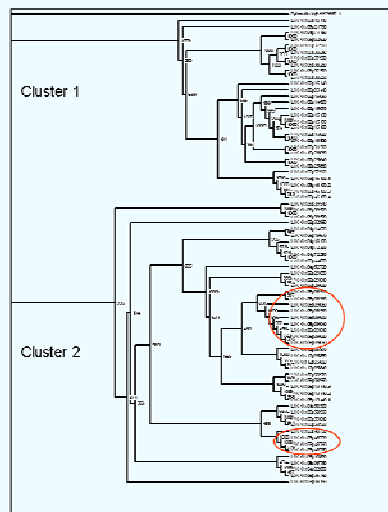
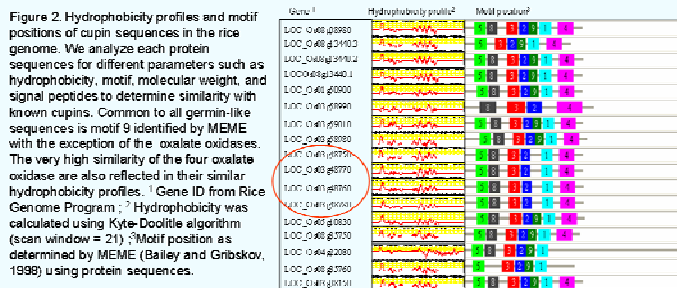


Fig. 3. Phylogenetic relationships of the cupin superfamily of proteins in the genome of rice cultivar Nipponbare. Oxalate oxidase is a member of the cupin superfamily of proteins with PFAM00190 domain. There are 74 cupin sequences in the rice genome. Two main clusters are derived based on protein sequences. Cluster 1 is composed of large cupins such as glutelins and globulins. Cluster 2 is composed of germin-like proteins such as oxalate oxidase. Two types of germin has been linked to blast resistance in rice, Chr3 oxalate oxidase and Chr8 oxalate oxidase-like protein both of which occur in multicopies in the genome. These oxalate oxidase gene sequences are located next to each other, with similarities ranging from 90% to 98%. For each gene, there was variation in the copy number of cis-elements related to biotic stress responses, such as W box, WNPR1, and WRKY, indicating that these genes have potential associations with the response of rice to pathogen infection, such as the blast fungus. The tree was generated by distance matrix using Clustal X and rooted with spherulin 1a.



## Future prospects

We continue to investigate the behavior of the QTLs in different genetic backgrounds. We also continue to develop markers derived from consensus candidate defense genes conferring partial resistance instead of just close linkage to resistance genes, and develop efficient and economical breeding procedures using molecular genotyping. Our research has therefore focused on these areas aimed at facilitating the selection of cultivars with broad-spectrum resistance against blast pathogen populations in diverse rice-growing environments.

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