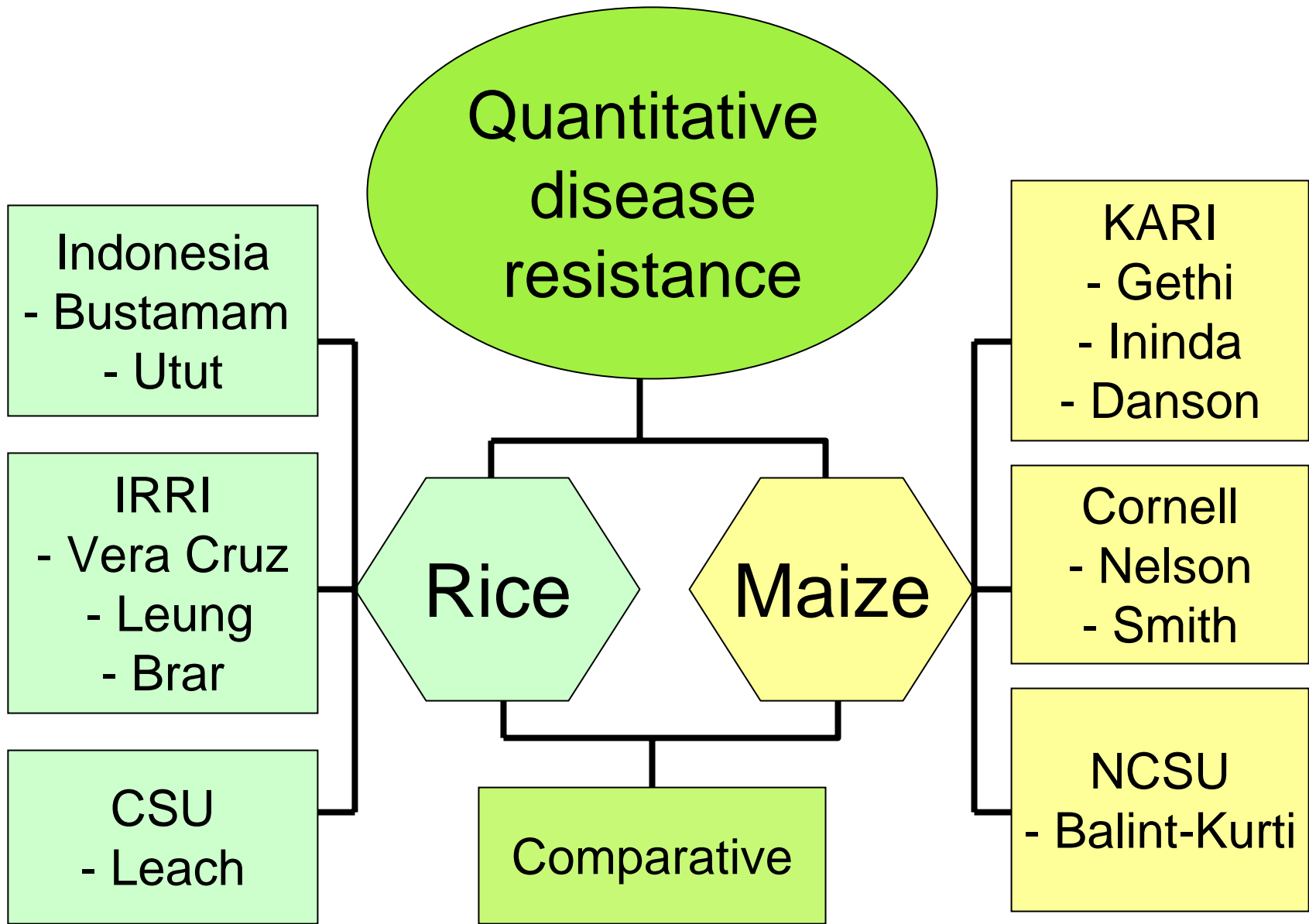


Targeted discovery  
of superior alleles for  
quantitative disease resistance  
in the maize and rice genomes

Generation Challenge Program  
Project #8



# General objectives

- Contribute to improvement of disease resistance in the cereals
- Address hypotheses concerning quantitative disease resistance
  - Does multiple disease resistance (MDR) exist? Identify such genomic regions in maize, rice and “cereals”
  - Contribute to a better understanding of the genes that condition quantitative resistance

Germplasm panel:  
disease resistance  
sources

Breeding resistant  
rice and maize

Recurrent selection  
mapping

dQTL synthesis  
maps

Near-isogenic  
lines

Genetic dissection  
of dQTL

Deletion mutants for  
gene validation

Phenotypic  
characterization  
of dQTL

# Resistance panels

- Rice
  - Advanced stocks derived from cultivars with durable resistance to blast
  - Mutants in IR64 with altered resistance phenotypes
  - Screening germplasm for sheath blight resistance
    - Wild species
- Maize
  - Maize diversity panels screened at NCSU; potential sources screened by KARI; recognized sources of resistance collected at Cornell
    - Screening in Kenya, NCSU, NY, VA
  - Lines with multiple disease resistance (CIMMYT, other)
    - Now shared by KARI and Cornell
  - Genetic stocks for isoline development

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# Disease QTL syntheses

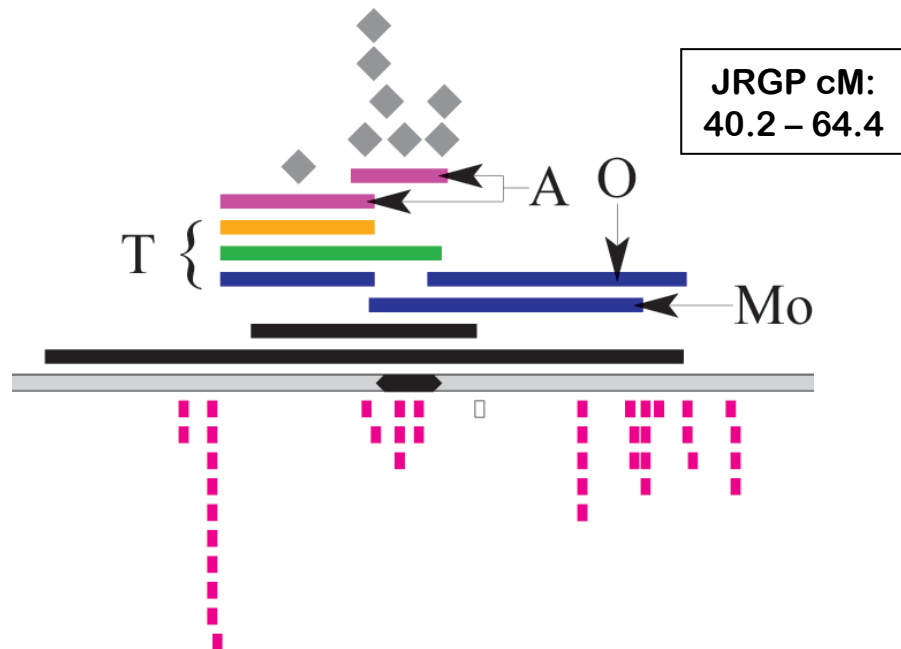
	Rice	Maize
No. disease QTL studies	16	34 (50 disease mapping studies)
% of genome covered by dQTL	54%	89%
QTL distribution	Non-random; clusters for multiple diseases	
For more info...	Wisser et al., Genetics 169: 2277	Wisser et al., Phytopath. (in press)

# 22 chromosomal segments associated with quantitative resistance to multiple diseases

11 chromosomal segments:

Co-localizing QTL for multiple diseases associated with the same parental allele

Pericentric section of chr. 12



# Significantly associated with the QTL fraction of the rice genome:

- All rice genes
- R-genes; RGAs
- Rice blast-responsive genes
- A few gene families ( $n = 4$ )
  - Glutathione S-transferase
  - UDP-glucosyltransferase
  - Peptidase

- A. Glutathione S-transferase
- B. UDP-glucosyltransferase
- C. Peptidase

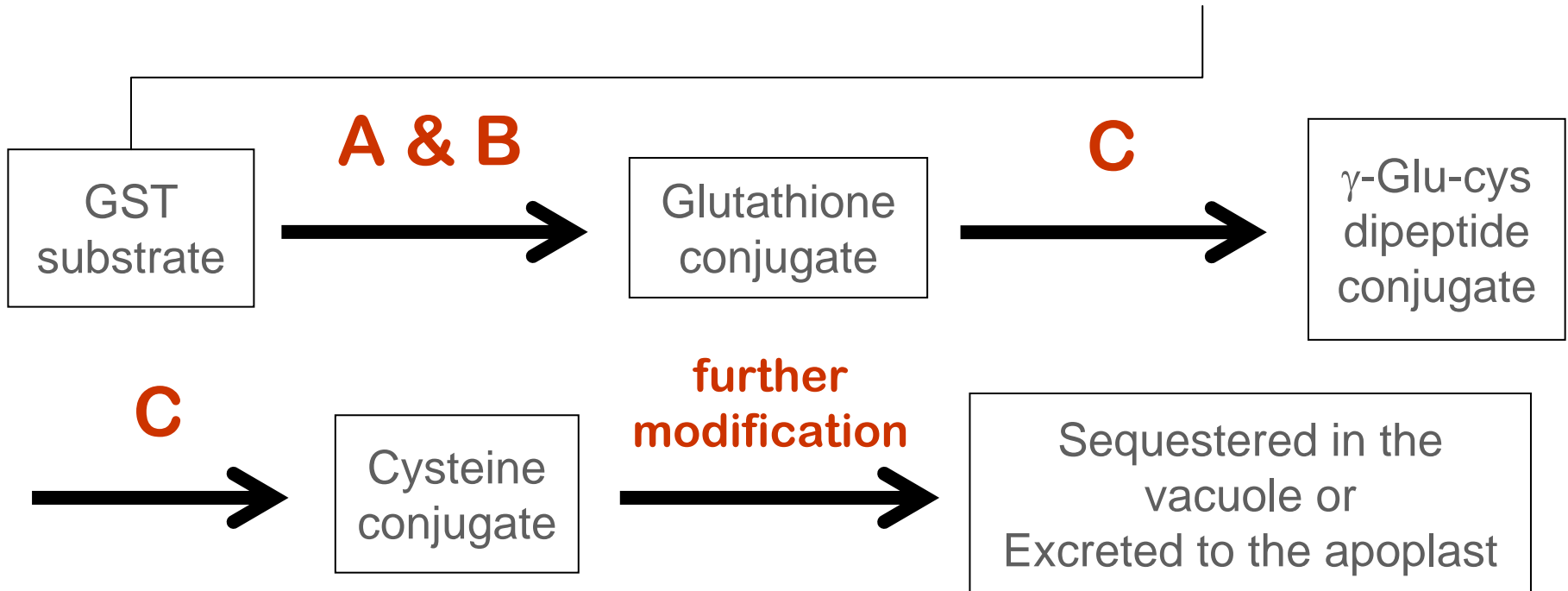
All involved in...

# Detoxification via glutathione metabolism

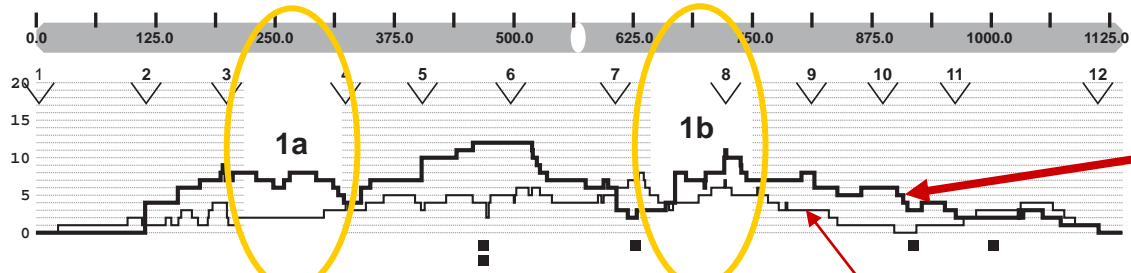
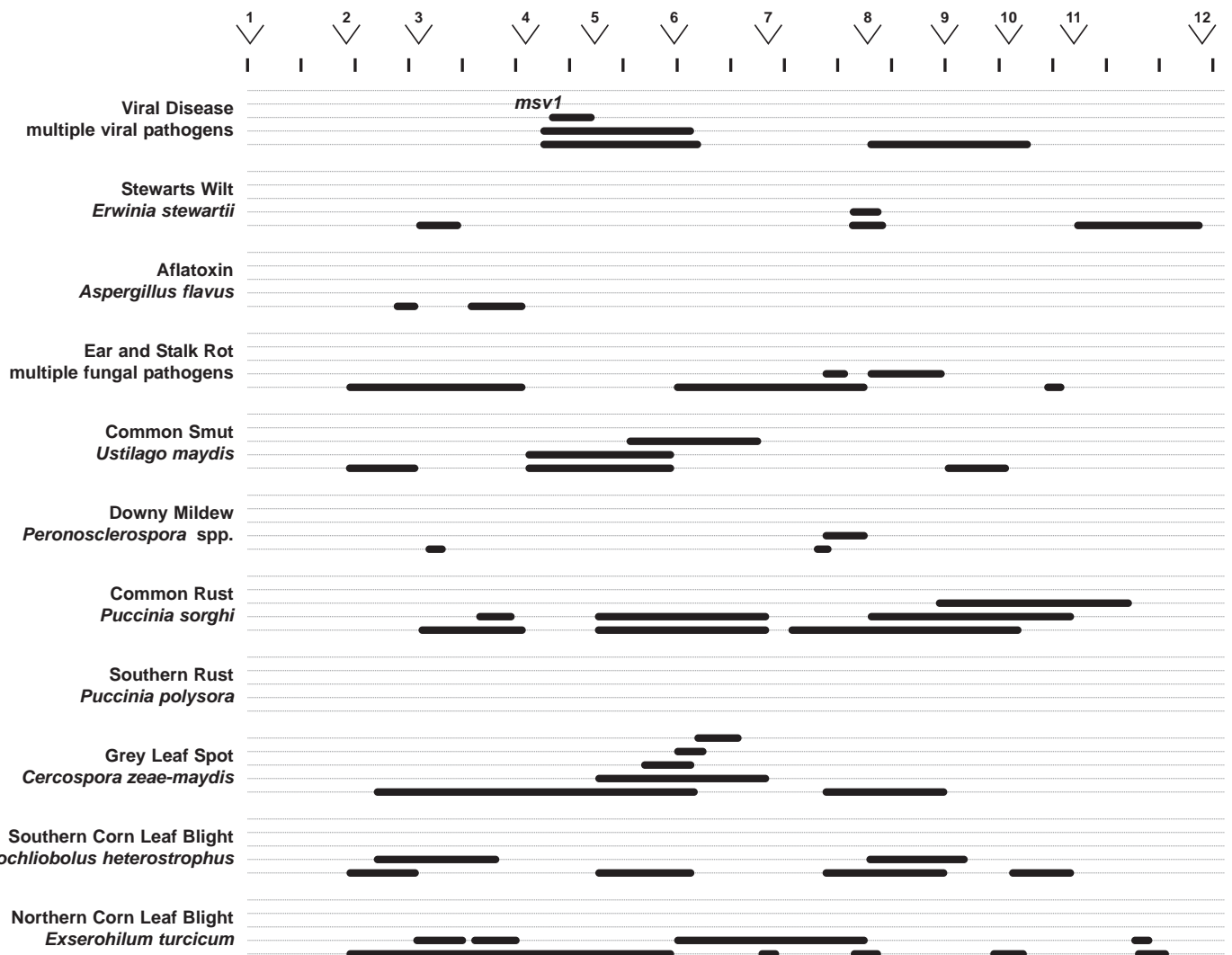
Tissue damage  
Wounding  
Pathogen attack

→ Oxidative burst →

CyP450 modification of  
electrophilic compounds  
(GST substrate)



# Integrated disease-QTL map of maize



← Chr. 1

Disease QTL

Flowering time QTL

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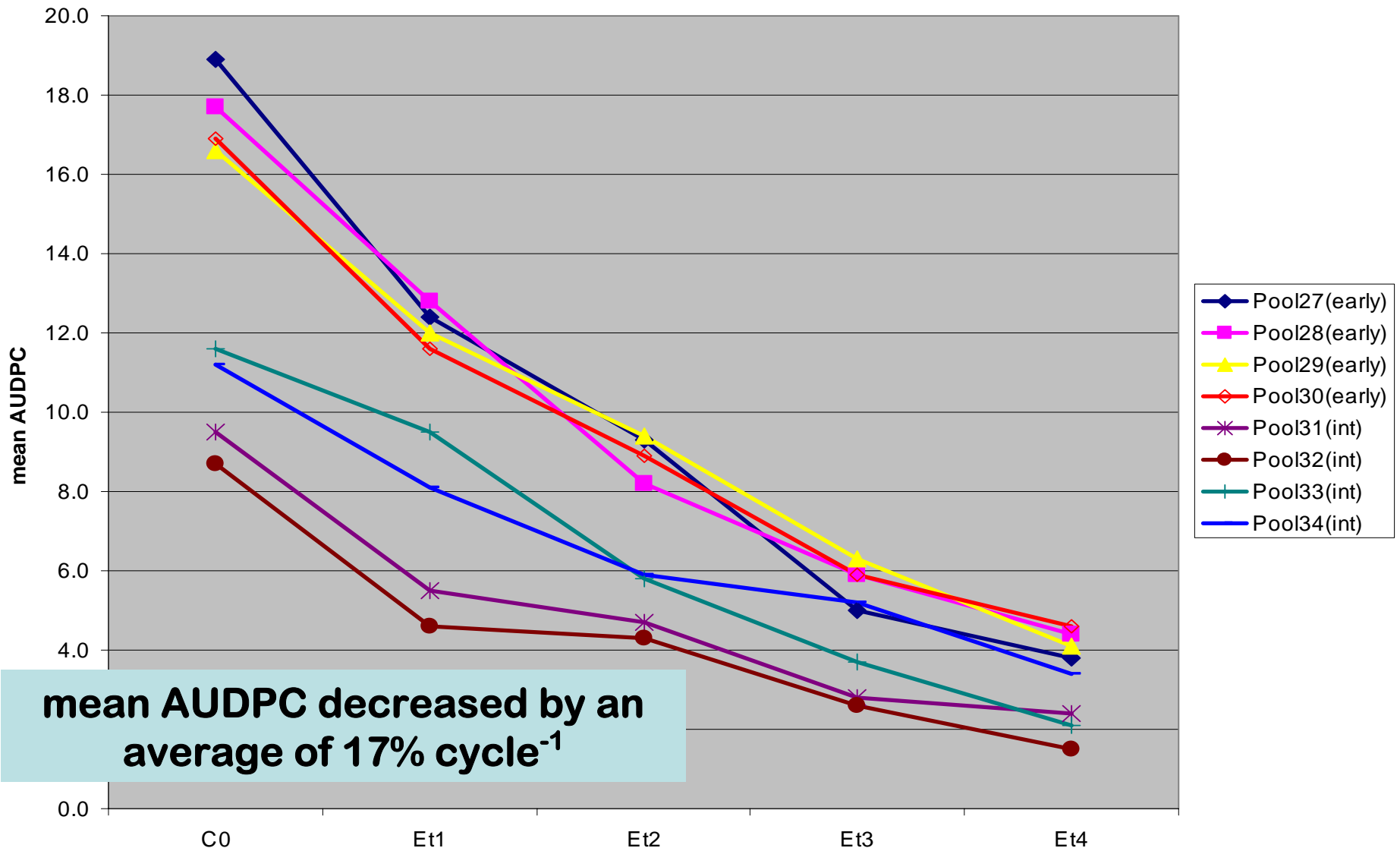
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# SOURCE of RESISTANCE: Recurrent selection for quantitative resistance to NCLB in 8 diverse maize pools



# Pool 30: One of 8 diverse sources of resistance used for recurrent selection

Materials resistant to downy mildew; Amarillo Bajío Precoz; Indonesian Comp.; Pairumani Syn.; Materials from Holland – Aurelio; Capella; Diana; Elina; Florina; Fronica; Fortha; Leopard; Pastora; Sandrina; Tamira; Rheintaler, (Ht1A; HT1B; to pool 30 only); Sabana Grande; Canda; Los Altos; El Viejo; Santa Ana; Maicillo; Tola Rivas; MV. MSC.342; MV.TC.281; MV.TC. 290; MV-MSC.201; MV.SC.202; MV.MSC.201; MV.MSC.262; BE-KE 270; UNCAC 242, Cornell Precoz; Compuesto L; Chihuahua Gpo. E; Pool 1; Syn. 107; Syn. MV.DC.520; MV.SC. MV.SC.620; MV.TC.6 MV.TC.540; MV.DC.3 Comp. Indonesia; Cor Shiao Sui Hua; Ki Ta Su; Hsiao Pai Chi; NEMP-5, BK-FS (Early), NEMP-5, BK-FS (Sel.); NEMP-2, BK-FS (Mount Lebanon); NEMP-2, BK-FS-Sel.; NEMP-1, BK-FS (Bekka 1); NEMP-1, BK, Yellow Dent segregates (Argentina 565, Arg. 567; Guatemala 246, Guat. 317, Guat. 313); Mex. Mix.; Super Mix; CBC 62A; GR-4; GR-12; GR-3; GR-7; GR-5; GR-8; GR-13; YB 8429; YB 8430, CBC 77: SJ 3120, 3080, 3443, 3773, Funks “D”; DDCSR1C0; DDCHT2C2; Tremesino, Queixale, 71A; Kohot; Bannu Yellow; Andaluz; Dent of Szeged; Rosero; Basto; MV.SC.47A; 74A; Gallego; Vasco; Puenfeareas; Blanco; “F” early yellow D; Red. King; Samsun 63; INRA 240; Seg. From 4; FB of Martonvasar; INRA 310; Hembrilla Norteña; Cuba; Enano Norteño; AT 209; BT; AT 633; Locar var. of Zala; HVM 424; Germany 504; USA Pool – YD segregates.

Average of 5.7 alleles  
detected per SSR locus

n=90 individuals, pool 30

# Recurrent Selection Mapping

Identify shifts in allele frequencies at mapped loci between the first and last cycle that cannot be explained by drift, mutation, or migration.

- **Genotype  $C_0$  and  $C_L$**
- **Test if allele frequency shifts exceed drift (Waples, 1989)**

- Isozymes: Stuber, C. W., and R. H. Moll (1972) *Crop Sci.* 12:337
- SSRs: Labate, J. *et al.* (1999) *TAG* 99: 1166
- RFLPs: De Koeyer, D. L., *et al.* (2001) *Crop Sci.* 41: 1228
- SSRs: Ching, A. *et al.* (2003) 45th Maize Genetics Conference, Poster 195



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# Near-isogenic lines for maize dQTL

## Why?

- Genetic dissection
- Phenotypic analysis

## What?

- Selected chr. segments
- Allelic series

Recurrent selection populations

QTL mapping population(s)

Other sources of resistance

dQTL-NILs

## How?

- Marker-assisted backcrossing → NIL series
- Selfing → marker-assisted extraction of NIL pairs

# Maize mapping populations in use

- B73 x Mo17 RIL (IBM) Lee
- B73 x Mo17 RIL (Stuber) Holland
- B73 x B52 RIL Lee
- B73 x DE811 RIL Lee
- B73 x Ki14 RIL Pratt
- B73 x NC250 F2:3 Goodman
- B73 x H99 RIL Pé
- B104 x NC300 RIL Goodman
- B73/Tx303 NIL Holland
- Mo17/Oh43 NIL Holland

# Near-isogenic lines for SCLB



**Mo17  
allele at  
3.04**



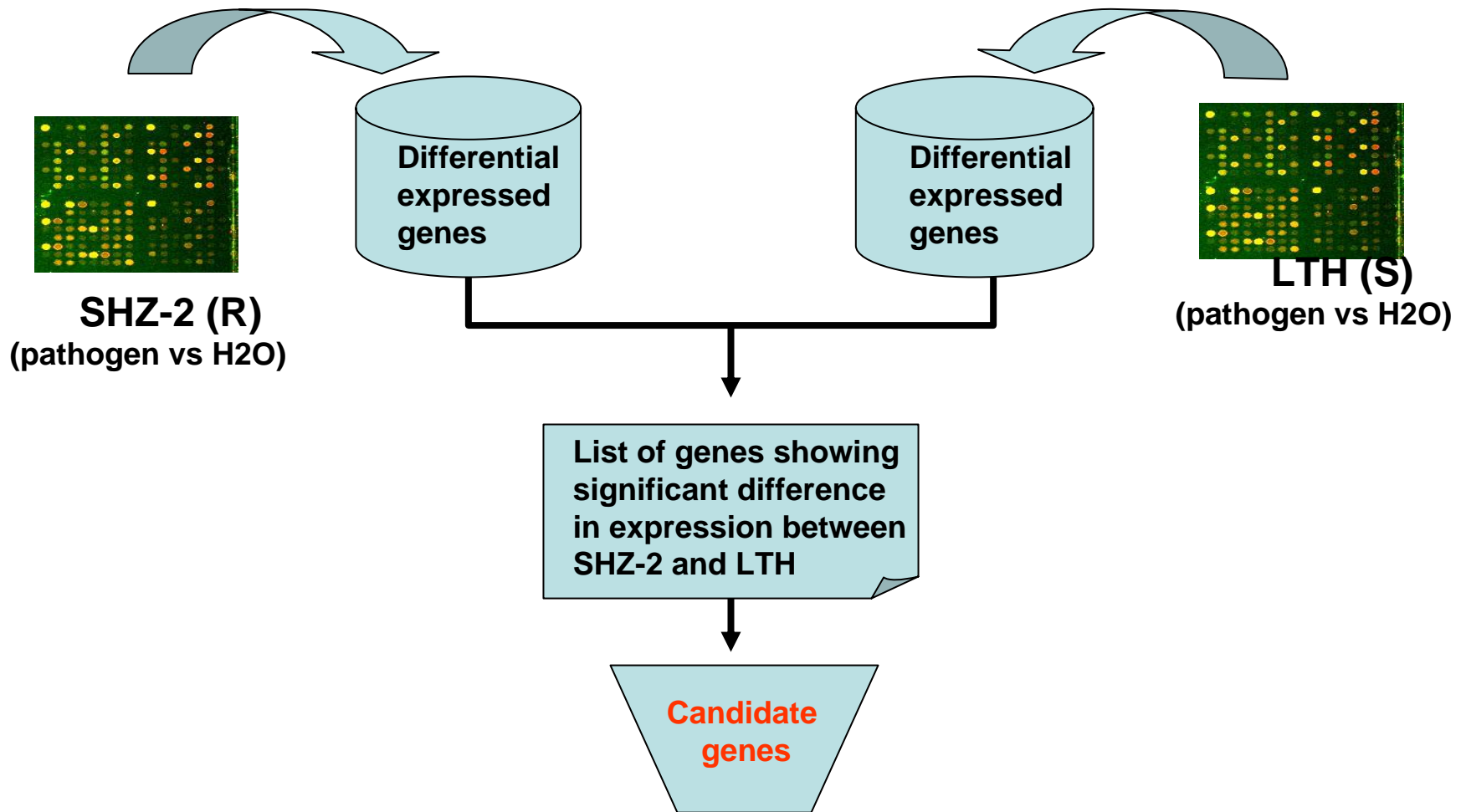
**B73  
allele at  
3.04**

# Resistance to rice blast and sheath blight in TXZ x SHZ2 derivatives

BC Line	Rice Blast						Sh Blight
	Lineage 4		Lineage 5		Lineage 6		Isol 1
	LT	%	LT	%	LT	%	% PAA
ShanHuang Zhan	3,4	50	3,4	48	3,2,4	39	30
TXZ/SHZ2 BC10-46	4	30	4	38	4	59	21
TXZ/SHZ2 BC10-10	4	19	4,3	12	4,3	37	8
TXZ/SHZ2 BC116	4	50	4,3	16	4	26	14

Blast: Lesion Type (1-2 = R, 3 = I, 4 = S); % Lesion Area Affected  
 Sheath Blight: % PAA (% Plant area affected)

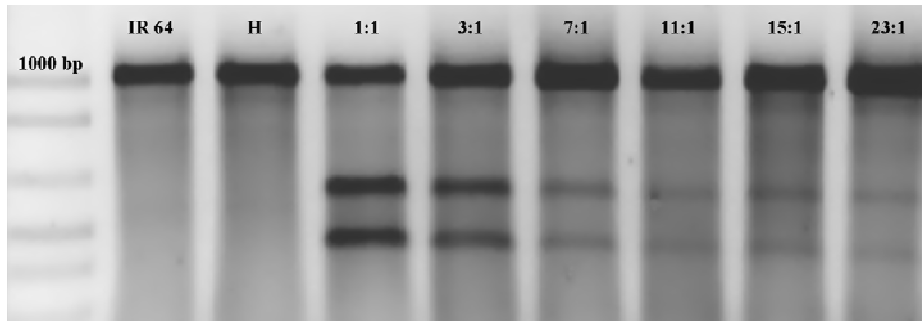
# Co-segregation analysis of differentially-expressed genes and quantitative resistance to blast disease



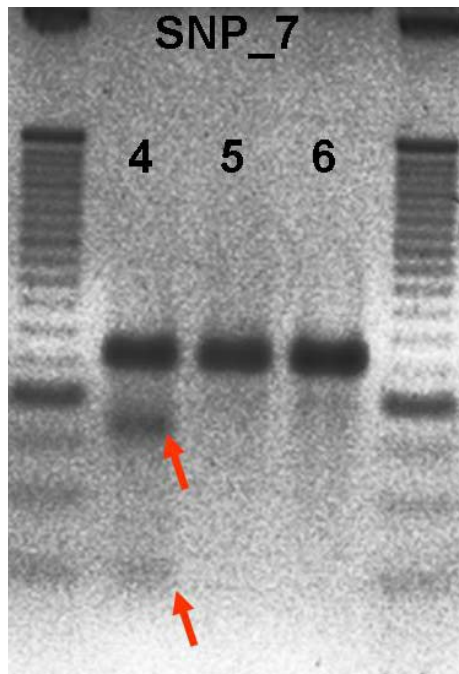
# The association with blast resistance and expression pattern of identified candidate genes

Gene ID	Gene name	Chr	Association with blast resistance		Expression pattern	
			R <sup>2</sup> (%)	P	Relative ratio at 48h	Relative ratio at 96h
AK062495	Subtilisin-cymotrypsin inhibitor	1	5.50	0.0215	1.59 <sup>***</sup>	0.49 <sup>**</sup>
AK111087	myb protein	2	7.26	0.0090	1.45 <sup>***</sup>	1.28 <sup>*</sup>
AK101841	Selenium-binding protein	2	6.88	0.0326	NS	2.15 <sup>***</sup>
AK109729	Unknown protein	3	3.61	0.0451	1.56 <sup>***</sup>	2.26 <sup>***</sup>
AK069447	Transketolase	4	3.33	0.0432	2.49 <sup>***</sup>	0.73 <sup>**</sup>
AK105947	Dehydration-responsive protein	5	3.15	0.0771	20.06 <sup>***</sup>	NS
AK107868	Unknown protein	8	27.2	0.0001	1.79 <sup>**</sup>	2.14 <sup>***</sup>
AK060990	Cytochrome P450	8	23.88	0.0001	10.27 <sup>***</sup>	NS
AK060251	Far-red impaired response protein	8	20.26	0.0001	0.42 <sup>***</sup>	NS
AK063193	Unknown Protein	10	3.49	0.0683	2.31 <sup>***</sup>	2.01 <sup>**</sup>
AK103834	Sumergence induced protein 2	10	2.87	0.0991	2.17 <sup>***</sup>	1.57 <sup>**</sup>

# SNP (heteroduplex) detection by conventional agarose gel electrophoresis



Heteroduplex detection  
in rice: *myb* dilution series



Heteroduplex detection  
in maize

Tool for seeking  
orthologs across cereals?

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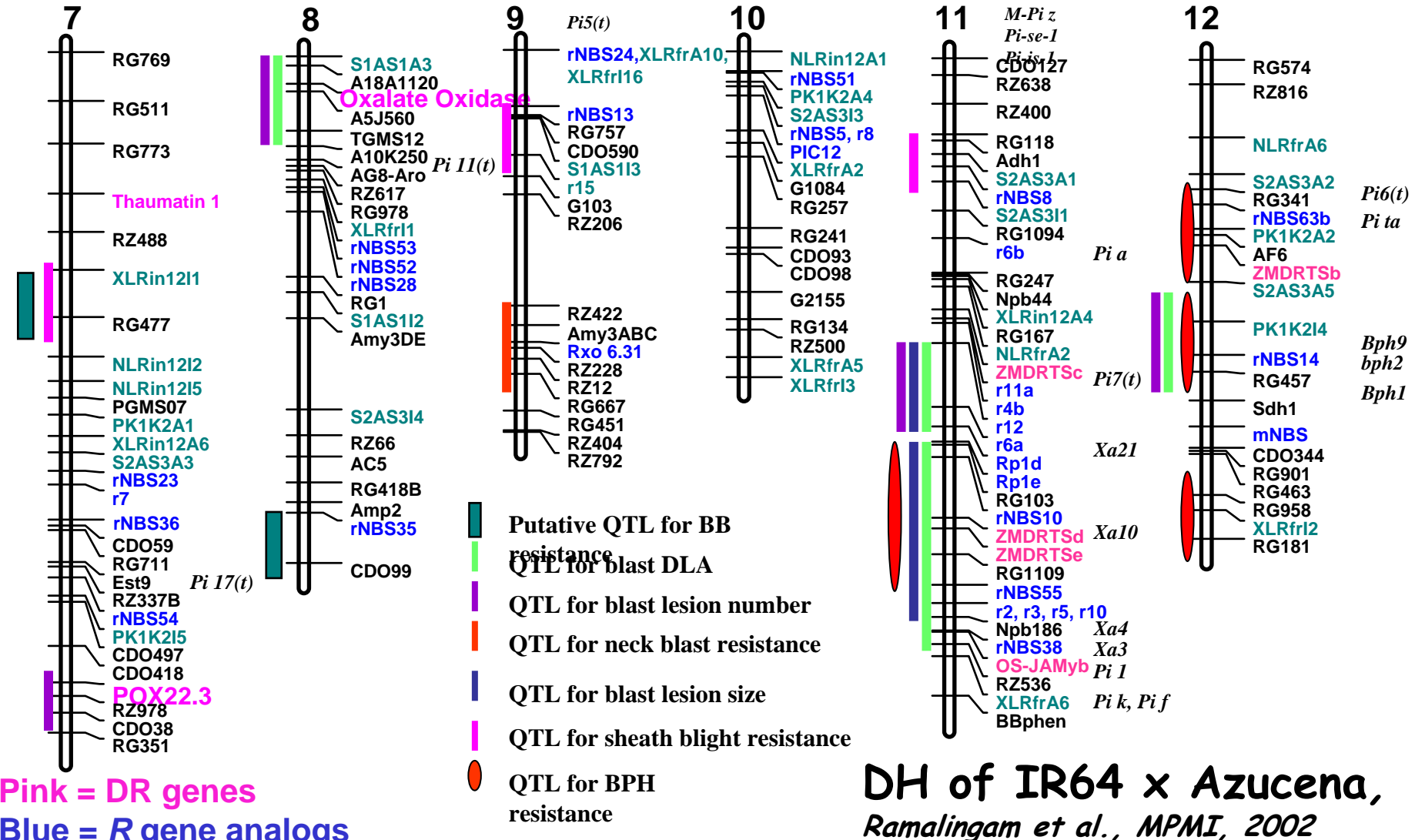
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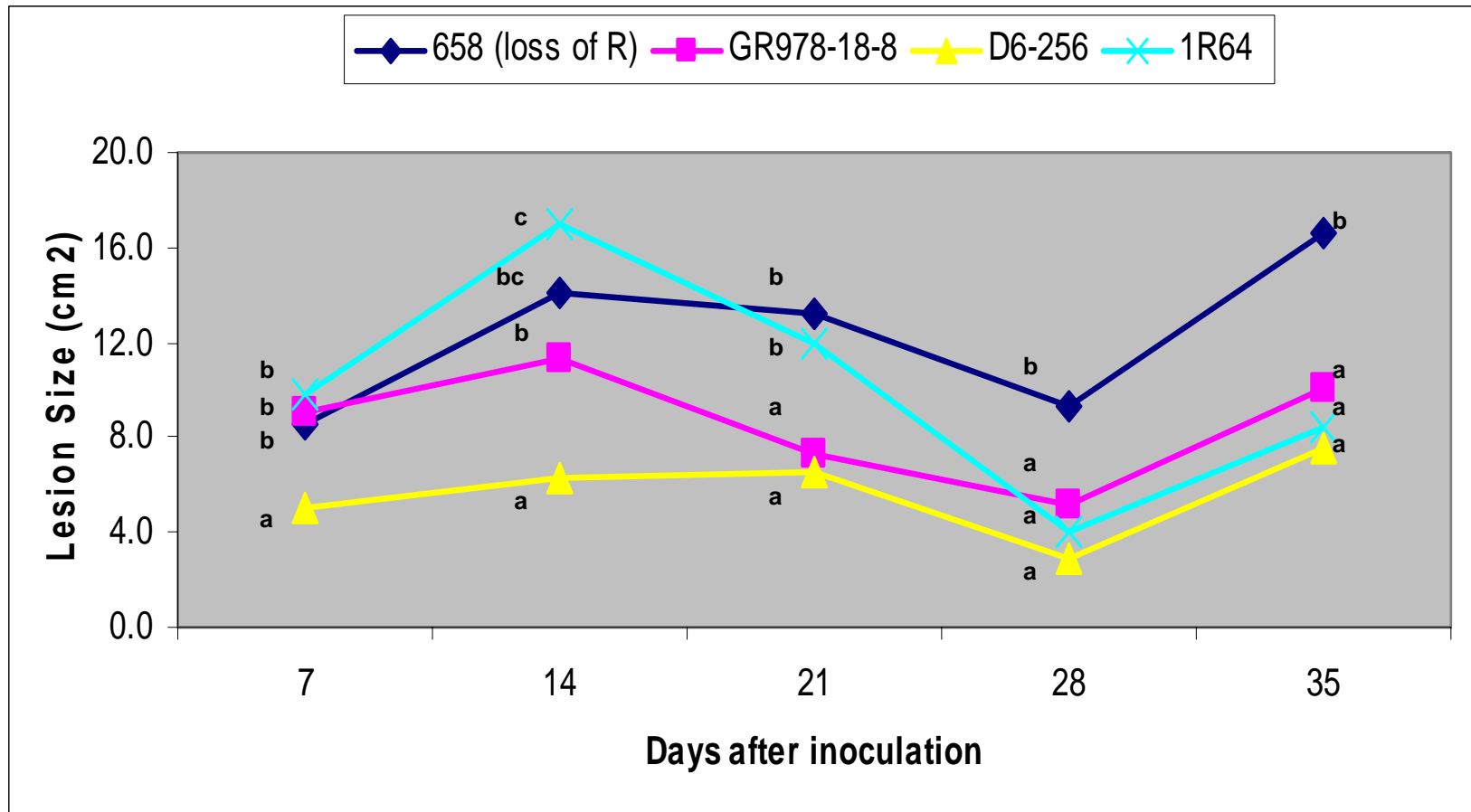
Phenotypic  
characterization  
of dQTL

# Disease resistance QTL identified in several rice populations



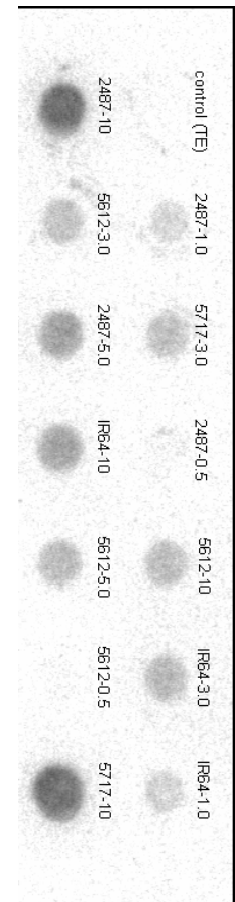
DH of IR64 x Azucena,  
 Ramalingam et al., MPMI, 2002

# Sheath blight progression on IR64 and mutants

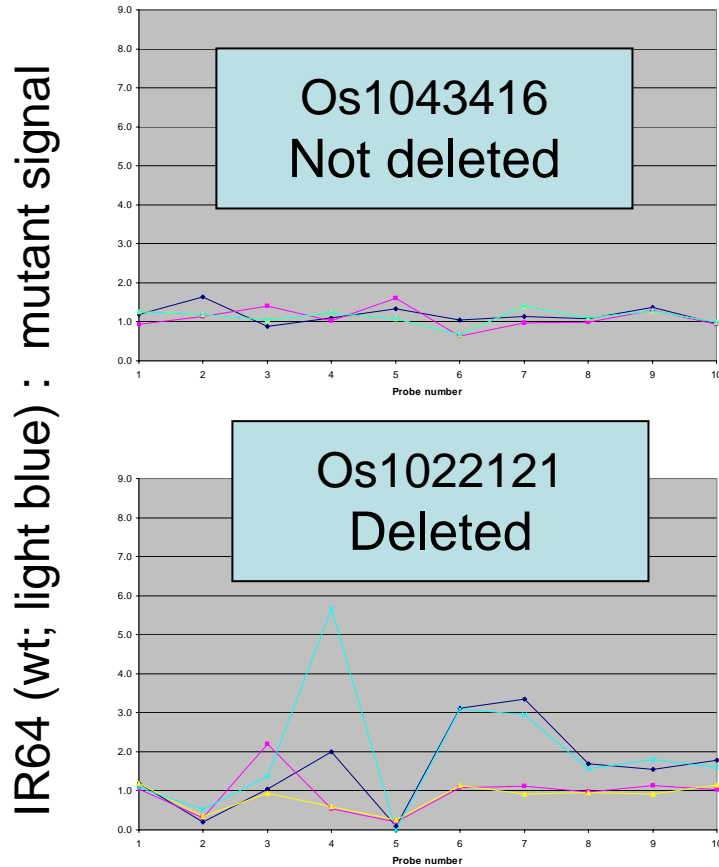


# Verifying candidate genes

- Identify deleted genes in selected mutants
  - *Gene to phenotype*: gDNA spotted arrays hybridized with overgo probes
  - *Phenotype to gene(s)*: Syngenta RiceChip oligonucleotide arrays for detection of deleted gene regions
    - Proofs-of-concept: *d1* gene, *Spl1* gene
    - Need to confirm utility of public oligonucleotide arrays
- Suppression of gene expression (VIGs and RNAi)



# Microarray meets deletion mutants



Oligo data across selected genes on 50K array

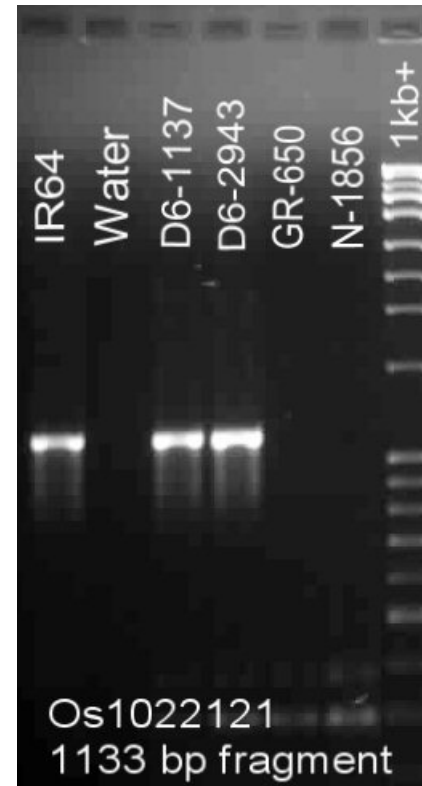
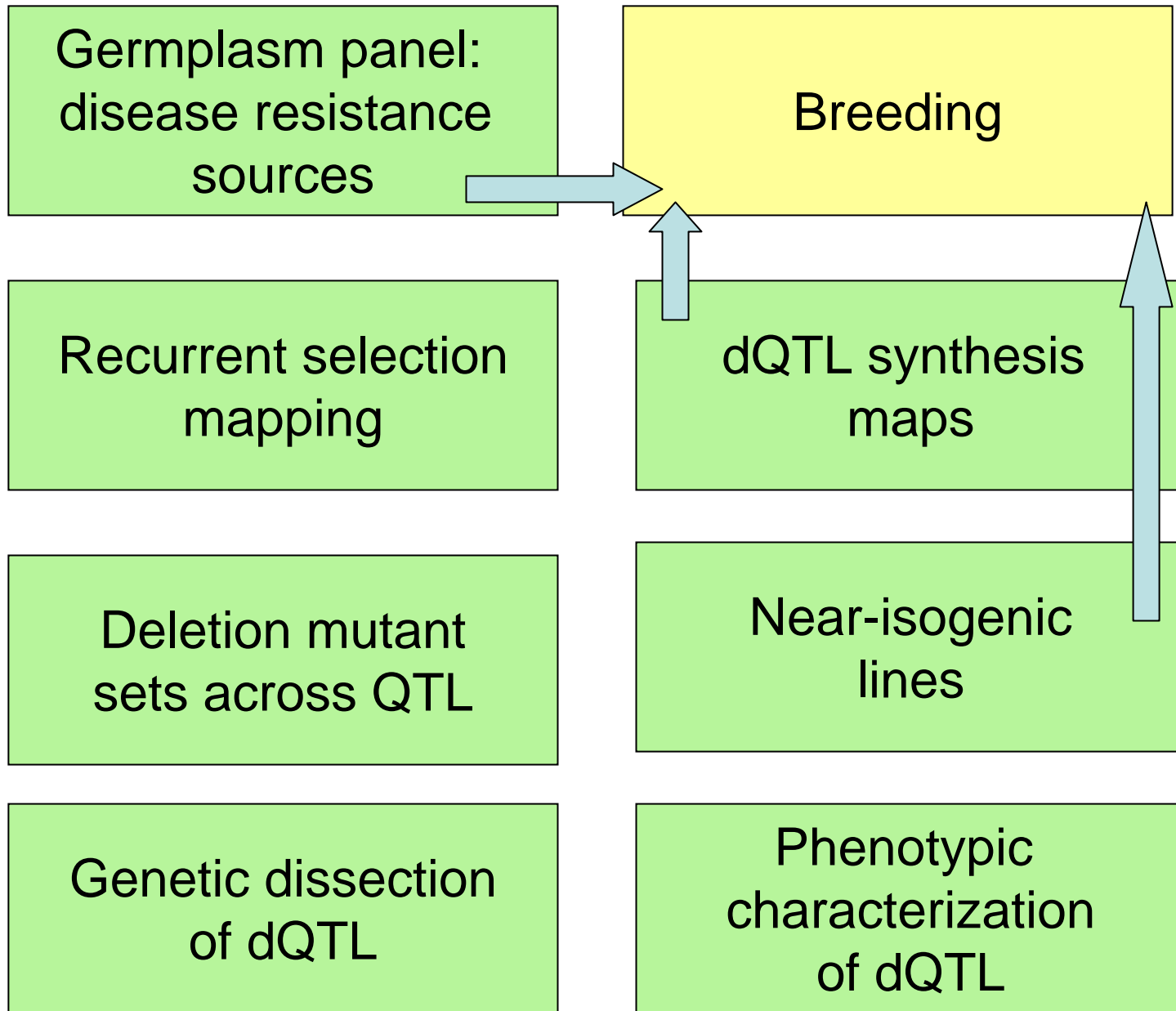


Image of PCR products confirms deletion in radiation mutants



Identify donors of potentially complementary dQTL

Resistance panel

Markers for GLS dQTL clusters

Inoculation studies

Lines with distinct dQTL

Crosses to CML373

BC1 → BC2 → BC3

SSD --> BC3F4

Extract NILs; confirm

Recombine dQTL

GLS-resistant breeding materials

# What's next?

- The Disease Panels
  - Continue to elaborate, increase seed, test
- Breeding
  - Utilize elite panel selections in non-conventional hybrids
  - Identify superior recombinants
- Disease QTL in rice and maize
  - Validate putative dQTL and capture in NILs
  - dQTL dissection through recombination, mutants
  - Detailed phenotypic analysis
- Comparative aspects
  - Identify “cereal” dQTL clusters through rice-maize comparisons
  - Identify orthologous candidate genes in rice and maize

# Acknowledgements

- Rice
  - Masdiar Bustamam, Utut Suharsono
  - Nollie Vera Cruz, Hei Leung, D.S. Brar, Gay Carrillo
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- Maize
  - Peter Balint-Kurti
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  - Rockefeller Foundation, McKnight Foundation, USDA-NRI, others