The background of the slide is a close-up photograph of rice leaves. The leaves are green and have several small, brown, necrotic spots scattered across them, which are characteristic of rice blast disease. The text is overlaid on this image.

Association of candidate defense genes with quantitative resistance to rice blast using molecular and *in silico* approaches

G. Carrillo, J. Wu, B. Liu, N. Sugiyama, I. Oña, M. Variar, B. Courtois, J.E. Leach, P. H. Goodwin, H. Leung and C.M. Vera Cruz

Putative QTL for blast partial resistance to PO6-6 in BC₃F₃ lines

Trait	Marker	Candidate Gene	Source	R ² (%)	F	P	TV (%)
DLA (%)	RGA8-4	NBS-LRR	Flax	11.79	9.36	0.0030	20.98
	RM215	SSR	Rice	9.19	7.09	0.0096	
LN	CG10d	Oxalate oxidase	Barley	28.65	27.7	0.0001	56.49
	CG17	Hv1433	Barley	14.07	11.3	0.0013	
	RGA1-10	LRR	Rice	9.39	7.25	0.0089	
	RM21	SSR	Rice	9.09	6.90	0.0100	
	RM168	SSR	Rice	10.73	8.42	0.0050	
LS	RM250	SSR	Rice	9.55	7.39	0.0082	9.55

^a DLA = % Diseased Leaf Area, LN = Lesion Number, LS = Lesion Size

^b Total variation explained by the traits

^c The model included 7 markers at P = 0.05

J. Wu et al., 2004

Phenotypic selection of Vandana x Moroberekan, IRRI & Cavinti



BC3F4 lines, IRRI Blast Nursery

***In India, have partial R across screening sites; V4M-5-3-B has good phenotypic acceptability.**

M. Variar

Line	Seedling blast* (%DLA)		Neckblast** (% Incidence)
	Cavinti	Blast Nursery	GH (PO6-6)
V4M-5-3-B*	3.7	21.9	33.0
V4M-6-1-B*	3.1	13.9	26.0
V4M-10-1-B	6.1	53.9	67.0
V4M-14-1-B	0.7	4.2	4.8
V4M-15-3-B	1.0	9.2	36.8
V4M-19-1-B	1.4	17.2	52.4
V4M-42-2-B	0.7	6.4	28.6
V4M-52-2-B	9.2	30.9	20.0
V4M-53-1-B	1.2	15.5	42.9
V4M-60-2-B	1.8	25.3	42.9
V4M-63-1-B	9.6	47.8	30.0
V4M-70-1-B	5.6	31.1	71.4
V4M-74-1-B	8.4	31.5	76.2
V4M-75-1-B	8.1	36.6	47.6
V4M-82-2-B	8.5	17.0	23.8
V4M-83-2-B	9.2	35.9	19.0

* BC3F4 gen

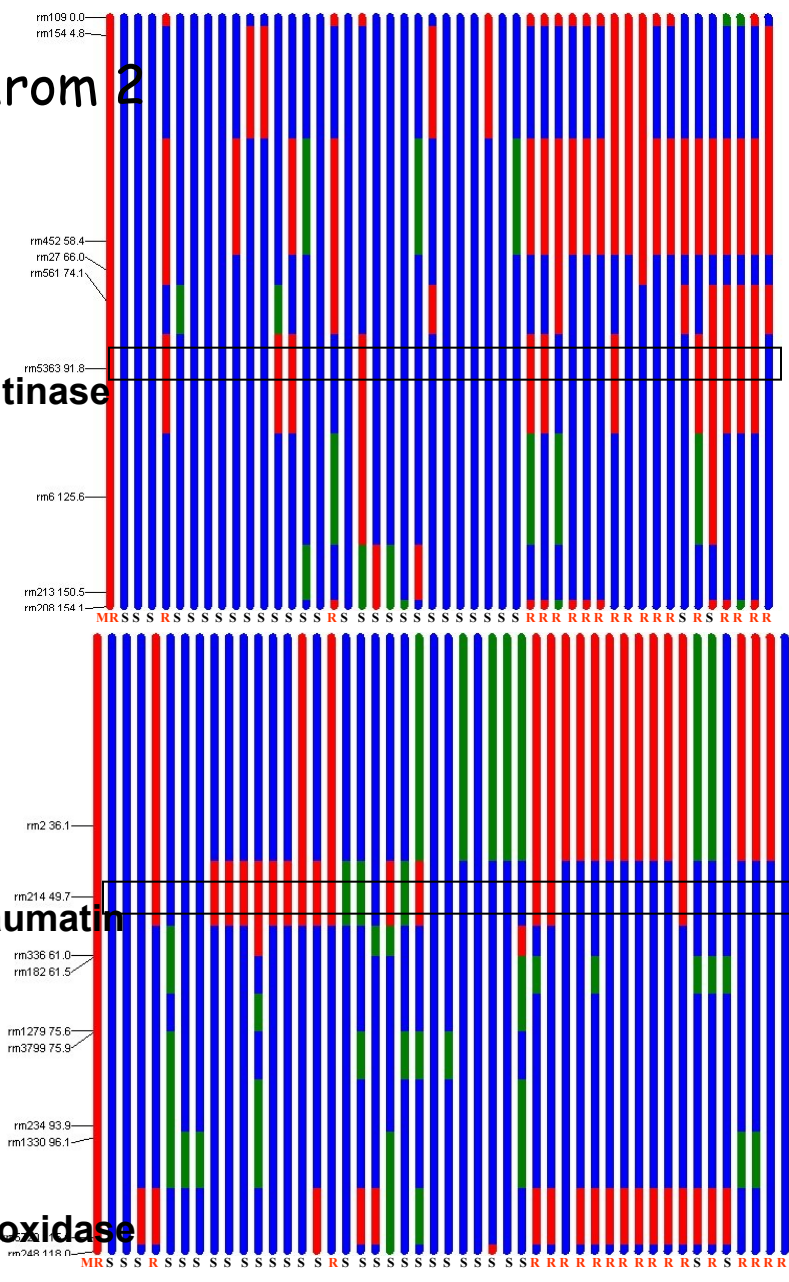
** BC3F5 gen

Chrom 2

Chitinase

Thaumatin

Peroxidase

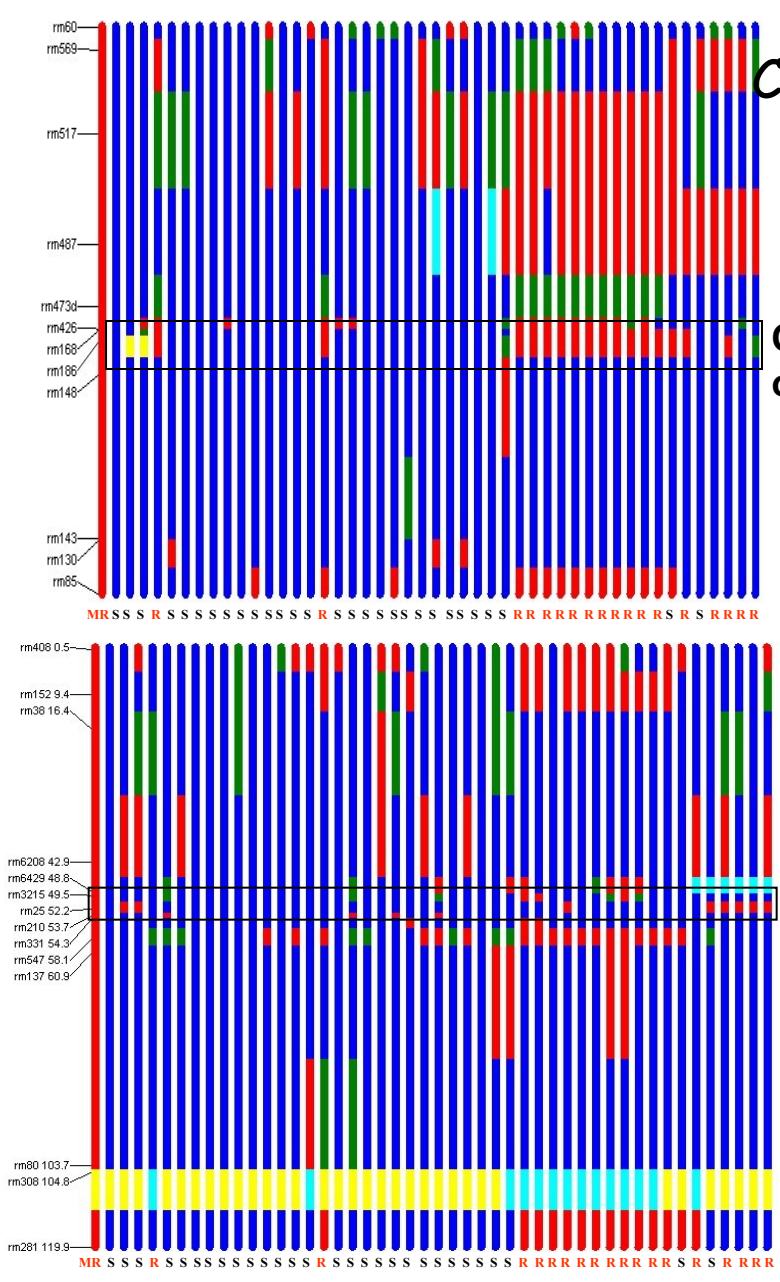


Chrom 7

Chrom 3

Oxalate oxidase

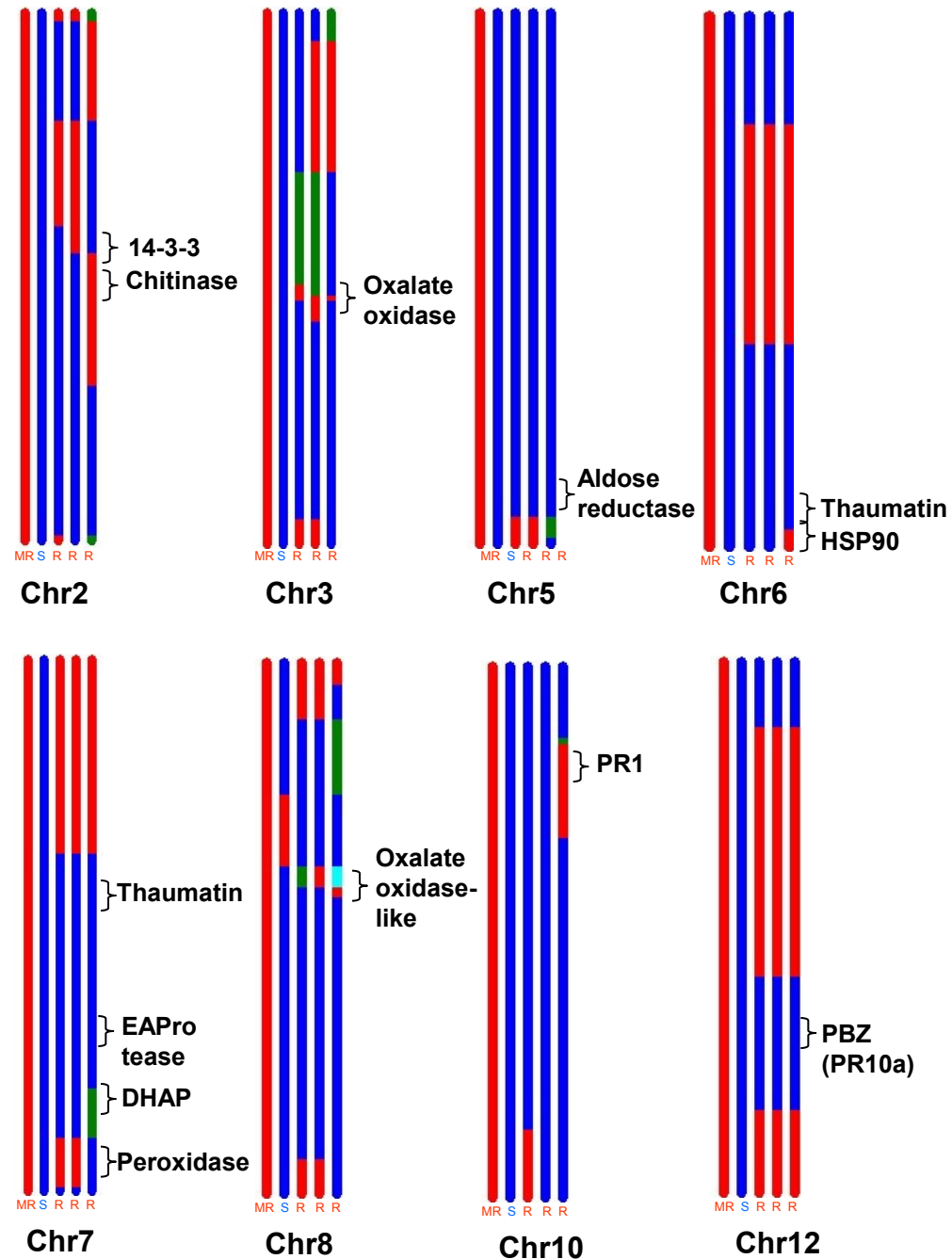
Oxalate oxidase-like protein



Chrom 8

Selected VxM lines

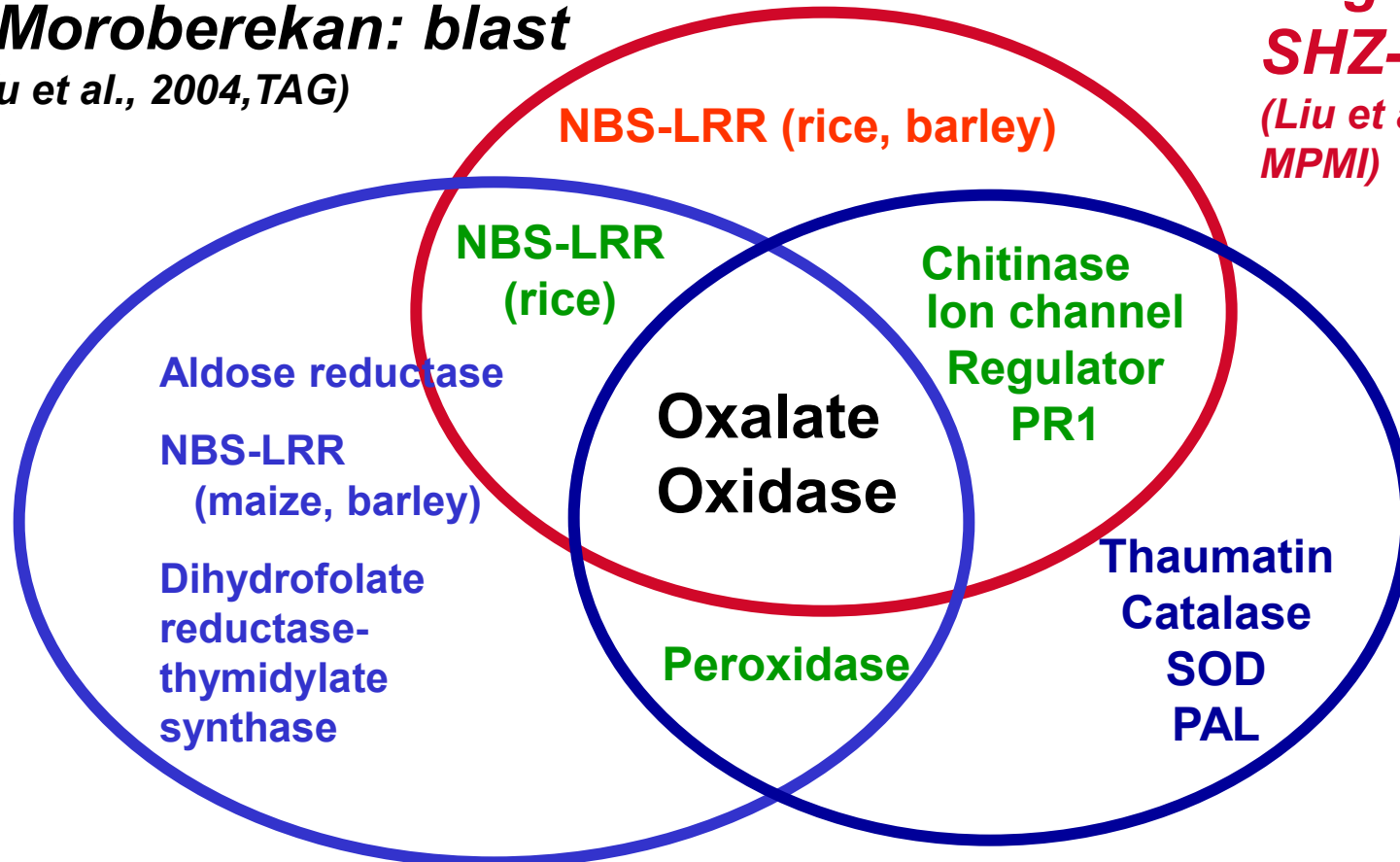
Candidate Gene	Mor	Van	IR78221-19-6-33-B	IR78221-19-6-90-B	IR78222-20-7-148-2-B
14-3-3	+	-	-	+	-
Chitinase	+	-	-	-	+
Oxalate Oxidase	+	-	+	-/+	+
Aldose reductase	+	-	-	-	-
Thaumatin (Chr6)	+	-	-	-	-
HSP90	+	-	-	-	+
Thaumatin (Chr7)	+	-	-	-	-
Eukaryotic aspartyl protease	+	-	-	-	-
Deoxyphosphohept onate aldolase	+	-	-	-	-/+
Peroxidase	+	-	+	+	-
Oxalate Oxidase-like	+	-	-/+	+	+
PR1	+	-	-	-	+
PBZ (PR10a)	+	-	-	-	-
Seedling blast (Philippines)	0.3	7	1	1	2
Seedling blast (Almora, India)	1.5	8.5	4	4	4



Identify "consensus" candidate genes

**Upland rice Vandana
x Moroberekan: blast**
(Wu et al., 2004, TAG)

**Irrigated rice
SHZ-2: blast**
(Liu et al., 2004,
MPMI)

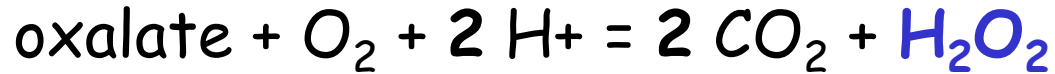


**DH Rice IR64 x Azucena:
BB, blast, ShB**

**Wheat: tan spot and/or
leaf rust (Faris et al. 1999, TAG)**

(Ramalingam et al., 2003, MPMI)

Role of oxalate oxidase in defense response



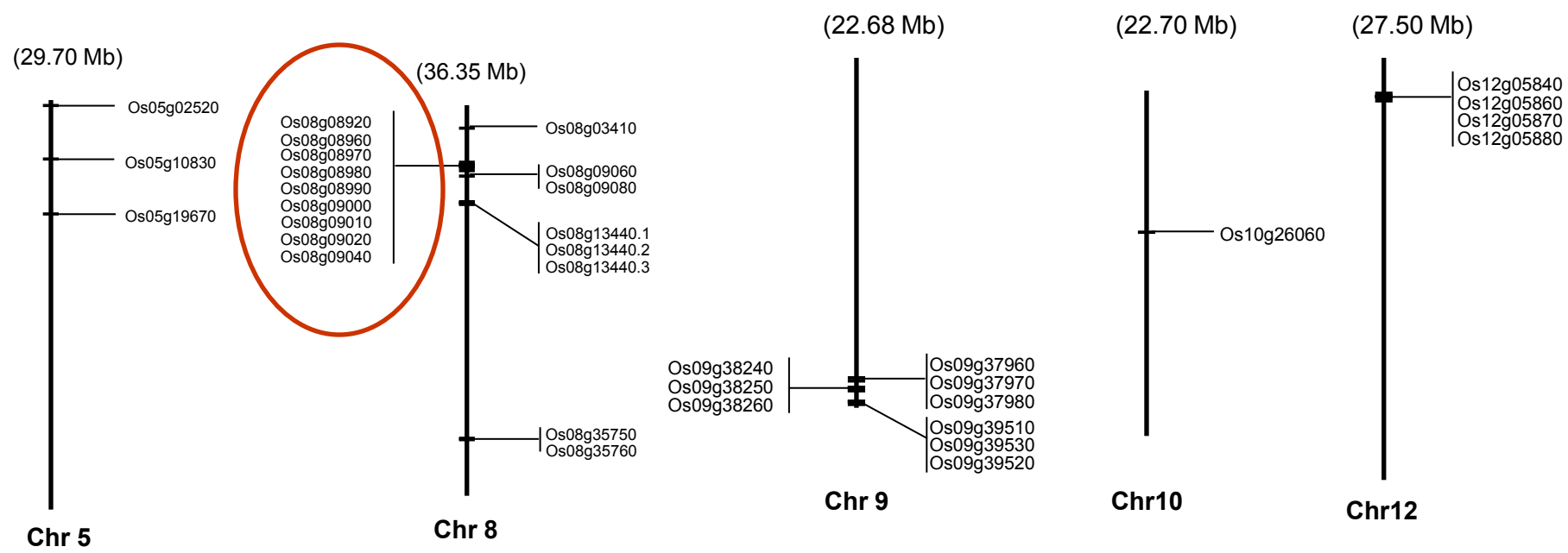
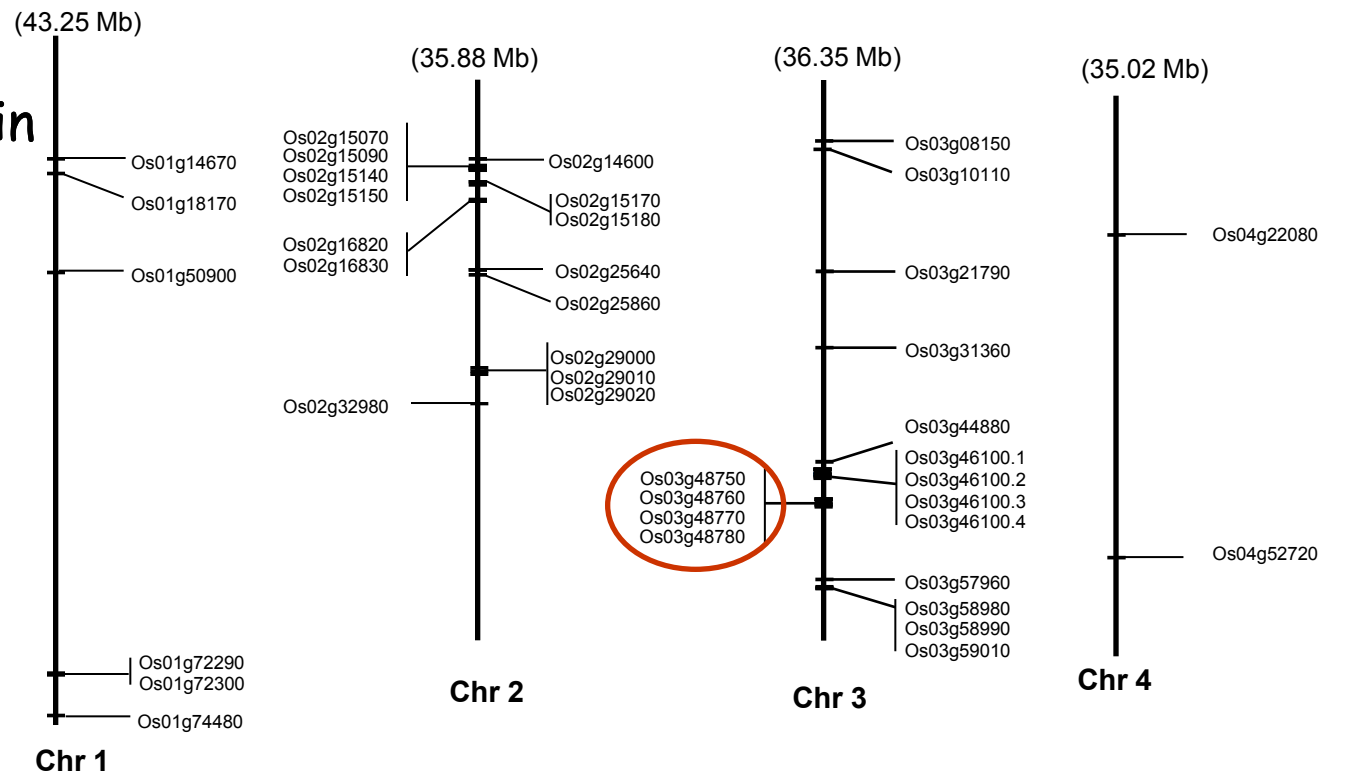
- rapid generation of superoxide and accumulation H_2O_2 is a hallmark of hypersensitive response in plants
- Oxidative burst
 - Direct antimicrobial activity
 - H_2O_2 as substrate for oxidative cross-linking in the cell wall
 - threshold trigger for HR
 - diffusible signal for induction of cellular response

Oxalate oxidase is a member of the cupin superfamily of proteins which is structurally conserved but functionally

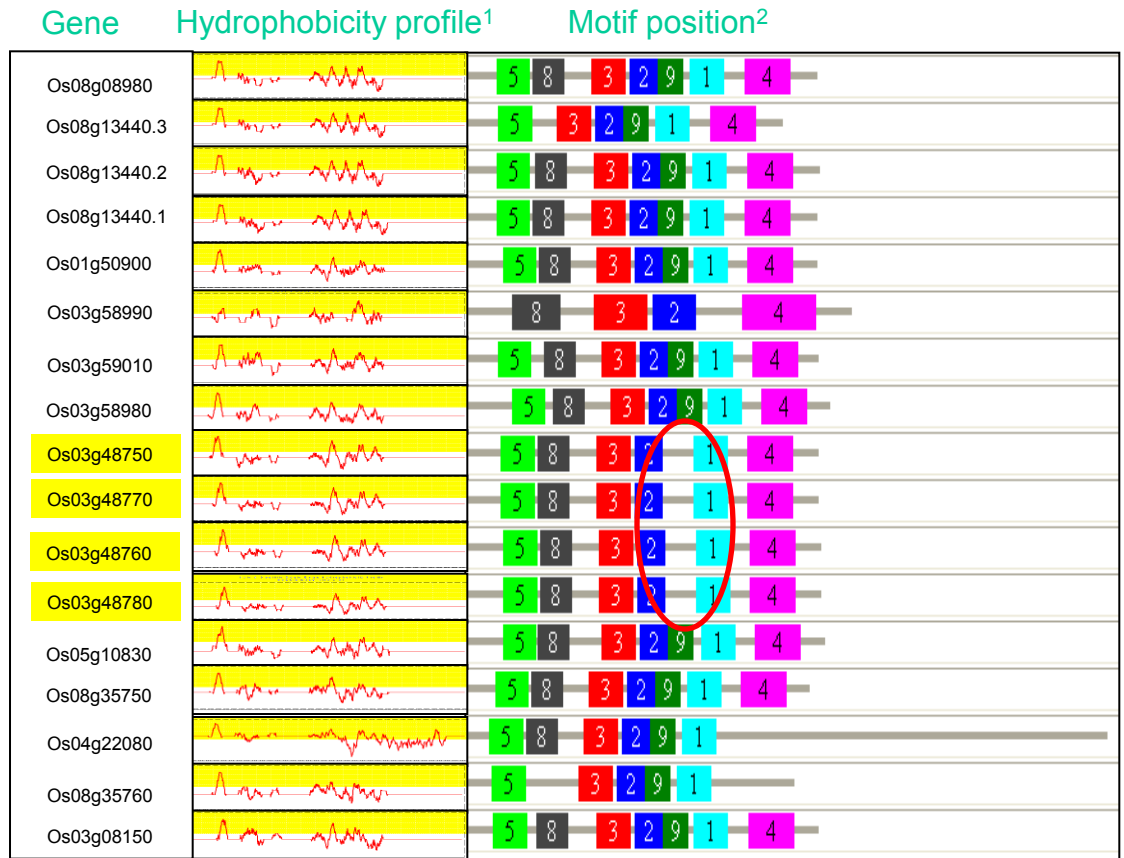
diverse

74 cupin sequences in the rice genome

Distribution of genes of the cupin superfamily in the genome of rice cultivar Nipponbare



Hydrophobicity profiles and motif positions of germin-like proteins in the rice genome



¹ Hydrophobicity was calculated using Kyte-Doolittle algorithm (scan window = 21).

² Motif positions 1-9 as determined by MEME (Bailey and Gribskov, 1998) using protein sequences.

Analysis of 1kb upstream sequence

- Plant cis-acting elements (PLACE) database
- Variation in the number of copies of cis-elements related to biotic stress
- Wbox, WNPR1, WRKY
- PCR primers based on the 1kb upstream region of Chr3 oxalate oxidases show polymorphism between Vandana and Moroberekan using primers based on Os03g48760 and Os03g48780

Summary and future prospects

- ✦ We have associated known sequences of candidate genes to phenotypes of Vandana x Moroberekan population
- ✦ We are testing the VxM materials for resistance to sheath blight and brown spot to identify lines with broad spectrum resistance to fungal pathogens of rice
- ✦ Using the available genetic and bioinformatic resources for rice coupled with efficient phenotyping tools, it is possible to relate QTLs to candidate genes and metabolic pathways