

# Associating candidate genes with quantitative resistance to rice blast

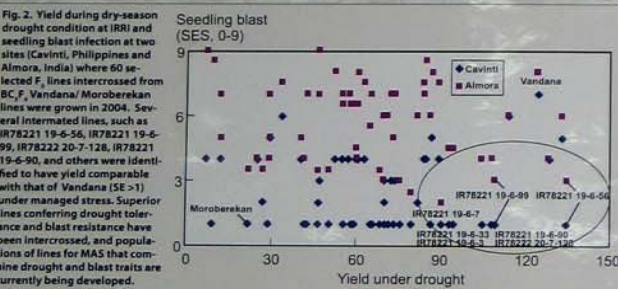
Many genetic traits exhibit quantitative inheritance. To characterize these quantitative traits, the candidate gene (CG) approach has been applied in several cereal crops such as maize, wheat, and rice because of the detection of multiple genes defining a complex trait, their partial effects on phenotypic variation, and their less precise localization on genetic maps in comparison with traits conferring qualitative inheritance (Ramalingam et al 2002, Liu et al 2004, Wilson et al 2004). The availability of the whole rice genome sequence as a result of public and private sequencing efforts has provided opportunities to predict putative functions of a gene based on sequence information, thus allowing the identification of CGs. Candidate genes are DNA sequences similar to known genes or conserved motifs, thus making inference on their biological functions possible. Through their association with disease resistance, they become candidate defense-response (DR) genes for conferring particular phenotypes.

## Methodology

- To accumulate different CGs involved in quantitative blast resistance, 15 BC<sub>2</sub>F<sub>3</sub> lines of Vandana/Moroberekan cross showing partial resistance at IRRI and Cavinti, Philippines and carrying CG alleles were selected and crossed in all pairwise combinations.
- The top 84 F<sub>4</sub> selections have been evaluated in multilocation trials with NARES collaborators (India, Philippines, and Indonesia) to determine resistance to blast and agronomic performance.
- The CG-based markers and SSRs co-localizing with the CGs were used to conduct a genome scan to determine the proportion of CG alleles from Moroberekan in 108 introgression lines (84 selected for blast resistance and 24 for drought tolerance).
- The CGs were characterized by *in silico* analysis to identify putative regulatory cis-elements in the 1-kb upstream region of each gene.



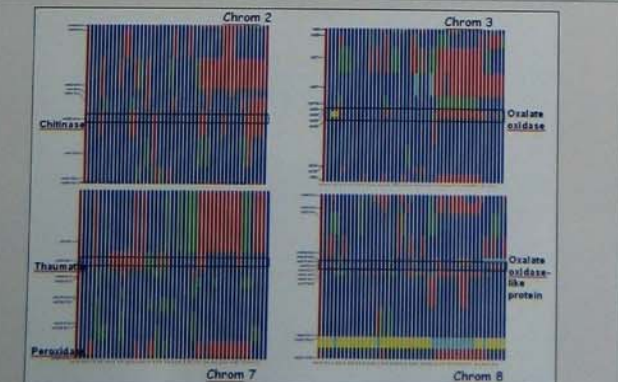
**Fig. 1.** Phenotypic selection of intermated lines derived from Vandana (V)/Moroberekan (M) BC<sub>2</sub>F<sub>3</sub> advanced lines at IRRI and Cavinti at different generations. Six hundred families of intermated V x M lines were assessed for reaction to rice blast in IRRI and Cavinti, Philippines. The top 10% of 568 lines scored by reaction to blast and agronomic qualities were further advanced and 84 F<sub>4</sub> selections were evaluated in multilocation trials with NARES collaborators (India completed, Philippines and Indonesia ongoing) to determine resistance to blast and agronomic performance.



**Fig. 2.** Yield under dry-season drought condition at IRRI and seedling blast infection at two sites (Cavinti, Philippines and Almora, India) where 50 selected F<sub>4</sub> lines intercrossed from BC<sub>2</sub>F<sub>3</sub> Vandana/Moroberekan lines were grown in 2004. Several intermated lines, such as IR78221 19-6-56, IR78221 19-6-99, IR78222 20-7-128, IR78221 19-6-90, and others were identified to have yield comparable with that of Vandana (SE > 1) under managed stress. Superior lines conferring drought tolerance and blast resistance have been intercrossed, and populations of lines for MAS that combine drought and blast traits are currently being developed.

**Table 1.** Candidate genes potentially involved in disease resistance. Converging evidence obtained from QTL mapping, microarray analysis, phenotyping, and selection for partial blast resistance points to their role in defense response against blast.

Gene	Bioprocess	Functional evidence
Oxalate oxidase/germin like protein	Oxidative burst, signaling, structure	Gene cluster as a disease resistance QTL
Aspartyl protease (Esi-16)	Early salt-induced	As disease resistance QTL
14-3-3	Signal cascade	As disease resistance QTL
PR-1	Stress response	As disease resistance QTL
PBZ (PR10a)	Could have ribonuclease activity	Pathogen-induced defense gene
Rice peroxidase 22.3	Oxidation of organic and inorganic substrates at the expense of H <sub>2</sub> O <sub>2</sub>	Pathogen-induced defense gene
Heat shock protein 90	Stress response	Pathogen-induced defense gene
Putative 2-dehydro-3-deoxyphosphoheptanate aldolase	Aromatic amino acid synthesis	Pathogen-induced defense gene
Thaumatin-like pathogenesis related protein	Binding to D-glucans of the type commonly found in fungal cell walls	Stress response
Glyoxalase 1 ( <i>Dryos sativa</i> )	Detoxification of the cytotoxic metabolite methylglyoxal that can be produced by increased levels of glycolysis under conditions of stress	Stress response
S-adenosyl L-homocystein hydrolase	Cytokinin-binding protein CBP57 (cytokinin-mediated signal transduction)	Pathogen-induced defense gene



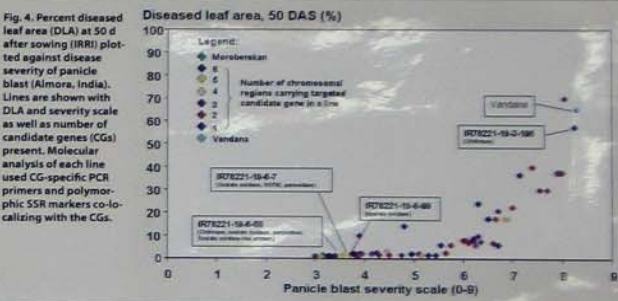
**Fig. 3.** A genome scan using candidate gene-based PCR primers. SSRs co-localizing with the candidate genes and SSRs randomly distributed across the genome was conducted to identify introgressed Moroberekan chromosome segments in the advanced backcross lines. M – Moroberekan V-Vandana, 3

**Table 2.** Candidate gene alleles contributed by Moroberekan in blast-resistant Vandana/Moroberekan lines, their yield and disease resistance at two blast hotspots. Disease pressure was higher in Almora, India than in Cavinti, Philippines.

Candidate gene	IR78221-19-6-56	IR78221-19-6-99	IR78222-20-7-128	IR78221-19-6-90	IR78221-19-6-33	IR78221-19-6-90	IR78222-20-7-128	IR78221-19-6-90	IR78221-19-6-56	IR78221-19-6-90
Chitinase	+	+	+	+	+	+	+	+	+	+
Oxalate oxidase	+	+	+	+	+	+	+	+	+	+
Aldolase	+	+	+	+	+	+	+	+	+	+
Thaumatin (Ork)	+	+	+	+	+	+	+	+	+	+
HSP90	+	+	+	+	+	+	+	+	+	+
Thaumatin (Ork)	+	+	+	+	+	+	+	+	+	+
Eukaryotic aspartyl protease	+	+	+	+	+	+	+	+	+	+
Deoxyphosphoheptanate aldolase	+	+	+	+	+	+	+	+	+	+
Peroxidase	+	+	+	+	+	+	+	+	+	+
Oxalate oxidase-like (P1)	+	+	+	+	+	+	+	+	+	+
PR10	+	+	+	+	+	+	+	+	+	+
Seedling blast (Philippines)	1	1	1	1	1	1	1	1	1	1
Seedling blast (Almora, India)	4.5	2.5	4.3	1	4	3	4	4	4	3
Yield based on biomass harvest index (%)	20.8	24.0	21.9	16.6	18.6	20.6	21.3	19.6	24.0	22.3

(+) = allele contributed by Moroberekan; (+/-) = heterozygote at the candidate gene locus.

Single marker analysis of variance identified three candidate genes significantly correlated with blast resistance. Oxalate oxidase and peroxidase were correlated with yield under blast as well as with panicle blast in Almora, a blast hotspot in India. Thaumatin is correlated with neck blast incidence in Cavinti, Philippines. Significant two-gene interactions ( $P < 0.001$ ) were observed in seedling blast (HSP90 x thaumatin and thaumatin x oxalate oxidase) and neck blast (chitinase x thaumatin and thaumatin x oxalate oxidase-like protein). Of the lines selected for blast resistance, 23 yielded more than Vandana under natural drought stress. These included two lines (IR78221-19-6-7-8-B and IR78221-19-6-99-8-B) that are also high-yielding under high blast pressure in Almora. IR78221-19-6-7-8-B has alleles of oxalate oxidase, peroxidase, and HSP90 from Moroberekan. IR78221-19-6-99-8-B has only oxalate oxidase allele from Moroberekan. We are testing if additional candidate defense genes are responsible for the observed resistance in these lines.



**Fig. 4.** Percent diseased leaf area (DLA) at 50 DAS after sowing (IRRI) plotted against disease severity of panicle blast (Almora, India). Lines are shown with DLA and severity scale as well as number of candidate genes (CGs) present. Molecular analysis of each line used CG-specific PCR primers and polymorphic SSR markers co-localizing with the CGs.

**Table 3.** Common cis-elements existing in multiple copies in the 1-kb upstream region of each candidate gene. The cis-elements were identified using the Database of Plant Cis-Acting Elements (PLACE, <http://www.dna.uhrr.jp/PLACE/>).

Cis-element	Description	Motif
ASF1/MOTIFCAVY	Involved in developmental activation of several genes by auxin or salicylic acid	TGACGG
PALBOXAPC	One of the cis-acting elements (boxes P, A, and L) of phenylalanine ammonia-lyase	CCGTCC
WBQKATNPR1	Wounding promoter of Arabidopsis thaliana (AtL) NPR1 gene	TTCAG
WBXXTEF3	Methyl jasmonate activation of ERF3 by wounding	TGTACT
ELRECORECP1	Five Element core of parsley (Pc) PR1 genes	TGTACC
WBBOXCWRY1	Wounding inducible parsley (Pc) WRKY1 gene promoter; also conserved in WRKY3 gene	TTTGACT
GCCGCC	Conserved in many pathogen-responsive genes such as PDF2.2, Thi2.1, and PR6	GCCGCC
MYB1LEPR	MYB1 regulates defense-related gene expression via GCC box and non-GCC box cis elements (Myb1/GTTAGTT), G box, (CAGCTG)	GTTAGTT
T/GBDATV	Stimulate induction of tomato proteinase inhibitor II (pin2) and leucine aminopeptidase (LAP) genes T/G-box	
AGCBOXNGLB	Conserved in most PR-protein genes	AGC
CACGTGMOTIF	Tomato P4[ERF] regulates defense-related gene expression via GCC box and non-GCC box cis elements (Myb1/GTTAGTT), G box, (CAGCTG)	CACGTG
AGMOTIFNTMYB2	AG-motif found at -114 of the promoter of NtMyb2 gene; NtMyb2 is a regulator of the tobacco retrotransposon To1 and the phenylalanine ammonia lyase	AG-motif

## Conclusions

- Lines with broad-spectrum blast resistance in Vandana plant type are available, which were identified from pyramided lines genotyped for blast loci with favorable recombinants.
- Candidate defense gene markers have been designed and SSR markers within the candidate defense genes were identified and made available.
- The effect of blast loci on yield has been established based on the evaluation of pyramided blast lines under managed drought.
- The candidate defense gene markers and SSR markers within the defense genes are currently being used in other mapping populations to identify broad-spectrum resistance to blast and other diseases.
- By combining multilocation tests and molecular analysis, we have identified gene-based markers strongly associated with partial blast resistance.
- We are now in a position to combine blast resistance with drought tolerance in advanced breeding populations for a target environment in India.

## References

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