

# Breeding for durable resistance to important fungal diseases in rice

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## Outline

- ❖ Review on the host *R* genes and pathogen *Avr* genes in rice and rice blast
- ❖ Research update on host plant resistance to blast and false smut
- ❖ Outlook for durable resistance to rice blast and false smut



# Rice blast is economically important to rice production

## Severity of rice blast

### Outbreak in super hybrid rice, China



### Rice blast in Nepal

Rice blast is a devastating disease and estimated to cost an estimated **\$66 billion** in annual losses worldwide Mitchell and Wang

### Rice blast in IRRI-ES



## Worldwide collaborative efforts



### JIRCAS Rice blast network



### BBSRC Africa rice blast network

### Rice blast WG in TRRC



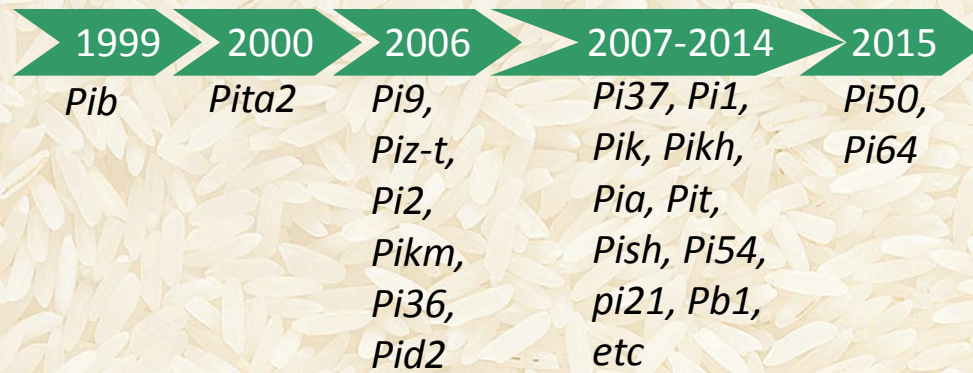
# Current status for breeding resistance to rice blast

- ❖ Utilization of host *R* genes is the most effective and environment-friendly strategy to control rice blast disease
- ❖ Rice and rice blast observe a typical gene-for-gene theory and resistance is not a single-player game
- ❖ Both *R* genes in host and *Avr* genes in pathogen have been extensively characterized, revealing a complicated scenario of interactions

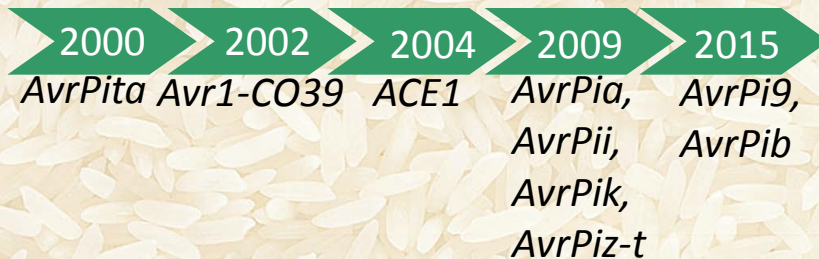


# Molecular characterization of *R* and *Avr* genes

## Host *R* genes



## Pathogen *Avr* genes

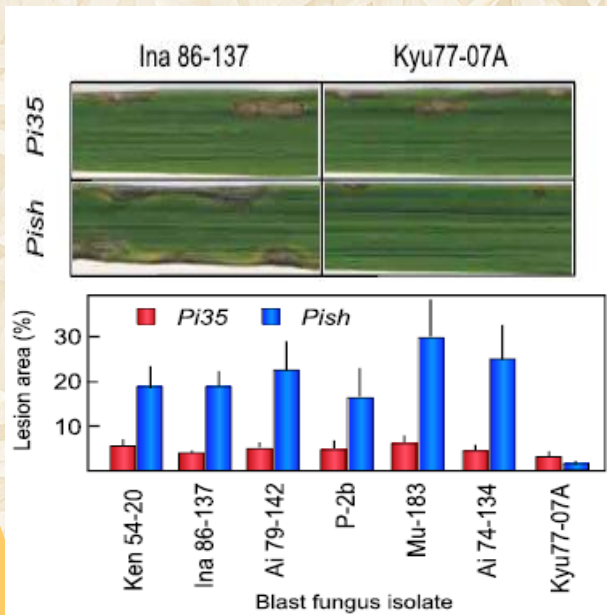


# Race dependent *R* genes to blast in rice genome

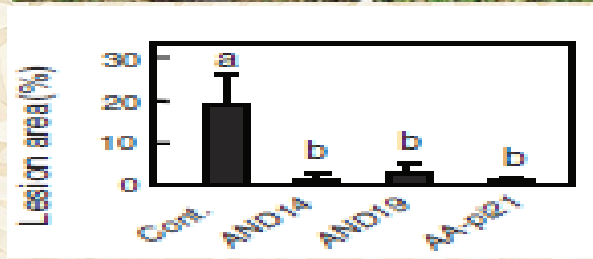
Locus	<i>R</i> genes	Chromosome	Functional mode	RGA type
<i>Pit</i>	<i>Pit</i>	1	S	NBS-LRR
<i>Pi37</i>	<i>Pi37, Pish, Pi64</i>	1	S	NBS-LRR
<i>Pib</i>	<i>Pib</i>	2	S	NBS-LRR
<i>Pi63</i>	<i>Pi63</i>	4	S	NBS-LRR
<i>Pi2/9</i>	<i>Pi2, Pi9, Piz-t, Pi50 [Piz,, Pi40, Pigm]</i>	6	S	NBS-LRR
<i>Pid3</i>	<i>Pid3, Pi25</i>	6	S	NBS-LRR
<i>Pid2</i>	<i>Pid2</i>	6	S	B-lectin
<i>Pi36</i>	<i>Pi36</i>	8	S	NBS-LRR
<i>Pi5</i>	<i>Pi5, Pii [Pi3, Pi15]</i>	9	D	NBS-LRR
<i>Pia</i>	<i>Pia/Pi-CO39</i>	11	D	NBS-LRR
AC134922	12 alleles	11	S	NBS-LRR
<i>Pi54</i>	<i>Pi-k<sup>h</sup> (Pi54)</i>	11	S	Atypical NBS-LRR
<i>Pik</i>	<i>Pik, Pikm, Pikp, Pi1, Pik*, [Pi7]</i>	11	D	NBS-LRR
<i>Pita</i>	<i>Pita [Pi12, Pita2, Pi19, Pi6, Pi20...]</i>	12	S	NBS-LRR

# Race independent R genes (QTLs) control partial but durable resistance

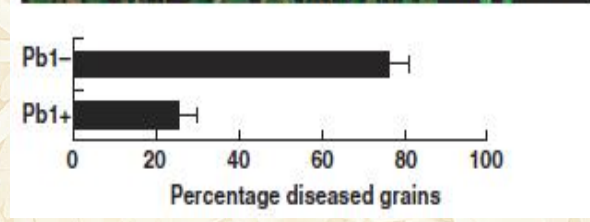
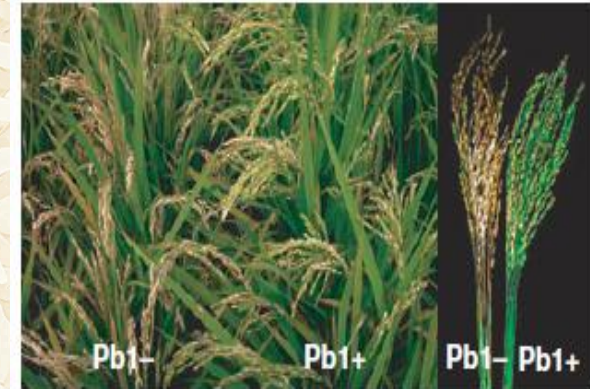
R-QTL	Chromosome	Feature of protein	Notes
<i>Pi35</i>	1	NBS-LRR	Allelic to <i>Pish/Pi37/Pi64</i>
<i>pi21</i>	4	Proline rich	
<i>Pb1</i>	11	Atypical NBS-LRR	Interacting with WRKY45



Fukuoka et al., 2014



Fukuoka et al., 2009



Hayashi et al, 2010

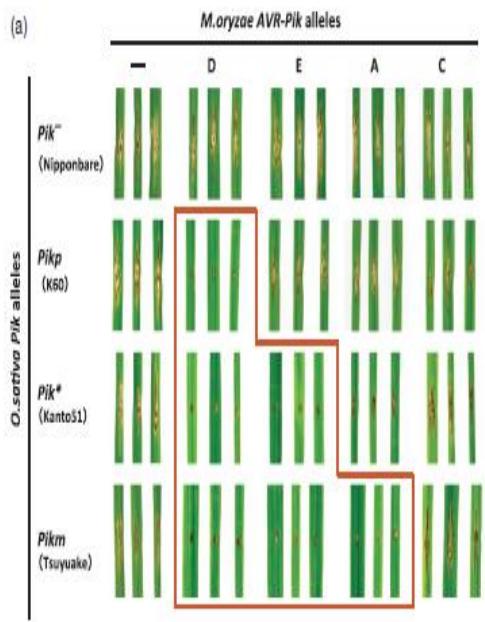
# Avr genes in *M. oryzae*

Avr genes	Avr products	Secreted proteins	Cognate R genes	Interaction mode
<i>AvrPi-ta</i>	Zinc-metalloprotease	Yes	<i>Pita</i>	Direct
<i>AvrPii</i>	Small unknown secreted protein	Yes	<i>Pii</i>	NA
<i>Avr-Pik/km/kp</i>	Small unknown secreted protein	Yes	<i>Pik/km/kp</i>	Direct
<i>AvrPiz-t</i>	Small unknown secreted protein	Yes	<i>Piz-t</i>	ND
<i>AvrPia</i>	Small unknown secreted protein	Yes	<i>Pia</i>	Direct
<i>ACE1</i>	PKS–NRPS	No	<i>Pi33(t)</i>	NA
<i>Avr1-CO39</i>	Small unknown secreted protein	Yes	<i>Pi-CO39</i>	Direct
<i>AvrPi9</i>	Small unknown secreted protein	Yes	<i>Pi9</i>	NA
<i>AvrPib</i>	Small unknown secreted protein	Yes	<i>Pib</i>	NA



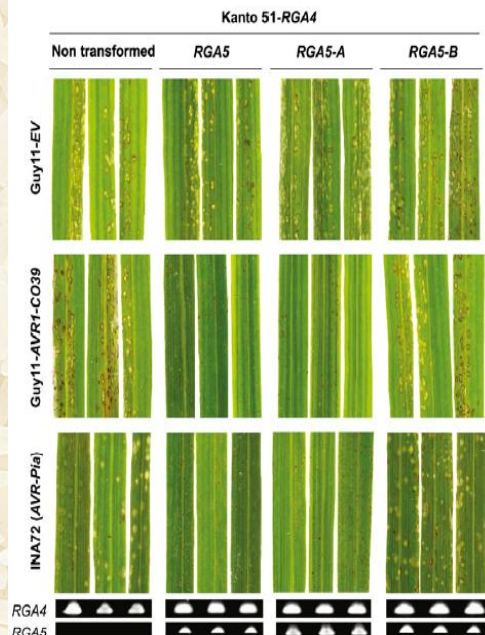
# Complicated recognition models beyond simple gene-for-gene theory

*Pik/Pikp/Pikm & AvrPik/km/kp*



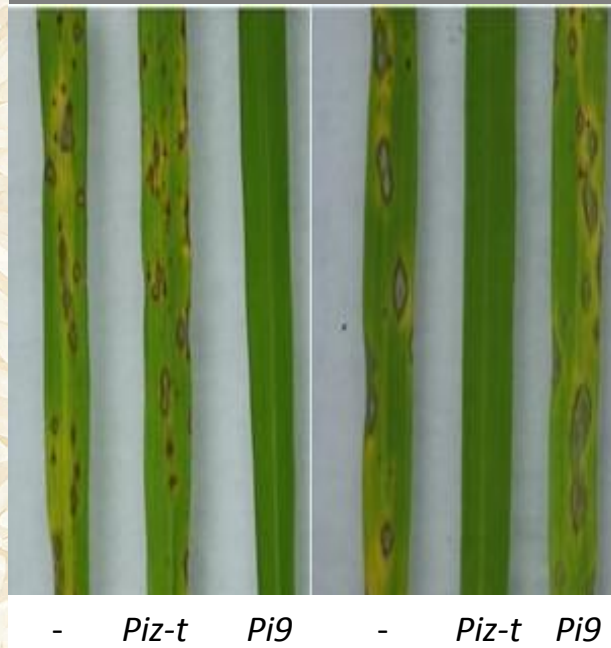
Different *R* alleles to different *Avr* alleles. (Kanzaki et al., 2012)

*Pia/Pi-CO39 & AvrPia/Avr1-CO39*



Same *R* gene to different *Avr* genes. (Cesari et al., 2013)

*Piz-t/Pi-9 & AvrPiz-t/AvrPi9*



Different *R* alleles to different *Avr* genes. (Li et al., 2009; Wu et al., 2015)

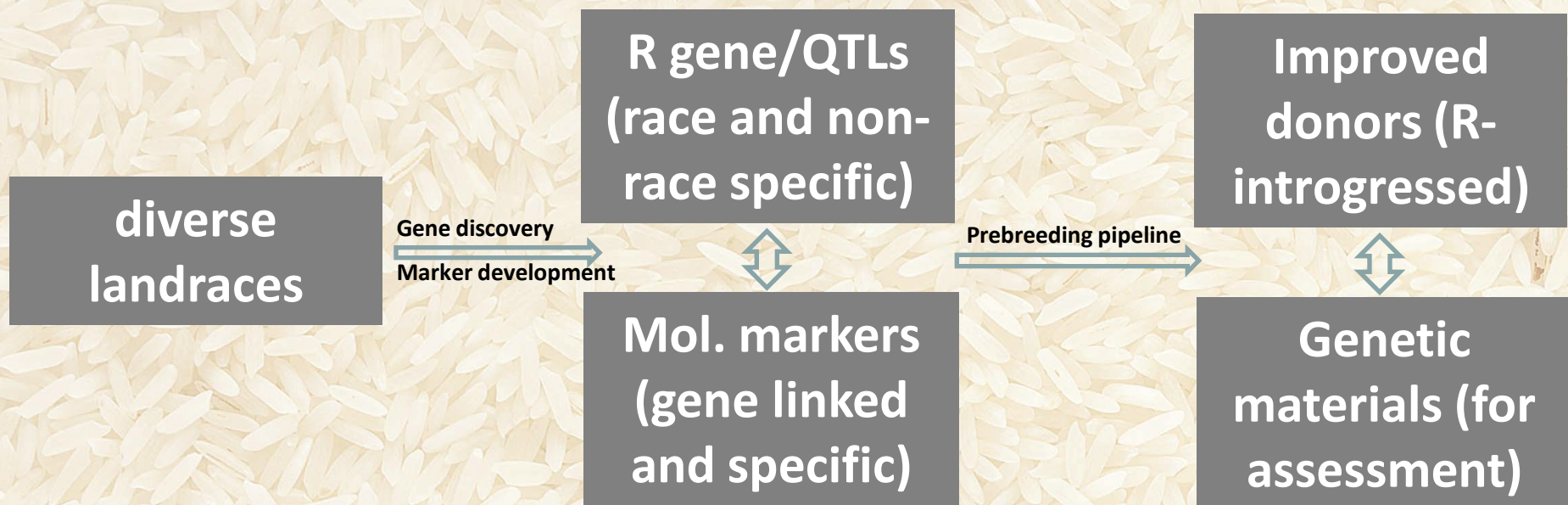
# Summary

- ❖ Blast *R* gene/QTLs predominantly encode NBS-LRR proteins
- ❖ Cluster of *R* gene/QTLs is prevalent in the rice genome
- ❖ The function of race-specific *R* genes is dependant on *Avr* genes in pathogen
- ❖ *Avr* genes in rice blast predominantly encode small secreted proteins and are main targets for mutations to escape the recognition by cognate *R* genes
- ❖ Different *R/Avr* gene pairs have evolved distinct recognition mechanisms

## Research update on host plant resistance to rice blast

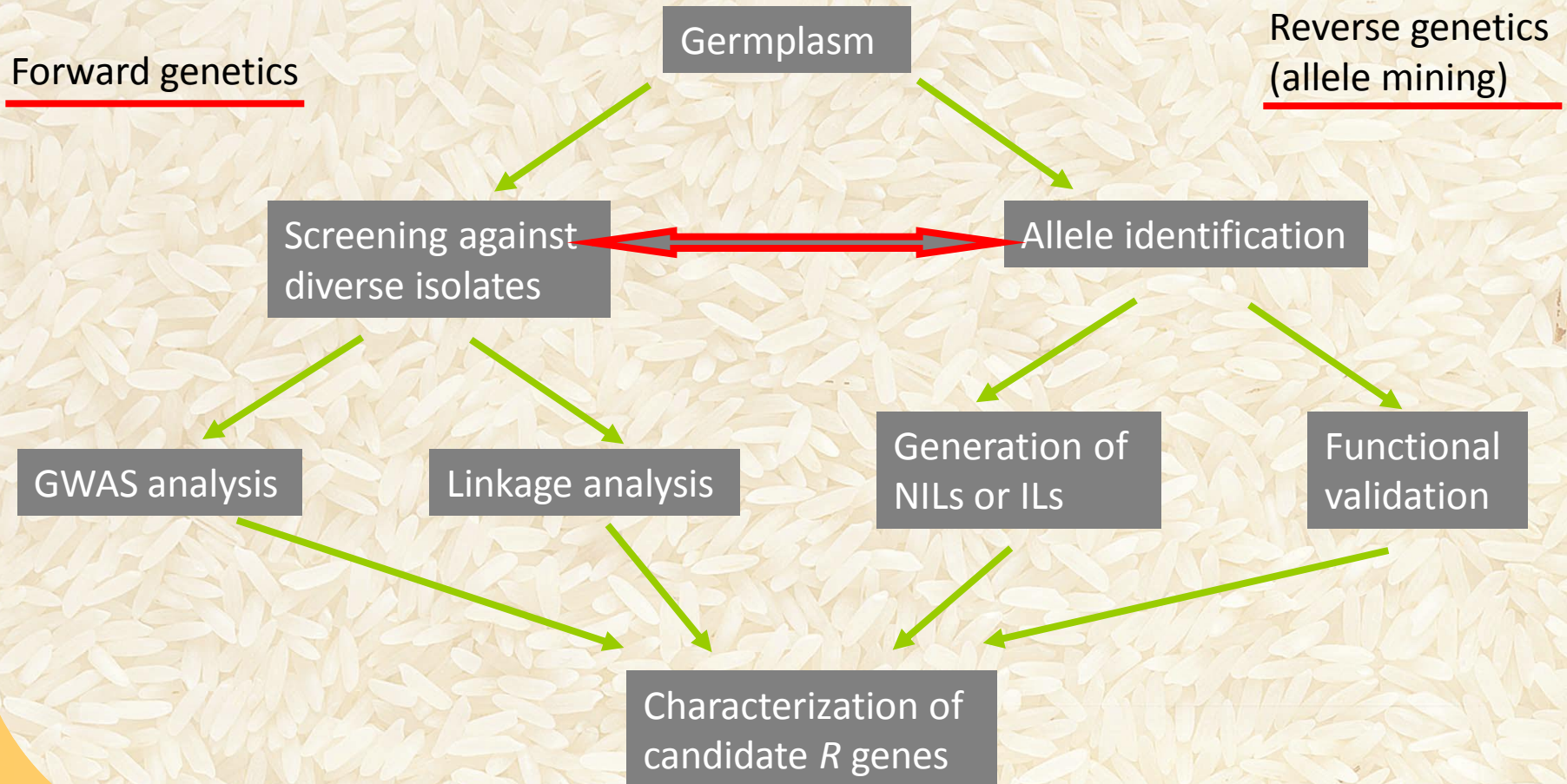
- ❖ Excavation of novel *R* gene/QTLs for increasing gene pool for breeding program
- ❖ Development of rice materials for resistance assessment and improved donors for breeding program
- ❖ Development of diagnostic tools for rice *R* gene/QTLs and race composition of blast population
- ❖ Understanding the stability of resistance under changing environment
- ❖ Development and validation of an integrated strategy for durable resistance to rice blast

# A pipeline for gene discovery and pre-breeding of R gene driven host plant resistance program



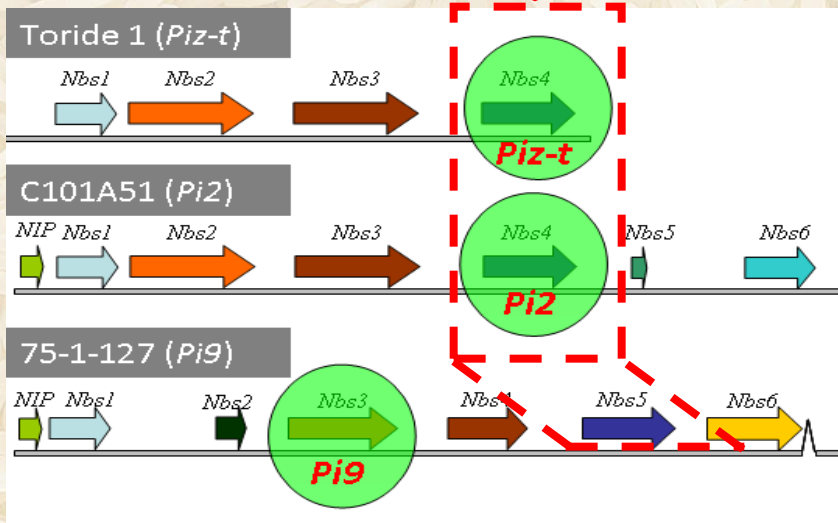
**Germplasm** → **Valuable gene pool** → **Improved pre-breeding and genetic lines**

# Excavation of novel *R* genes in diverse germplasm



# Novel *Pi2/9* alleles in 3K genomes

RGA4-specific  
sequence tag



Differential strains used for phenotyping

Strains	Differential lines			
	<i>Pi2</i>	<i>Piz-t</i>	<i>Pi9</i>	<i>Piz</i>
5167-1	S	R	R	R
6006-1	R	S	R	S
IK81-25	R	S	R	R
M101-1-2-9-1	S	S	R	R

2926 lines (3K panel)

Analysis using a  
bioinformatics tool

580 positive lines

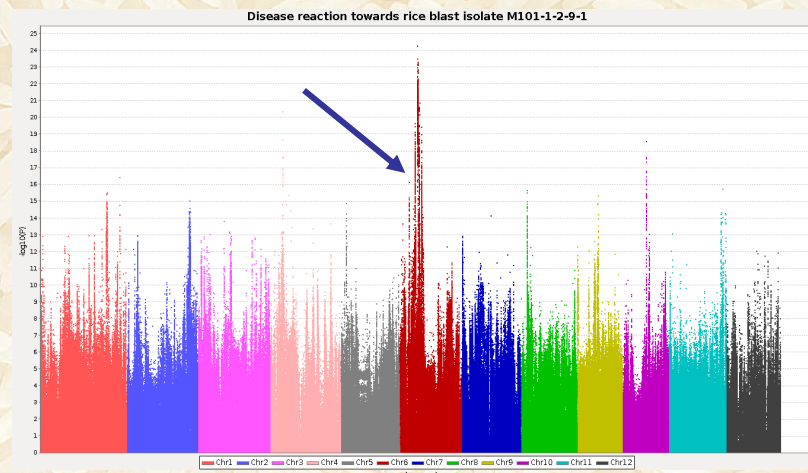
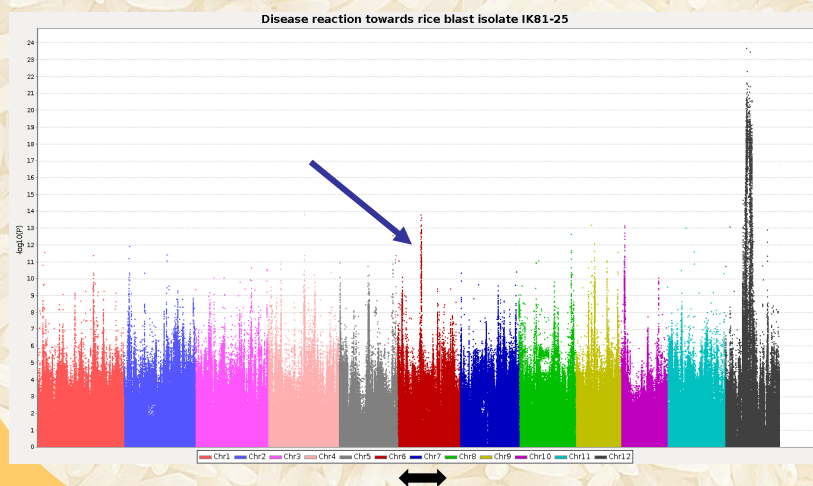
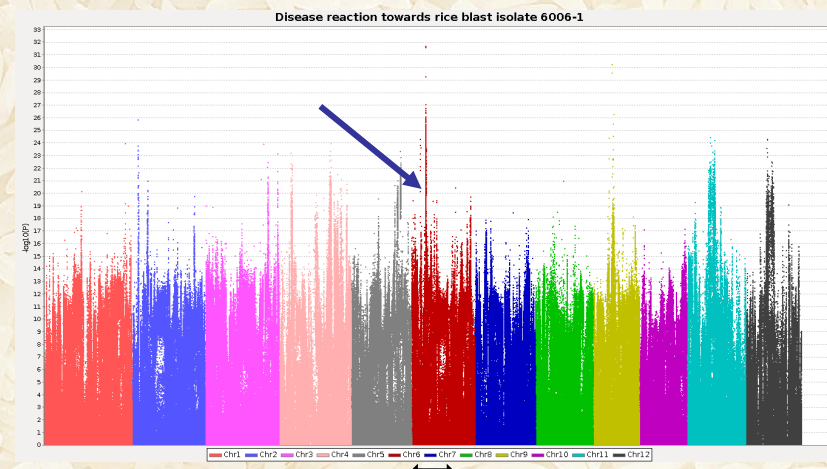
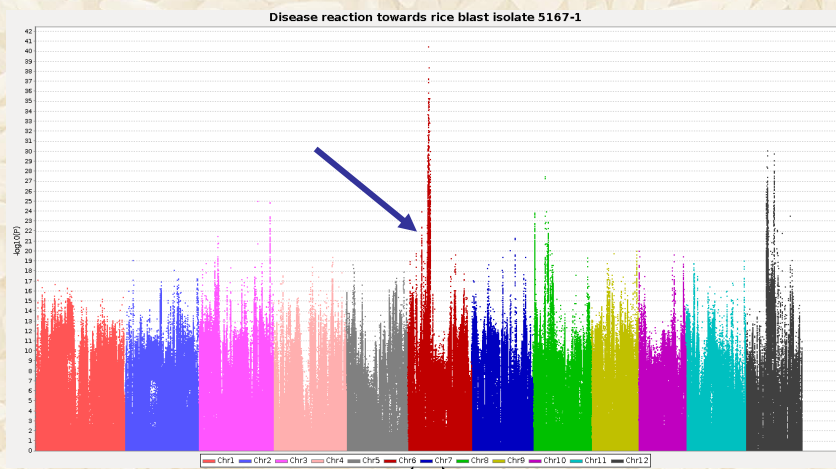
Phenotyping using 4  
differential strains

161 *R* lines to at  
least 1 strain

GWAS

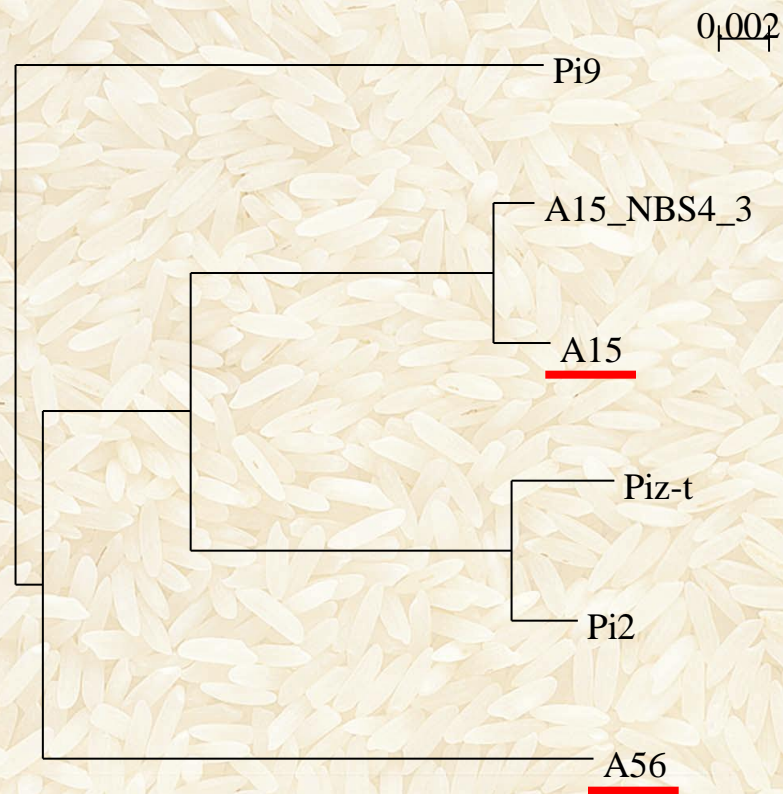
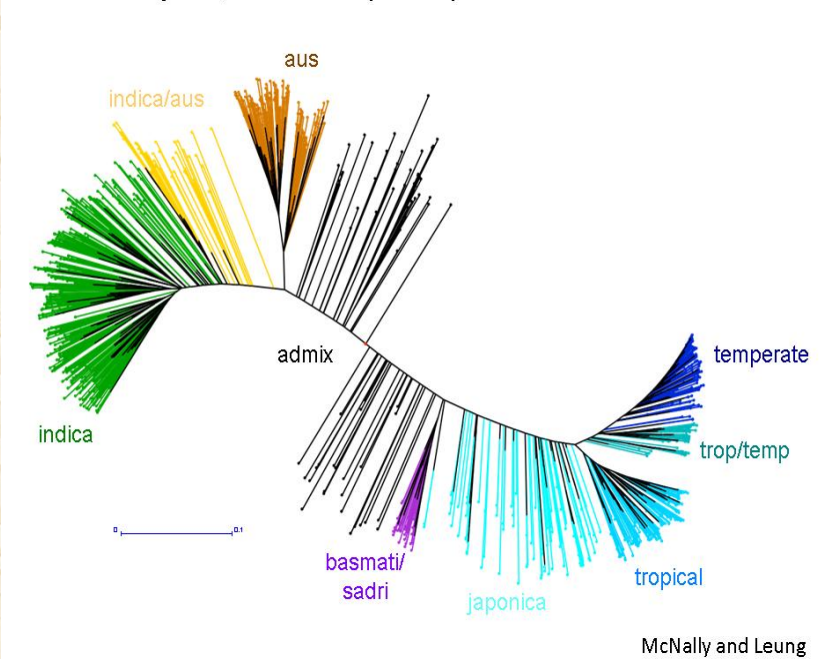
Putative new  
alleles

# Identification of novel *Pi2/9* functional alleles using GWAS



# Novel *Pi2/9* alleles in 2K panel

Subset of 2K panel, 130K SNPs (filtered)





## 4 new introgression lines with different *R* genes



**CO39**

**Pi2-A15**

**Pi2-A35**

**Pi2-A43**

**Pi2-A56**

Table 1. Resistance frequency analysis of *Pi2-A15* and *-A56* to different isolates

Isolates	Donor and derivative introgression lines (BC3F3)				
	CO39	A15	NIL-A15	A56	NIL-A56
JMB8401	S	R	R	R	R
IK81-3	S	R	R	R	R
5008-3	S	R	R	R	R
9497-3	S	R	R	R	R
9475-1-3	S	R	R	R	R
5167-1	S	NA	NA	R	R
9126-1	S	R	R	R	S
6161-1	S	R	R	R	S
6003-3	S	R	R	R	S
5092-3	S	R	R	R	S
9406-3	S	R	R	R	R
Ca89	S	R	R	S	S
BN111	S	R	R	R	R
M64-1-3-9-1	S	R	R	R	R
M101-1-2-9-1	S	R	R	R	R
2769273	S	R	R	R	R
JMB840610	S	R	R	R	R
5167-1	S	R	R	R	R
JMB8401	S	R	R	R	R
<b>RF(%)</b>	<b>0</b>	<b>100</b>	<b>100</b>	<b>95</b>	<b>74</b>

# A promising *R* QTL (*Pi-A35*) identified by allele mining



PCR positive

PCR negative

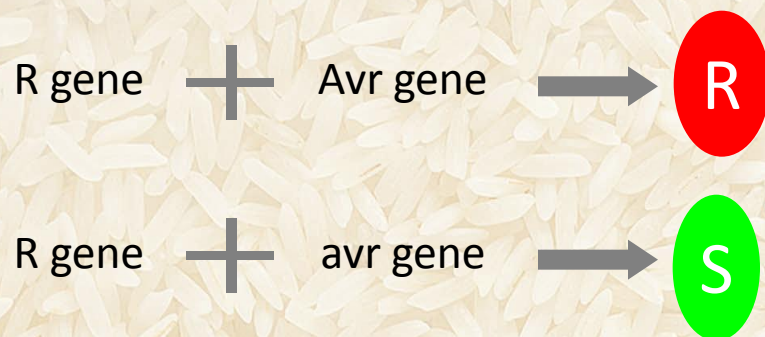
# Is it sufficient to have only *R* genes for resistance breeding?



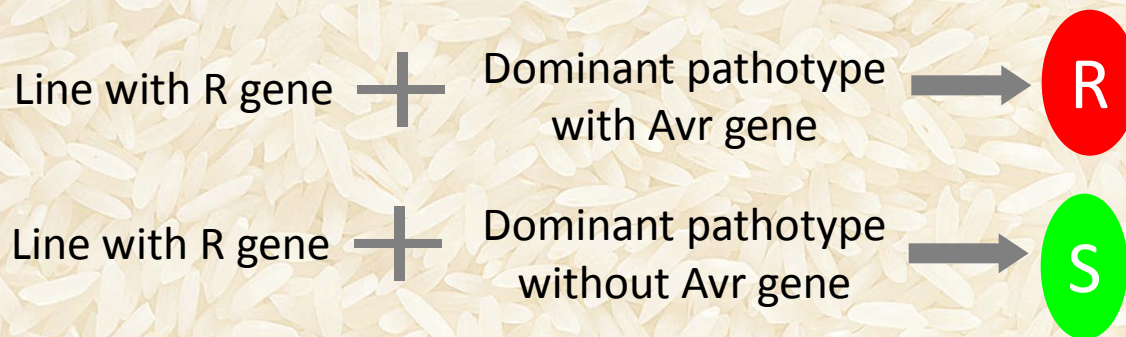
Observation: *Pik* is effective in ES-IRRI but not in Bohol

Reason: the frequency of *AvrPik* in ES-IRRI and Bohol is 88% and 32%, respectively

## Individual plant to individual isolate



## Plants to pathogen population



*Avr* genes are main targets under strong selection for quick mutations rendering pathogen virulence to host with cognate *R* genes

# Reshuffling of *Avr* genes in pathogen population



Reshuffling of *Avr* genes among isolates could be random. However, the frequency of avirulent isolates in population is largely selected by deployed *R* genes, which resulted in the quick dominance of virulent isolates and resistance erosion in 2-3 years in general.

## Genetic events in *Avr* gene mutations

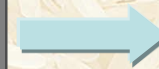
<b>Avr genes</b>	<b>Cognate <i>R</i> genes</b>	<b>Variation patterns</b>
<i>AvrPi-ta</i>	<i>Pita</i>	InDels, SNPs, TE insertion, Presence/absence
<i>AvrPii</i>	<i>Pii</i>	Presence/absence
<i>Avr-Pik/km/kp</i>	<i>Pik/km/kp</i>	SNPs, Presence/absence
<i>AvrPiz-t</i>	<i>Piz-t</i>	TE insertion, SNPs
<i>AvrPia</i>	<i>Pia</i>	Presence/absence
<i>ACE1</i>	<i>Pi33(t)</i>	Presence/absence, TE insertion
<i>Avr1-CO39</i>	<i>Pi-CO39</i>	Presence/absence, InDels
<i>AvrPi9</i>	<i>Pi9</i>	Presence/absence, TE insertion
<i>AvrPib</i>	<i>Pib</i>	Presence/absence, TE insertion

## Tools for pathogen surveillance

**Field tests**  
(NILs or breeding materials in hotspot, MET etc)



**Greenhouse tests**  
(Pathotype and virulence)



**Laboratory tests**  
(*Avr* genes/genome)

Surveillance tool	Needs for trial	Advantages	Disadvantages
Field tests	Differential lines, and hotspots	Visualized, fast	Sketchy, relatively lagged, and IRBL dependent
Greenhouse tests	Differential lines and single spores	Visualized, quantitative, predictive	Time consuming, less scalable, and IRBL dependent, biosafety concern
Lab tests	DNAs of single spores or lesion samples	Specific, scalable, quantitative, predictive, real-time, movable	Small no. of known <i>Avrs</i> , multiple haplotypes

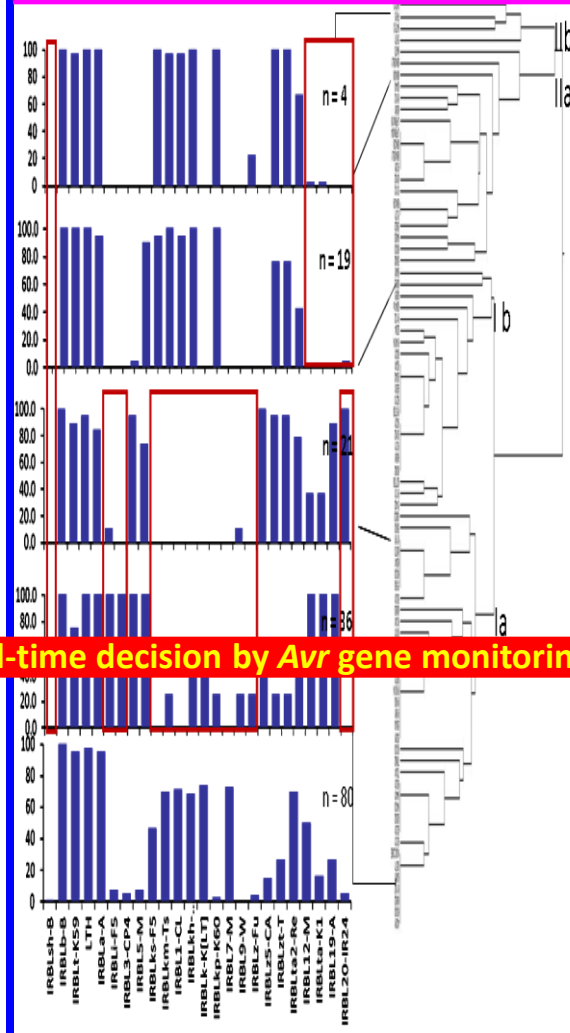


# A trial for pathogen surveillance in Bohol

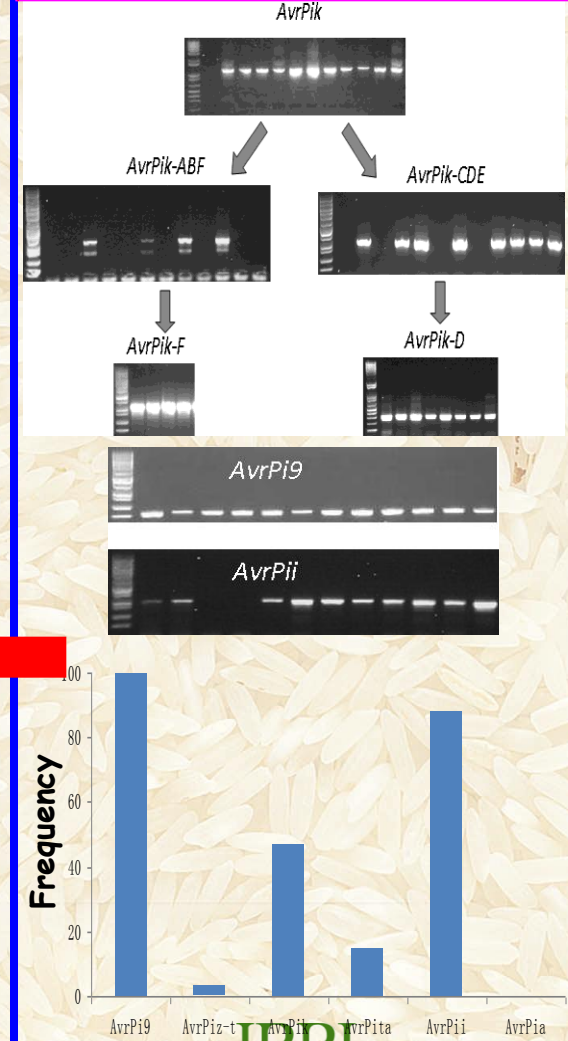
## Field assessment



## Pathotype test



## Avr gene monitoring



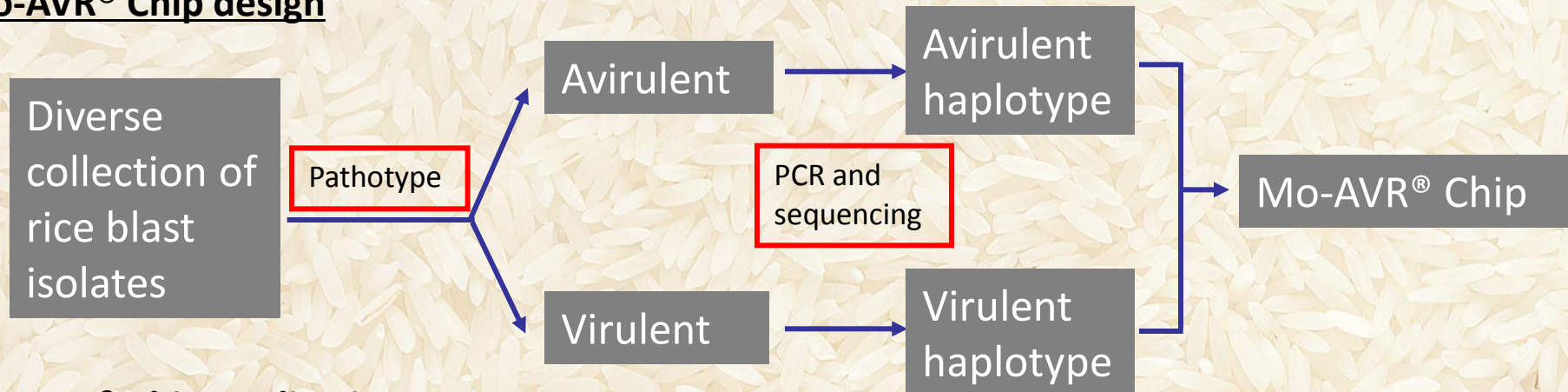
Real-time decision by Avr gene monitoring

# The Avr-based diagnosis provides a more precise profiling of race composition

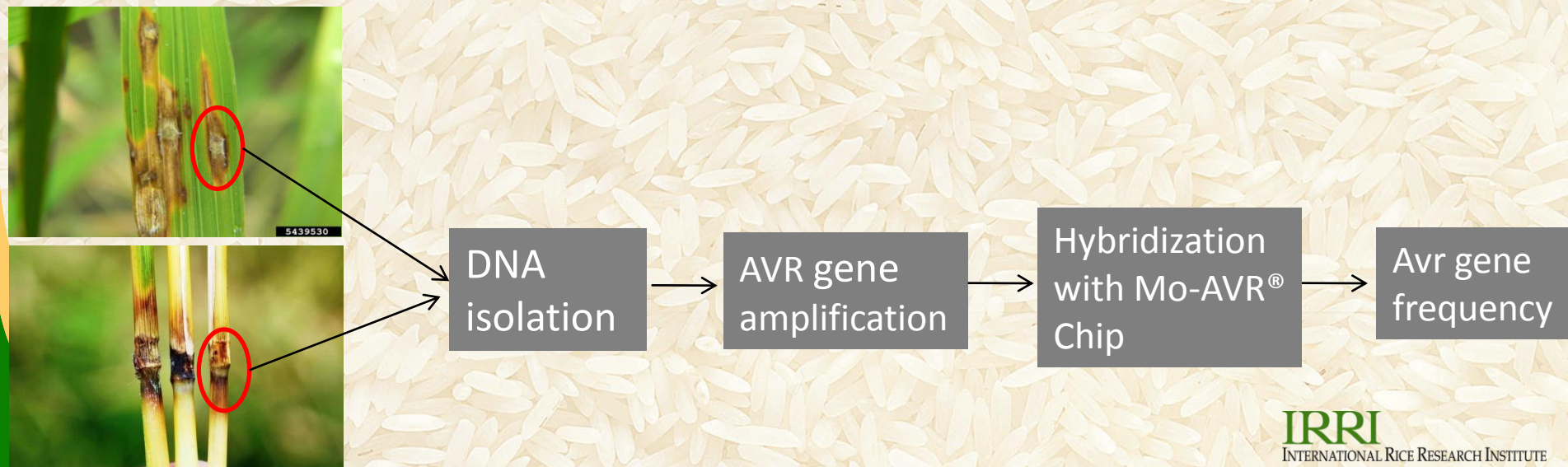
IRBLs	Target <i>R</i> genes	Pathotype (%)	Avr gene diagnosis (%)	Additional genes
IRBL9-W	<i>Pi9</i>	100	100	
IRBLz-Fu	<i>Piz</i>	95.9		
IRBLz5-CA	<i>Piz-5</i>	80.8		
IRBLzt-T	<i>Piz-t</i>	80.8	2.7	Pi19 or its allele in CO39
IRBLks-F5	<i>Piks</i>	0		
<b>IRBLkp-K60</b>	<b><i>Pik-p</i></b>	<b>98.6</b>	<b>D: 20.5</b>	<b>Pi19 or its allele in CO39</b>
IRBL7-M	<i>Pi7</i>	20.5	D: 20.5	
IRBLk-Ka	<i>Pik</i>	20.5	D: 20.5	
IRBLkh-K3	<i>Pik-h</i>	23.3	D: 20.5, E: 1.3	Additional gene to only one isolate
IRBLkm-Ts	<i>Pik-m</i>	21.9	D: 20.5, E: 1.3	
IRBL1-CL	<i>Pi1</i>	23.3	D: 20.5, E: 1.3	Additional gene to only one isolate
IRBLsh-S	<i>Pish</i>	97.3		
IRBLt-K59	<i>Pit</i>	9.6		
IRBL5-M	<i>Pi5</i>	95.9	86.3	Additional gene to 7 isolate
IRBL3-CP4	<i>Pi3</i>	95.9	86.3	Additional gene to 7 isolate
IRBLi-F5	<i>Pii</i>	93.2	86.3	Additional gene to 6 isolate
IRBLb-B	<i>Pib</i>	0		
IRBLa-A	<i>Pia</i>	6.8	0	Additional gene to 7 isolate
<b>IRBLta-K1</b>	<b><i>Pita</i></b>	<b>78.1</b>	<b>0</b>	<b>Pi19 or its allele in CO39</b>
IRBL12-M	<i>Pi12</i>	45.2		
IRBL20-IR24	<i>Pi20</i>	97.3		
IRBLta2-Re	<i>Pita-2</i>	21.9		
IRBL19-A	<i>Pi19</i>	78.1		
IRBL11-Zh	<i>Pi11</i>	5.5		
<b>CO39</b>	<b>S check</b>	<b>78.1</b>		<b>Pi19 or its allele in CO39</b>
LTH	S check	0		

# MoAVR<sup>®</sup>-chip for diagnosis of rice blast pathogen

## Mo-AVR<sup>®</sup> Chip design

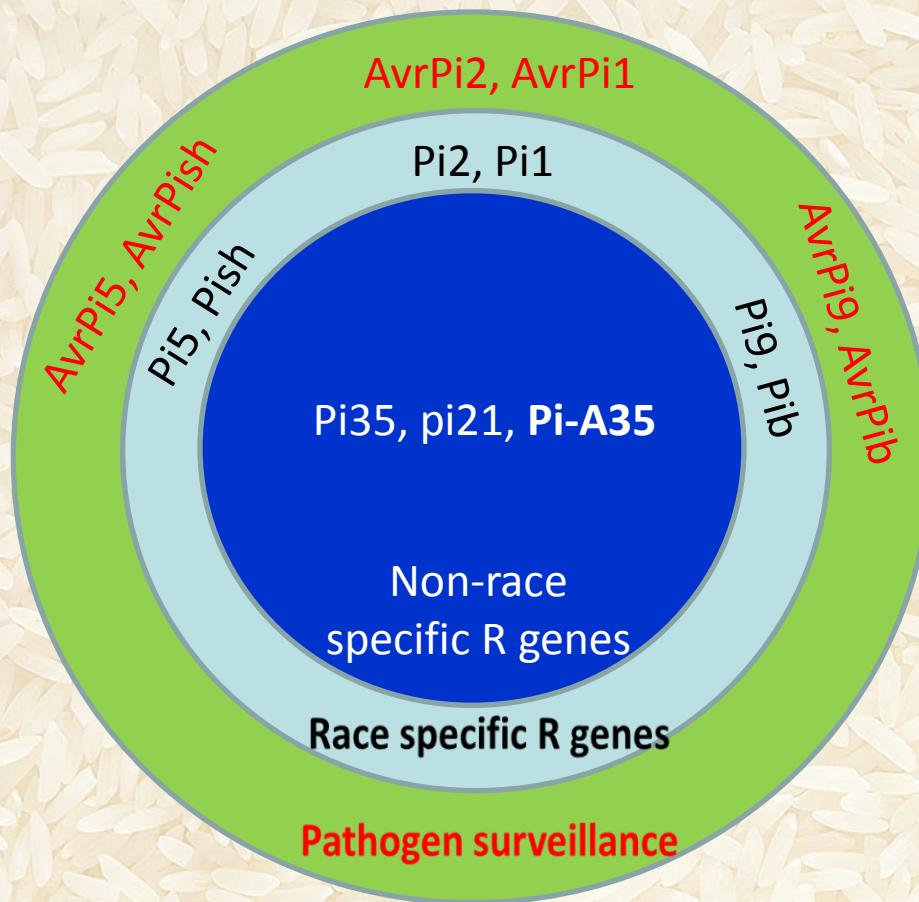


## Mo-AVR<sup>®</sup> Chip application



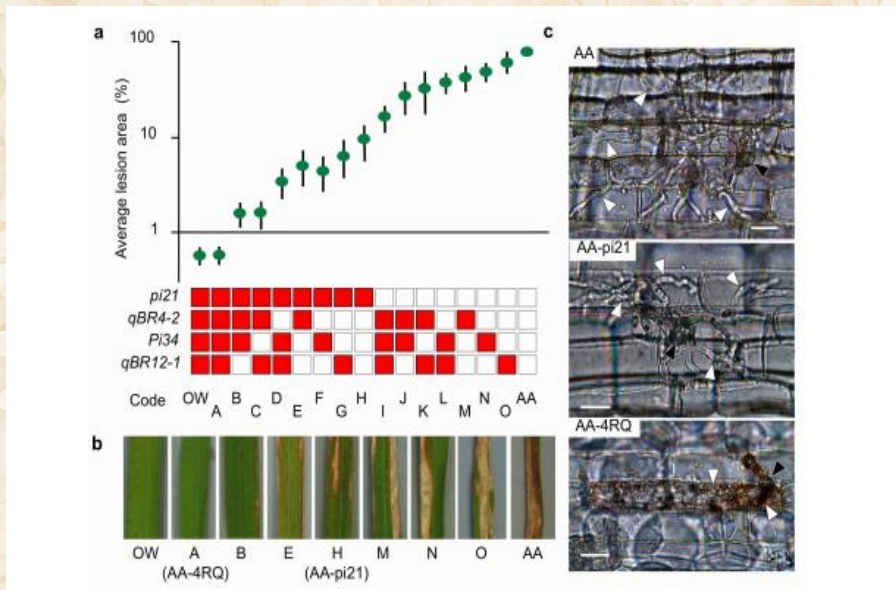
# How to breed durable resistance to rice blast?

# An integrated strategy for durable resistance to rice blast



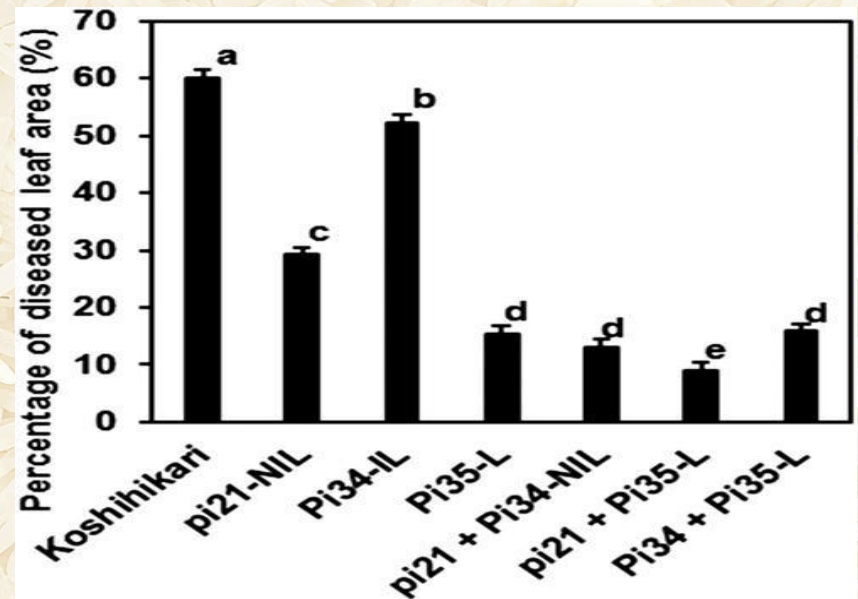
# Pyramiding of multiple *R* QTLs controls strong resistance to virulent isolates of rice blast

Pyramiding of *pi21*, *Pi34*, *qBR4-2*, *qBR12-1*



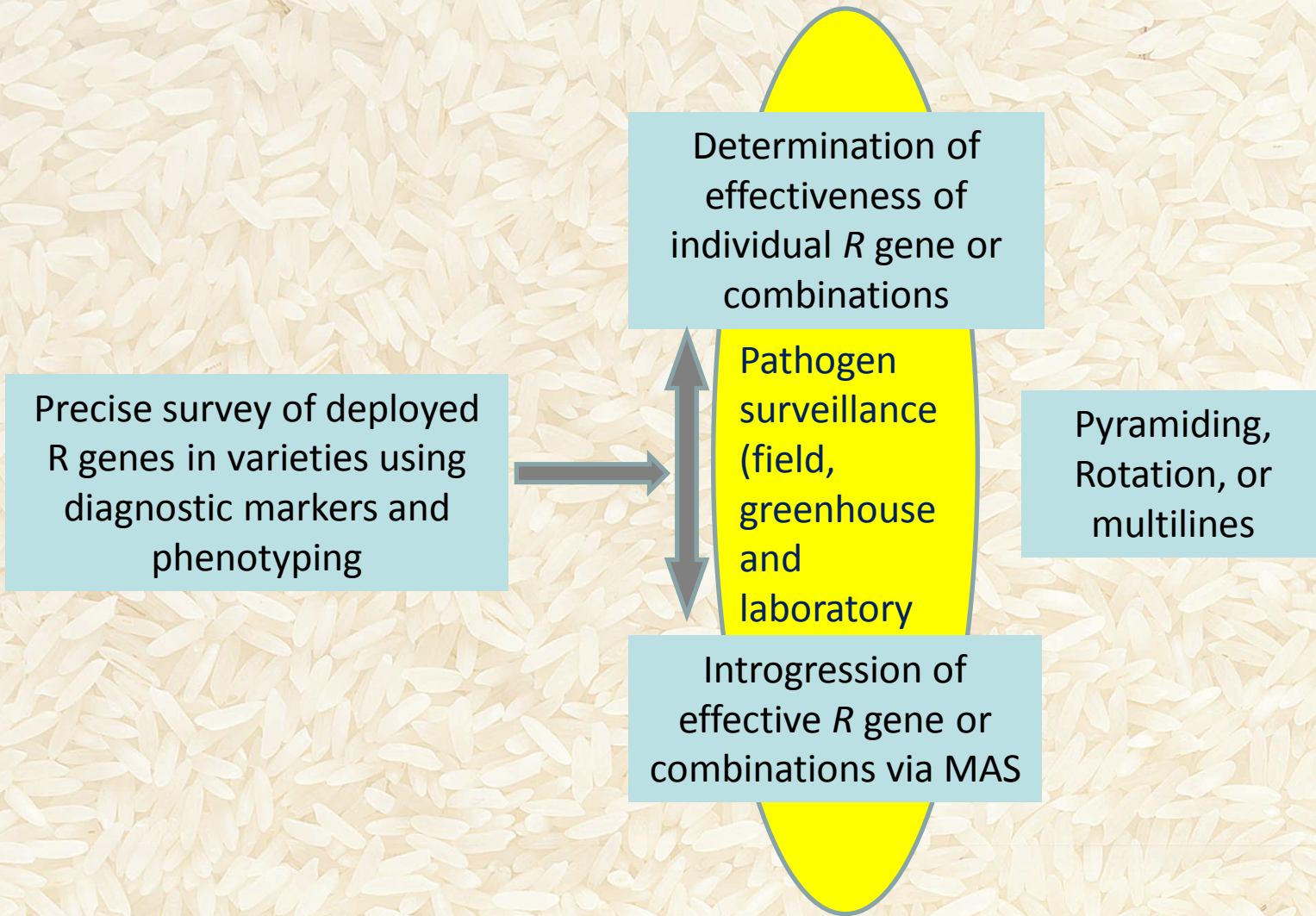
Fukuoka et al., 2015

Pyramiding of *pi21*, *Pi34*, *Pi35*



Yasuda et al., 2015

# efficient utilization of *R* genes (race specific)



## Current resistance breeding program



## Sustainable resistance breeding program

- ❖ Blind utilization of race specific R genes
- ❖ Neglected utilization of non-race specific R genes with large effects
- ❖ Reliance on resistance donors and traditional breeding together with field selection
- ❖ Less-economical utilization of R genes

- ❖ Combination of non-race and race specific R genes
- ❖ Pathogen-informed deployment of race specific R genes or combinations
- ❖ R-gene orientated molecular breeding
- ❖ Economical utilization of R genes to save resource for other traits, e.g., yielding
- ❖ Climate resilience of resistance to blast disease (high nitrogen and extreme temperature and rainfall)



## Research activities on host resistance to false smut

- To develop a highly reproducible and scalable phenotyping protocol using artificial inoculations
- To develop hotspots for field assessment and epidemiology modeling
- To evaluate disease resistance of a diverse panel of germplasm and identify lines with high levels of resistance
- To identify *R* gene/QTLs to false smut and develop gene linked molecular markers

# Optimization of the boot-injection method for evaluating rice resistance to false smut

## Inoculum preparation



Inoc. Of culture medium (PSB)



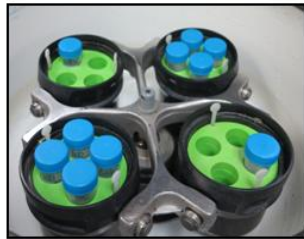
7 days Incubation (Shaker)



Mycelial inoculum



Prep. for spores alone inoculum



Centrifugation (Spores alone)

## Injection at the booting stage (3-7 days before exsertion)



Injection of plants at booting stage



Smut ball formation, 2 weeks after inoculation

## Distinct specificities in the interactions between rice and false smut

Rice entry	Number of smut balls/hill (PFS: Philippine False smut)								
	PFS 23-1	PFS 25-1	PFS 31-1	PFS 34-1	PFS 36-1	PFS 44-2	PFS 99-2	PFS 102-1	PFS 109-1
1	19	0	2	-	7	7	-	-	<b>123</b>
2	93	20	-	-	6	17	2	<b>128</b>	73
3	96	29	25	<b>215</b>	6	25	-	-	153
4	0	0	4	36	<b>47</b>	18	0	44	33
5	225	67	-	222	33	208	295	<b>309</b>	312
6	<b>352</b>	29	61	23	224	50	133	311	110
7	0	0	3	15	-	7	0	<b>97</b>	19
8	28	<b>43</b>	-	-	-	-	0	5	9
9	40	48	5	-	0	-	0	<b>246</b>	16

**Line #3**



**Line #5**



**Line #6**



**Line #9**



# Acknowledgements

## RGDR group

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## Host-Plant Interaction cluster

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Dr. Simon Krattinger, ZUH, Switzerland



Research  
Program on  
Rice  
Global Rice  
Science  
Partnership



**Bill & Melinda Gates Foundation**

**Switzerland Science Foundation**