CEREALIMMUNITY: an integrated project to unveil immunity mechanisms to fungal pathogens in rice and wheat
Generation Challenge Program – SP2
2005-2007

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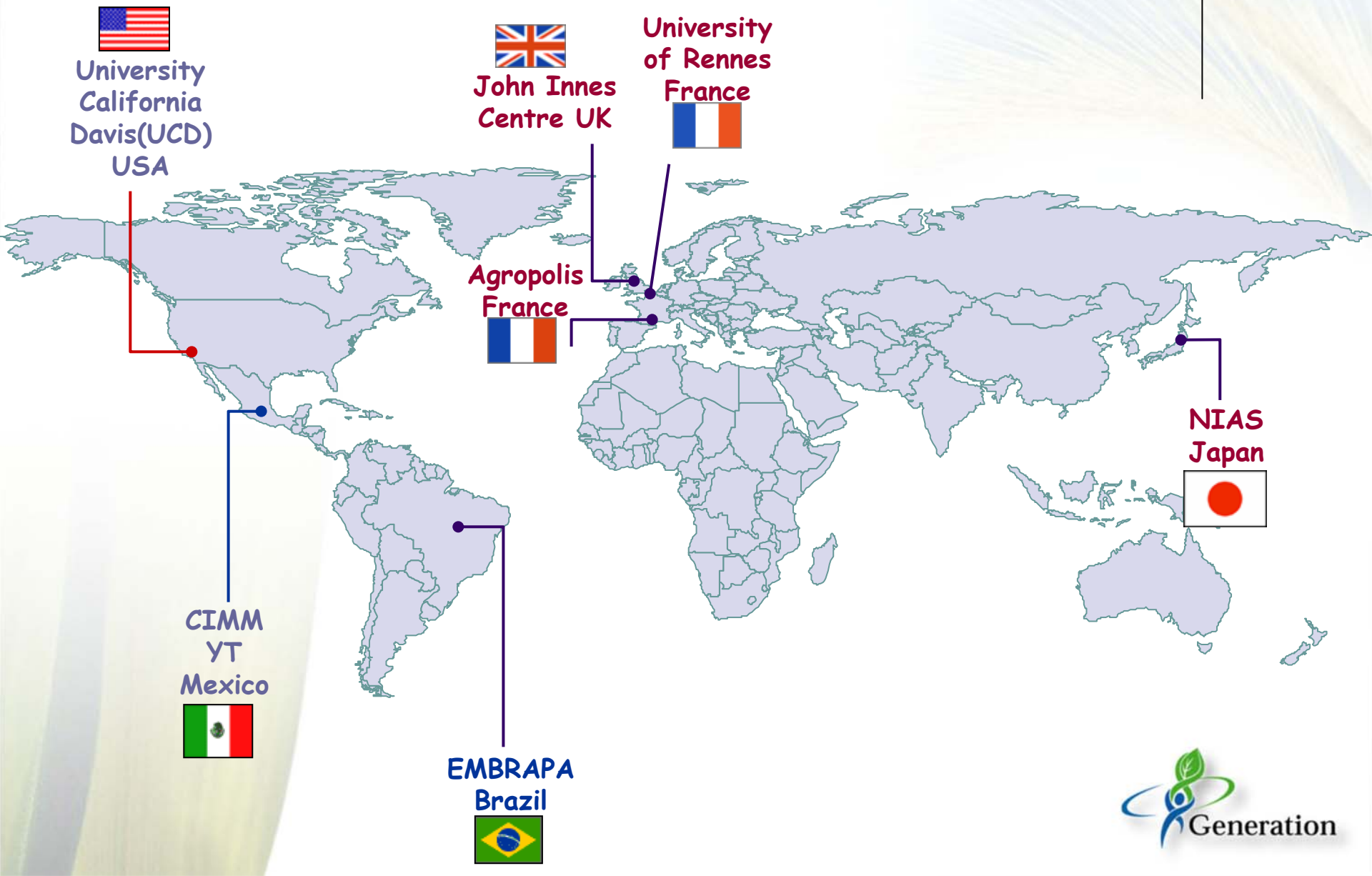
Generation
Cultivating Plant Diversity for the Resource-Poor

Cerealimmunity Participants



Generation

Non-host resistance: the plant's immune system

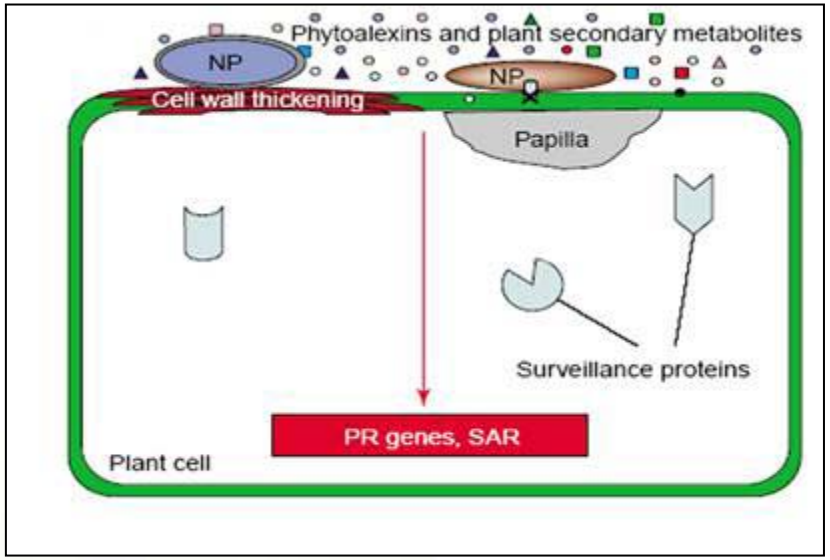
- Resistance shown by a plant species to the majority of potentially pathogenic microbes is known as **non-host resistance**
- Non-host resistance represents one of the most promising defence mechanisms in developing durable resistance against plant pathogens, namely due to its effectivemenss against a broad range of pathogen species and its durability in nature
- The majority of identified and characterised **non-host specific genes** have novel functions when compared to known host-specific resistance and defence-related genes – pointing to the existence of novel signalling and effector components and at the existence of novel sources of broad-spectrum resistance in plants

Generation

Non-host resistance

Type 1

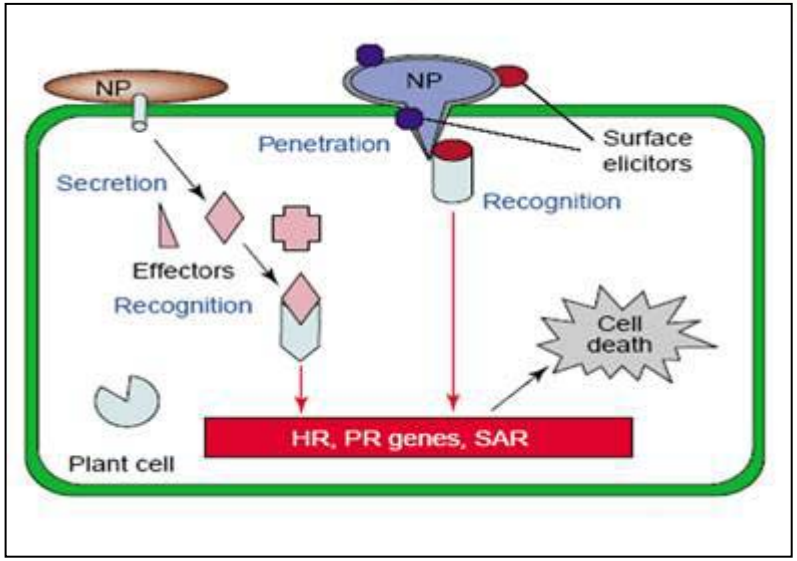
no visible phenotypes



Early block of penetration
Early detection
of the micro-organism

Type 2

associated to HR-like phenomena

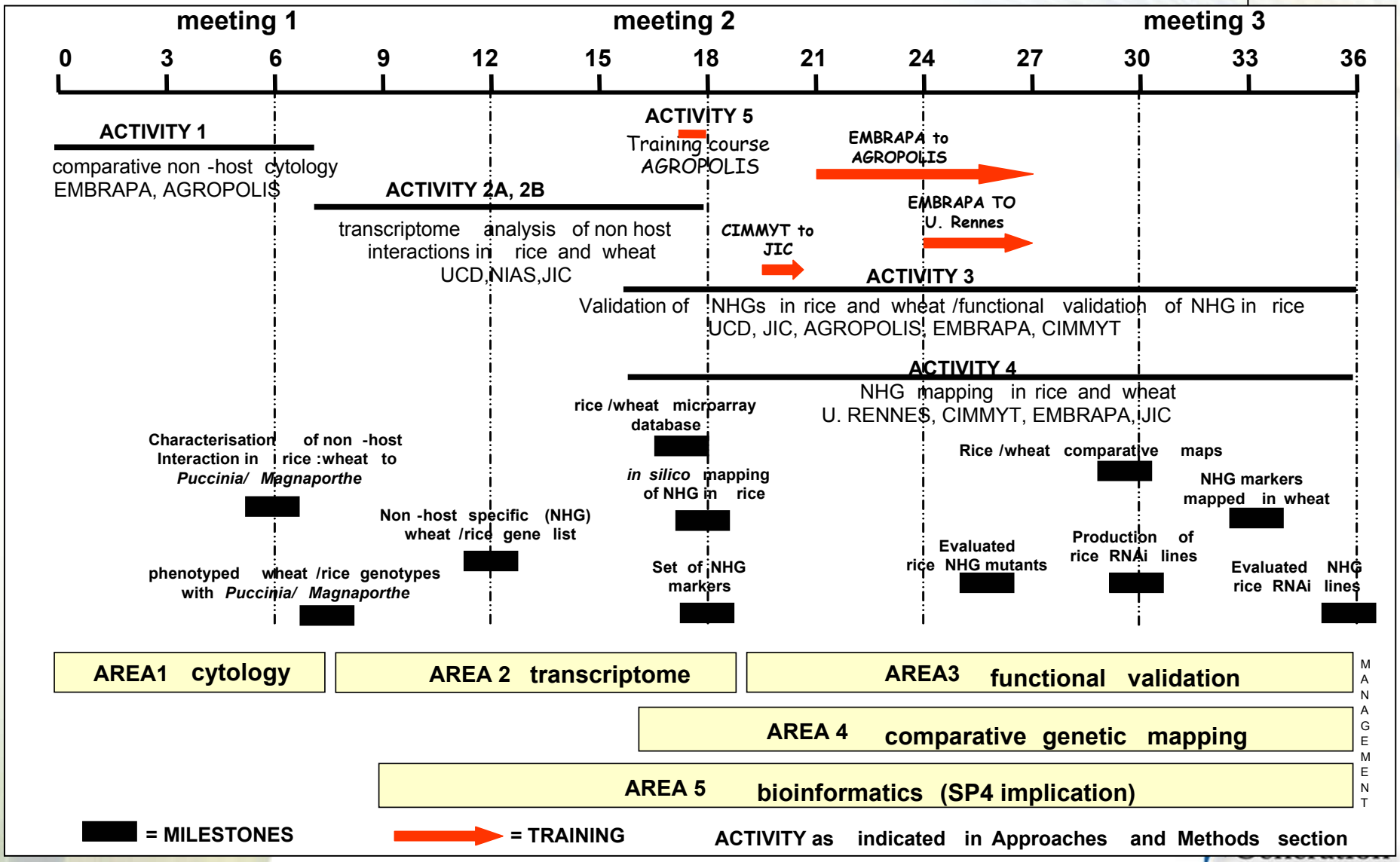


Elicitor recognition
Possible convergence to R gene-mediated resistance

NH resistance involves a combination of preformed and inducible defense responses

Generation

Cerealimmunity Timeline/Milestones



Cerealimmunity objectives

- **Objective 1** comparative phenotypic and cytological characterisation of non-host interactions in rice and wheat with Magnaporthe and Puccinia strains
- **Objective 2** identify and validate a set of differentially expressed non-host specific genes (NHG) in rice and wheat
- **Objective 3** functional analysis of NHGs in rice
- **Objective 4** genotyping and phenotyping of wheat and rice mapping populations/germplasm for non-host resistance to M. grisea and Puccinia strains
- **Objective 5** enhance NARS/CGIAR capacity in functional genomics and molecular marker technology

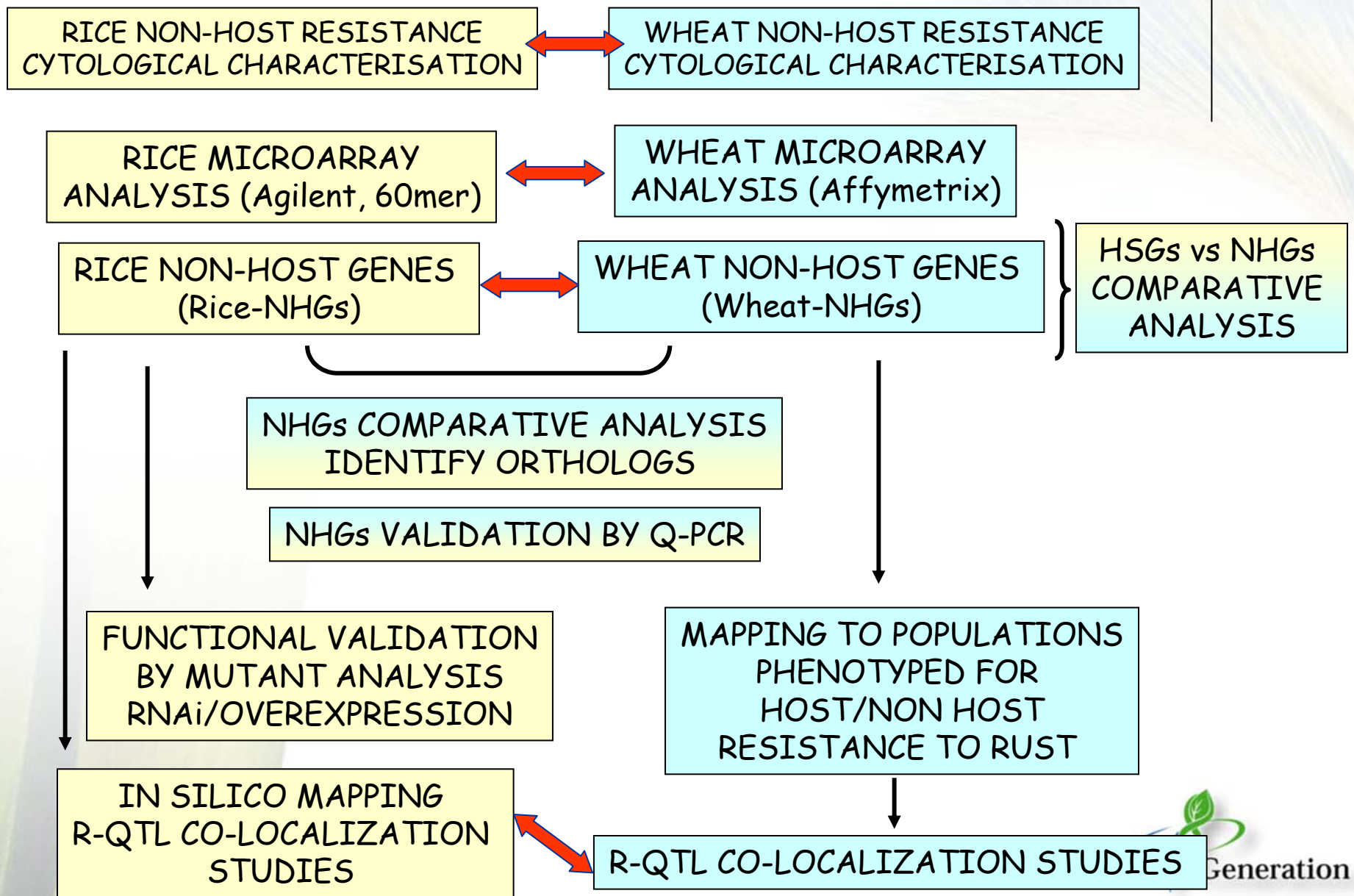
Generation

Cerealimmunity project outline

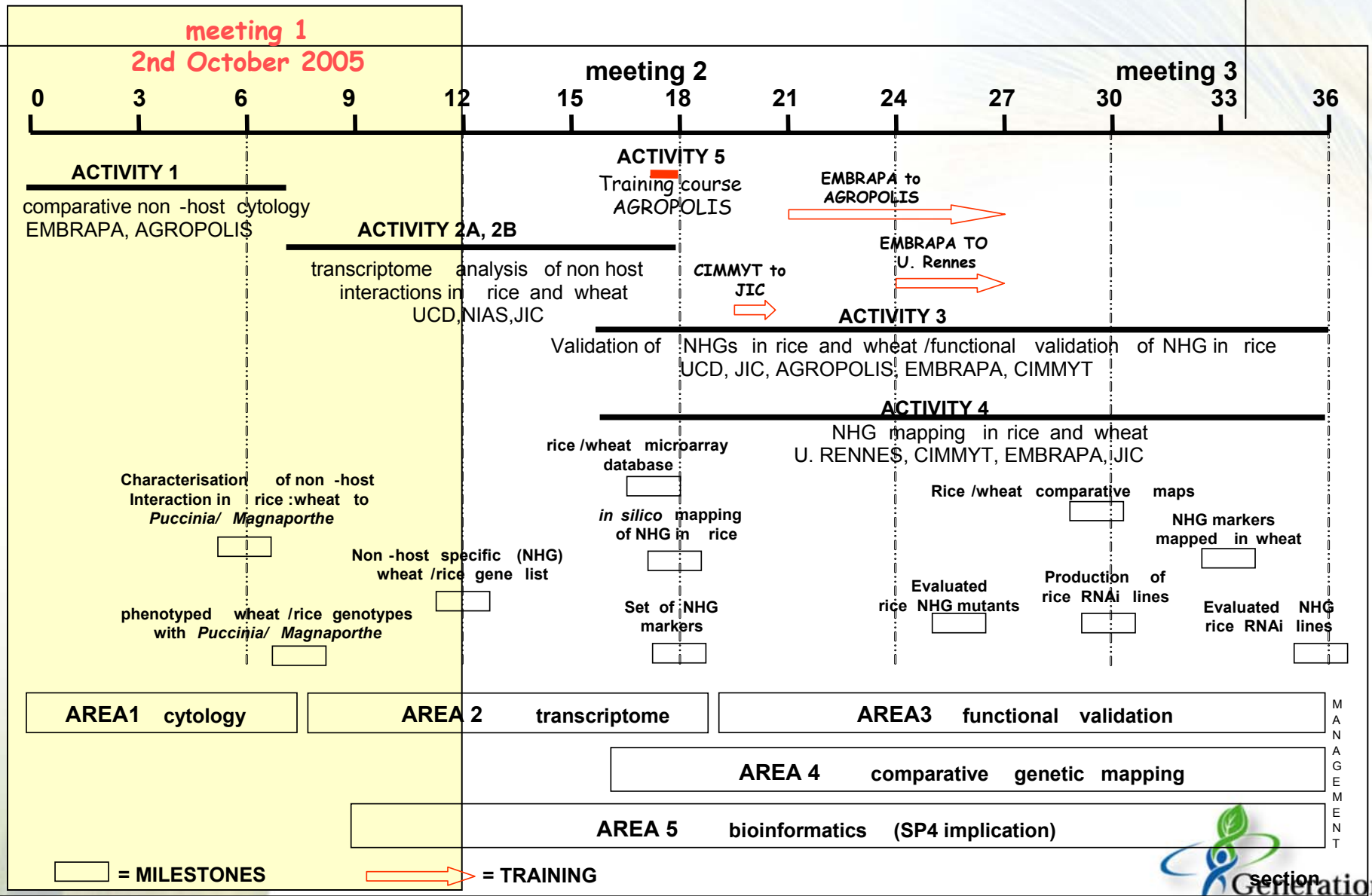
- ACTIVITY 1 Comparative cytological analysis of non-host interactions to *Magnaporthe grisea* and *Puccinia* strains in rice and wheat
- ACTIVITY 2A, 2B Non-host transcriptome analysis in rice and wheat
- ACTIVITY 3A NHGs validation
- ACTIVITY 3B Functional analysis in rice/ *in silico* mapping
- ACTIVITY 4 Genetic mapping in wheat

Generation

Cerealimmunity experimental approach



Cerealimmunity 1st year outline



section Generation

Cerealimmunity YEAR 1

Objective 1 comparative phenotypic and cytological characterisation of non-host interactions in rice and wheat with *Magnaporthe* and *Puccinia* strains

Activity 1 phenotypic and cytological characterisation of non-host interactions in rice and wheat with M. grisea and Puccinia strains

AIMS

- Identify most suitable strains to carry out transcriptome analysis, rice mutant analysis, wheat phenotyping
- Define cellular events leading to non-host resistance to M. grisea and Puccinia in rice and wheat
- Provide suitable material for activity 2 (transcriptome analysis)

INSTITUTIONS INVOLVED: AGROPOLIS, EMBRAPA, JIC

Generation

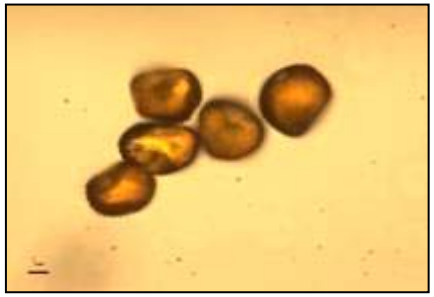
Cerealimmunity YEAR 1

Objective 1 comparative phenotypic and cytological characterisation of non-host interactions in rice and wheat with *Magnaporthe* and *Puccinia* strains

Activity 1 phenotypic and cytological characterisation of non-host interactions in rice and wheat with M. grisea and Puccinia strains

AGROPOLIS	focus on rice-Magnaporthe grisea non-host interaction
	focus on rice-Puccinia triticina non-host interaction
EMBRAPA	focus on rice-Magnaporthe grisea non-host interaction
	focus on rice Puccinia non-host interaction
JIC	focus on wheat-Puccinia striiformis non-host interaction
	focus on wheat-Magnaporthe grisea non-host interaction

Generation



Non-host pathosystem

Rice - leaf rust

Oryza sativa – Puccinia triticina

Puccinia triticina

BASIDIOMYCETE

Uredinales

Obligate biotrophic fungus

Pathogenic agent of leaf rust in wheat

Oryza sativa L.

MONOCOT

Poales

Whole genome sequenced

Model species for monocots

Generation

NON-HOST RESISTANCE
the Rice-*Magnaporthe* model system

Magnaporthe grisea

Model phytopathogen

Intracellular hemybiotroph

Fully sequenced genome

Attacks a large spectrum of cereal crops and other monocots

Oryza sativa

Model crop for monocots

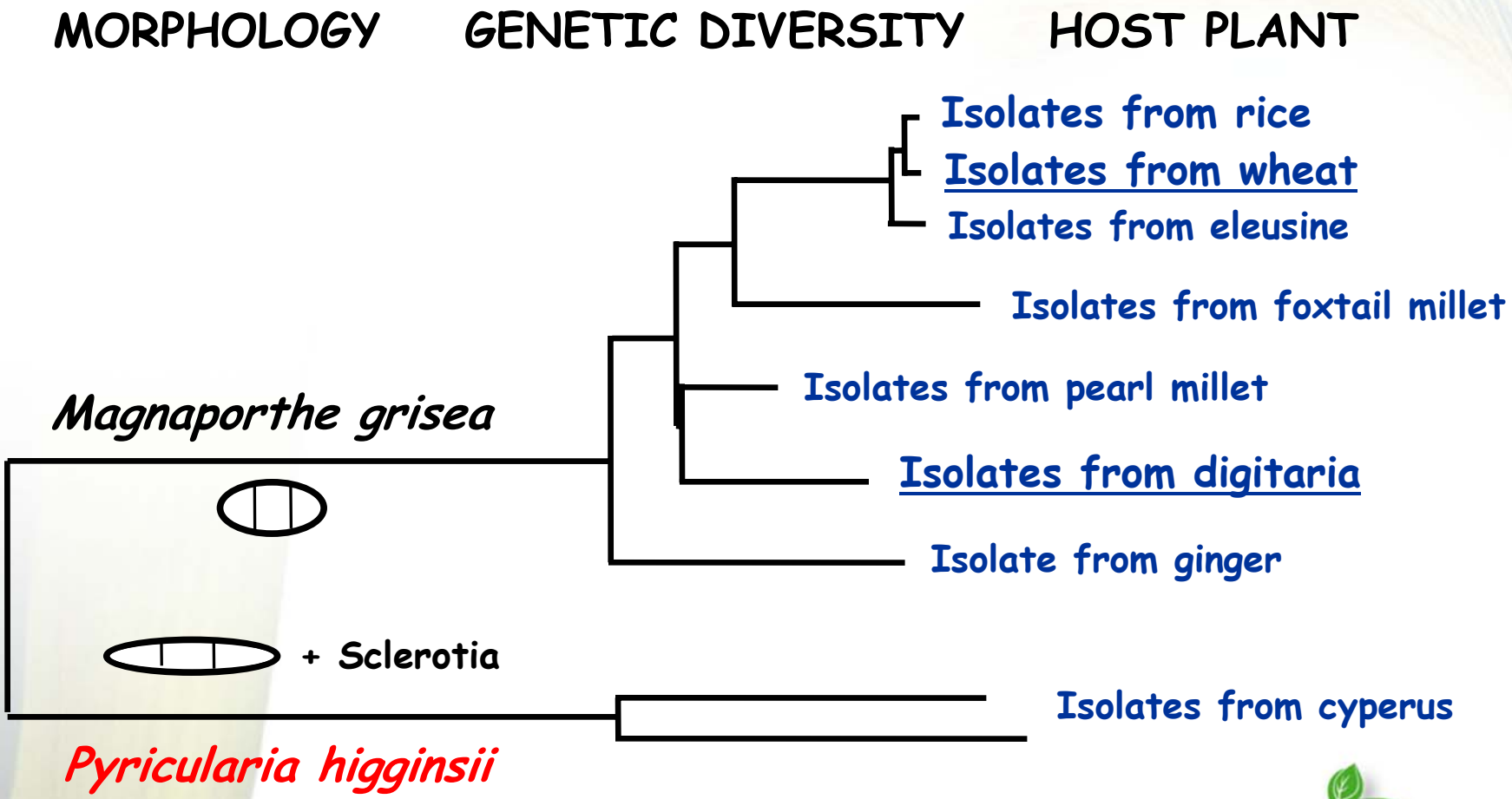
Fully sequenced genome

Large spectrum of genetic and molecular tools

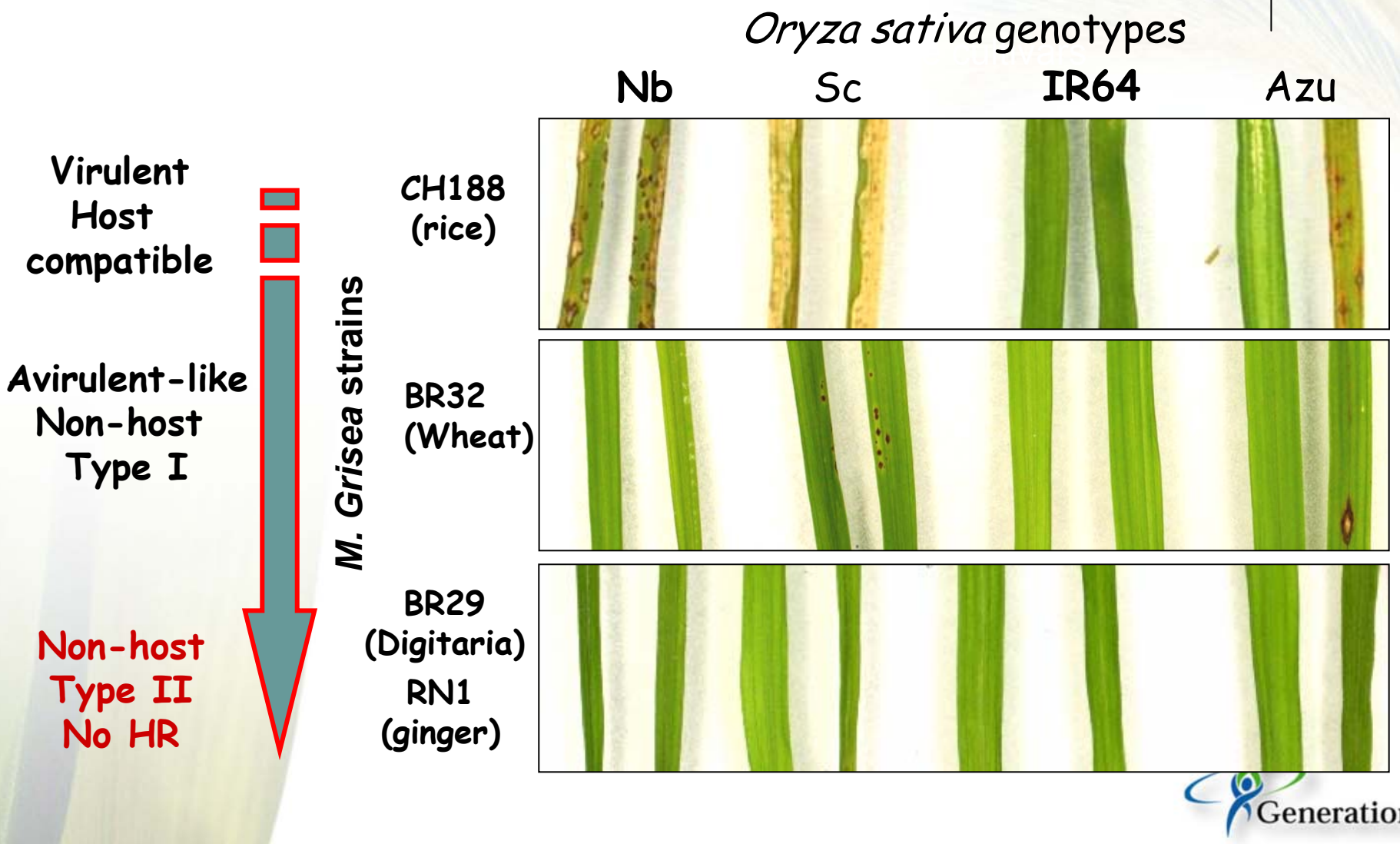
Syntenic relationships with other cereal crops

Generation

NON-HOST RESISTANCE
the Rice-*Magnaporthe* model system



Characterisation of non-host phenotypes in rice

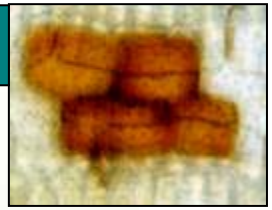


Characterisation of non-host cellular phenotypes in rice

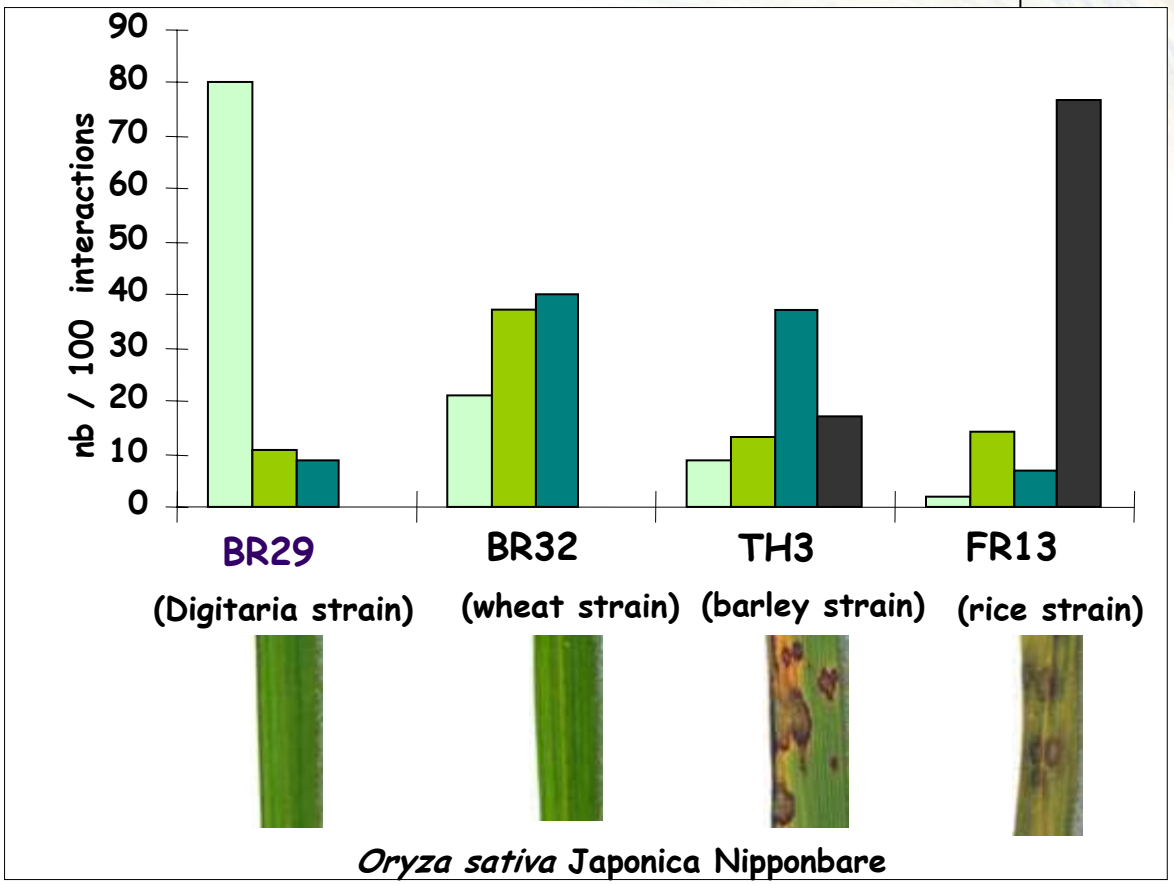
Type I

Type II

Type III



Type IV



Non-host

Host

EMBRAPA DATA

Isolates utilized in the inoculation tests, their orign, collection site and year of collection

Isolates	Host/Cultivar	Location/State	Year
Py 182	Wheat /Anahuac	Mato Grosso do Sul	1988
Py 5994	Wheat /-	Rio Verde/Goiás	2002
Py 7596	Wheat / PF 89375	Montividiu / Goiás	2004
Py 7608	Wheat /BR 17	Costa Rica / Mato Grosso do Sul	2004
Py 183	Wheat /Anahuac	Mato Grosso do Sul	1988
Py 5996	**Wheat /-**	**Rio Verde/Goiás**	**2002**
Py 7601	Wheat /BR 17	Costa Rica / Mato Grosso do Sul	2004
Py 201	Wheat / Anahuac	Mato Grosso do Sul	1995
Py 7612	Wheat / BH 1146	Alto Taquari / Mato Grosso	2004
Py 7618	Wheat / BH 1146	Alto Taquari / Mato Grosso	2004
Py 204	Wheat / Anahuac	Mato Grosso do Sul	1988
Py 7599	Wheat / PF 89375	Montividiu / Goiás	2004
Py 7606	Wheat / BR 17	Costa Rica / Mato Grosso do Sul	2004
Py 7600	Wheat / BR 17	Minas Gerais	2004
Py 212	Grass / *Digitaria horizontalis*	Goiás	1989
Py 195	Grass / *Eluesine indica*	Goiás	1989
Py 5990	Barley /-	Rio Verde/Goiás	-
Py 3970	Rice / Bonança	Goiás	2002

Generation

EMBRAPA DATA

14 wheat cultivars, one rice cultivar and one barley cultivar were utilized for inoculation tests. Inoculations were made with aqueous spore suspension ($3x10^5$ conidia per ml) on 21-day old plants, using 18 isolates of *M. grisea*

These 14 wheat cultivars will be inoculated with 40 races of *Puccinia recondita* at Passo Fundo to select two virulent races and three wheat cultivars

Test 8 rice cultivars including IR64 with *P. recondita* races

This work is underway.

Cultivars	Mean of severity(%)
Ágata	95,41
Aliança	89,37
Brilhante	76,59
BR 18	56,11
Embrapa 22	85,34
BR 33	86,73
Embrapa 42	90,13
BRS 207	68,33
BRS 208	89,86
BRS 210	81,52
BRS 234	68,75
BRS 254	81,87
BRS 264	87,98
Pioneiro	88,33
Barley	95,48
Rice (Bonança)	0,00

Disease severity on wheat cultivars in inoculation tests with18 isolates of *Maganaporthe grisea*

Generation

Cerealimmunity YEAR 1

- **Objective 1** comparative phenotypic and cytological characterisation of non-host interactions in rice and wheat with *Magnaporthe* and *Puccinia* strains

Activity 1 phenotypic and cytological characterisation of non-host interactions in rice and wheat with M. grisea and Puccinia strains

AGROPOLIS

***Rice-Magnaporthe grisea* non-host interaction**

JUNE 2005 defined 2 *M. grisea* strains (wheat and Digitaria) and 2 rice genotypes (Nipponbare and IR64) to carry out detailed cytological and transcriptome analysis – two time-points 16HPI, 24HPI

NIPPONBARE/wheat M. grisea strain

NIPPONBARE/Digitaria M. grisea strain

IR64/wheat M. grisea strain

IR64/Digitaria M. grisea strain

Generation

Cerealimmunity YEAR 1

- **Objective 2** identify and validate a set of differentially expressed non-host specific genes (NHG) in rice and wheat

Activity 2 »in depth » transcriptome analysis of non-host interaction in rice and wheat

INSTITUTIONS INVOLVED: AGROPOLIS, NIAS, UCD, JIC

AGROPOLIS generation of RNA sample to be sent to NIAS and UCD
NIAS transcriptome analysis – Rice IR64 samples
UCD transcriptome analysis – Rice Nipponbare samples
JIC transcriptome analysis – wheat samples

To discuss during 1st Year Cerealimmunity Meeting October 2nd:

- experimental design microarray analysis (biological replicates)
- bioinformatic data analysis

Generation

Cerealimmunity YEAR 1

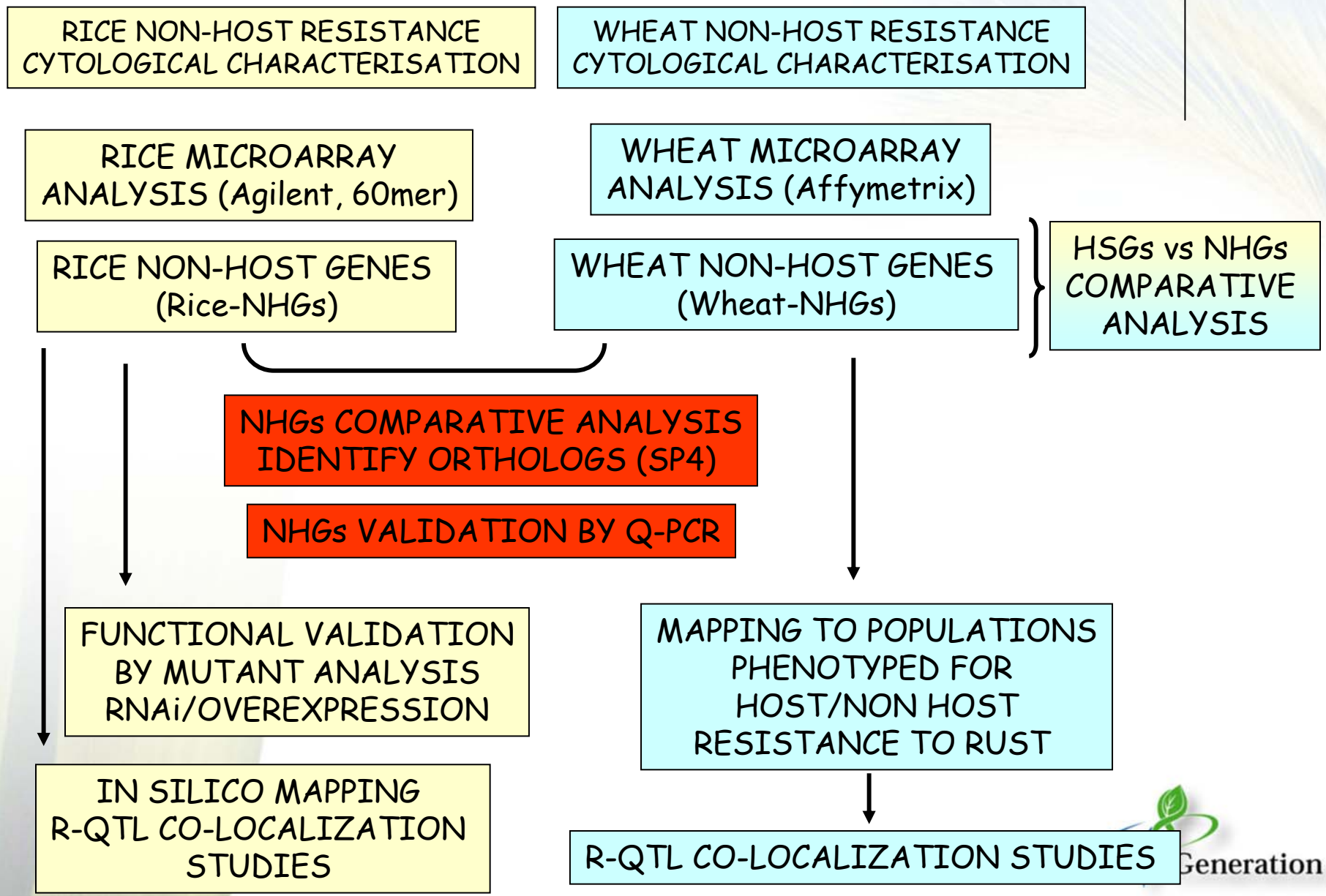
Objective 2 identify and validate a set of differentially expressed non-host specific genes (NHG) in rice and wheat

Activity 2B validation of NHGs identified by microarray analysis using Q-PCR technology – comparative analysis in host and non host interactions in rice and wheat

INSTITUTIONS INVOLVED: AGROPOLIS, JIC

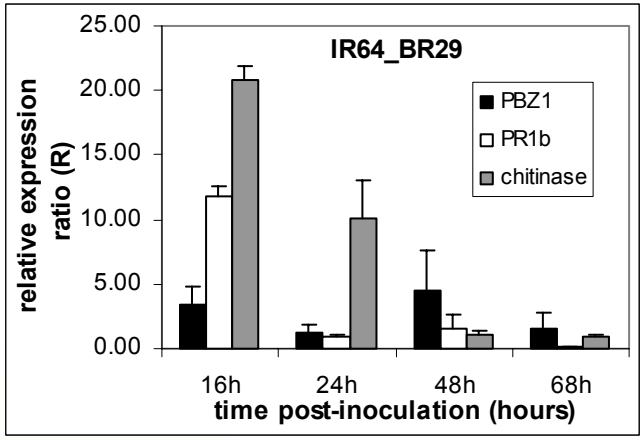
Generation

Cerealimmunity experimental approach

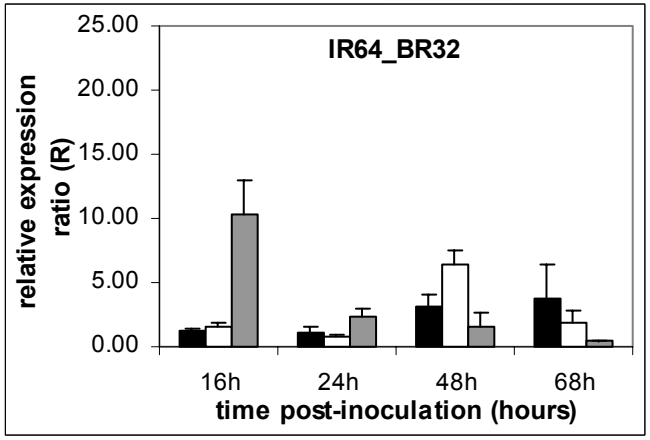


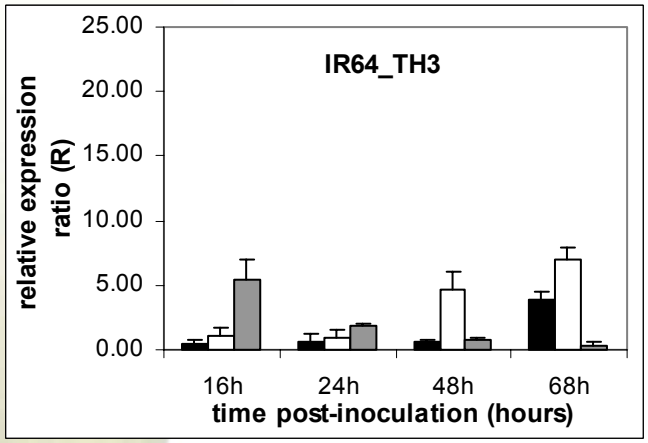
Comparative Q-PCR analysis for host and non-host resistance to *M. grisea* in rice

NON-HOST TYPE II

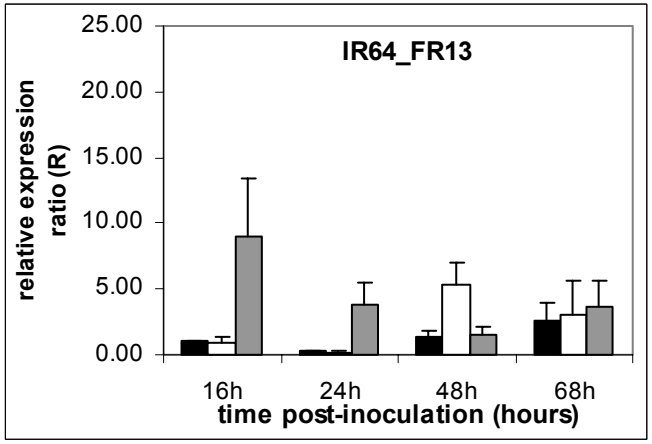


NON-HOST TYPE I





NON-HOST TYPE I



HOST-SPECIFIC

Generation

Cerealimmunity experimental approach

RICE NON-HOST RESISTANCE CYTOLOGICAL CHARACTERISATION

WHEAT NON-HOST RESISTANCE CYTOLOGICAL CHARACTERISATION

RICE MICROARRAY ANALYSIS (Agilent, 60mer)

WHEAT MICROARRAY ANALYSIS (Affymetrix)

RICE NON-HOST GENES (Rice-NHGs)

WHEAT NON-HOST GENES (Wheat-NHGs)

HSGs vs NHGs COMPARATIVE ANALYSIS

NHGs COMPARATIVE ANALYSIS IDENTIFY ORTHOLOGS

NHGs VALIDATION BY Q-PCR

FUNCTIONAL VALIDATION BY MUTANT ANALYSIS RNAi/OVEREXPRESSION

MAPPING TO POPULATIONS PHENOTYPED FOR HOST/NON HOST RESISTANCE TO RUST

IN SILICO MAPPING R-QTL CO-LOCALIZATION STUDIES

R-QTL CO-LOCALIZATION STUDIES

Generation

Phenotyping for compromised non host resistance to *M. grisea* in rice

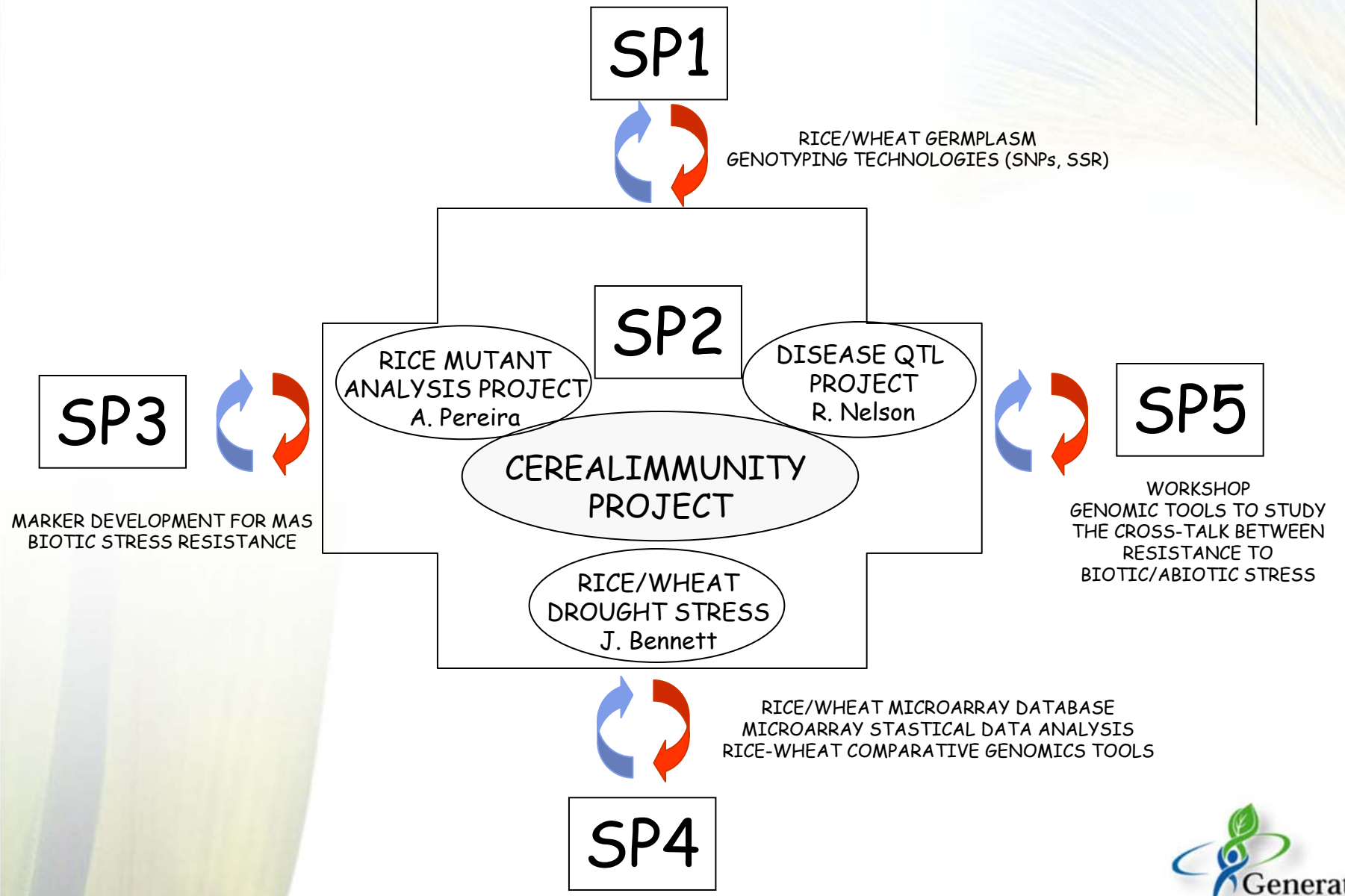
Sari Celtic Positive Control

Nipponbare Control

T-DNA lines with compomised non-host resistance to M. grisea strain BR32 (wheat strain)

Generation

GCP Networking



Generation

Future Prospects

• Comparative transcriptome analysis of non-host interaction to Magnaporthe and Puccinia in rice and wheat

CHALLENGES AHEAD

- suitable bioinformatics tools for comparative analysis of rice and wheat transcriptomes (implication of SP4)

- co-localization studies QTL quantitative/broad-spectrum resistance in rice and wheat (implication of SP4)

- define cross-talk between biotic and abiotic stress resistance signalling and effector components (Workshop 2006, SAG database)

Generation

Presentation Agropolis Team

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Head Rice Phytopathology Team

D. Tharreau didier.tharreau@cirad.fr AGROPOLIS – CIRAD
Researcher – M. grisea diversity

J-B Morel jbmorel@cirad.fr AGROPOLIS – INRA
Researcher – rice/Magnaporthe grisea host interaction – molecular approach

E. Guiderdoni guiderdoni@cirad.fr AGROPOLIS – CIRAD
Head of Rice functional genomics Unit

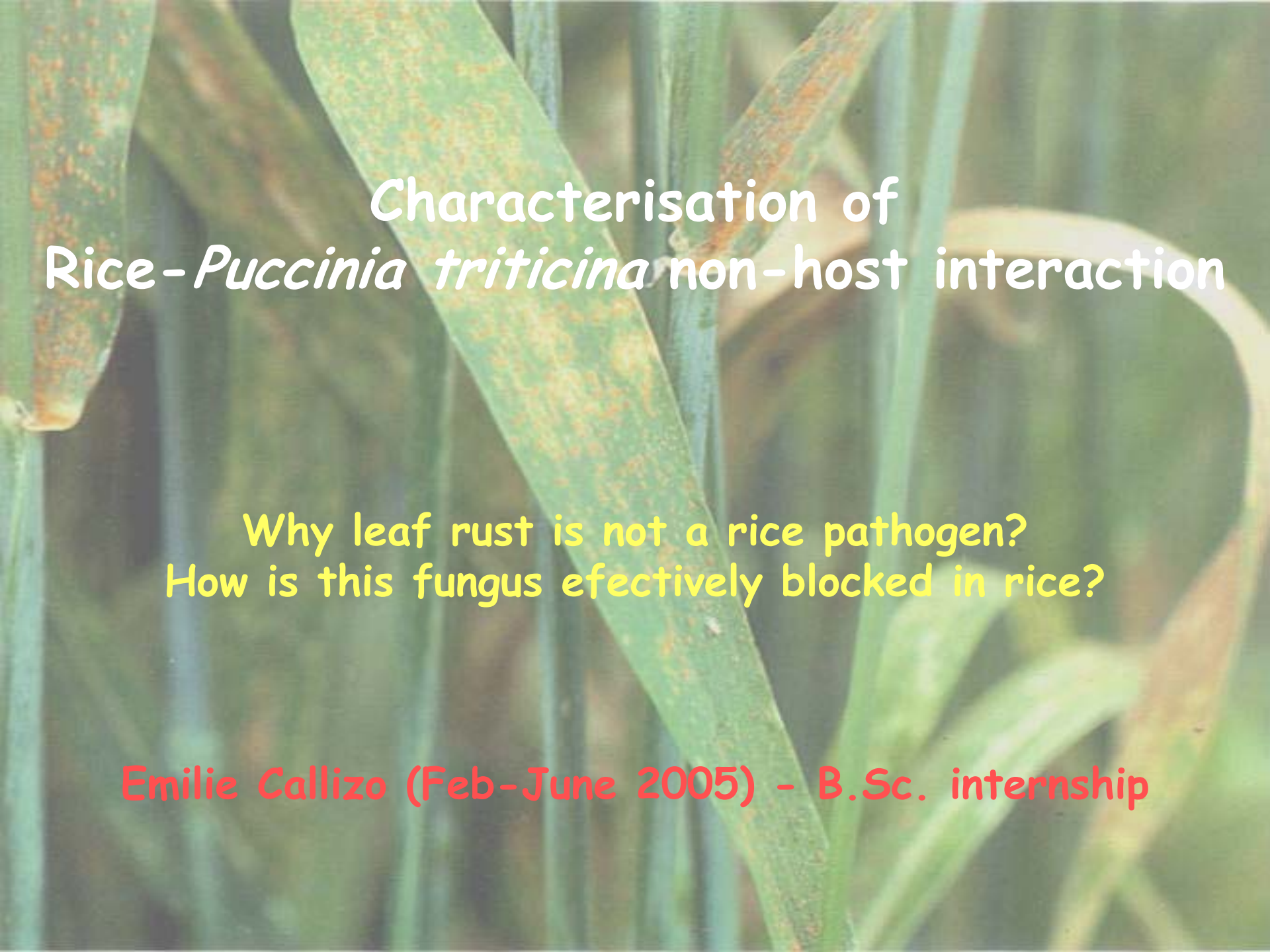
P. Piffanelli piffanelli@cirad.fr AGROPOLIS- CIRAD
Researcher – rice non-host interaction – cytology/molecular approach

Generation

Presentation Agropolis Team ongoing research activities

- Rice-Magaporthe grisea non-host model system
- Rice- Puccinia non-host interaction system

Generation

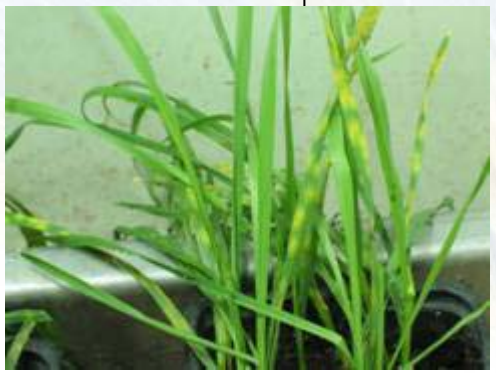


Characterisation of Rice-*Puccinia triticina* non-host interaction

Why leaf rust is not a rice pathogen?
How is this fungus efectively blocked in rice?

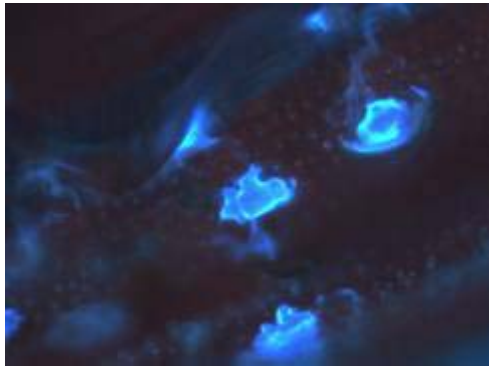
Emilie Callizo (Feb-June 2005) - B.Sc. internship

Analysis of Rice-Puccinia triticina non host interaction



- **Phenotypic analysis**

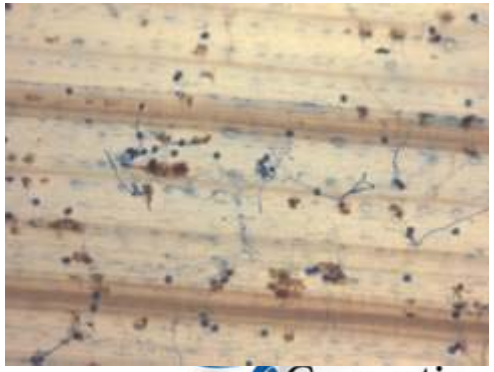
• macroscopic observation at different time points following inoculation with hig doses of P. triticina spores.



- **Microscopic analysis at 24 and 48 hours following inoculation**

Calcofluor

• observation of germination and leaf surface events



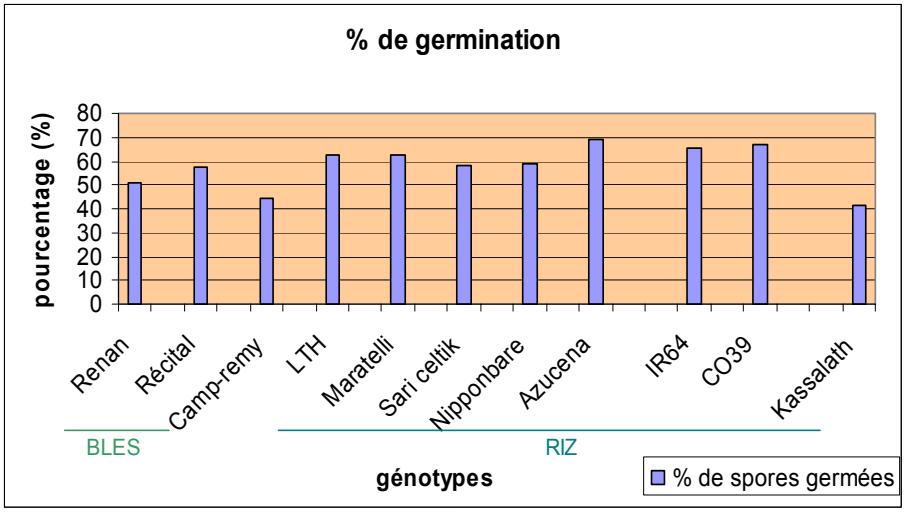
DAB + Methyl Blue

• imaging of ROI production
• staining of fungal structures (appressorium, etc.)

Generation

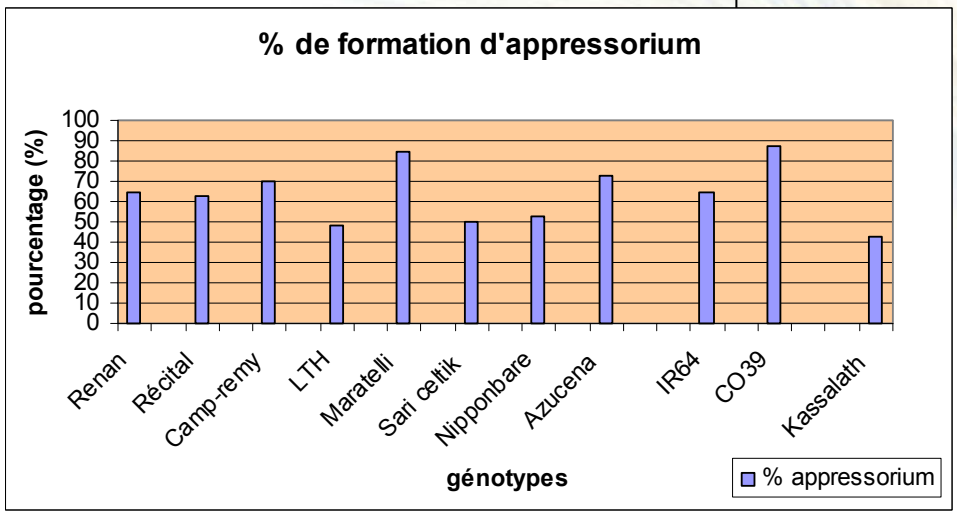
➢ *Au niveau microscopique*

taux de germination



- légèrement supérieur sur les riz
  riz : 60% blé : 50%

taux de formation d'appressorium



- de 60 à 70% chez les blés
- de 50 à 80% chez les riz

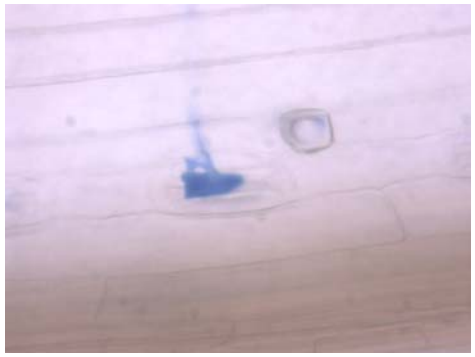
D'une manière générale :

➢pas de grande différence entre le riz et le blé

➢la résistance n'est pas liée à une inhibition de la germination ni de la formation des appressoria.

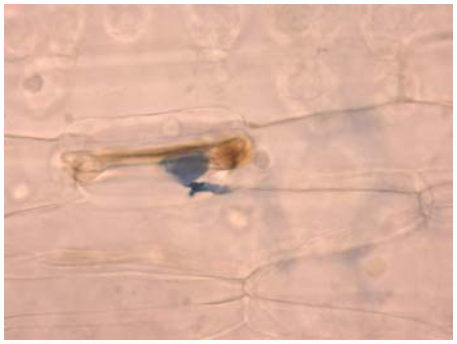
3 « types » de coloration

Type 1



Type 2

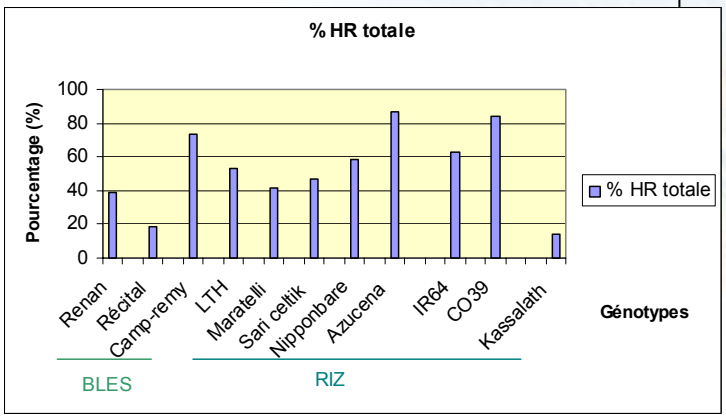
HR locale



Type 3

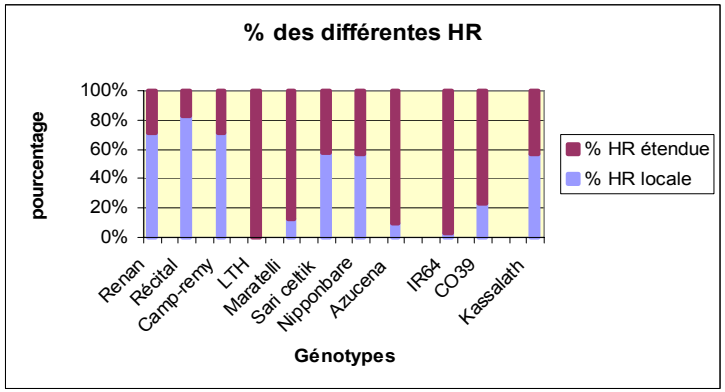
HR étendue

Taux de HR



- Récital: le moins de HR des blés

- forte HR chez les riz // Camp-rémy



- Blé HR surtout **locale** (70 à 80%)

Camp le plus résistant, un peu plus de HR étendue

- Riz : variable mais + de HR **étendue** que les blés

DISCUSSION POINTS

- INTERACTION WITH OTHER PROJECTS

- Genoplante – analysis of T-DNA mutants in NHGs
  - define with E. Guiderdoni (Agropolis)
- GCP project 8 – Coordinated by rebecca Nelson

- YEAR 2 MEETING – date and venue
  - June 2006/Europe?

Generation

Cerealimmunity YEAR 1

- **Objective 2** identify and validate a set of differentially expressed non-host specific genes (NHG) in rice and wheat

Activity 2 »in depth » transcriptome analysis of non-host interaction in rice and wheat

INSTITUTIONS INVOLVED: AGROPOLIS, NIAS, UCD, JIC

AGROPOLIS generation of RNA sample to be sent to NIAS and UCD
NIAS transcriptome analysis – Rice IR64 samples
UCD transcriptome analysis – Rice Nipponbare samples
JIC transcriptome analysis – wheat samples

Generation

DISCUSSION POINTS ACTIVITY 2 TRANSCRIPTOME ANALYSIS

1) focus on Puccinia/comparative analysis M.grisea/Puccinia

Magnaporthe and Puccinia have two different modalities of penetration – difficult comparisons

2) time-frame

3) experimental design microarray analysis

Same genotype with different strains/time points/importance of mock inoculated controls

4) bioinformatic data analysis – gene lists

5) creation of database – implication of SP4 (Theo van Hintum) implication of TIGR – implication of JIC?

Generation

OTHER USEFUL MATERIAL FOR THE MEETING

Global Budget full proposal/Institution (annual and summary)

	AGROPOLIS	CIMMYT	EMBRAPA	JIC	U RENNES	NIAS	UCD	TOTAL
Salaries/Benefits	80	60	70	100	49	40	75	474
Supplies/Services	35	20	30	64	24	26	41	240
Travel	9	10	10	8	9	9	9	64
Training/Workshops	11	5	10	5	5	0	5	41
Equipment	0	0	20	0	0	0	0	20
Indirect Costs	10	10	10	8	3	10	10	61
Total Costs	**145**	**105**	**150**	**185**	**90**	**85**	**140**	**900**
In-kind contribution	**160**	**100**	**100**	**160**	**90**	**90**	**150**	**850**
Total	**305**	**205**	**250**	**345**	**180**	**175**	**290**	**1750**

Budget is expressed in K$ (x1000)

Generation

OTHER USEFUL MATERIAL FOR THE MEETING

Budget Full proposal by Partner - Annual basis

	AGROPOLIS			CIMMYT			EMBRAPA			JIC			U RENNES			NIAS			UCD		
	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3	Yr 1	Yr 2	Yr 3
Salaries/Benefits	40	25	15	0	30	30	35	20	15	60	40	0	0	15	34	40	0	0	35	20	20
Supplies/Services	15	15	5	0	10	10	15	10	5	32	18	14	0	12	12	21	5	0	21	10	10
Travel	3	3	3	3	3	4	3	4	3	3	3	2	3	3	3	3	3	3	3	3	3
Training/Workshops	9	2	0	0	3	2	3	5	2	0	5	0	0	5	0	0	0	0	3	2	0
Equipment	0	0	0	0	0	0	10	6	4	0	0	0	0	0	0	0	0	0	0	0	0
Sub-total	67	45	23	3	46	46	66	45	29	95	66	16	3	35	49	64	8	3	62	35	23
Indirect Costs	5	3	2	1	4	5	4	4	2	5	2	1	0	2	1	7	2	1	5	3	2
Total costs	**72**	**48**	**25**	**4**	**50**	**51**	**70**	**49**	**31**	**100**	**68**	**17**	**3**	**37**	**50**	**71**	**10**	**4**	**67**	**38**	**35**
In kind contributions	**60**	**60**	**40**	**5**	**50**	**45**	**40**	**40**	**20**	**80**	**60**	**20**	**10**	**40**	**40**	**50**	**30**	**10**	**90**	**40**	**20**
Total	**132**	**108**	**65**	**9**	**100**	**96**	**110**	**89**	**51**	**180**	**128**	**37**	**13**	**77**	**90**	**121**	**40**	**14**	**157**	**78**	**55**

Budget is expressed in K$ (x1000)

Generation

FIRST YEAR MEETING
GENERATION CHALLENGE PROGRAM CEREALIMMUNITY PROJECT
JULY 17th FROM 8.30 TO 16.30
Coral Garden 2 ROOM
CORAL BEACH HOTEL – CANCUN

SCIENTIFIC PROGRAM

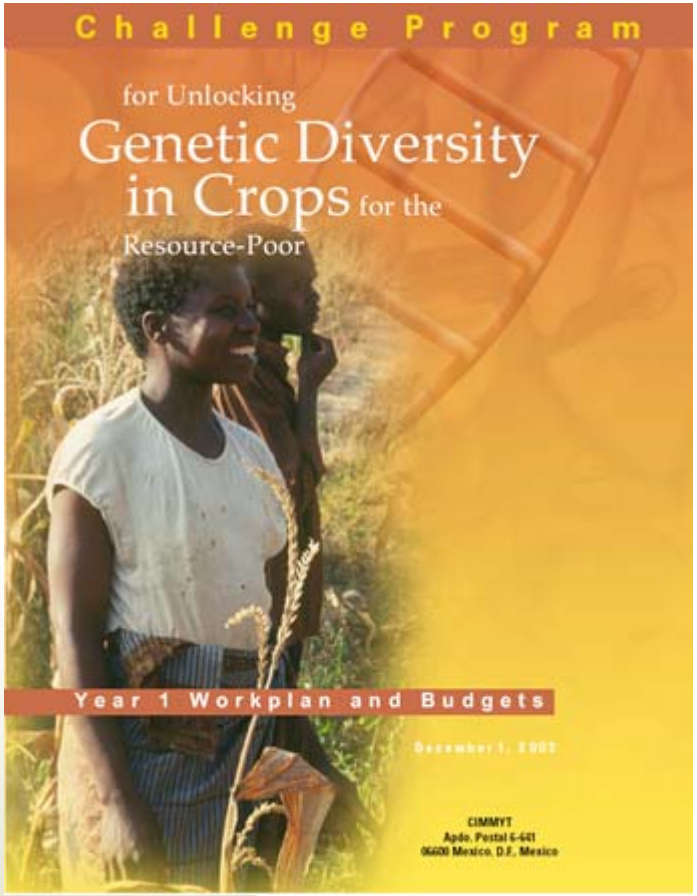
8.30-9.15 P. Piffanelli – Agropolis – CEREALIMMUNITY PROJECT OVERVIEW
9.15-9.30 P. Piffanelli – Agropolis
9.30-9.45 S. Kikuchi – NIAS
9.45-10.0 Kihong Jung – UCD
10.00-10.15 S. Prabhu – EMBRAPA
10.15-10.30 L. Boyd – JIC
10.30-11.0 Coffee
11.00-11.15 F. Dedryver – U. Rennes (represented by P. Piffanelli)
11.15-11.30 M. William - CYMMIT
11.30-12.0 P. Piffanelli – first Year Plan
12.00-12.30 Discussion
12.30-14.0 Lunch
14.00-15.0 Discussion cytology work
14.45-15.30 Discussion transcriptome work
15.30-16.0 Discussion 2nd and 3rd Year Plan – wheat mapping/wheat and rice comparative mapping work
16.00-16.30 Conclusions and Perspectives – date/venue of the 2nd Year meeting

Generation
Cultivating Plant Diversity for the Resource-Poor

Cerealimmunity project overwiew Outline

- The Generation Challenge Program
- The Cerealimmunity project
- Scientific plan and objectives
- Non-host resistance
- First Year Plan

The Generation Challenge Program: Unlocking Genetic Diversity in Crops



- Apply tools of comparative biology and genomics to access the useful genetic diversity in land races and crop relatives or progenitors

- Leverage relatively large investment in crop genomics (>$120 million/yr invested in US plant genome research)

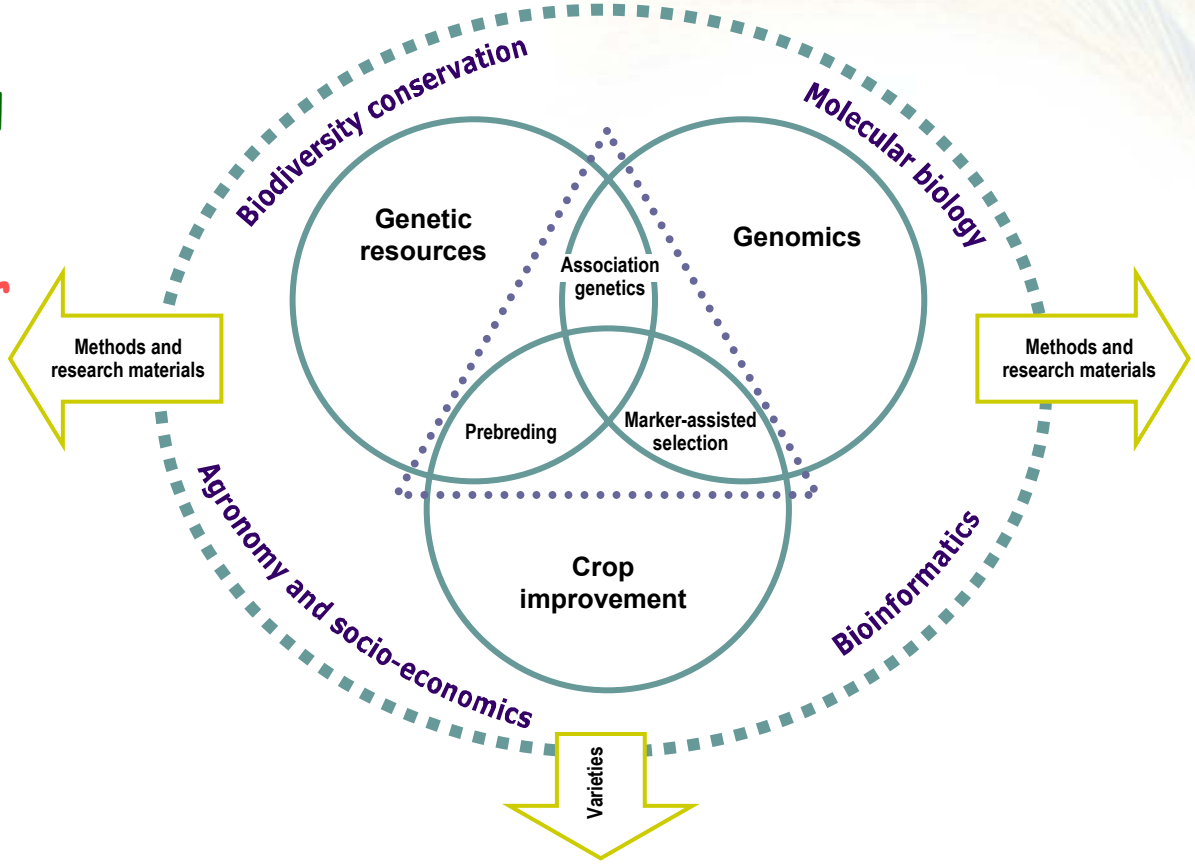
- Capture the imagination of policy makers and legislators with the potential for advanced science to help solve intractable problems that sustain abject poverty

Generation

The Generation Challenge Program Conceptual Framework: Linking Advanced Genetics to Breeding Applications

Five Sub-Programs (SP):

1. Genetic diversity of global genetic resources
   J. C. Glaszmann CIRAD
2. **Comparative genomics for gene discovery**
   H. Leung IRRI
3. Trait capture for crop improvement
   J. Crouch ICRISAT
4. Genetic resource, genomic and crop information systems
   T. van Hintum WUR
5. Capacity Building
   C. de Vicente IPGRI



Address Intractable Problems Through Comparative Biology

Generation

What is the Generation Challenge Program?

- A mechanism to direct and adapt basic science in functional & comparative genomics to crop improvement in developing countries
- *Not only* Discovery Science
- *Not only* Breeding or Education
- *Is* a means to link the two,
  - filling the gulf between and adding value to national competitive basic research programs, international development oriented crop improvement research and the private sector

Focus on drought and other intractable abiotic/biotic stresses

Generation

Growing Support for Generation CP

- US$ 13.5 M committed for 2005
- Largest donors from Europe
  - European Commission
  - UK (DFID)
- Other donors include: World Bank, Pioneer – DuPont, Syngenta Foundation, Austria, Sweden, Kirkhouse Trust, Rockefeller Found.
- Discussing several significant projects with RF 2005 – 2008: Africa and South Asia
- Discussions ongoing with others

Generation

Non-Host specific Genes (NHGs)

- PEN1 syntaxin
- NHO1 glycerol kinase
- PEN2

Generation

Cerealimmunity - Financial matters

- 3 years project – starting date: March 2005
- Total budget 900 K$
- Year 1: 387 K$, Year 2: 300 K$, Year 3: 213 K$
- Money Year 1 transferred to CIRAD March 2005
- Budget allocated to CoPIs transferred upon receipt of signed contract
- Contact person:

  Alain Chauchard – CIRAD

  alain.chauchard@cirad.fr

	Yr 1	Yr 2	Yr 3	TOTAL
Salaries/Benefits	210	150	114	474
Supplies/Services	104	80	56	240
Travel	21	22	21	64
Training/Workshops	15	22	4	41
Equipment	10	6	4	20
Indirect Costs	27	20	14	61
Total Costs	**387**	**300**	**213**	**900**
In kind contribution	**335**	**320**	**195**	**850**
Total	**722**	**620**	**408**	**1750**

Generation