Gall midge- and brown planthopper-rice interaction — models for understanding insect-plant interaction

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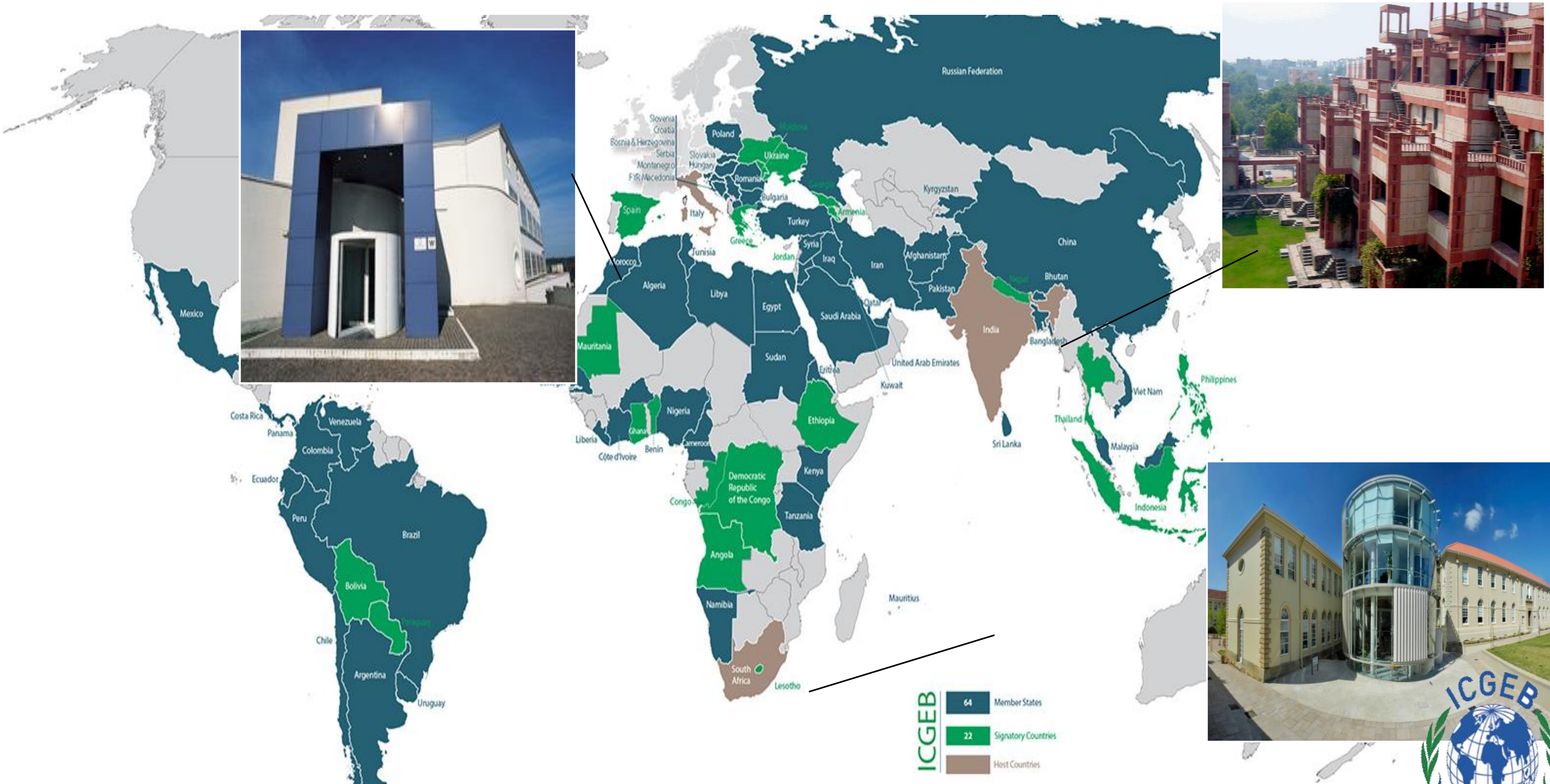
ICGEB

80+ Signatory States, 60+ Member States, 3 Components: Trieste (Italy) –
New Delhi (India) - CapeTown (South Africa) and a network of 40+ Affiliated Centres



The 5 instruments of ICGEB action

- Cutting-edge scientific **research** in its laboratories in Trieste, New Delhi and Cape Town
- Advanced training supported by long- and short-term **fellowships** for PhD students and post-docs
- Organisation of **Meetings**, Courses and Workshops at the international level
- Competitive research **grants** for scientists in Member Countries, including Early Career Return Grants
- **Technology transfer** to industry for the production of biotherapeutics and diagnostics

Introduction

- Plants face abiotic and biotic stress
  - Each confronts the plant with a particular set of challenges
- Plant adjusts its metabolism- Acclimation
  - Altered growth pattern – avoid sustained exposure to stress
- Abiotic stress due to environmental factors like
  - High or low temperatures
  - High soil salinity
  - Excess or depletion of water in soil
- Biotic stress due to pathogens-bacterial, fungal or viral and/or pests
- Crop yield affected – Rice being no exception
- India is one of the largest producer of rice which is a staple food here
- Apart from abiotic stress factors, rice yield threatened by major pests such as stem borer, brownplant hopper, the **rice gall midge**, the rice leaf roller.

Plant insect interaction

- Plants and insects have co-existed for >350 million years
- Some interactions beneficial to both
- But by and large the most common interaction involves insect predation of plants
- Plants on its part build up defences against these herbivores

↓

Leads to species diversity in both insect herbivores and hosts

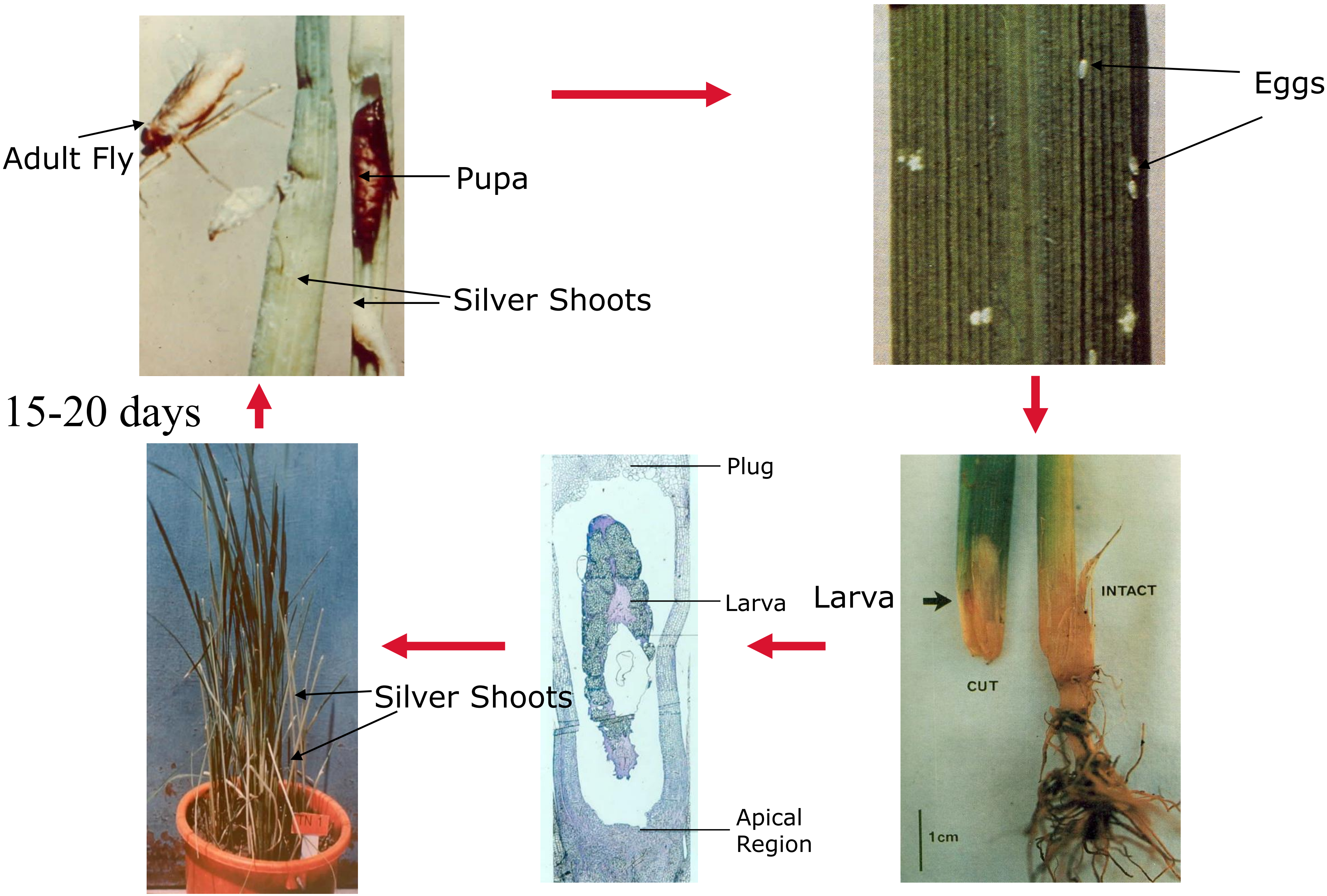
Crop lost to insect pests

About 14-18% of crops lost to insect pests (pre-harvest)!

- Decrease in arable land
- Pressure to use less pesticides
- Urgent need to increase food productivity

Host based resistance is the best way to solve this problem

Life Cycle of the Rice Gall Midge



Gall Midge Resistance genes tagged and mapped at ICGEB

Genes		Reference	Chromosome
Gm2	Mapping	**Theor Appl Genet (1994) 87:782**	**4**
	MAS	**Theor Appl Genet (1995) 91: 68**	
Gm4	MAS	**Theor Appl Genet (1996) 92:660**	**8**
	Mapping	**Theor Appl Genet (1997) 95:777**	
Gm7	Mapping & MAS	**Theor Appl Genet (2002) 105:691**	**4**
Gm8	Mapping & MAS	**Theor Appl Genet (2004) 109:1377**	**8**

METABOLIC PROFILING WITH GC-MS

Biological samples

- GMB1 challenged tissues from Resistant varieties Kavya (HR-) and RP2068 (HR+) and Susceptible variety TN1 and un-infested tissues from all 3 var.

Extraction using

- Organic solvent system- methanol: chloroform: water mixture

Extraction using

- Methanol

Derivatisation

- Of polar functional groups
- Using Methoxyamine hydrochloride and N-Methyl-N-(trimethylsilyl) trifluoroacetamide

Separation by GC

- On an RTX-5MS GC column (Restek Corporation) with 0.25mm thickness, 30m length, and 0.25mm diameter.

Ionization for MS

- To produce fragments and molecular ions

Detection of molecular ions

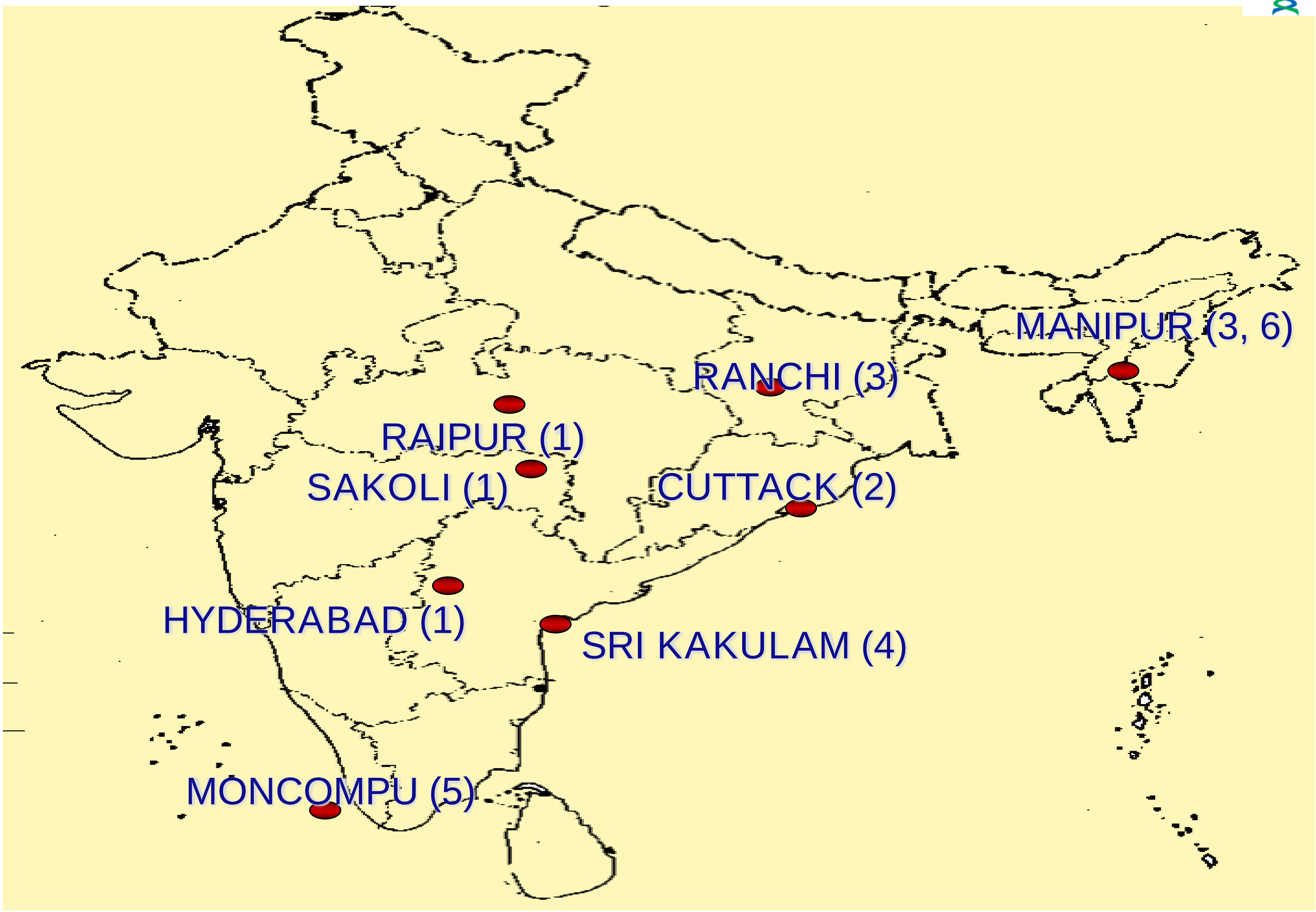
- By quadrupoles

EVALUATION OF DATA

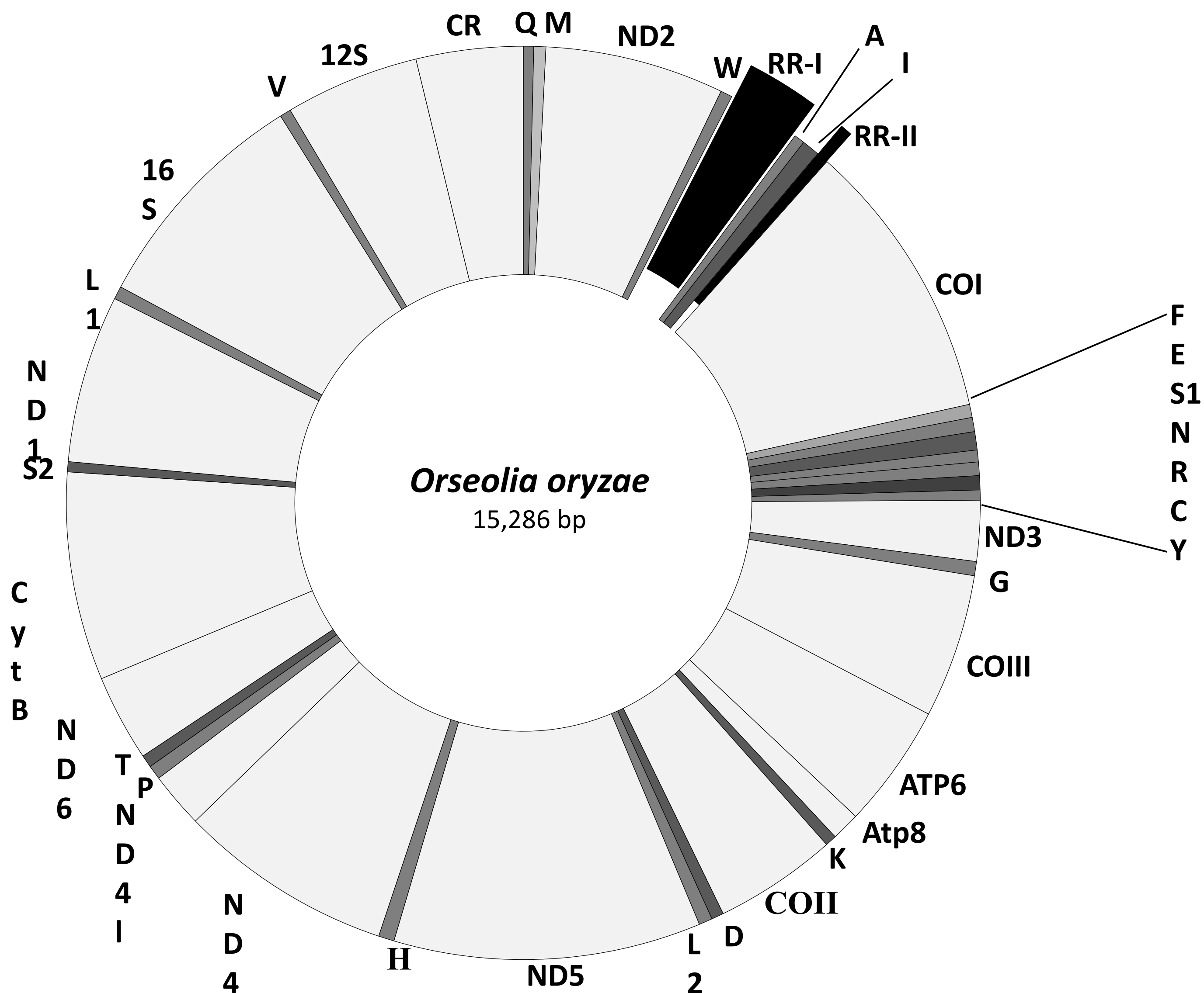
- Statistical analysis

Agarrwal et al 2016. Rice 9:5

DISTRIBUTION OF GALL MIDGE BIOTYPES



Mitochondrial Genome



Atray et al. 2015 PLoS ONE 10 (7): e0134625

Biotype differentiation based on Mt repeat regions

Repeat 1

Biotype/Species	**Repeat Motif Present**		
	TAAAA	**AAATT**	**TAAAT**
GMB 1	25	49	-
GMB 2	11	61	-
GMB 3	11	16 + 29	-
GMB 4	44	-	30-33
GMB 4M	41	28-37	-
GMB 5	-	-	-
GMB 6	5-7	52-58	-
Orseolia oryzivora	-	-	-
Orseolia fluvialis	-	-	-

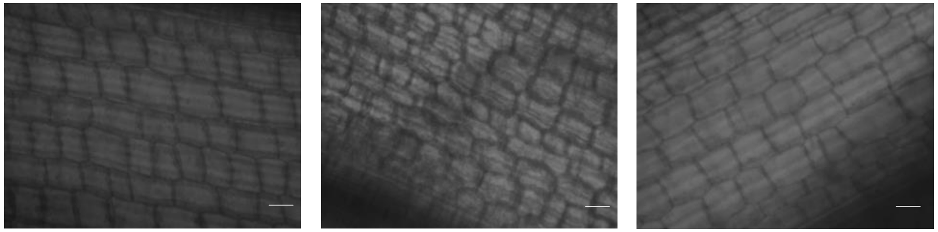
Repeat 2

Biotype/Species	**No. of Repeats**
GMB 1	5.4
GMB 2	None
GMB 3	None
GMB 4	3.4
GMB 4M	4.4
GMB 5	None
GMB 6	5.4*
Orseolia oryzivora	None
Orseolia fluvialis	None

*Not a perfect repeat

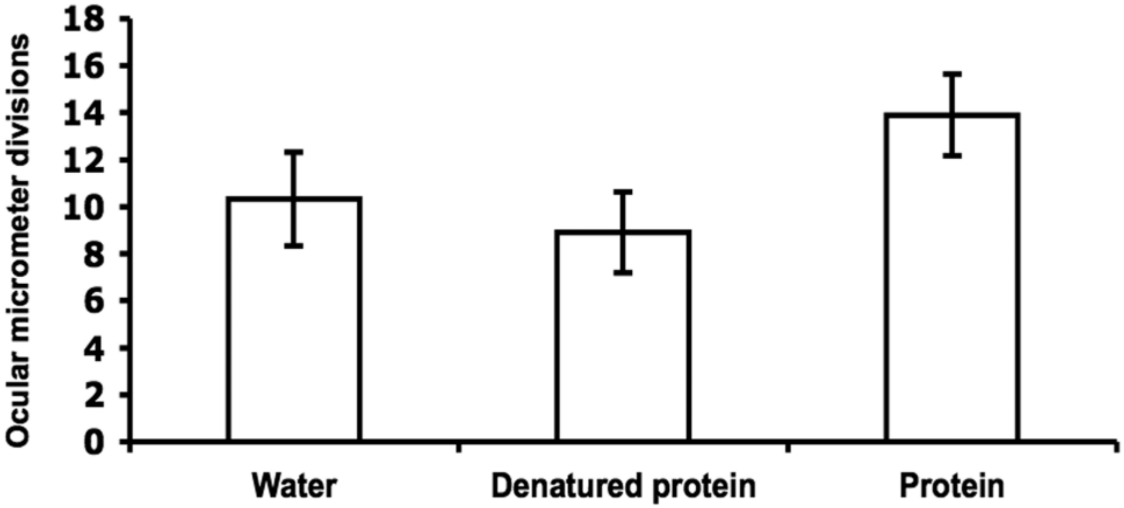
Atray et al. 2015 PLoS ONE 10 (7): e0134625

Coleoptile cell elongation assay



(A) (B) (C)

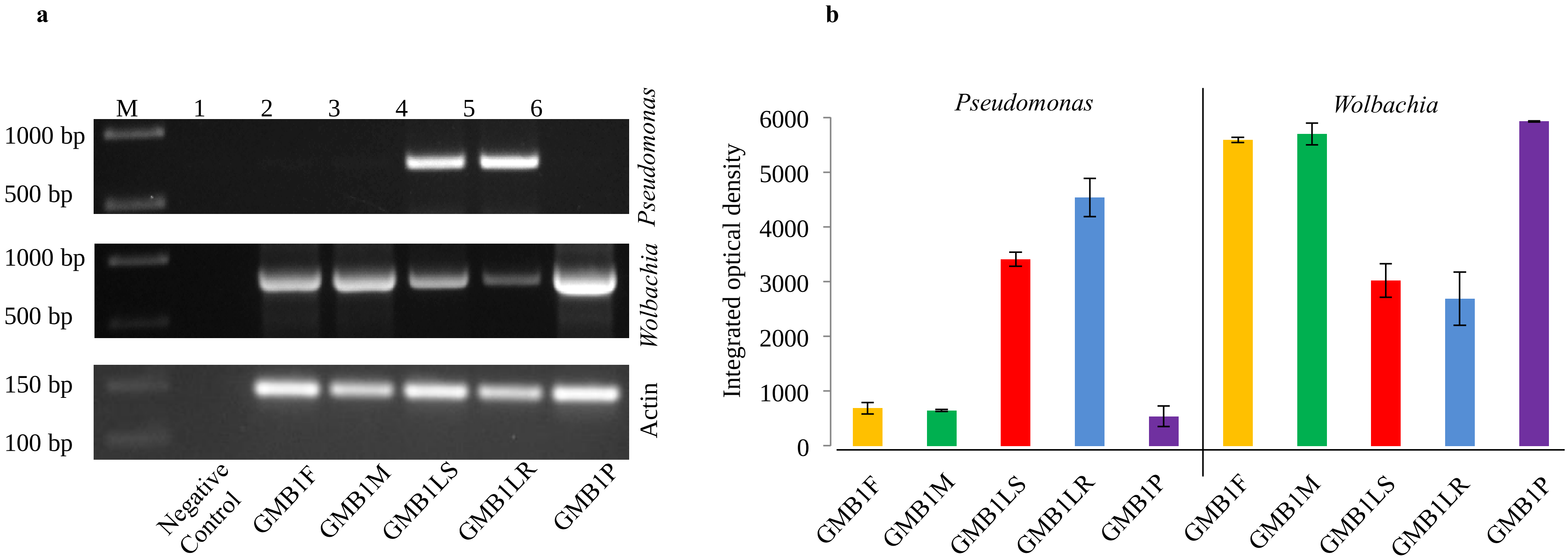
White bar=20µm



Susceptible rice variety used: Jaya

Sinha et al 2012. Insect Mol. Biol. 21: 593–603.

Rice gall midge metagenome

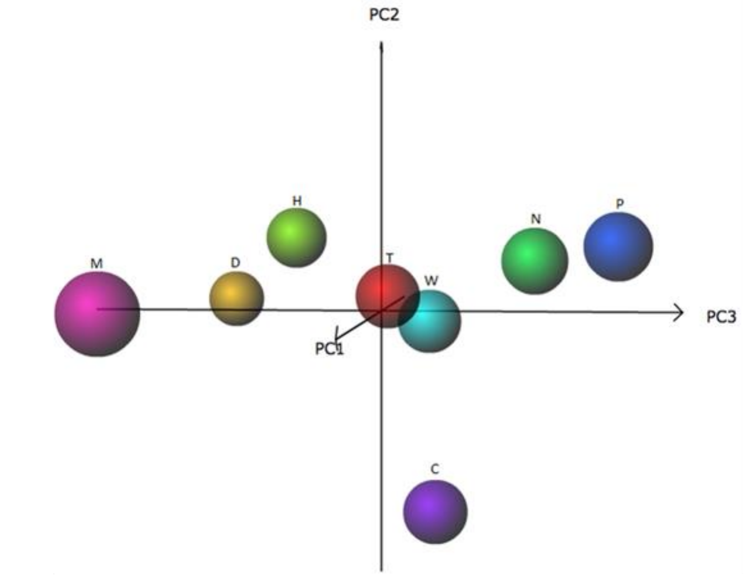


a, Semi quantitative PCR and **b**, image analyses of the agarose gel in 'a', for quantifying abundance of *Pseudomonas* and *Wolbachia* in different GMB1 samples. Actin gene served as the internal control.

Ojha et al 2017. Sci Rep 7, 9424

BPH-Rice interaction

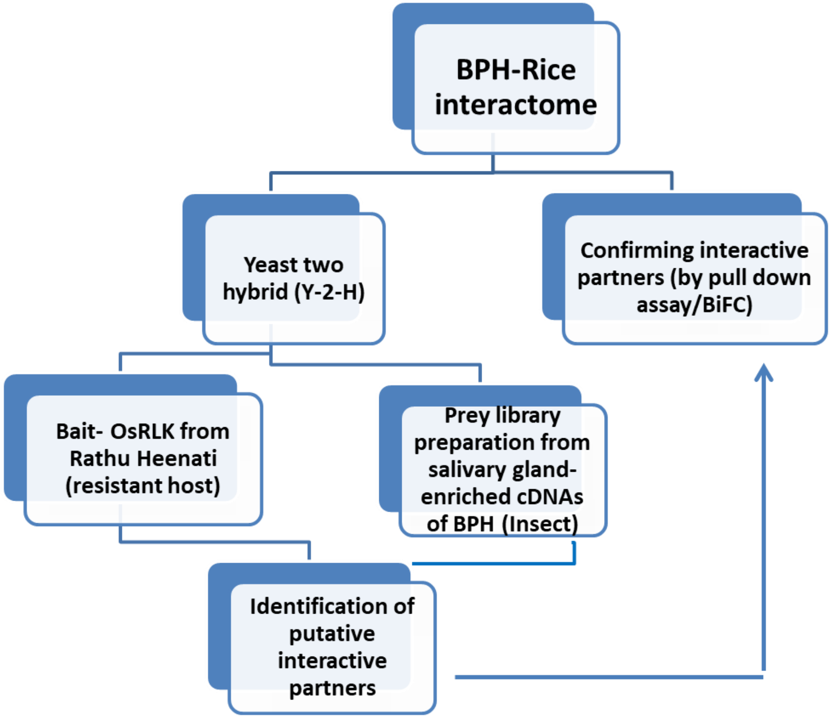
- Resistance to BPH is known to be due to combination of antibiosis and antixenosis.
- It is very important to identify the BPH population prevalent in an area so that rice varieties with the corresponding BPH resistance gene could be deployed in the field.
- Moreover, due to the migratory nature of BPH, populations are always in a state of flux.



C: Cuttack, D: Delhi, H: Hyderabad, M: Manipur, N: Nalgonda, P: Punjab, T: Tripura and W: Warangal.

Combining PCR markers, for tandem repeats present in the control region of the BPH mitochondria, along with digital restriction fragment length polymorphisms (d-RFLP), eight BPH populations obtained from different rice growing regions of India

BPH-Rice interactions



BPH resistance gene *Bph3* (*LecRK1*, *LecRK2*, *LecRK3*)
Rice variety: Rathu Heenati

Candidate gall midge resistance genes identified and being validated through transformation of the susceptible rice variety TN1

- *Gm4* (type: NBS-LRR) (**Divya et al 2015**).

- *Gm8* identified in rice variety Aganni, by quantitative PCR suggested that Aganni has a deviant form of inducible resistance that is salicylic acid (SA)-mediated, but without invoking HR (**Divya et al 2016**).

Divya et al 2015 Euphytica 203, 185–95

Divya et al 2016 Funct. Integr. Genomics 16, 153-69

Current interests

1. **Whole genome sequencing of the rice gall midge:**
- Help in dissecting the molecular events occurring inside the insect during its interaction with rice host
- Various factors that synchronize the interaction

2. **Study the mitochondrial genome:**
- Increase the amount of information present on phylogenetic relationships with other insects
- Interpret broader aspects of genome evolution

3. **Transformation of susceptible rice variety TN1 with *Gm4* candidate resistance gene** (in collaboration with other PMB Group PIs)
4. **Metagenome of the rice gall midge and BPH**
5. **Rice-brown planthopper (BPH) interaction**

To Sum up……..

- Less reliance on and use of pesticide will not only slow down the degradation of the ecosystem but will also contribute to the prevention of loss of biodiversity
- Moreover, using natural resistance we can produce more in terms of crop yield; then there will be less pressure to convert forestland to farmland. This will also prevent loss of biodiversity. Green cover loss is slowed down.
- Continued screening of germ plasm will ensure that new sources of insect resistance genes are identified and ensure that these donors are conserved before they become extinct.

My Reccomendations

1. Carry out vigorous screening of crop germ plasm to identify appropriate resistance genes against major pests

2. Encourage strategic research to get a better understanding of key insect plant-interactions, specially those of extremely important crop plants such as rice, wheat and maize

3. Device molecular tools to get a better understanding of insect pests specially with regard to their population structure, migratory patterns and breeding and feeding behaviour.

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