Saint Petersburg

CSIRO

Borlaug Global Rust Initiative

# Understanding the molecular basis of rice non-host immunity to rust

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# Nearly all agricultural cereals and grasses have rust pathogens

| Species | Examples of Rust Pathogens |
| --- | --- |
| Wheat | *Puccinia graminis, P. triticina, P. striiformis* |
| Barley | *Puccina graminis, P. striiformis, P. hordei* |
| Maize | *Puccinia sorghi, P. polysora* |
| Sorghum | *Puccina purpurea* |
| Rye | *Puccinia graminis, Puccinia striiformis, Puccinia triticina* |
| Millet | *Puccinia substriata* |
| Sugarcane | *Puccinia melanocephela, Puccinia kuehnii* |
| Oats | *Puccinia coronata, Puccinia graminis* |

# Rice has no known rust disease despite large production areas overlapping with wheat

**Objective 9 DRRW:**

- **What is the basis of the apparent immunity of rice to cereal rusts?**
- **Can this rice NHR to rust be transferred to wheat?**

# Model of nonhost resistance mechanisms

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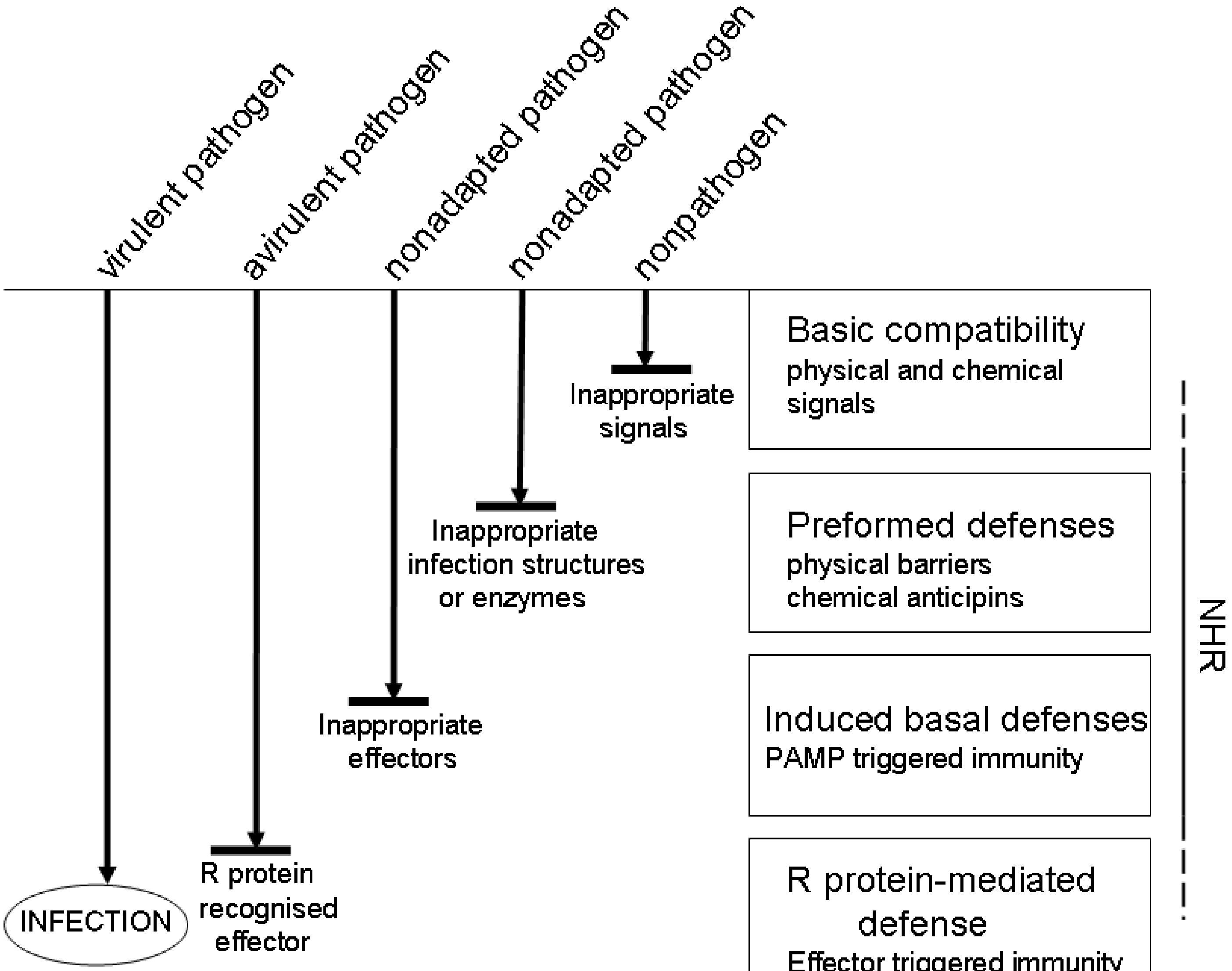
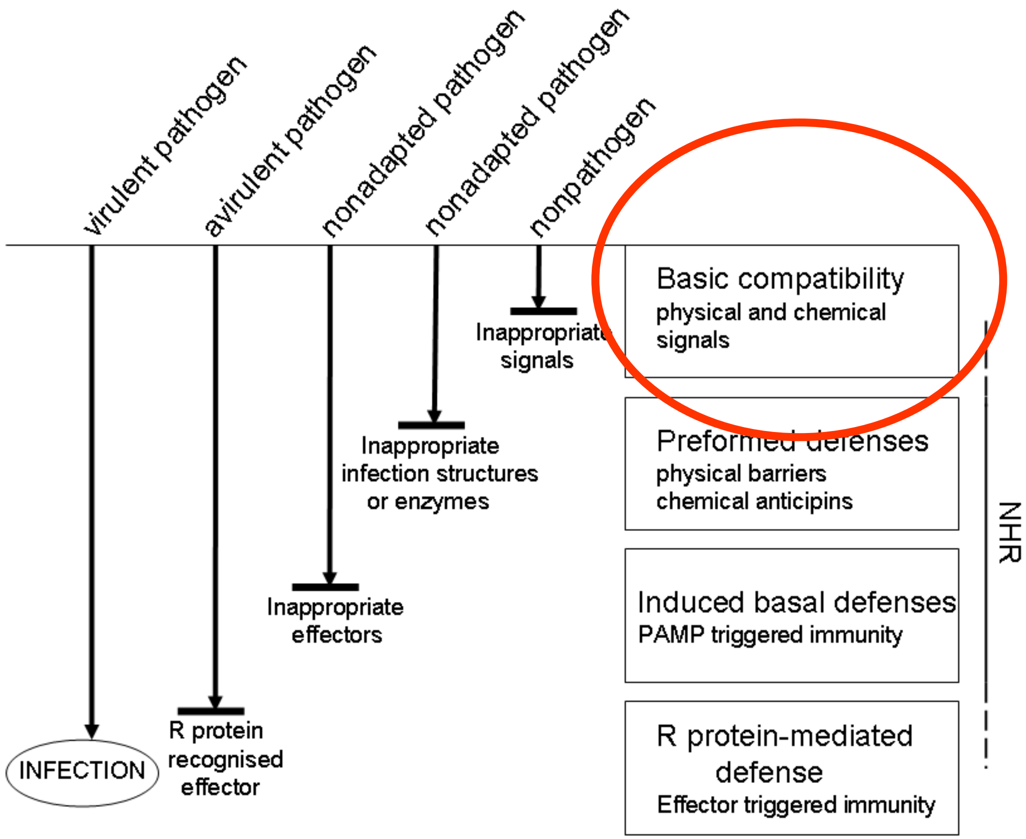
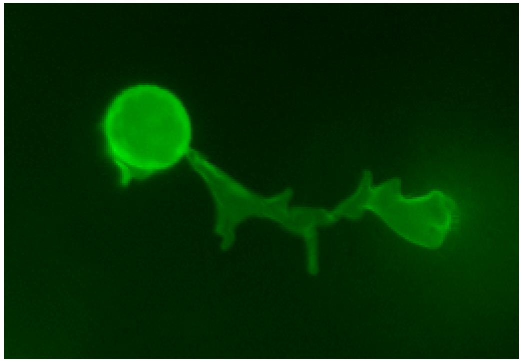


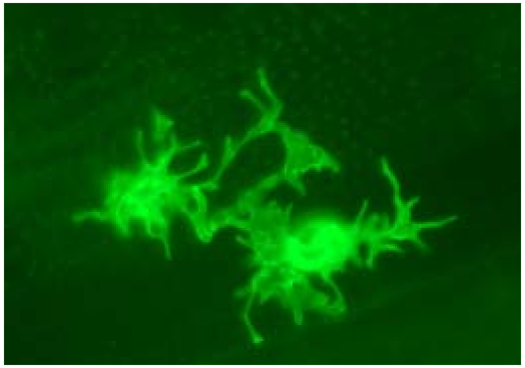
# Can cereal rust actually infect rice?




Flax rust infection of flax

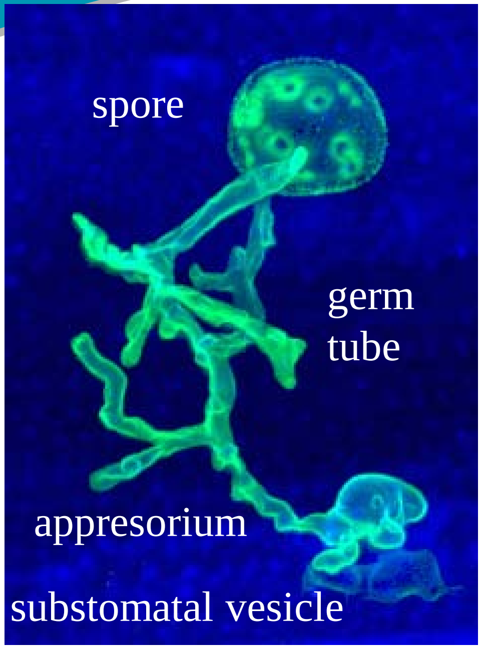


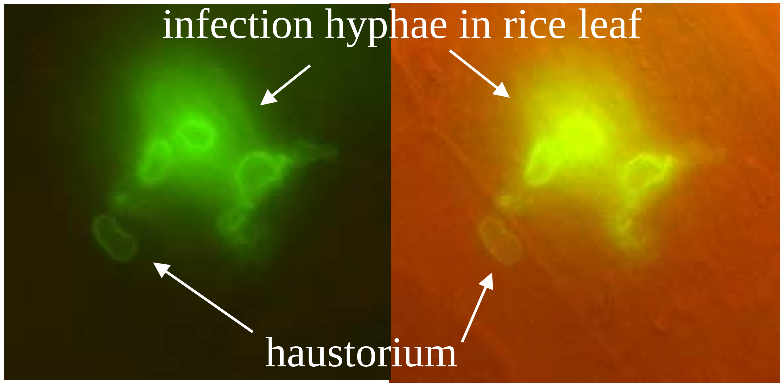
Flax rust infection of rice



**Only 2.5% of germinated flax rust spores actually infect the leaf**

# Cereal rusts can infect rice leaves



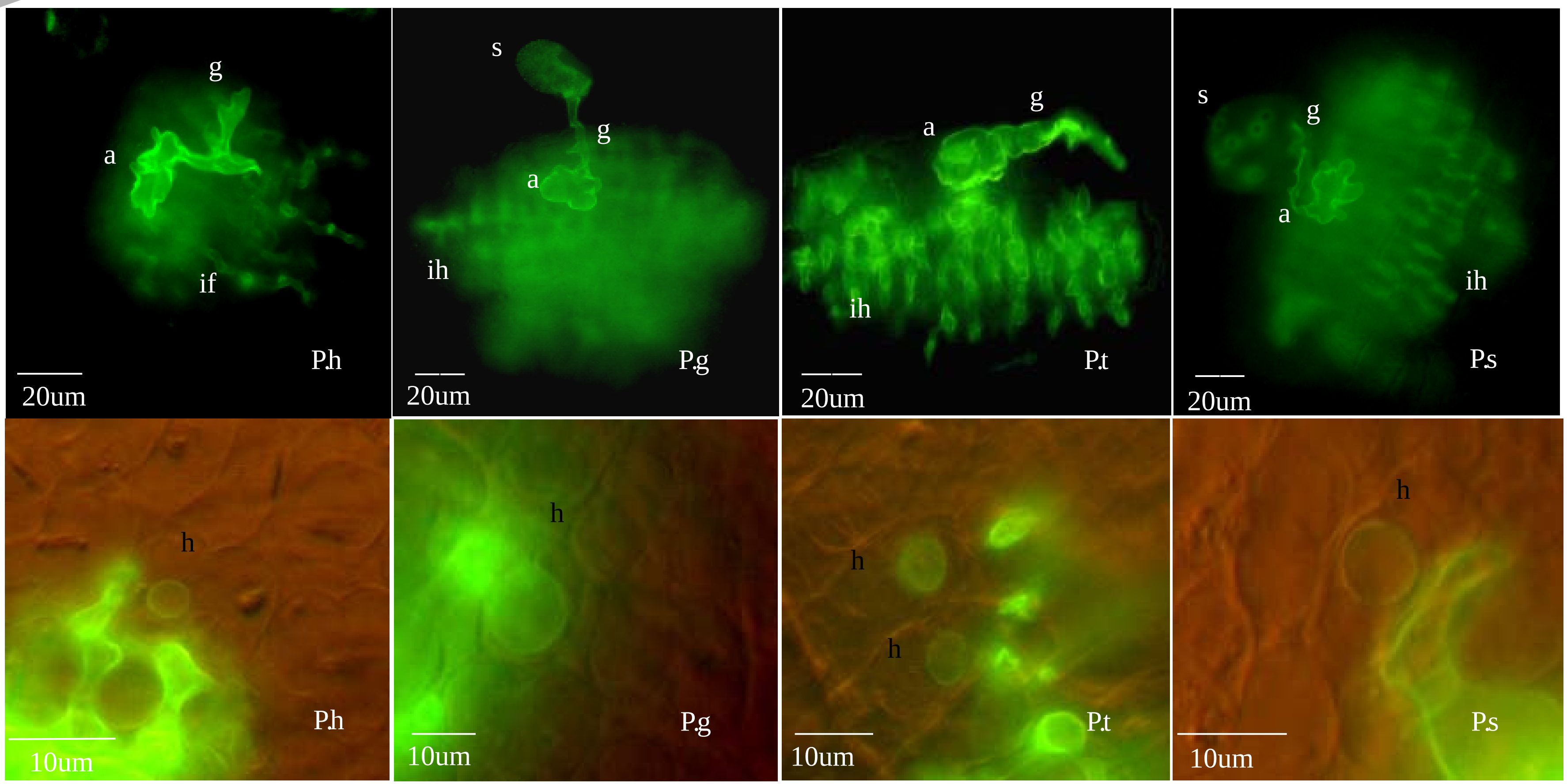

Wheat stem rust on wheat
-80% of germinated spores produce infection hyphae

Wheat stem rust on rice
- 50% of germinated spores produce infection hyphae

Barley leaf rust on barley
- 88% of germinated spores produce infection hyphae

Barley leaf rust on rice
- 40% of germinated spores produce infection hyphae

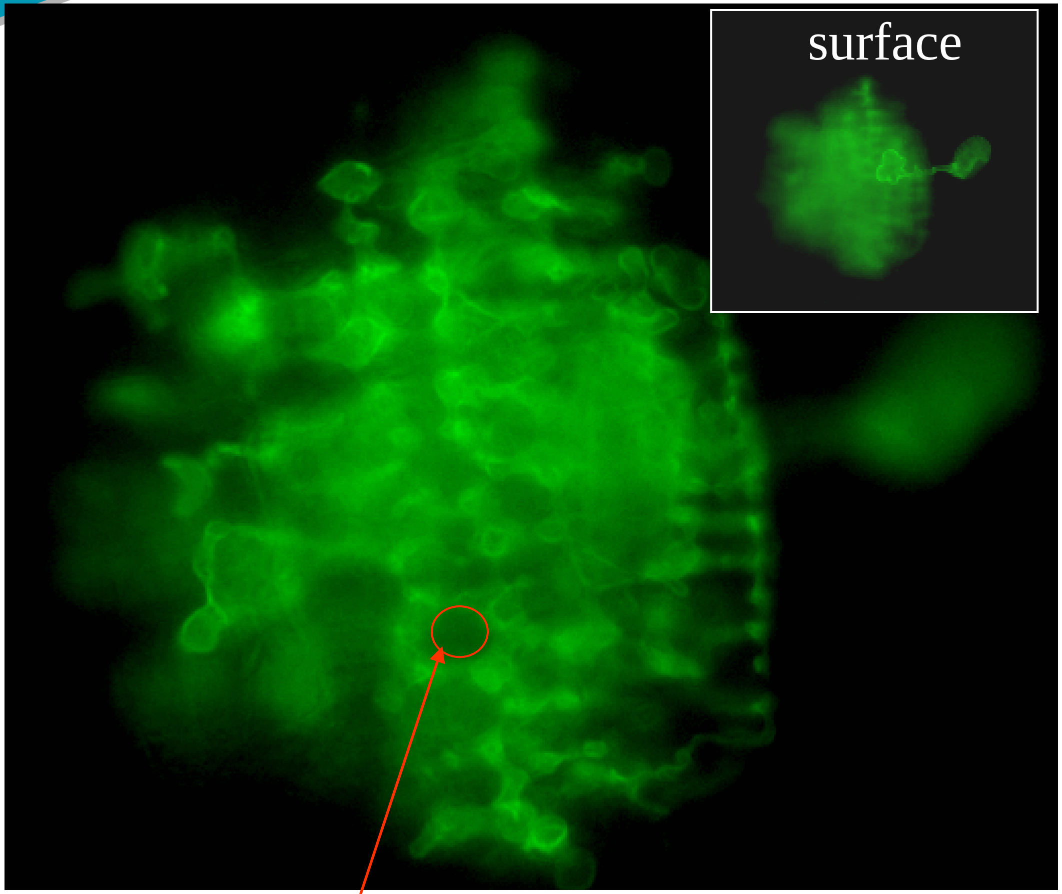
# In some cases cereal rust infection sites on rice are large and encompass many mesophyll cells




*Puccinia hordei* *Puccinia graminis* *Puccinia triticina* *Puccinia striiformis*

# Inferences from large cereal rust infection sites on rice

WSR on rice

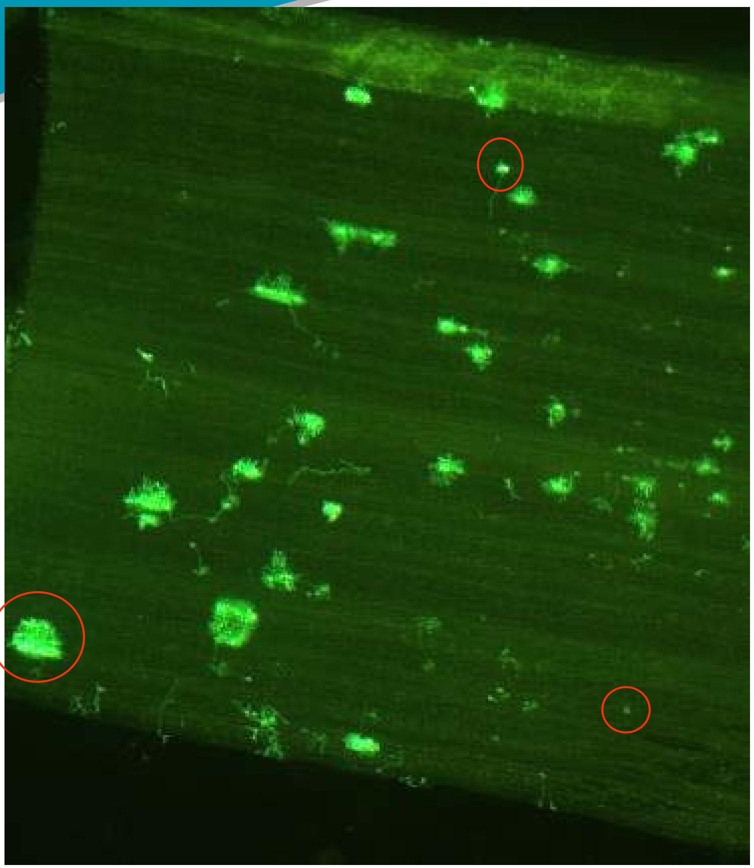



**Direct nutrient uptake from the infected rice leaf by cereal rusts**
**- presence of haustoria**

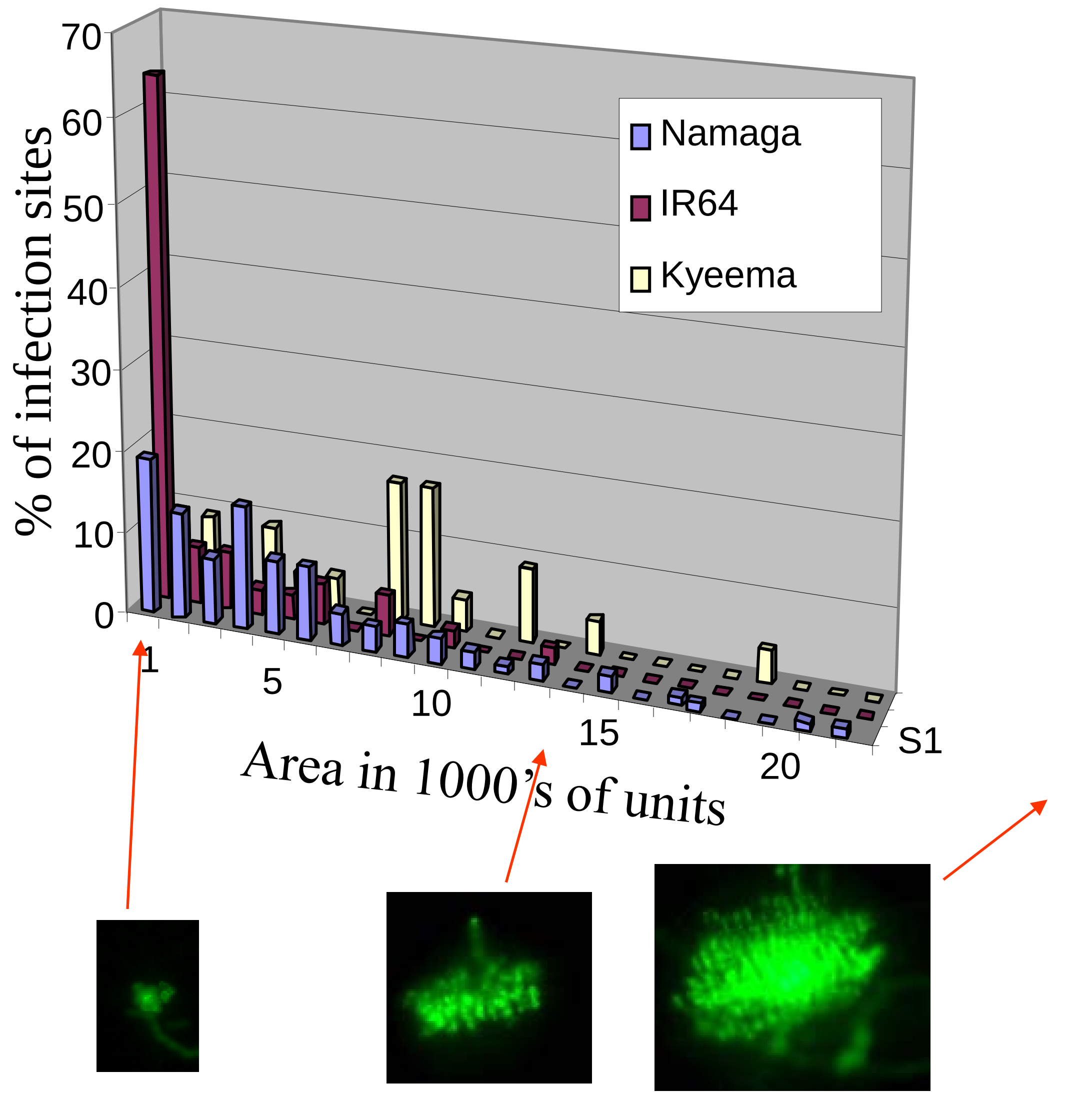
**An absence of basic incompatibility between cereal rusts and rice**

Single mesophyll cell surrounded by infection hyphae (i.e. 10 X 6 = 60 cells)

# For all rust species, a distribution of infection site sizes is observed on a single leaf



Considerable variation in wheat stem rust infection site sizes are apparent on the same rice leaf

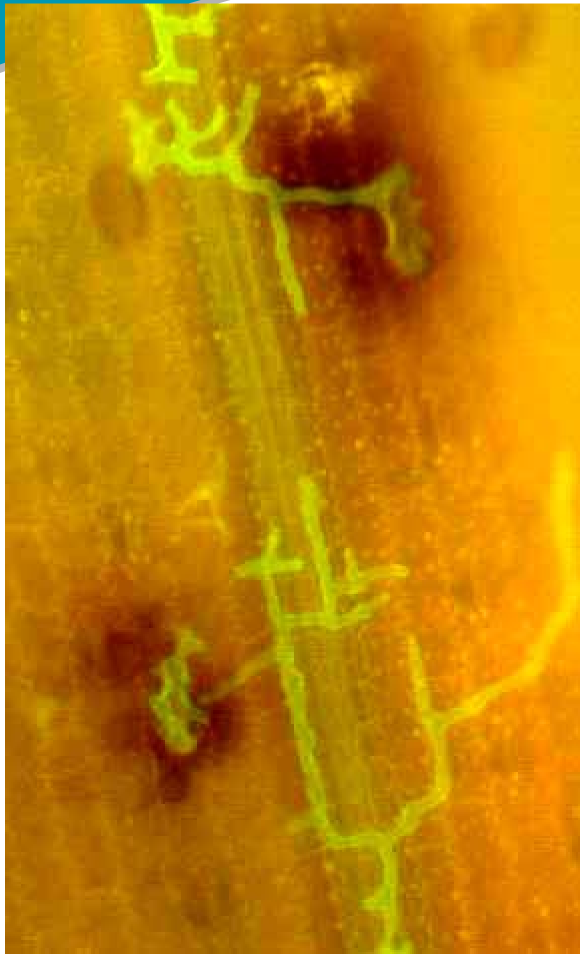



314

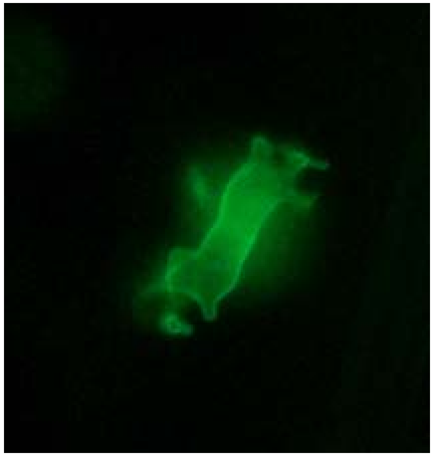
13, 870

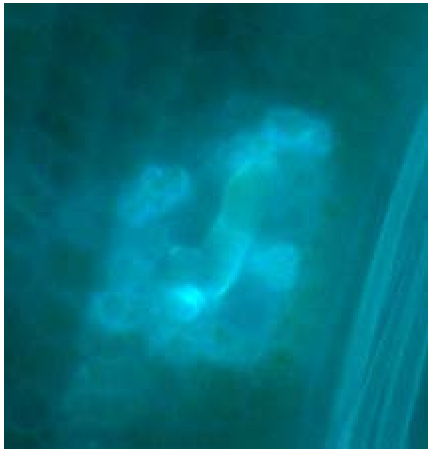
54, 023

# Rice mounts an active defense response to both WSR and BLR

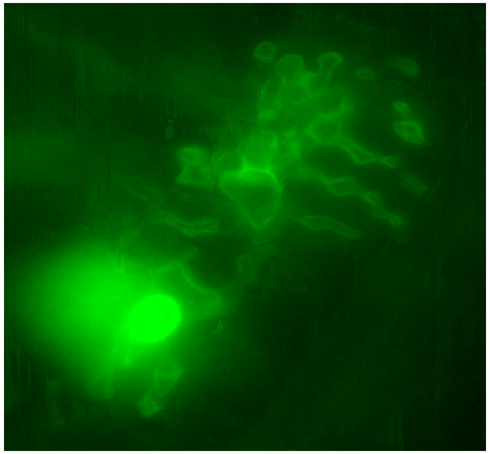


$H_2O_2$ production
(DAB stain)



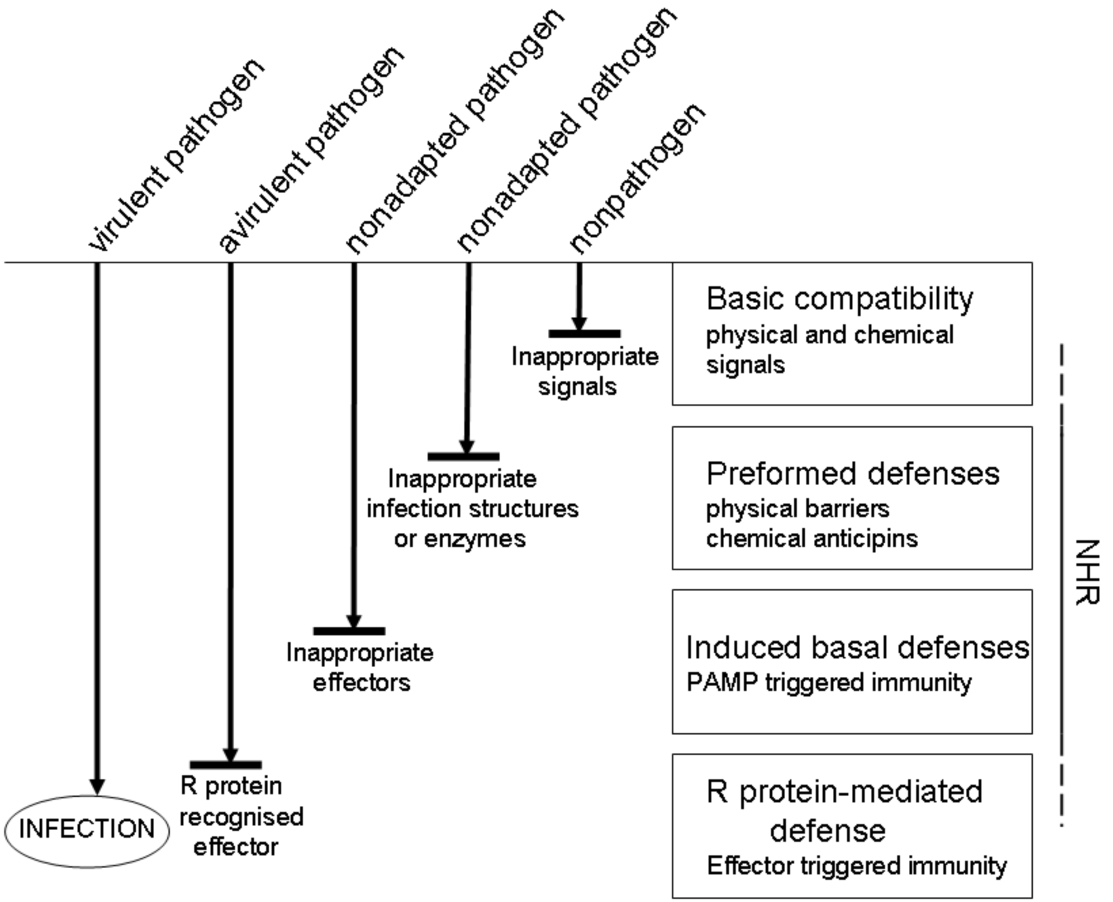


callose deposition (aniline blue)



autofluorescence (UV)

# Immunity of rice to cereal




**Large rust infection sites that contain haustoria**

**Active rice defense response**

# Dissecting rice immunity to cereal rust

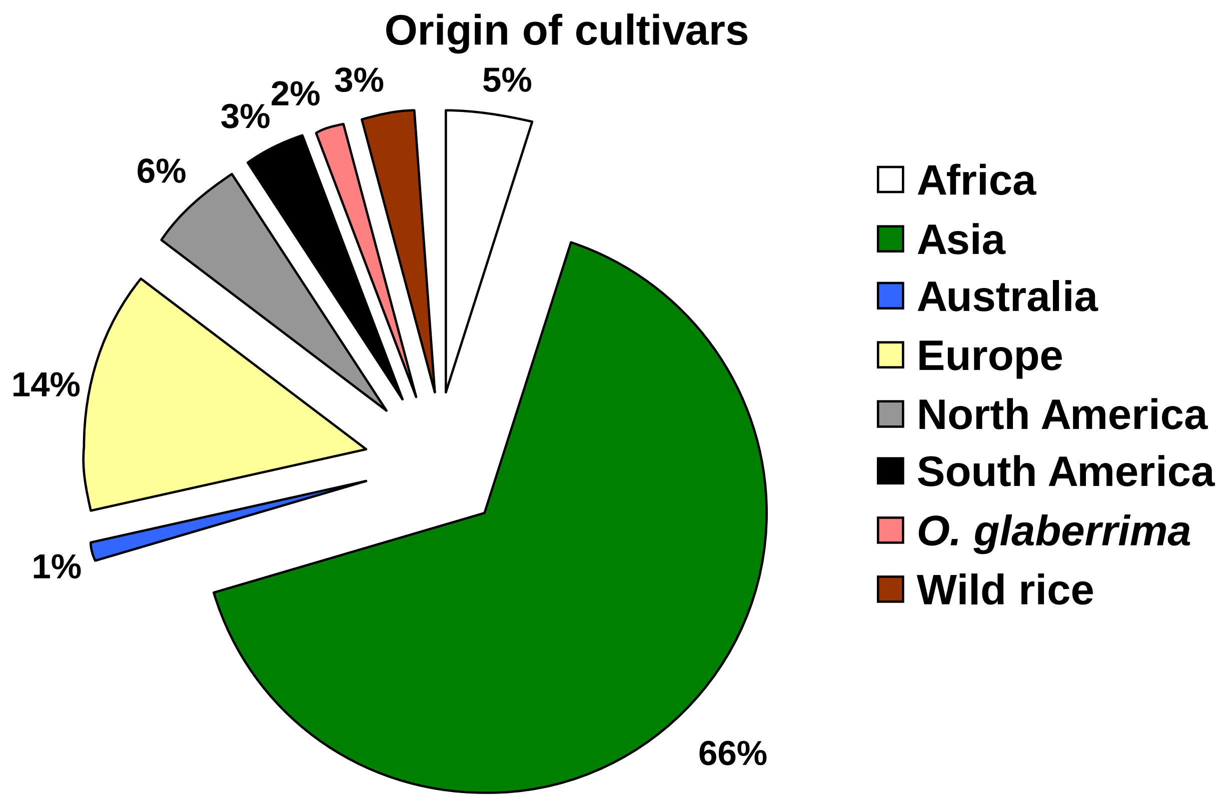
1) Genetic variation amongst rice cultivars for NHR response to cereal rust

2) Candidate gene approach

3) Mutant screening

# Genetic variation amongst rice cultivars for NHR efficacy to cereal rust

**9,000 rice cultivars/accessions screened for altered rust infection phenotypes when challenged with oat and rye stem rust**



# Genetic variation amongst rice cultivars for NHR efficacy to cereal rust

Typical immune response

Accession 127, JANTROCEROS KORN, Portugal

Accession 414, ETYM, USSR

Accession 4575, APIN-APIN, Malaysia

1. For most accessions, no macroscopic evidence of rust infection structures – rice is true nonhost

2. Macroscopic infection symptoms in 33 lines

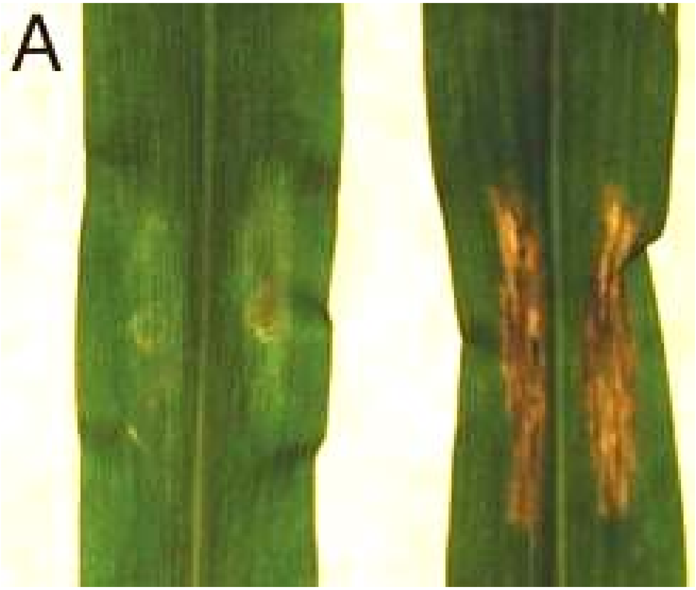
| Macro-phenotype | Number |
|---|---|
| Chlorosis-like flecks | 3 |
| Dark green distinct areas | 2 |
| White flecks | 17 |
| Brown spots/flecks | 11 |

3. Microscopic analysis showed no increased rust growth

4. Inheritance ?

# The maize *Rxo1* gene

Zhao et al (2005) A maize resistance gene functions against bacterial streak disease in rice. PNAS 102: 15383-15388.




(A) Transgenic maize leaf (right) and nontransgenic maize after infiltration with *X. o.* pv. *oryzicola* carrying avrRxo1.

**In maize, difference between strong HR and apparent immunity to a non-adapted *Xanthomonas* bacterial pathogen was a single NBS-LRR gene, *Rxo1*.**

**First example of transfer of NHR**

# Genetic variation amongst rice cultivars for NHR efficacy to cereal rust

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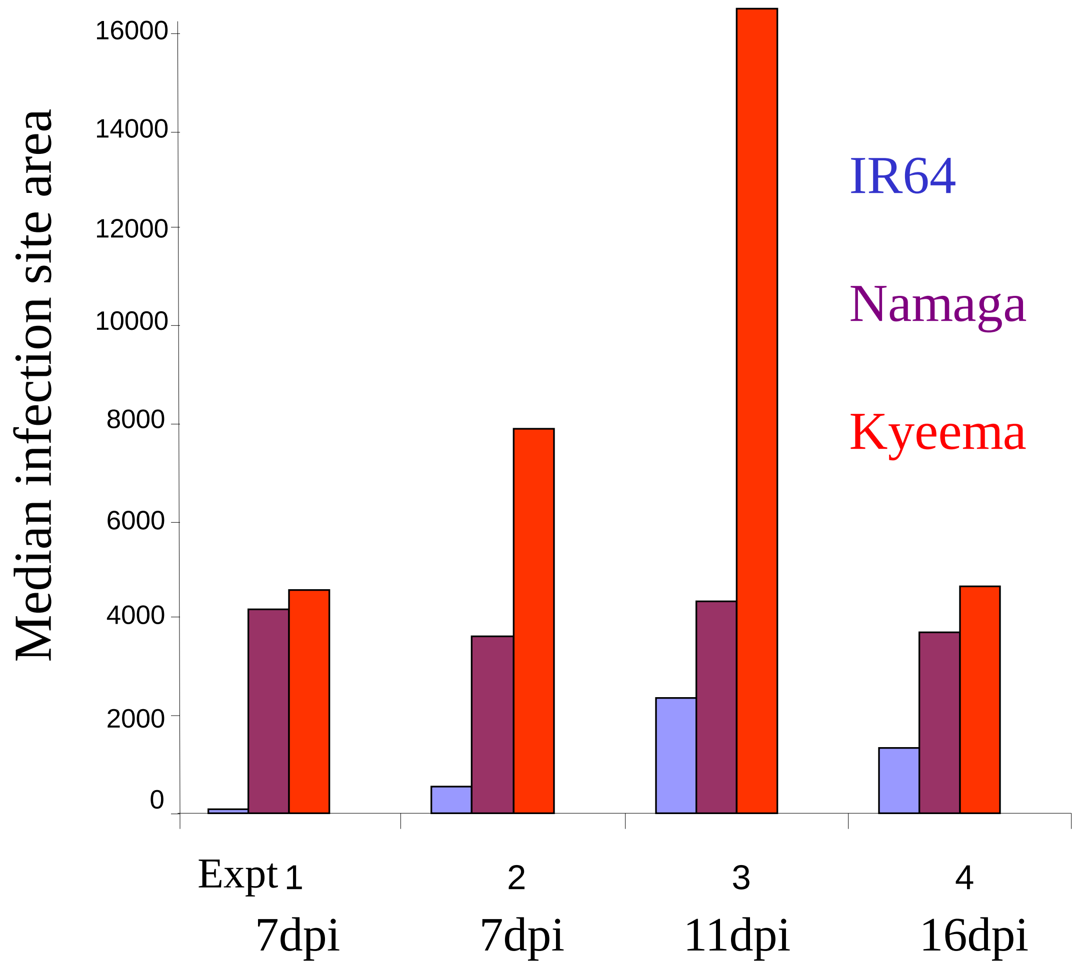
Quantitative microscopic analysis between 12 rice cultivars identified two lines showing reproducibly greater growth of WSR

Mann-Whitney U test

| Expt 1 | Kyeema | IR64 (36) |
|---|---|---|
| Namaga (91) | 0.24<P<0.47 | P<0.0001 |
| Kyeema (71) | - | P<0.0001 |
| | | |
| Expt 2 | Kyeema | IR64 (61) |
| Namaga (95) | P<0.01 | P<0.0001 |
| Kyeema (23) | - | P<0.0001 |
| | | |
| Expt 3 | Kyeema | IR64 (113) |
| Namaga (101) | P<0.0001 | P<0.007 |
| Kyeema (117) | - | P<0.0001 |
| | | |
| Expt 4 | Kyeema | IR64 (75) |
| Namaga (147) | P<0.015 | P<0.0017 |
| Kyeema (72) | - | P<0.0001 |



# Rust infection of rice

**Wheat stem rust**

**Kyeema > Namaga > IR64 – stem rust 21-0**
**Kyeema > Namaga > IR64 – stem rust 326**

**Wheat leaf rust**

**Namaga > Kyeema > IR64**

**Barley Leaf rust**

**No consistent difference in infection site size**

**Differences in NHR interactions between different rice cultivars and different rust species**

# Can the variation in WSR on rice cultivars be mapped?

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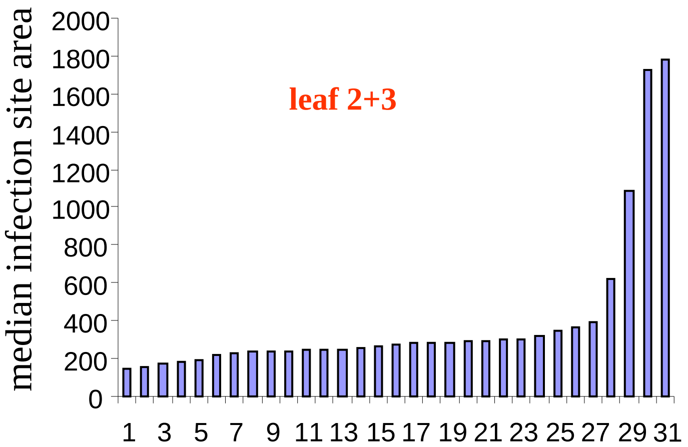
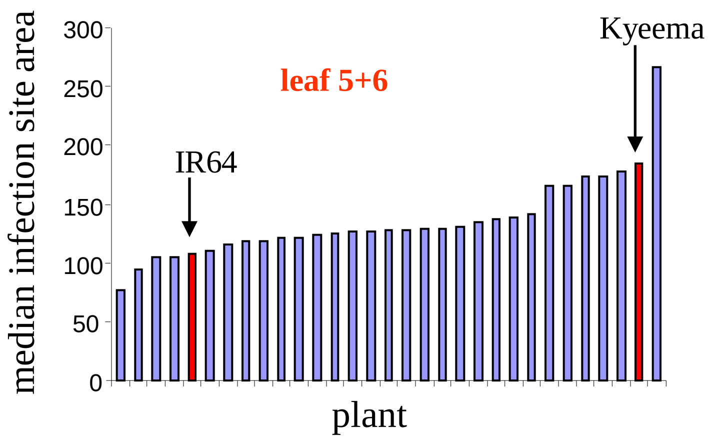
**$F_2$ progeny produced between Kyeema (permissive) and IR64 (restrictive)**



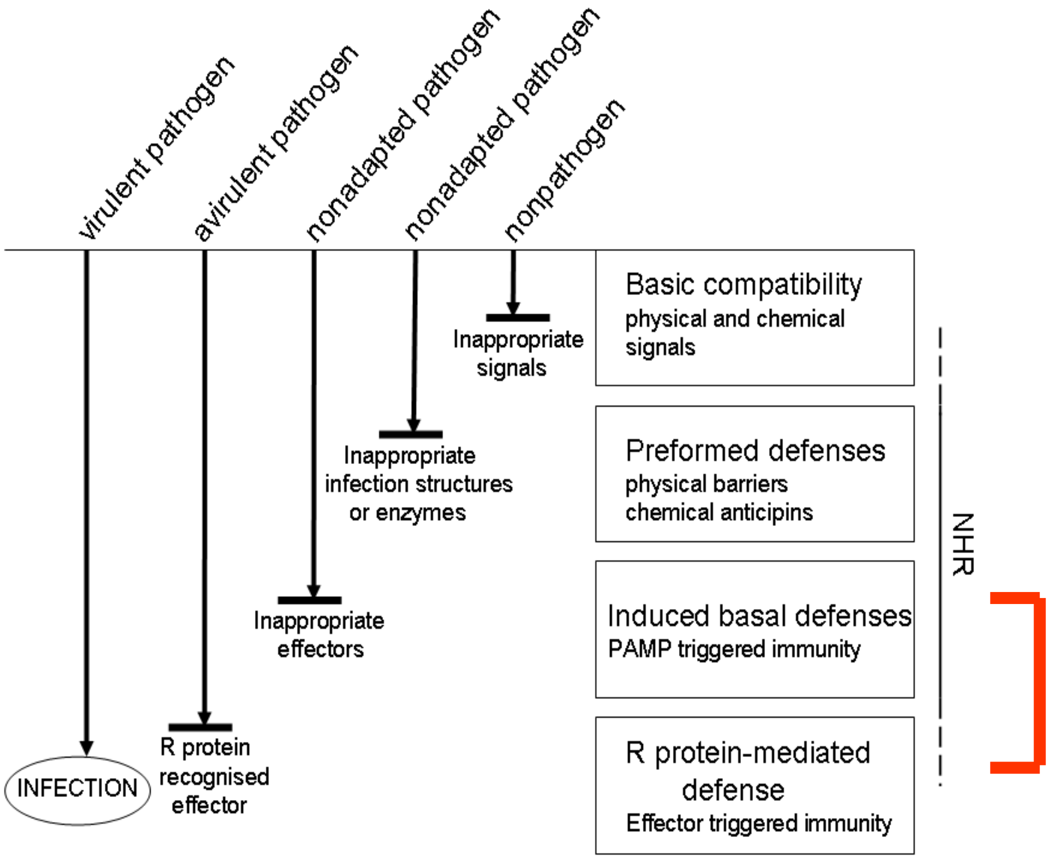

Leaf 2 and 3 of 31 seedlings infected with WSR and average infection site area calculated for each plant

Leaf 5 and 6 infected and average infections site area calculated for each plant

Spearman Rank Correlation

Correlation = 0.477
P= 0.008

# Candidate gene approach




**Potential roles for**

**-PAMP receptors**

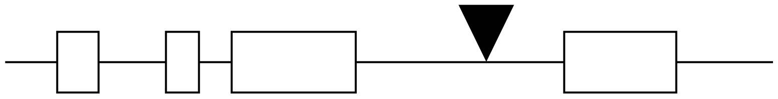
**-Basal defense signalling**

**-R proteins**

# Rice T-DNA insertions – infected with WSR

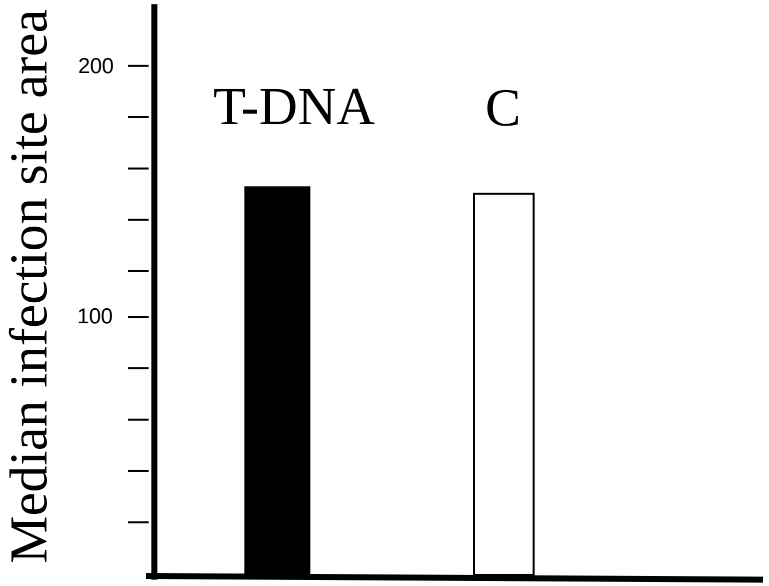
PAMP receptor

Chitin elicitor binding protein



Demonstrated chitin receptor in rice

Arabidopsis CERK1/LysMRLK1 KO impairs resistance to avirulent *Alternaria* (Miya et al, 2007) and increases susceptibility to virulent *Erysiphe* (Wan et al, 2008).

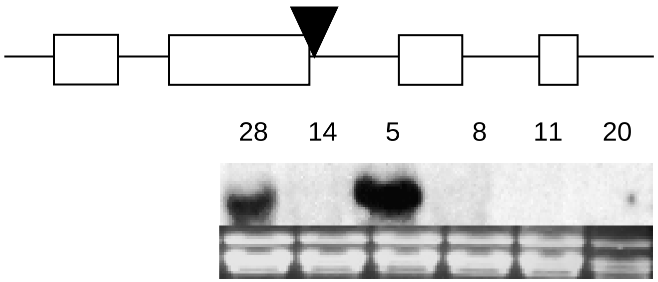



Mann Whitney U test  P= 0.28

# Rice T-DNA insertions – infected with WSR

R protein chaperonin

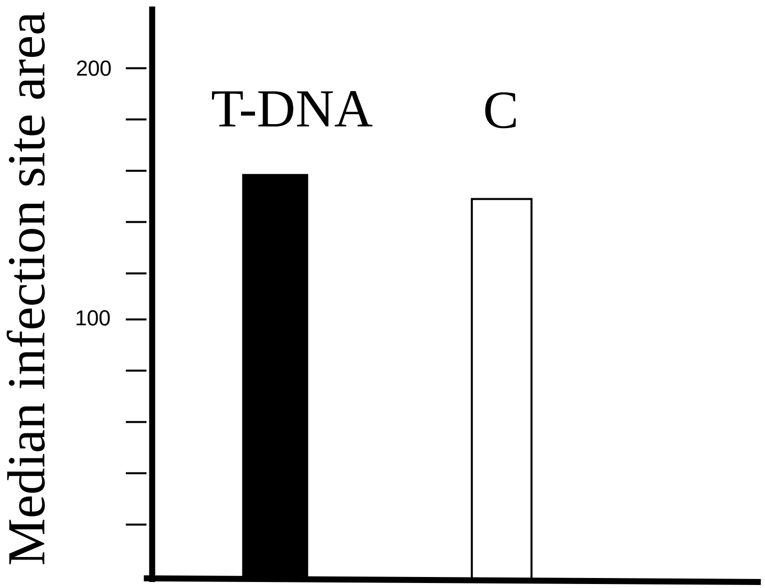
RAR1




Rice RAR iRNA impairs basal resistance to virulent rice blast and *Xanthomonas* (Thao et al, 2007).

RAR1 required for basal resistance to rice blast in mlo barley (Jarosch et al, 2005)

RAR1 required for R gene signalling in barley (Mla) and wheat (Lr21)

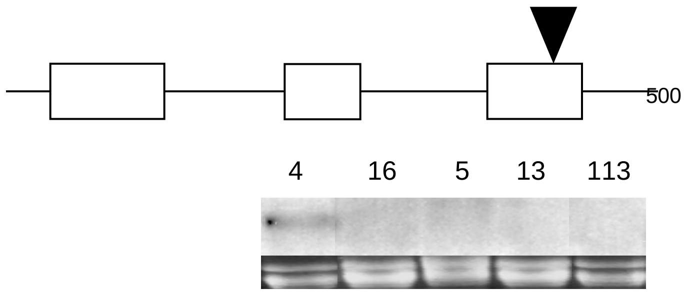



Mann Whitney U test P= 0.23

# Rice T-DNA insertions – infected with WSR

Basal defense pathway

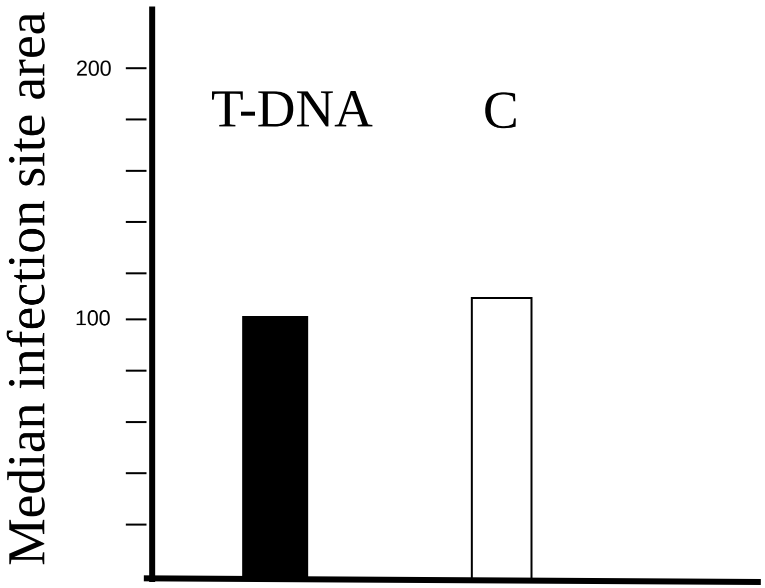
EDS1 homologue




Role in rice unknown

Implicated in basal defense, SA signalling and TIR-NBS-LRR function in Arabidopsis

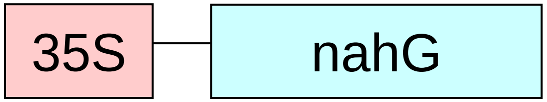
Required for NHR to wheat mildew in Arabidopsis



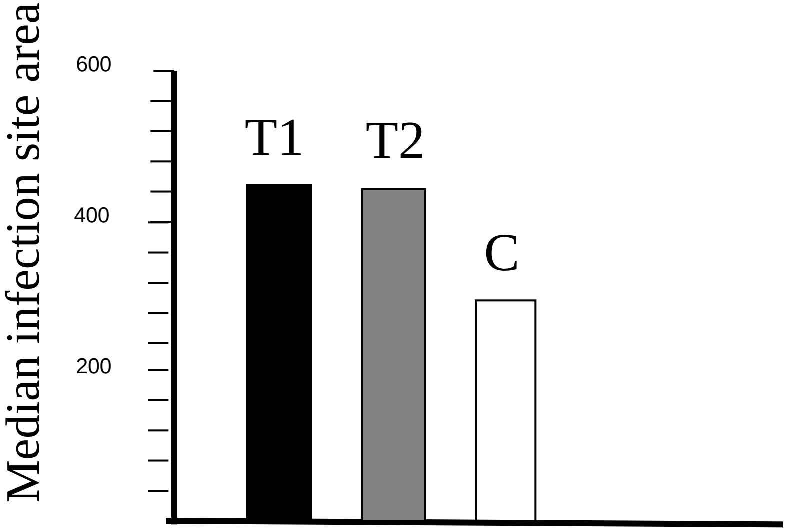

Mann Whitney U test P= 0.28

# Modest increase in WSR growth on nahG rice lines?

**Salicylic acid degradation**



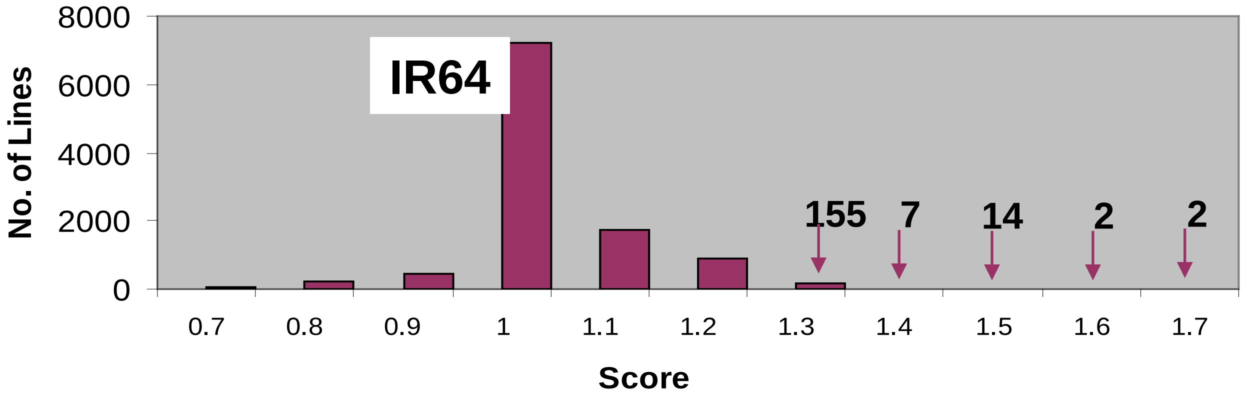

| | T2 | Control |
|---|---|---|
| T1 | P = 0.7 | P= 0.001 |
| T2 | | P = 0.0004 |




Transgenics provided by Professor Yinong Yang, Pennsylvania State Uni

# Mutation

**Normalized Frequency Distribution of M4 IR64 Mutants Screened in BN 2007**





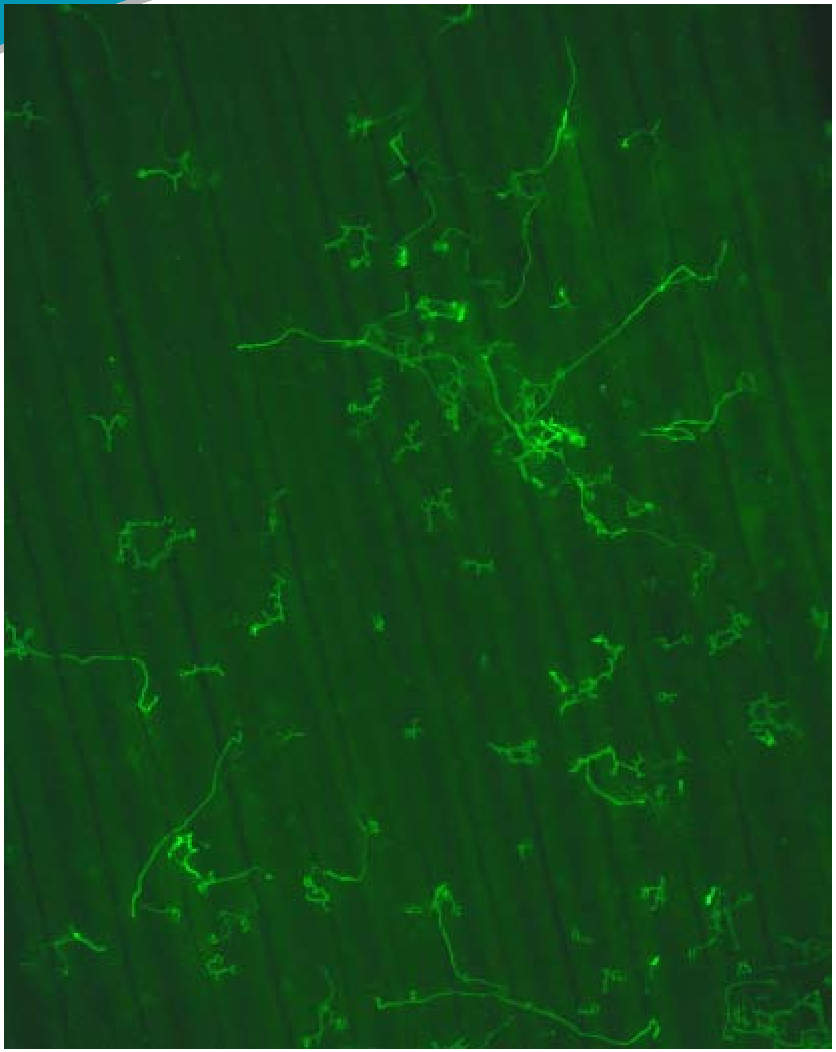
**300 rice mutant lines previously identified as showing increased rice blast susceptibility**

**More resistant**

**IR64**

**More susceptible**

# Mutation – microscopic screening

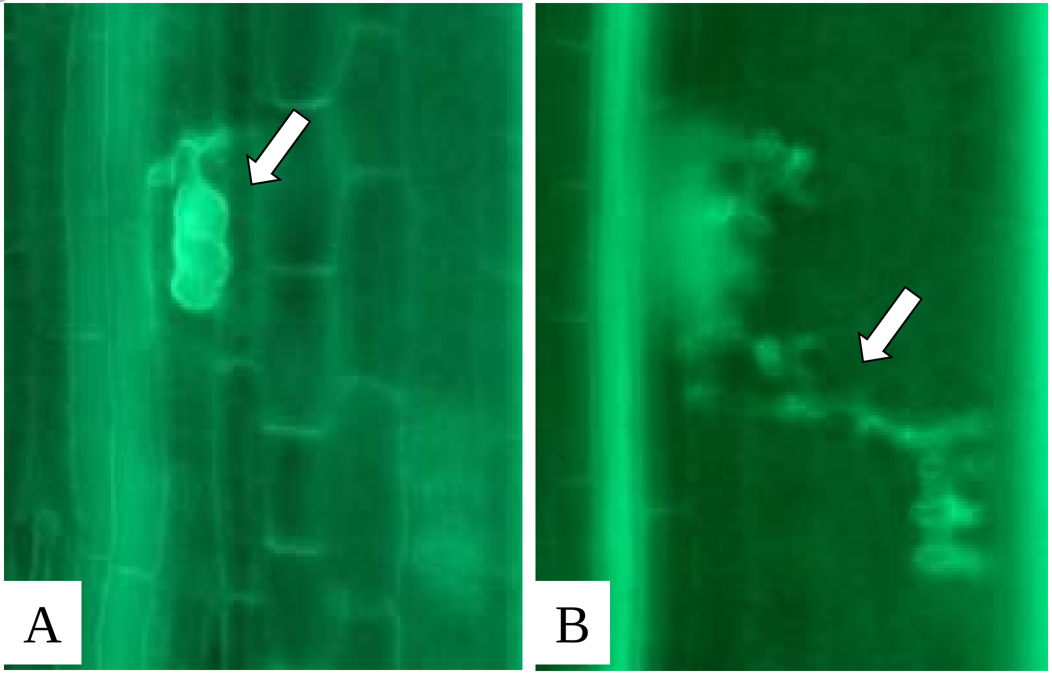


**No comprised NHR to wheat stem rust amongst**
**300 rice blast susceptible mutant lines.**

**No obvious overlap between rice basal defense to rice blast and NHR to wheat stem rust.**

**Random mutagenized M4 rice lines are being screened for compromised NHR to WSR.**

# Mutation screening – stripe rust



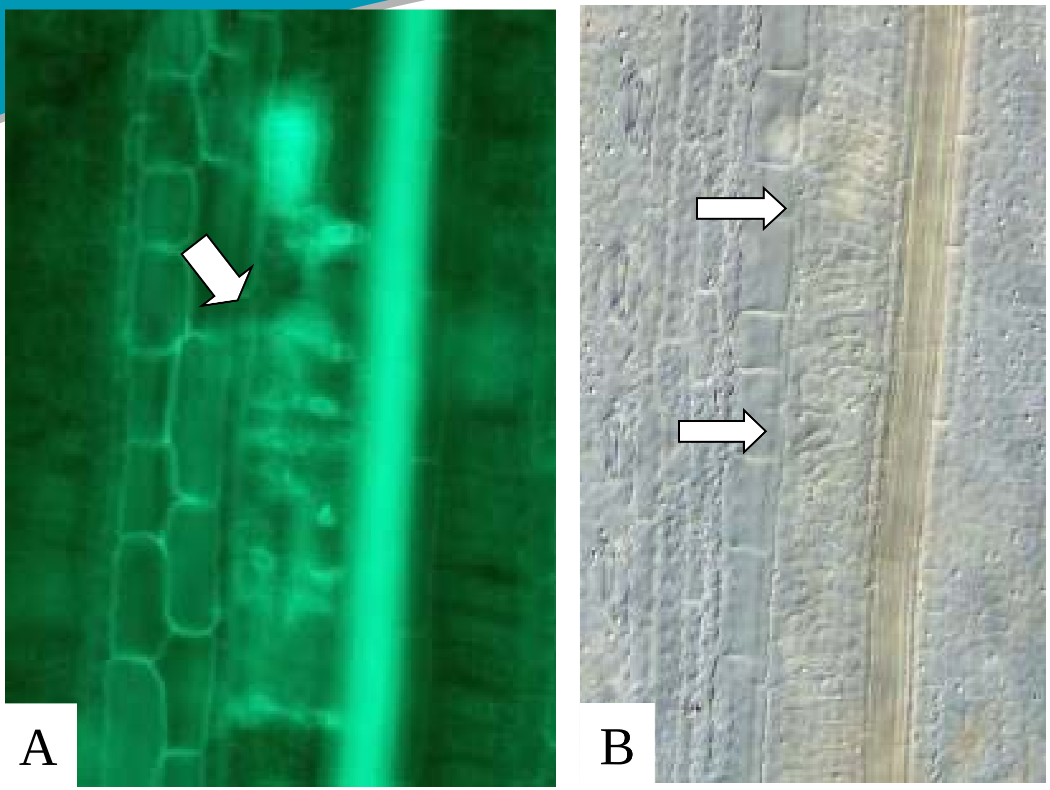

**A T-DNA insertion rice mutant (2611-E-2) showing increased growth of *Puccinia striiformis* f. sp. *tritici (Pst)*. (A). Substomatal vesicle (arrow) in inoculated leaf tissue 20 days after inoculation. (B) Growth of hypha in inoculated leaf tissue 20 days after inoculation (arrow). Tissues were stained by Calcofluor White M2R and observed under UV light**

**Screening of 5229 rice T-DNA insertion lines in Zhonghua 11 background**

**3 potential increased rust growth mutants identified: 2611, 2430 and 2782**

**Zhensheng Kang, Northwest A&F University**
**Shiping Wang, Huazhong Agricultural University**

# Mutation - screening stripe rust




IR64 mutant (B12-2) showing increased growth of *Puccinia striiformis* f. sp. *tritici* (*Pst*). A) Colonization by *Pst* hypha (arrow) 20 days after inoculation. Leaf tissue was stained with Calcofluor White M2R and observed under UV light. B) Same leaf surface as (A) shown by Nomarski interference optic. Arrows indicate altered cell structure due to hyphal colonization of mesophyll tissue

- **Screening of 5000 IR64 mutant lines in pools (50 plants/pool)**
- **3 potential increased rust growth mutants identified amongst two pools**
- **Inheritance and confirmation of phenotypes currently being investigated**

**Zhensheng Kang et al.**

# Summary

- Rice is a true nonhost of cereal rust and not an occasional host.
- At least 5 rust species are capable of infecting rice and producing large infection sites.
- Rice NHR response - reactive oxygen species, callose deposition, cell death
- Macroscopic phenotypic variation to rust infection
- Different rice lines show different responses to different rust species
- Loss of basal resistance to rice blast does not seem to affect the NHR response to stem rust (continuing with other rusts).
- Role for salicylic acid in NHR response to WSR ?
- Potential mutants with altered response to stripe rust identified.

**Priority:**

- Screen rice germplasm with additional stem rust races to look for large effect suitable for QTL analysis
- Screen additional rice mutants (target 10,000) with stem and stripe rusts
- Confirm phenotypes of promising mutants (T-DNA and IR64 mutants)
- Determine genetic inheritance of increased rust growth phenotypes

# Acknowledgements


**Macroscopic screen**

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Uni Minnesota, USA
**Yue Jin**
USDA/ARS, Uni Minnesota, USA

**Microscopic screen**

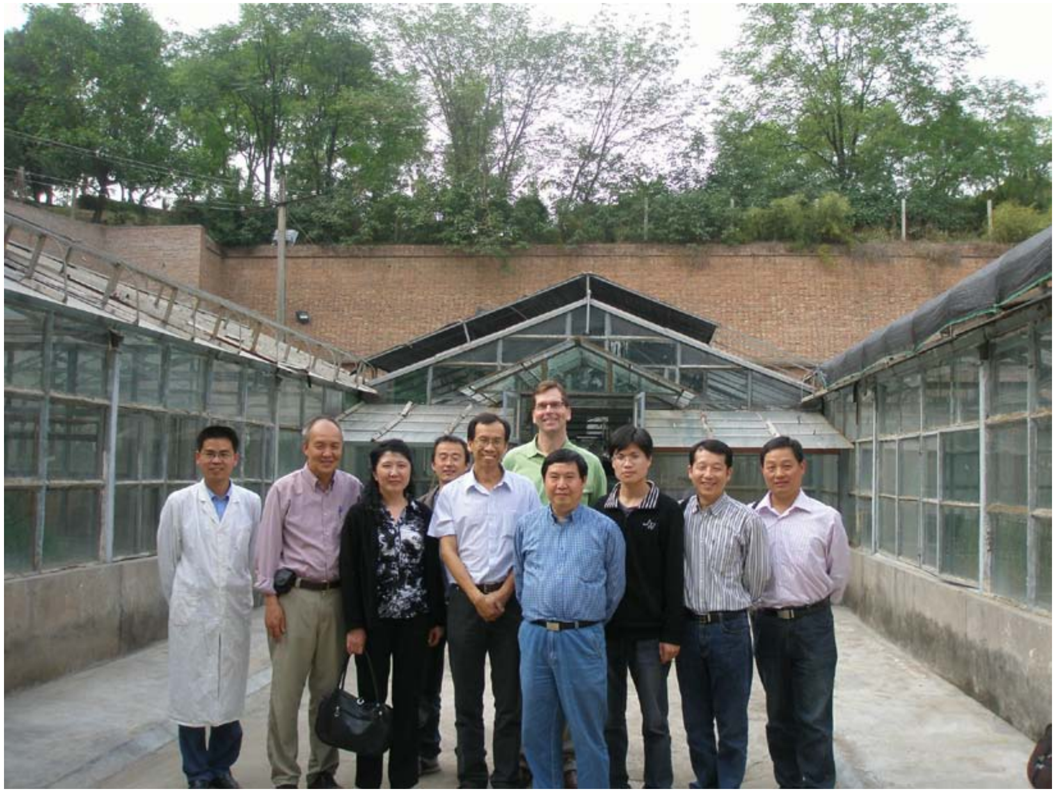
**Mick Ayliffe**
CSIRO Plant Industry, Australia
**Lachlan Lake**
CSIRO Plant Industry, Australia

**Stripe rust screen**

**Zhensheng Kang**
Northwestern A& F University
**Shiping Wang**
Huazhong Agricultural University

**Rice mutants/germplasm**

**Hei Leung**
IRRI, Philippines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