



# Tomorrow's Rice

**Jan E. Leach**  
**Colorado State University**

USAID  
July, 2013





**Food  
Safety**



**Plant  
Health**



**Energy**

# **Tomorrow's Rice**



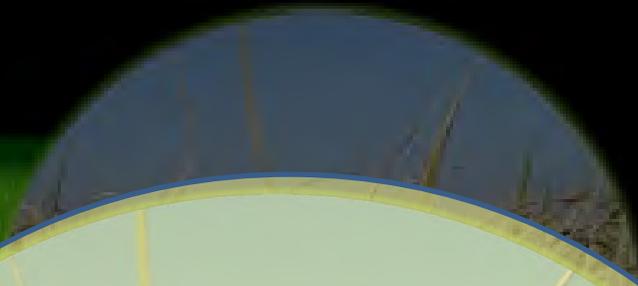
**Human  
Health**



**Good  
Agronomic  
Traits**



**Food  
Safety**



# **Plant Health**

- **What is out there?**
- **Broad spectrum resistance**



**Energy**

**T**



**Agronomical  
Traits**

*“...up to 40% of plant productivity in Africa and Asia, and about 20% in the developed world, is lost to pests and pathogens.”*

Somerville & Briscoe,  
Science 2001 292:2217

*“In rice production, estimates for loss potentials (for pathogens)... averaged 15% ...worldwide.”*

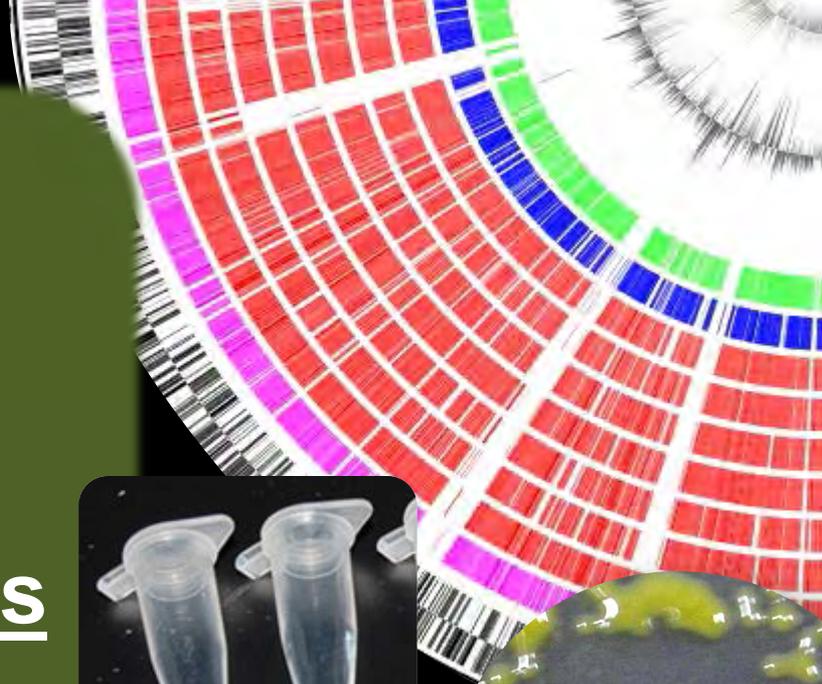
E. C. Oerke, 2006.  
*Crop Losses to Animal Pests,  
Plant Pathogens, and Weeds*



# What is out there?

**Need:** Reliable, simple assays to detect rice pathogens in field surveys and seed testing

J. Lang, L. Triplett, V. Verdier, C. Vera Cruz, G. Ash, A. Djikeng, J. E. Leach



Colorado State University

Charles Sturt University

IRD  
Institut de recherche  
pour le développement

biosciences  
eastern and central africa

IRRI  
INTERNATIONAL RICE RESEARCH INSTITUTE

**Step 1: Develop a one-step computational tool that will design specific diagnostic primers from draft genome sequence \*UniPrimer\***

Input: Target sequences

+

Input: Non-target sequences

~ 15-30 min. on laptop

**Output:**

- Primers specific to all target sequences
- Primer information (optimal conditions, etc)

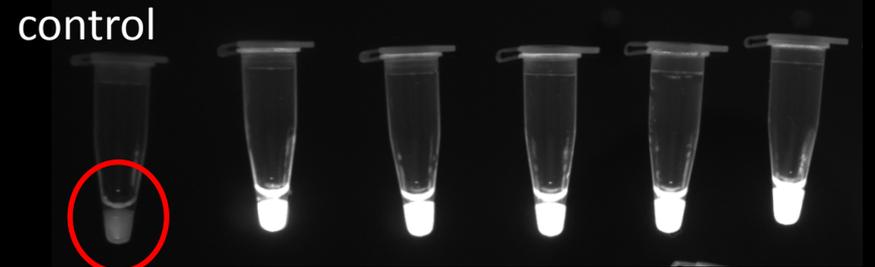
# Diagnostic primers adapted to LAMP\* assay for field surveys

Sequence genomes

Run Uniprimer

Adapt primers for LAMP

Test primer specificity & sensitivity



Lang et al., 2010  
Triplett et al., 2011  
Lang et al., in prep  
Triplett et al., in prep  
Ash et al., in prep

**Diagnostic Primers  
ready for field use**

\*Loop Mediated Isothermal Amplification

# Diagnostic primers adapted to LAMP\* assay for field surveys

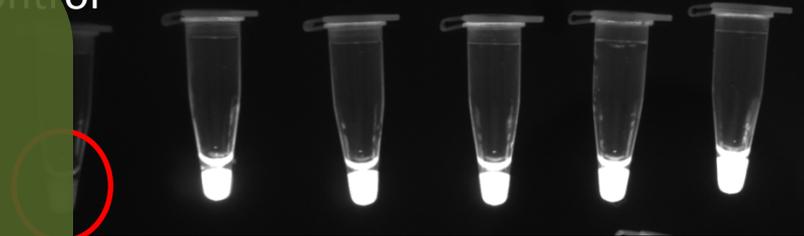
Sequence genomes

Adapt primers for LAMP

Run Uniprimer\*



control



Once draft genome available, primers designed and tested within 6 weeks

Diagnostic Primers ready for field use

\*Loop Mediated Isothermal Amplification

Lang et al., 2011  
Triplett et al., 2012  
Lang et al., in prep  
Triplett et al., in prep  
Ash et al., in prep

# Primers/LAMP ready or in development\* for:

- *Xanthomonas oryzae* pv. *oryzae* (bacterial blight)
- *X. oryzae* pv. *oryzicola* (bacterial leaf streak)
- *Pseudomonas fuscovaginae* (sheath brown rot)
- \**Burkholderia glumae* (bacterial panicle blight)
- \**Burkholderia gladiolae* (bacterial panicle blight)
- *Xanthomonas translucens* (black chaff & leaf streak wheat)





**Food  
Safety**



**Energy**



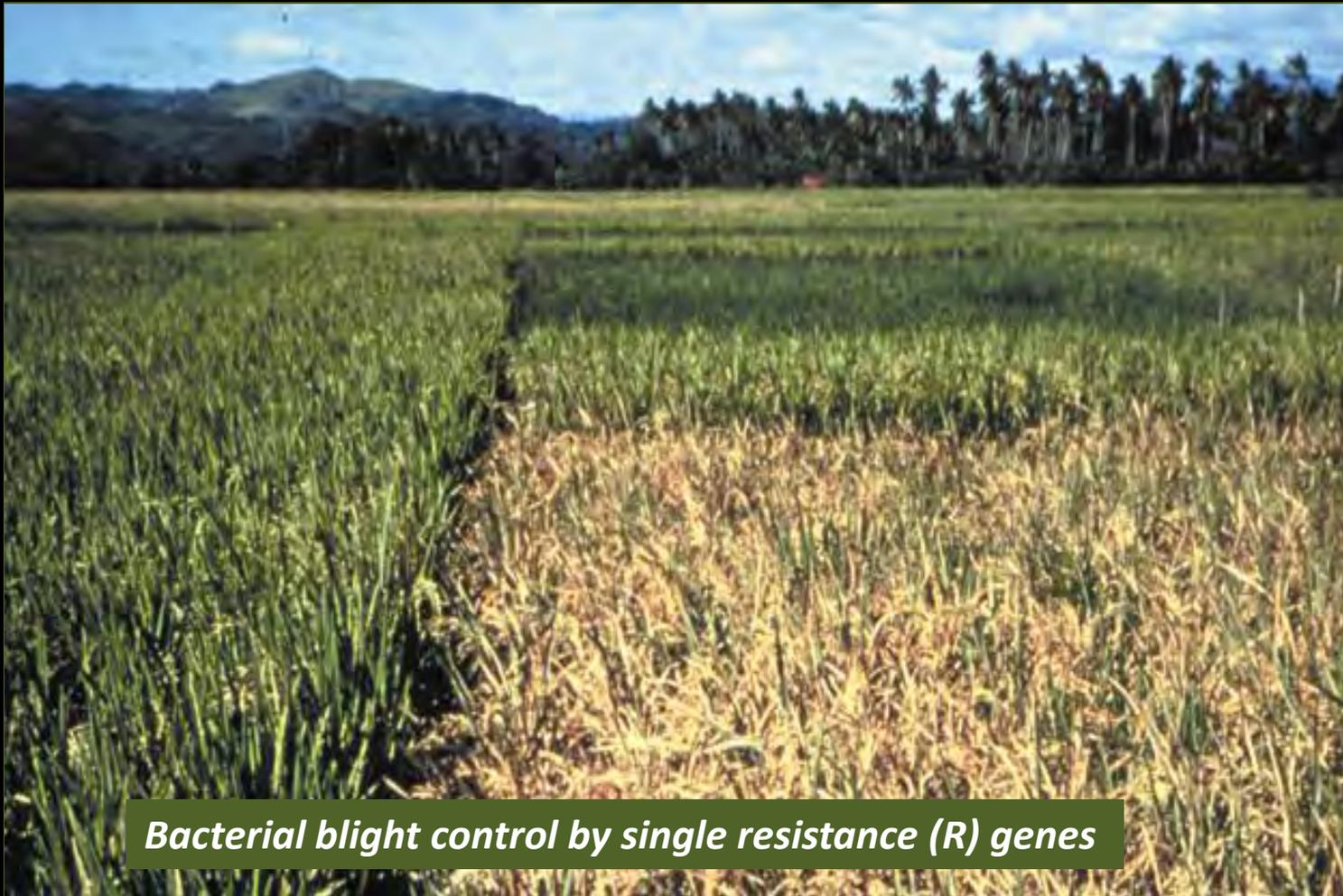
# **Plant Health**

- **What is out there?**
- **Broad spectrum resistance**



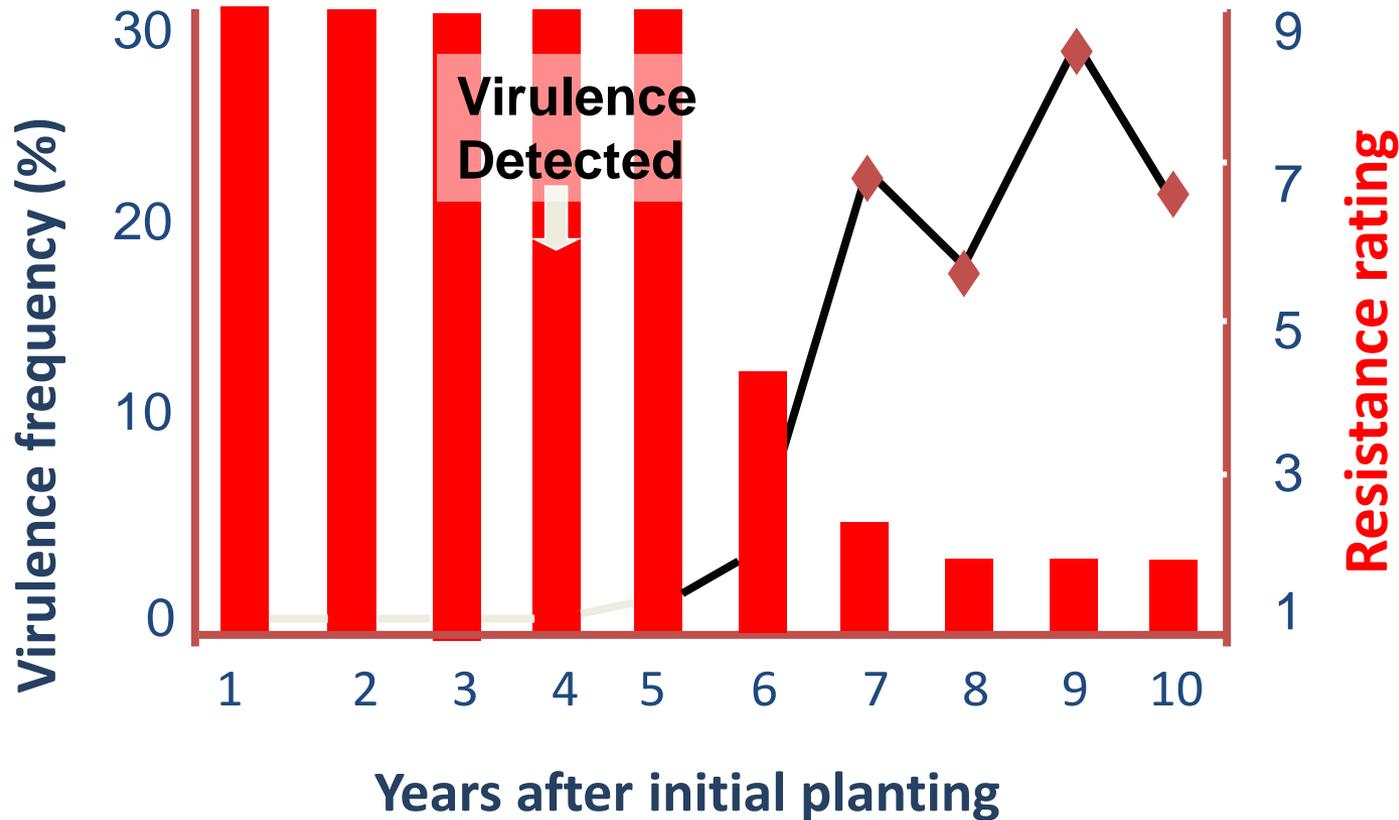
**Agronomic  
Traits**

*Current disease control practices  
largely exploit **single R gene resistance***



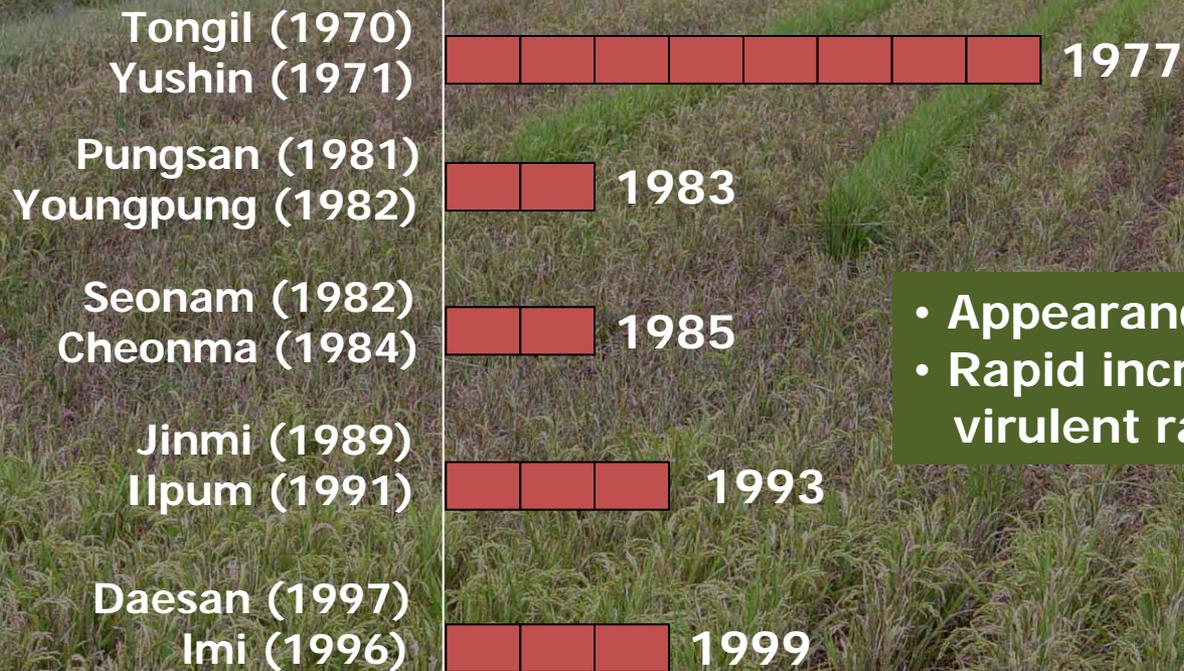
*Bacterial blight control by single resistance (R) genes*

# Problem with use of single gene resistance: *Pathogen changes and overcomes resistance*



# Single Gene Resistance Frequently Not Durable: Rice Blast

Cultivar (year of commercialization)      Year of breakdown



- Appearance of new races
- Rapid increase of virulent races

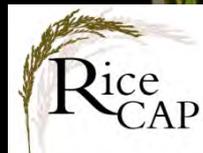
**Need to increase durability of resistance of rice cultivars**

# *Approaches to broad-spectrum, durable resistance*

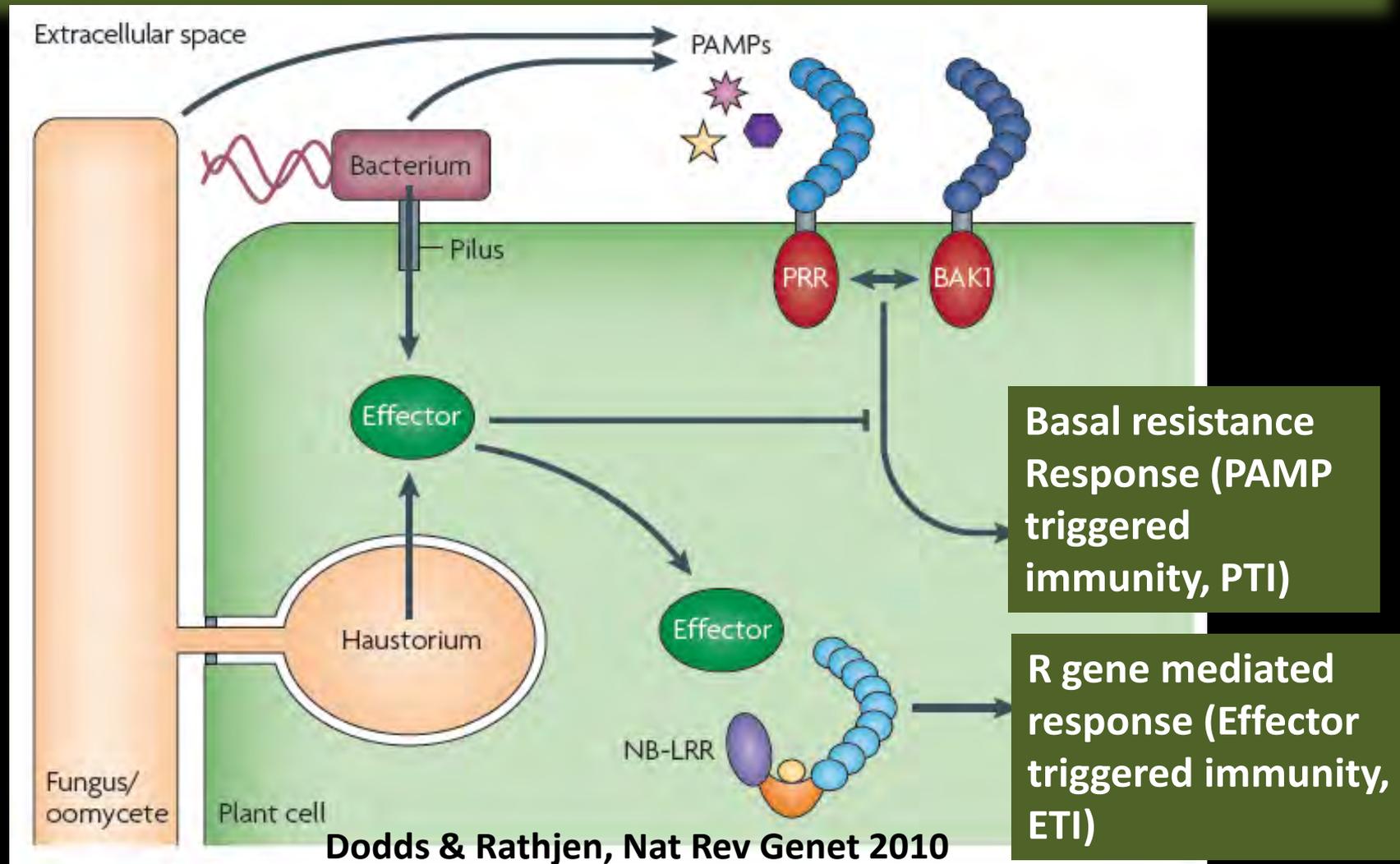
- Identify resistance governed by multiple genes (quantitative resistance)
  - Do defense response (DR) genes contribute to resistance QTL?
  - Does accumulation of those genes result in durable resistance?
  - Can accumulation of QTL be facilitated?



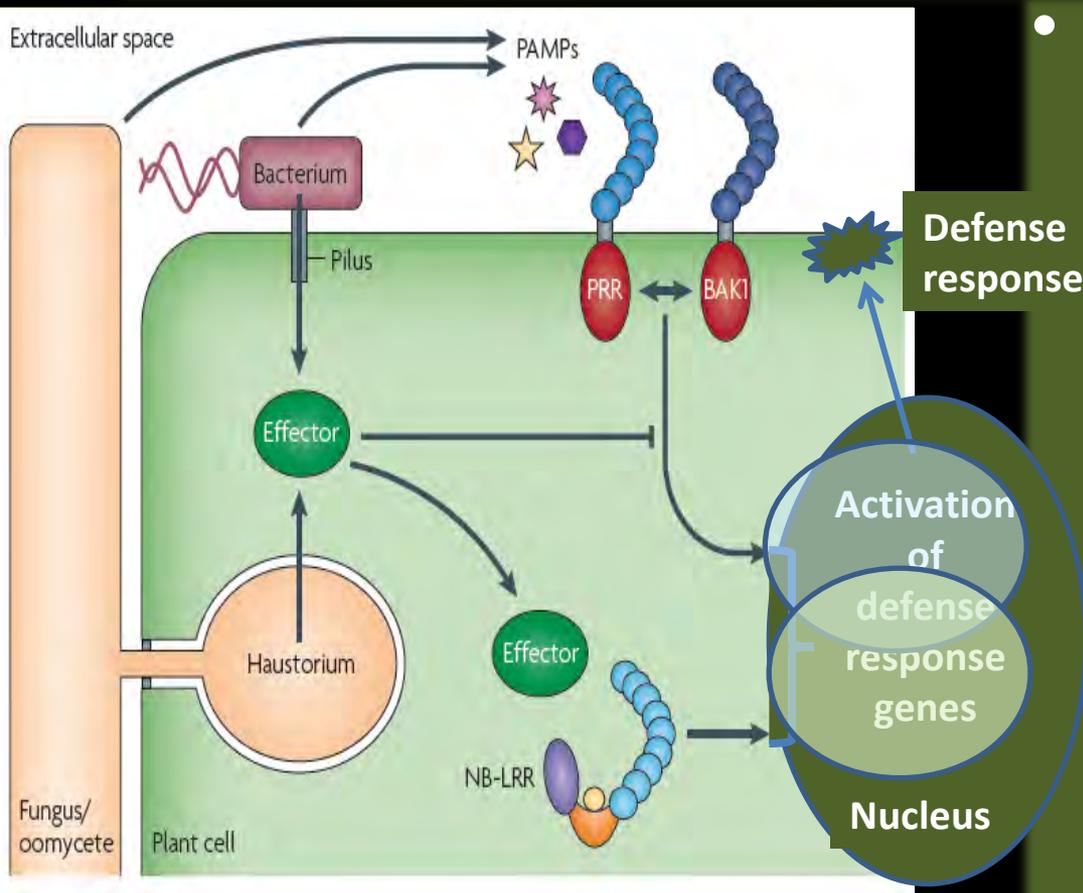
USAID-IRRI  
Linkage  
Program



# *Basal resistance and R gene mediated resistance in plants*



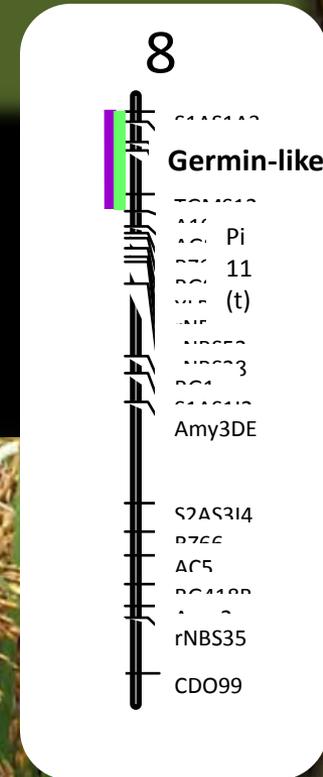
# Transcript profiles of PTI and ETI pathways overlap



- Induced Defense Response (DR) genes encode:
  - enzymes or proteins that collectively inhibit pathogen multiplication and/or spread, and are broadly functional in combating diverse pathogens
  - e.g.
    - Structural proteins
    - Enzymes of secondary metabolism
    - Enzymes directly involved in defense
    - Regulatory functions

# Basal responses governed by QTL: predicted to be long-lasting and broad spectrum

- *QTL are controlled by many genes (multigenic):*
  - Broad spectrum = effective against diverse types of pathogens
  - Durable = long lasting
- *Difficulty:*
  - Because the genes responsible are not known, difficult to target for breeding
  - Because multiple genes with partial effects, difficult to score phenotypes



***Rationale: Exploit natural diversity to identify genetic variation in defense response genes activated in basal resistance***

- Plants exhibit diversity in the levels of basal resistance
- Predict diversity for resistance is associated with variation in DR gene content and/or their expression
- If true, then identifying genetic variation associated with phenotypic diversity could:
  - Reveal genetic mechanisms of basal resistance
  - Provide molecular markers to build more durable, broad spectrum resistance
  - Expose novel strategies to engineer resistance

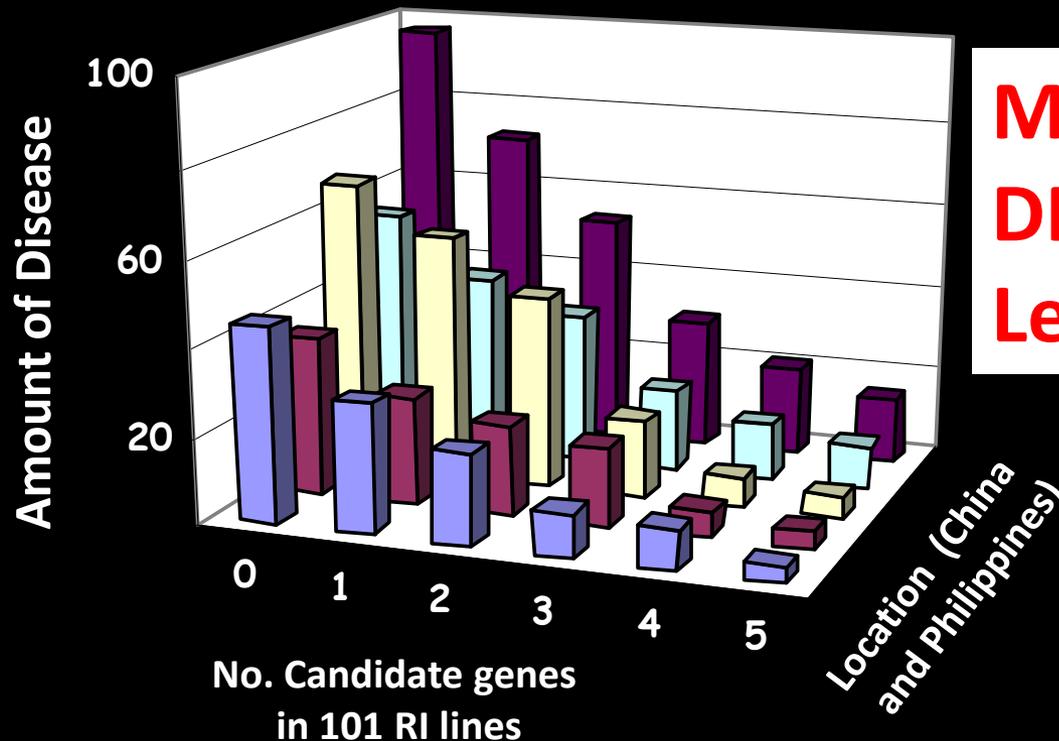


# Do DR genes contribute to disease resistance QTL function? YES!

DR Gene(s) (silenced/mutated)	Location	Effect of Silencing/Mutation
Germin-like Proteins ( <i>OsGLP8-1</i> through <i>OsGLP8-12</i> )	Chr 8	Enhanced susceptibility to blast and sheath blight diseases (Manosalva et al., 2009; Davidson et al., 2010)
Phenylalanine ammonia lyase ( <i>OsPAL4-1</i> through <i>OsPAL4-4</i> )	Chr 2	Enhanced susceptibility to bacterial blight and sheath blight disease (Manosalva, Tonnesen, Lang <i>et al.</i> , in prep)
Oxalate Oxidase ( <i>OsOXO3-1</i> through <i>OsOXO4</i> )	Chr 3	Enhanced susceptibility to blast and <i>Sclerotinia</i> diseases (Carillo <i>et al.</i> , 2009; Davidson <i>et al.</i> , in prep)
Chitinase ( <i>OsCHI2-1</i> )	Chr 2 Minor effect QTL	Enhanced susceptibility to sheath blight and bacterial blight (Snelling, Lee <i>et al.</i> , in prep)
**14-3-3 protein ( <i>OsGF14e</i> )	Chr 2	**Enhanced resistance (GF14e is a negative regulator of resistance) (Manosalva <i>et al.</i> 2011)

# Can accumulation of QTL enhance disease resistance?

(accumulate candidate defense response genes =  
markers for QTL)



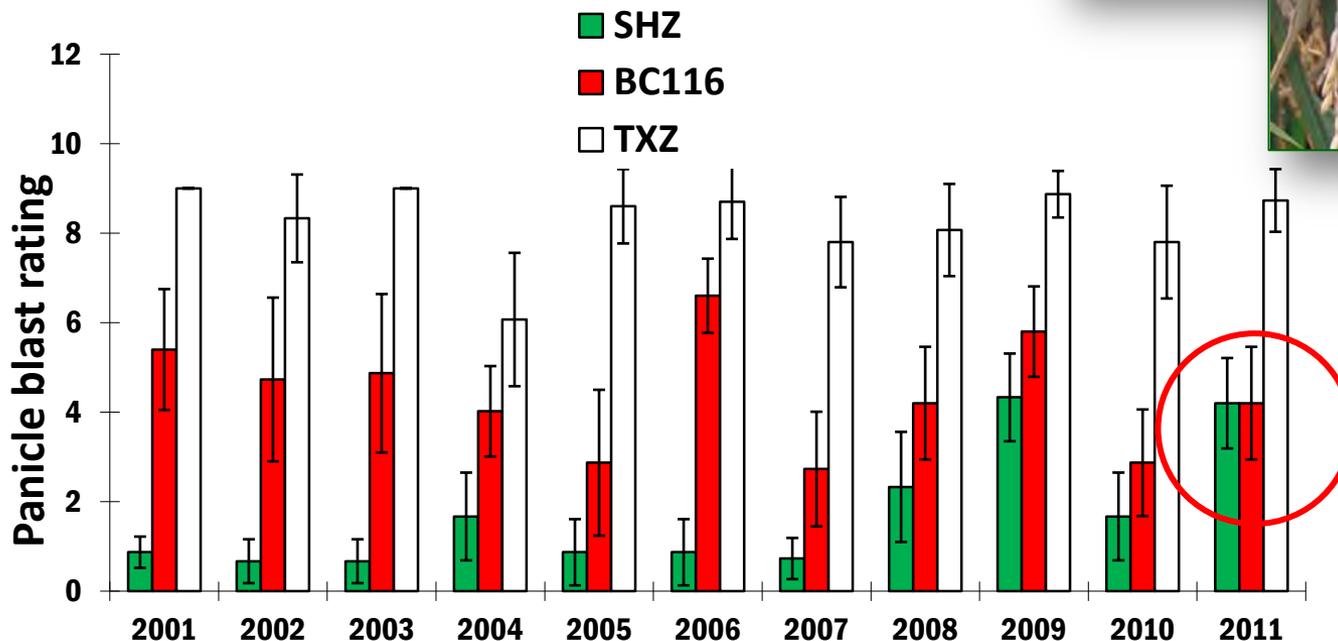
**More regions with  
DR genes (QTL) →  
Less blast disease**

Liu, Leung et al., 2004, MPMI

Rice Research Institute, Guangdong, China

# Is the resistance durable (long-lasting)?

11 years (22 seasons)  
consecutive tests on leaf and  
panicle blast resistance in two  
locations.....so, looking good!



Bin Liu and Xiaoyuan Zhu  
Rice Research Institute,  
Guangdong Academy of  
Sciences

Manosalva et al., 2009.  
Plant Physiol.



Food  
Safe



Plant



# Human Health

- Nutrition
- Health benefits



Energy



an  
alth



Agrochimic  
Traits

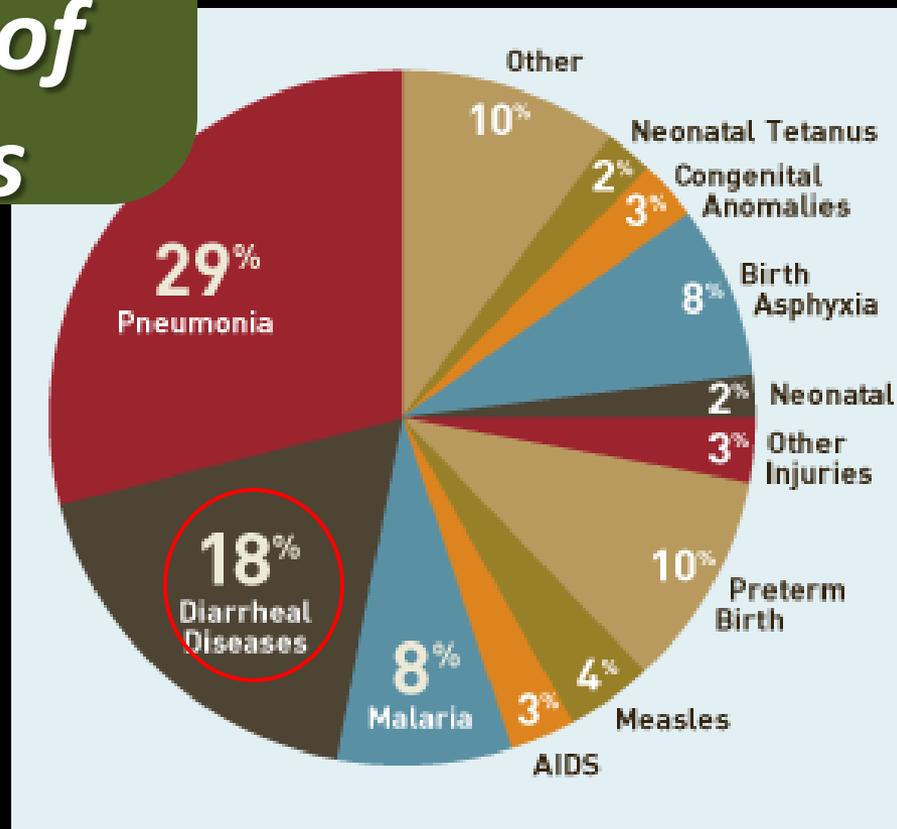
# *A Healthy Bowl of Rice: Improving Rice Bran to Reduce the Impact of Diarrheal Diseases*



*“Dietary rice bran supplementation for gut mucosal immunity & healthy rice crop improvement”*

- E. Ryan, S. Dow, J. Leach

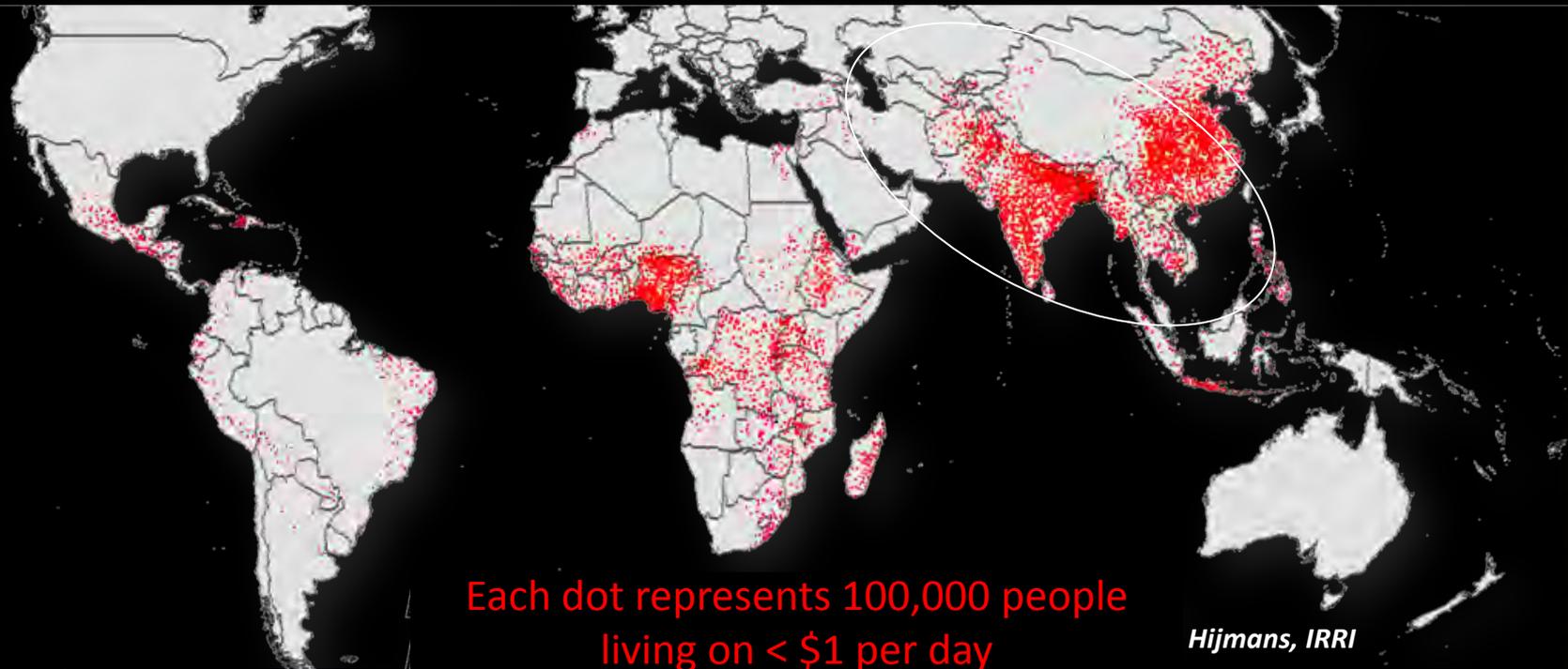
*Funding: Bill & Melinda Gates Foundation*



*Diarrhea kills ~1.5 million children annually.*

# Why rice?

*Rice supplies more than half the calories for the world's population, and is the staple food for most of the world's poor*



- ~ 560 million people in extreme poverty live in rice-producing areas, including areas where diarrheal diseases are prevalent

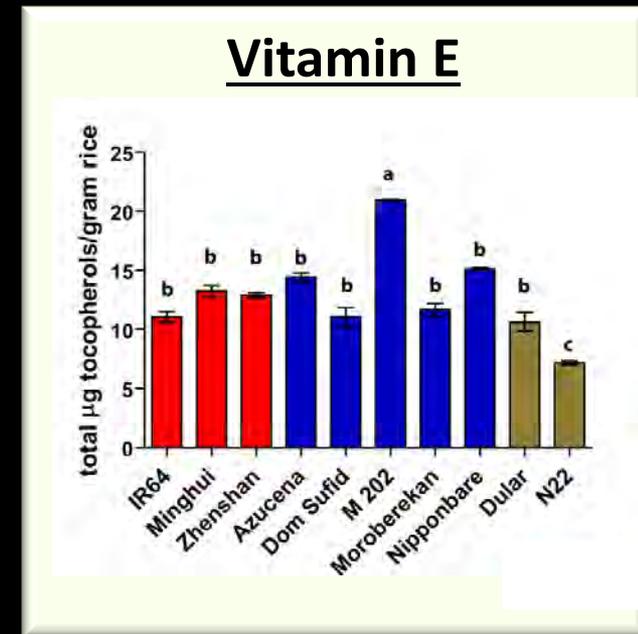
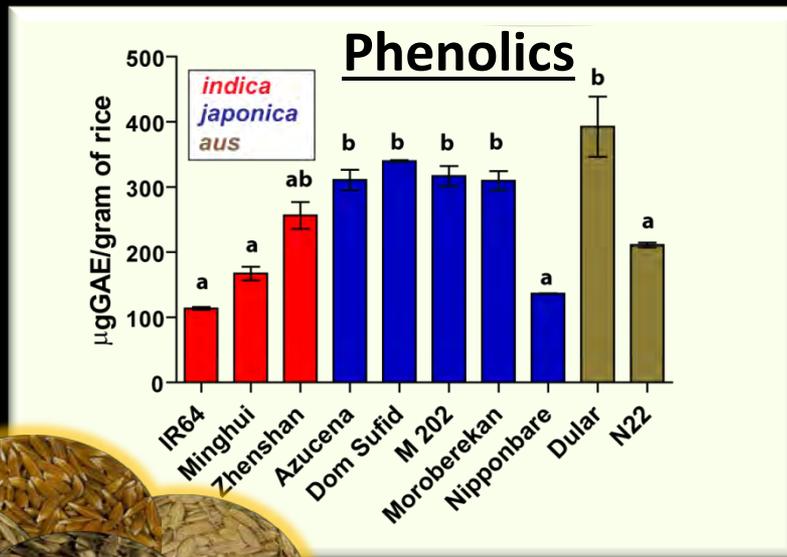
# What rice components to use?

## Rice Processing



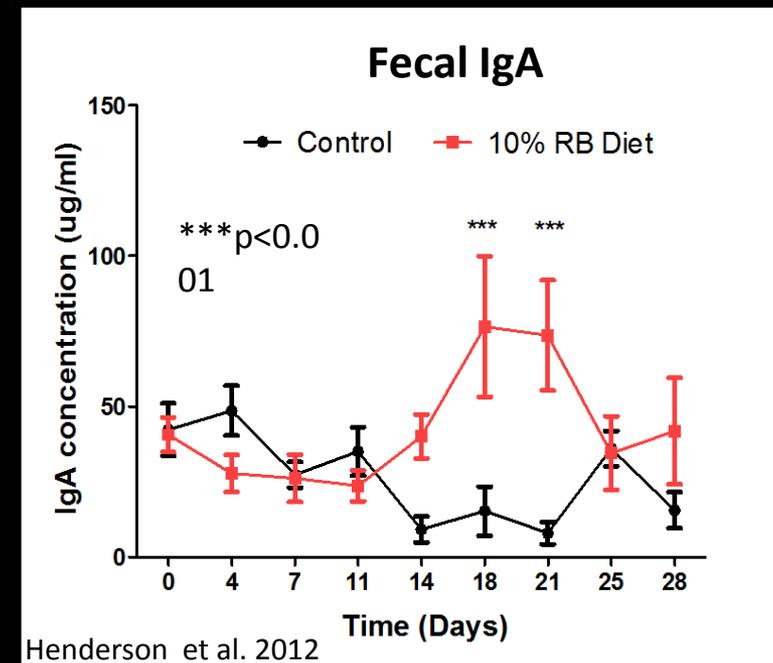
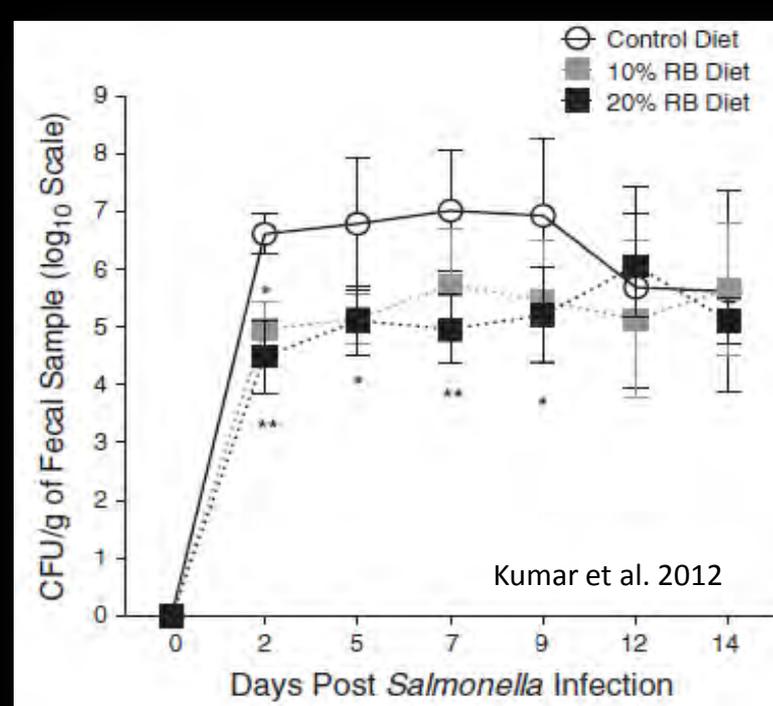
# Why rice? Rice contains bioactive compounds

- Cooked brown rice contains bioactive compounds
- Content differs among varieties



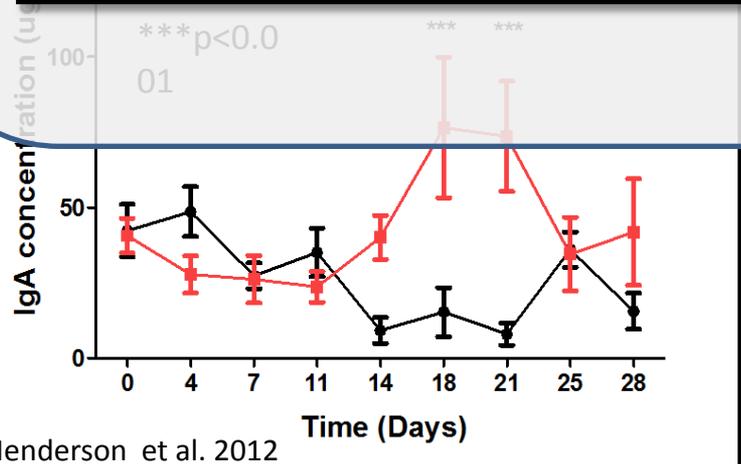
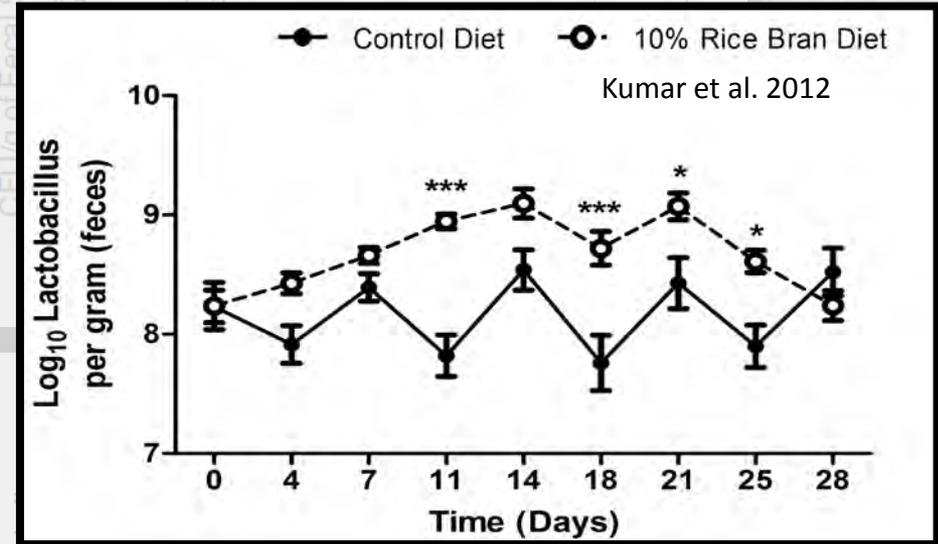
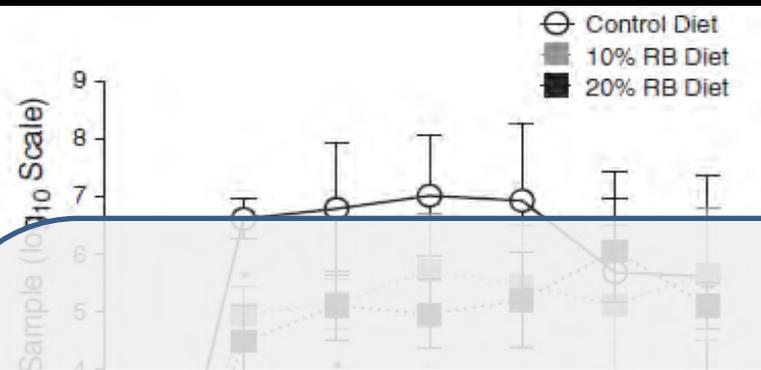
# Why Rice Bran?

- In mice, dietary rice bran:
  - Reduces susceptibility to *Salmonella*
  - Induces fecal and serum non-specific IgA content, etc.
  - Increases fecal *Lactobacillus* spp.



# Why Rice Bran?

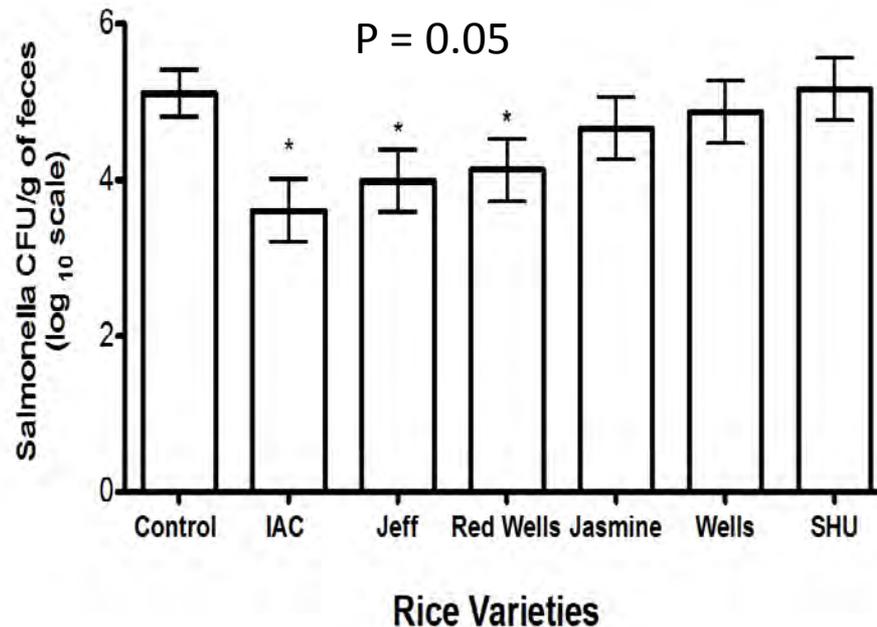
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Henderson et al. 2012

# Why Rice Bran?

- Variation in rice germplasm for rice bran bioactivity



## *Improvement of rice bran for health traits:*

- *Identify genetic basis for variation in loci for synthesis of metabolites important for nutrition/health*





# Energy

- Value added trait
- Optimize wall composition for energy process

Plant  
Health

Human  
Health

Agronomic  
Traits

# Rice residue (straw and hulls), now burned as waste, are a valuable resource



Punia et al., *CURRENT SCIENCE*, 94: 1185

<http://news.mongabay.com/bioenergy/2008/07/burning-issue-satellite-data-show-very.html>

# Rice residue (straw and hulls), now burned as waste, are a valuable resource

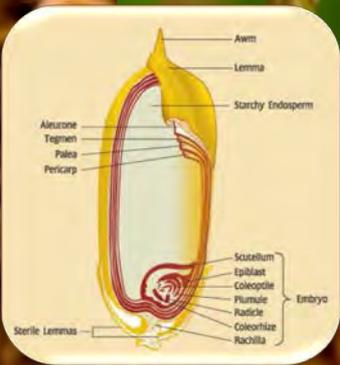
- ❑ In Asia, >700 million tons of rice straw & husks are produced annually
- ❑ Punjab yield roughly 100 million tons of rice straw per year, of which  $\frac{3}{4}$  is burned
- ❑ Equivalent of 35 to 40 million tons of coal. (US consumes 2.74 million tons coal/day)
- ❑ This is only one state in one rice producing country....



Rice burning in Punjab  
May 15, 2005

# Rice husk (hull) is the most prolific agricultural residue in rice producing countries

- 1 ton of rice paddy produces 220 kg rice husk
- 1 ton rice husk is equivalent to 410- 570 kWh electricity



# Cambodia has over 30 syngas plants to convert rice hulls



Stephen Haefele et al.  
International Rice Research Inst

## ■ SME RENEWABLE ENERGY

### Rice husks converted to cash make rice sector more competitive

**B**y operating a biomass gasifier with only 120 kg of rice husk, or 96 kg of wood, you can save up to 24 liters of diesel fuel per hour! Biomass gasification is a

Using a gasifier, biomass can be converted to a gaseous fuel with high efficiency. This gas can drive a modified engine to run on 100% producer gas, or can replace up to 75% of fuel

husk is equal to \$0.119 and 1 kg of wood to \$0.148, when compared with the current price of diesel (October 2005).

A biomass gasifier system generally consists of a:

and water cooling system. The ANKUR gasifiers we use and supply are simple to operate, have excellent variable load capacity (with 3.1 turndown), have a short start-up time.

# Tapping Genetic Variation to Improve Biomass Traits

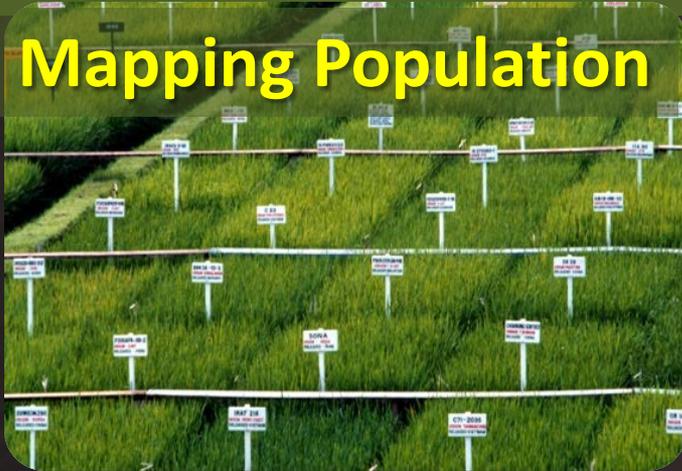
Jan E. Leach  
Colorado State University

CoPIs: H Leung, D Bush,  
A Kern, J McKay, BY Zhao

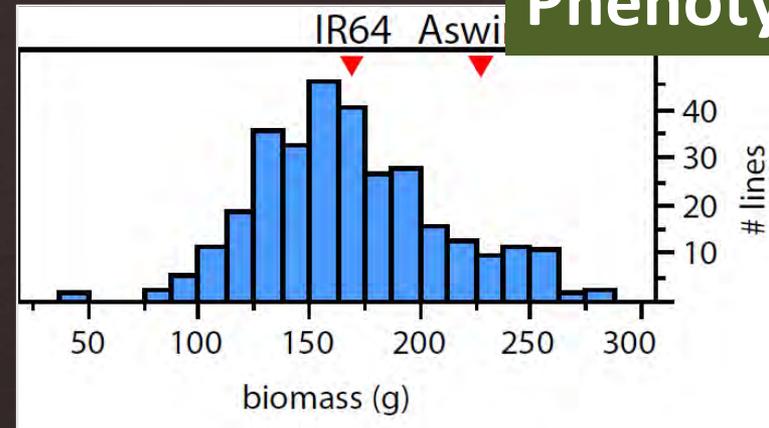


# Discovery of genes controlling biomass

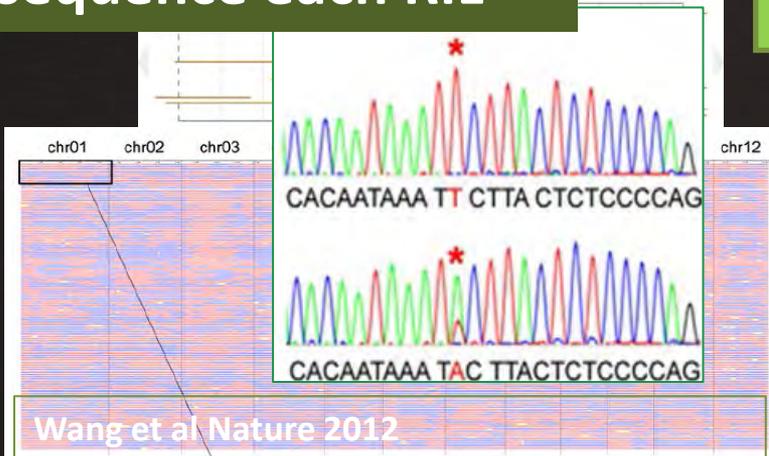
## Mapping Population



## Phenotype

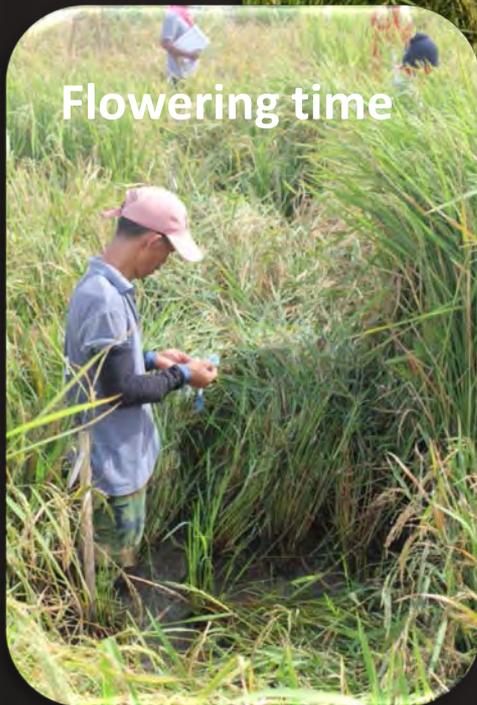


Genotype:  
sequence each RIL



QTL/genes  
controlling  
optimal biomass

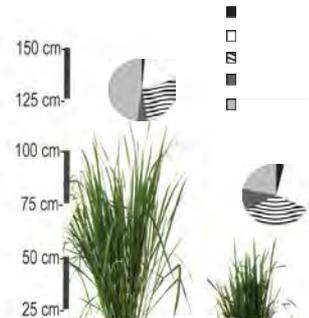
# Field level phenotyping to identify biomass QTL



Flowering time

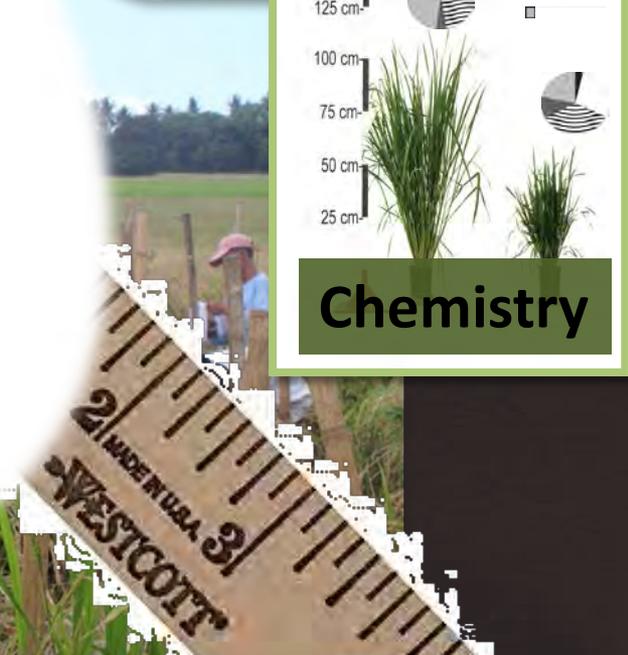
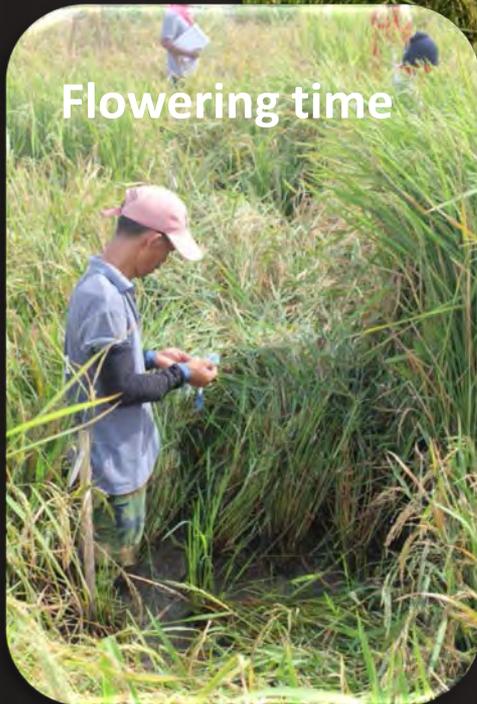


Plant Height



Chemistry

# Field level phenotyping to identify biomass QTL

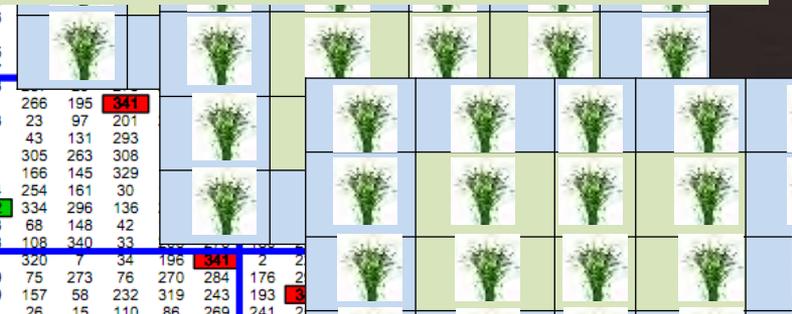


# OryzaPhenome1: 2013

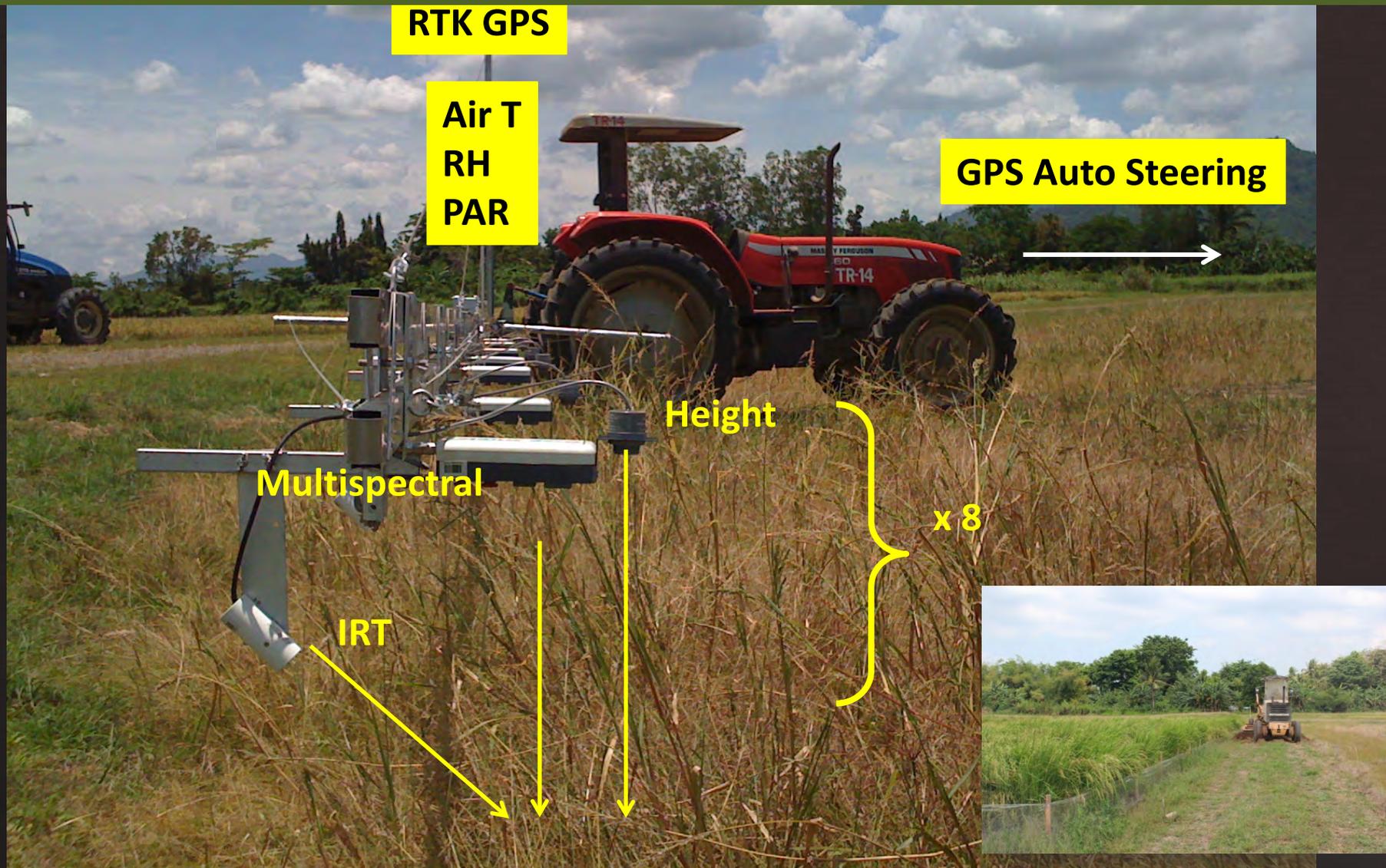
- ~1,500 Rice recombinant inbred lines
- 3 replications
- Semi-automated phenotyping + traditional
- Genotype all 1,500 RIL by sequencing

Row	1	2	3	4	5	6	7	8	9	10	11	12	13
1	113	126	96	112	167	322	199	282	38	21	234	277	227
2	28	105	444	473	446	380	310	44	443	430	310	330	64
3	39												
4	249			1	2	3	4	5	6	7	8	9	
5	133												
6	59												
7	259												
8	97	1		113	126	96	112	167	322	199	282	38	
9	207	2		28	195	141	173	146	289	312	11	143	
10	159	3		39	206	315	137	11	111	272	325	290	
11	58	4		249	200	110	241	80	12	278	220	170	
12	41	5		133	4								
13	22	6		59	6								
14	120	7		259	2				1	2	3	4	5
15	17	8		97	3								
16	305	9		207	2								
17	269	10		159	3	1							
18	24	11		58	2	2			113	126	96	112	167
19	224	12		41	2	3			28	195	141	173	146
20	88	13		22	1	4			39	206	315	137	11
21	94	14		120	2	5			249	200	110	241	80
22	254	15		17	2	6			133	4			
23	16	16		305	1	7			59	6			
24	95	17		269	1	8			259	2			
25	177	18		24	1	9			97	3			
26	168	19		224	3	10			207	2			
27	104	20		88	3	11			159	3			
28	40	21		94	1	12			58	2			
29	235	22		254	2	13			41	2			
30	7	23		16	1	14			22	1			
31	314	24		95	2	15			120	2			
32	198	25		177	2	16			17	2			
33	317	26		342	2	17			305	1			
34	66	27		168	5	18			269	1			
35	61	28		104	2	19			24	1			
36	115	29		40	1	20			224	3			
37	282	30		7	5	21			88	3			
38	180	31		314	1	22			94	1			
39	80	32		168	5	23			254	2			
40	188	33		314	1	24			16	1			
41	93	34		198	8	25			95	2			
42	155	35		317	1	26			177	2			
43	325	36		66	9	27			342	2			
44	281	37		61	1	28			168	5			
45	62	38		115	1	29			104	2			
46	277	39		282	5	30			40	1			
47	130	40		180	7	31			235	1			
48	31	41		80	1	32			7	5			
49	165	42		188	2	33			314	1			
50		43		93	2	34			198	8			
		44		155	2	35			317	1			
		45		325	4	36			66	9			
		46		281	3	37			61	1			
		47		62	2	38			115	1			
		48		277	2	39			282	5			
		49		130	1	40			180	7			
		50		31	2	41			80	1			
				165	1	42			188	2			
						43			93	2			
						44			155	2			
						45			325	4			
						46			281	3			
						47			62	2			
						48			277	2			
						49			130	1			
						50			31	2			
									165	1			

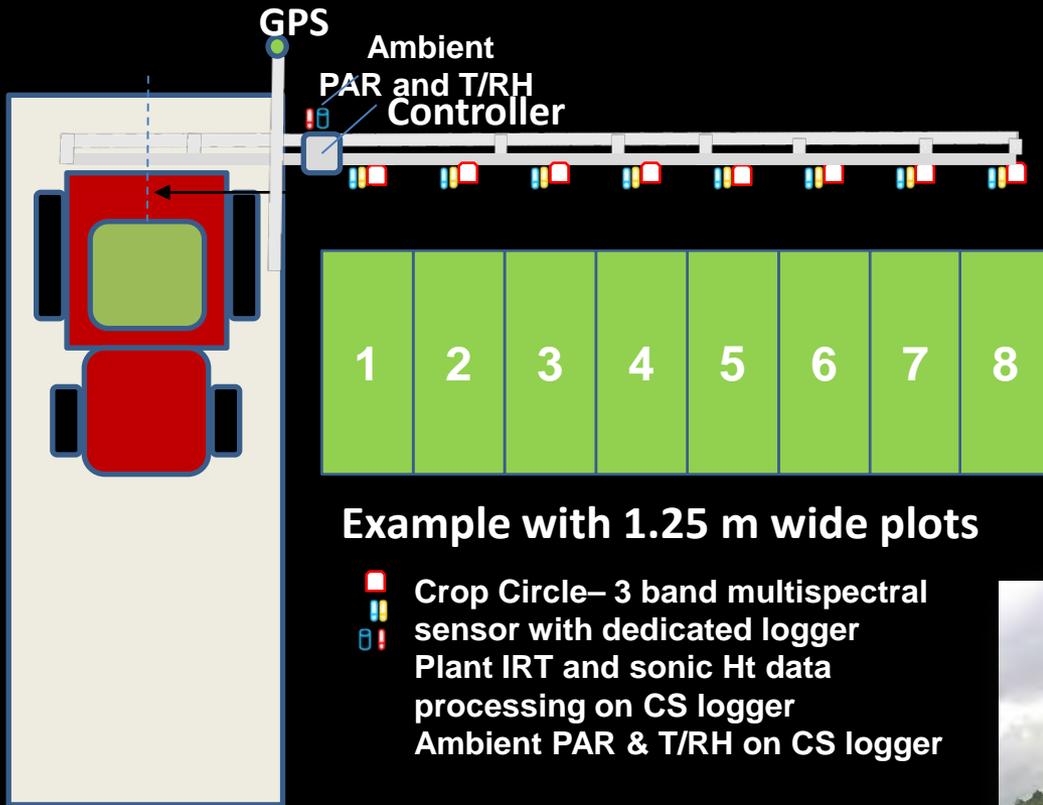
168	57	328	150	114	318	290	142	146											
104	227	283	274	225	205	341	114	74											
40	140	116	100	73	136	5	119	125											
235	163	324	56	196	174	179	167	247											
314	121	101	85	13	23	134	78	22	266	195	341								
198	86	48	21	30	342	102	194	283	23	97	201								
317	123	243	336	49	299	74	224	44	43	131	293								
66	90	329	142	296	258	291	231	14	305	263	308								
61	153	341	67	306	81	191	226	40	166	145	329								
115	106	4	293	197	18	276	12	154	254	161	30								
282	50	122	144	264	246	273	182	342	334	296	136								
180	76	245	132	216	44	99	285	203	68	148	42								
80	157	270	2	311	217	230	54	158	108	340	33								
188	294	337	181	284	51	129	144	9	320	7	34	196	341	2	2				
93	265	105	135	342	38	213	130	179	75	273	76	270	284	176	2				
155	27	326	268	176	204	304	126	309	157	58	232	319	243	193	3				
325	45	118	37	319	83	288	129	65	26	15	110	86	269	241	2				
384	323	170	30	14	245	464	123	100	241	340	10	3	267	208					



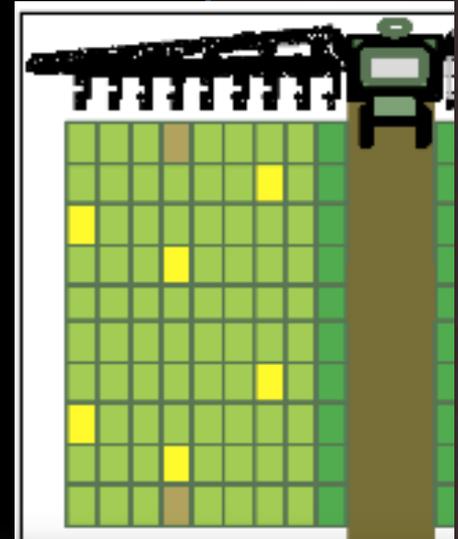
# Semi-Automated Data Capture: (spectral, temp, RH, ...)



# OryzaPhenome1: Automated data collection

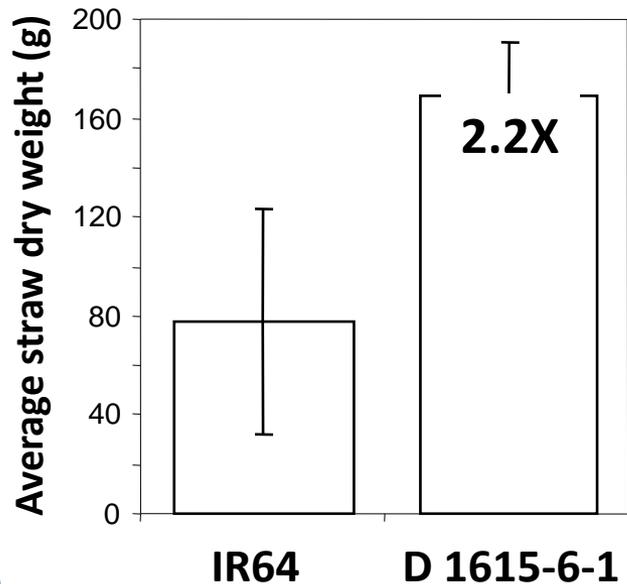


Example with 1.25 m wide plots

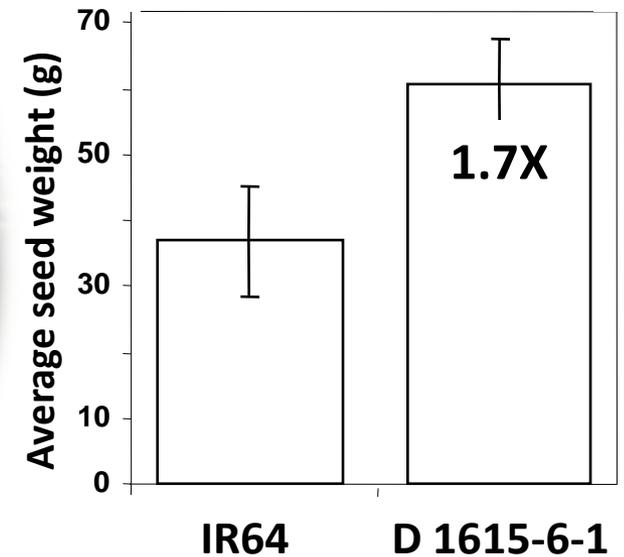


# Mutant shows increased biomass and seed weight

## Dry biomass



## Seed weight



# Tomorrow's Rice will:

- Be durably resistant to a broad spectrum of diseases and pests
- Promote human health
- Provide energy (value added trait)
- And.....

# Be delicious!

*"Without rice, even the  
cleverest housewife  
cannot cook."*

Ancient Proverb

