

# **SP3 #18: Development of Low Cost Gene-Based Trait Assay Technologies in Cereals**

**Workshop on Product Management and Delivery of GCP Rice Research in Asia**

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**Bangkok, Thailand**



**IRRI**  
INTERNATIONAL RICE RESEARCH INSTITUTE



C.M. Vera Cruz, E. Mercado, M. Reveche, J. Chen, D. Skinner, M. Bernardo, D. Sanchez, B. Collard, J.H. Chin, P. Virk, Y. Xu, M. William, J. Wu, D. Joshi, M. Bustamam, V. Verdier, J. Agarcio, M. N. Ndjondjop, K. McNally, J. Crouch, D. Mackill

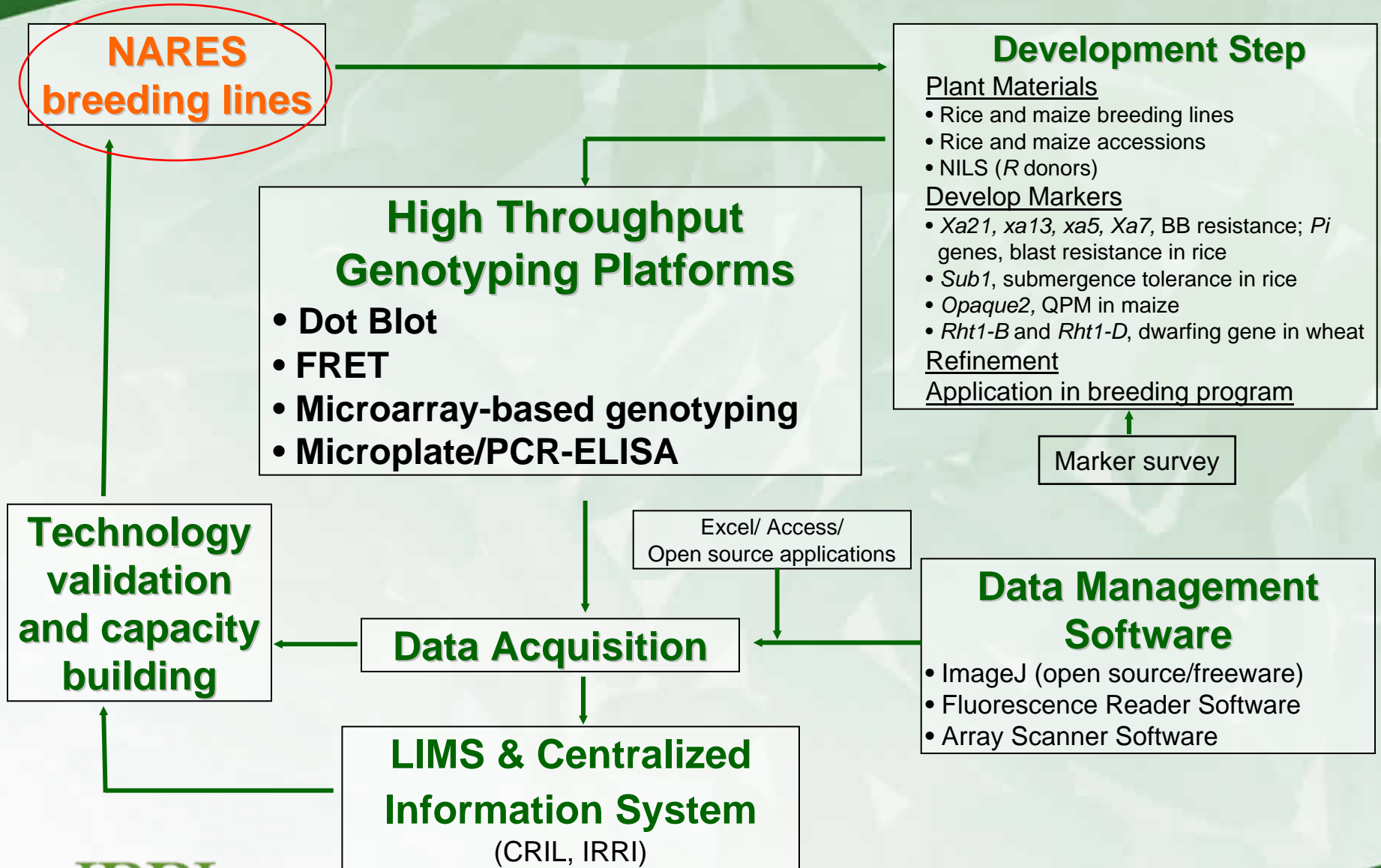
# Project Objectives

- ✓ To develop, refine and validate allele-specific **gel-free assays** for biotic stress and quality trait in cereals
  - ❖ low cost, low technology assays that require low capital set-up and unit costs for NARS and SMEs, and
  - ❖ low cost, high throughput assays for shuttle genotyping regional hubs in collaboration with national partners

## TRAITS :

- **Bacterial blight resistance (rice) [*Xa21* genes]**
- **QPM (maize) [Opaque2 (*O2*) alleles ]**

# Overall strategy



# Target Crop Types

## Rice

- **Bacterial blight resistance in hybrid parental lines**



- Hybrid rice – 15% yield advantage over inbred varieties (Yuan et al, 1994)

- Covers 50% of China's rice acreage and accounts for 60% of production

- Under tropical conditions, hybrids from indica subspecies increased yield potential by 9-15% (Virmani 1996, Redona et al, 1998)

- Yield reduction can be as high as 30- 50% due to BB

- Increasing diversity of CMS germplasm

- Improving resistance in restorer lines

- Potential for increase in area of cultivation in India, Indonesia, Philippines, Vietnam and other Asian countries, other than China

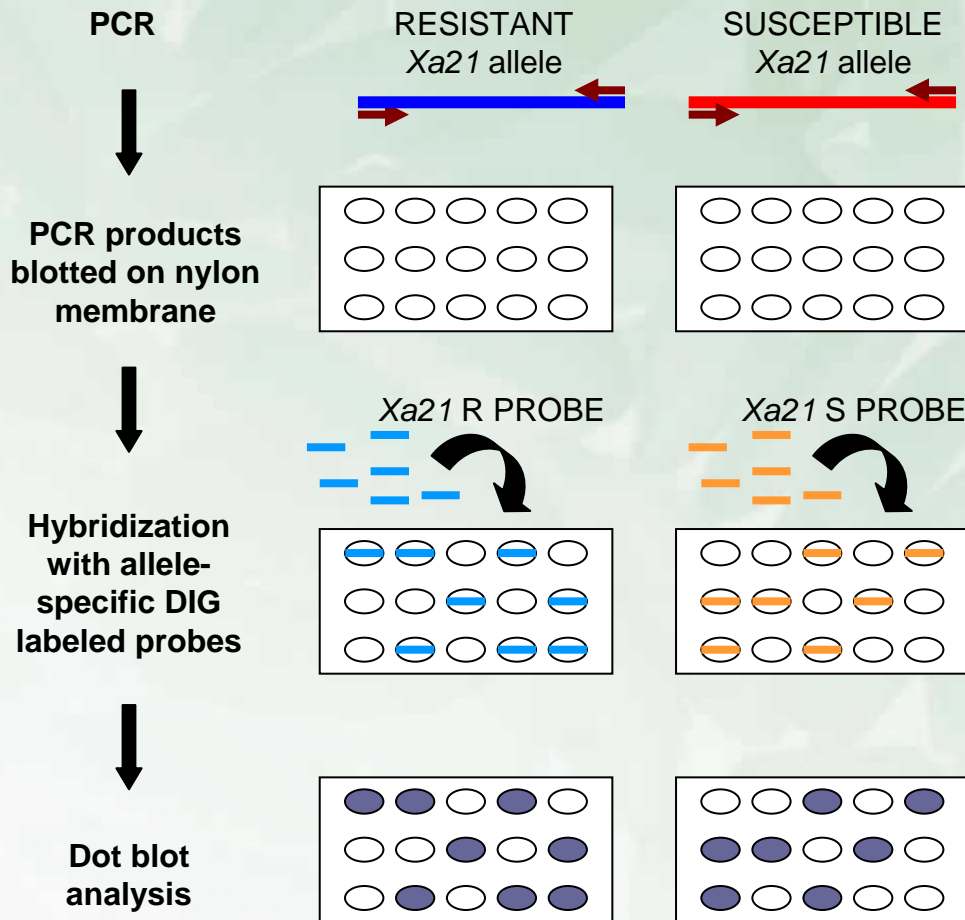
## Target regions and recipients for introgression of BB resistance in NARES rice breeding program

Country	Variety	Gene of interest		
		<i>xa5</i>	<i>Xa7</i>	<i>Xa21</i>
China –	Hui 161	n*	y*	y
Dr. J. Wu	Hui 333	n	y	y
	Hui 593	n	y	y
	Hui 811	n	y	y
Philippines –	IR58025B	n	y	y
Dr. D. Tabanao	IR68888B	n	y	y
Indonesia –	Simacan	y	n	n
Ms. M. Bustamam	Sintanur	n	y	n
	Setail	y	y	n
	BP364	y	n	y
India –	IR58025B	n	y	y
Dr. P. Kadirvel	Pusa 6B	n	y	y
Dr. Shashidar	Samba Mahsuri	y	y	y
	Swarna	y	y	y
Africa –	Adny	y	y	y
Dr. V. Verdier	Kogony	y	y	y

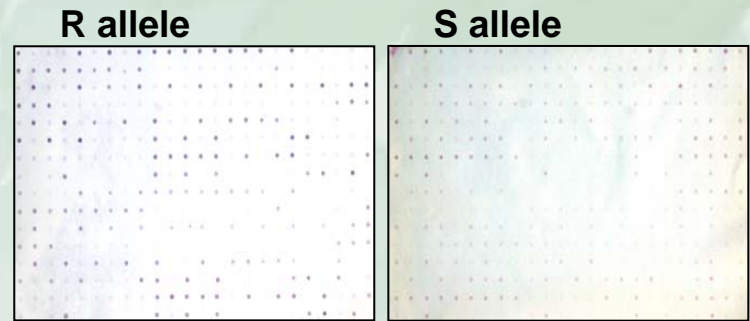
\*y = used in breeding program for a particular recipient; n = not used for a particular recipient.



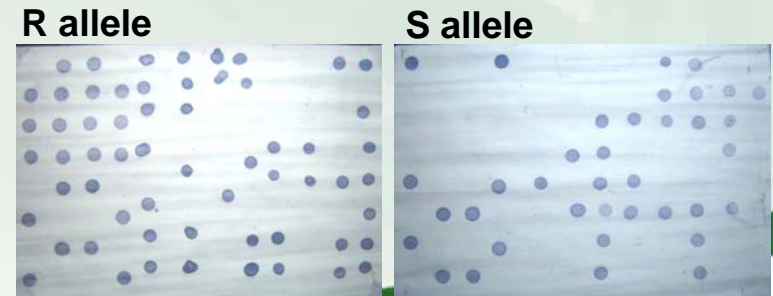
# Dot blot-based detection



- PCR products spotted on membranes
- Probed with allele-specific R and S alleles
- DIG-based detection on dot-blot
- Cost



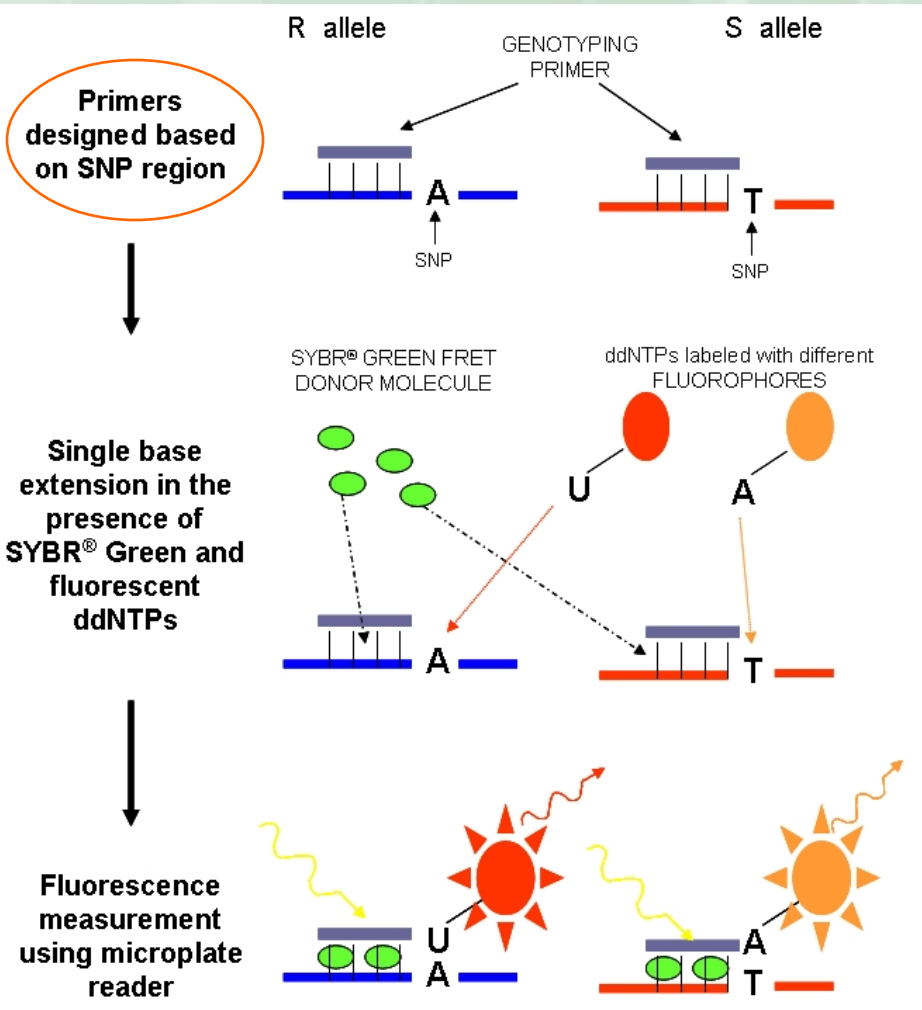
High throughput spotting (Biomek® HDRT)



# Genes in the pipeline

- ✓ Submergence tolerance gene in rice, *Sub1*  
(DL Sanchez, DJ Mackill, IRRI)
- ✓ Bacterial blight resistance genes in rice, *xa5*, *xa13*, *Xa7* (marker/probes designed for *xa5* and *xa13*, and conditions being optimized)
- ✓ Rice blast resistance genes, *Piz*, *Pi2*, *Pi9*, *Pikh*, *Pik*, *Pita2*
- ✓ [Wheat dwarfing genes, *Rht1-B* and *Rht1-D*]  
(M William, CIMMYT)

# Fluorescence Resonance Energy Transfer (FRET)-based SNP genotyping

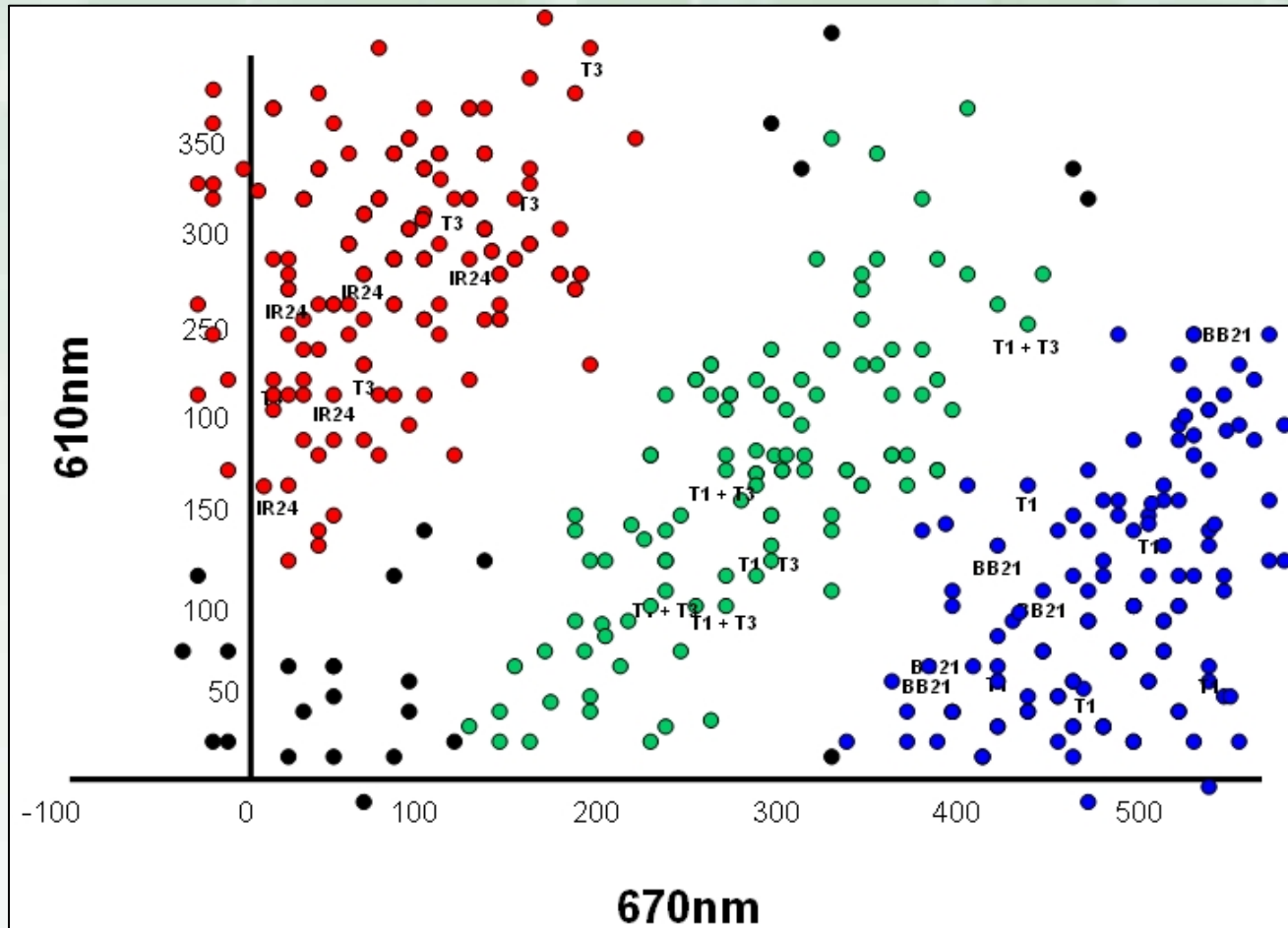


## *Xa21* primers

- target region of the gene in breeding lines
- R and S primers on SNP region
- Relies on distance-dependent transfer of energy from a donor molecule to an acceptor molecule.
- Single base extension in the presence of SBYR Green and fluorescent ddNTPs
- Fluorescence measurement using microplate reader
- Cost

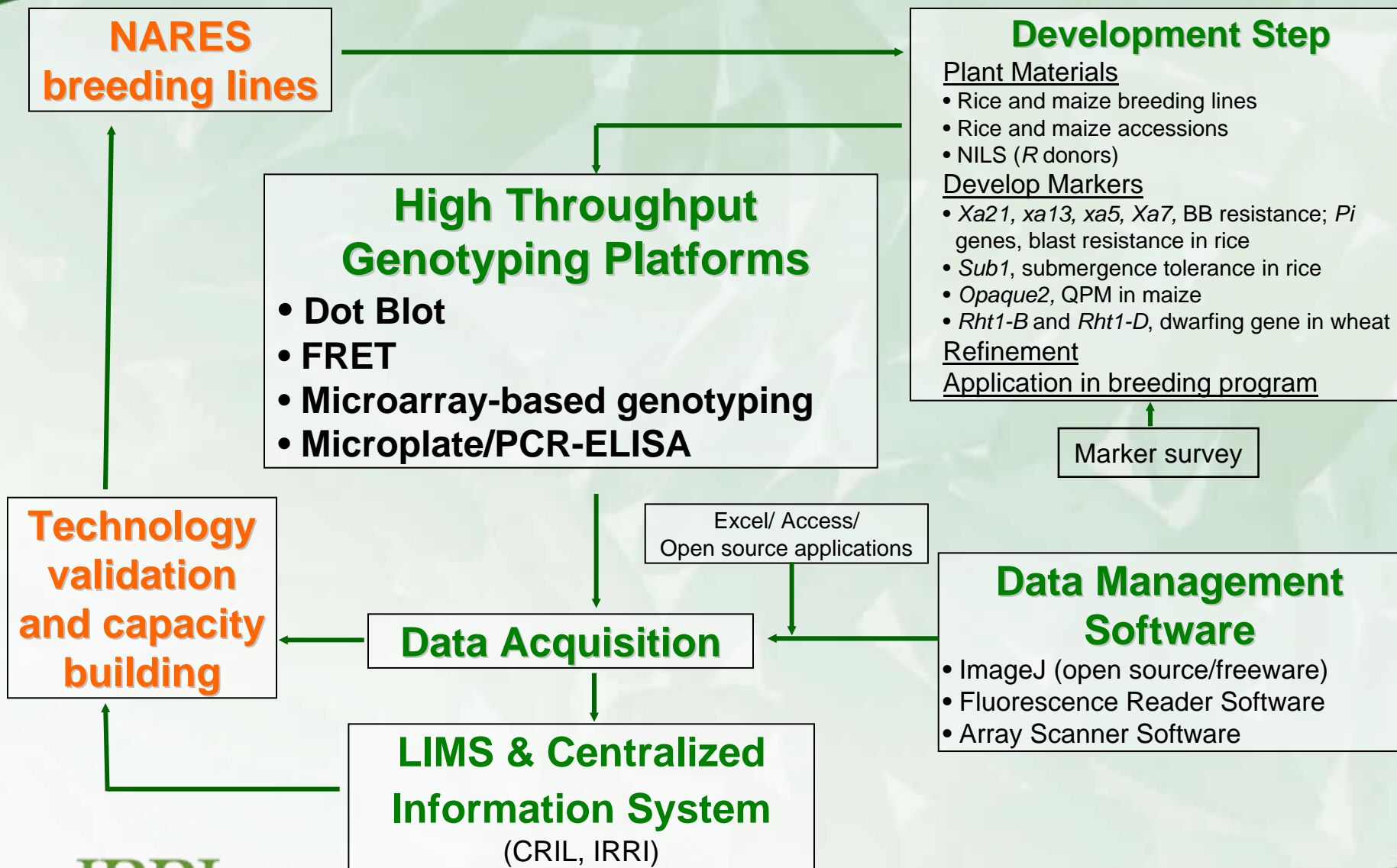
Takatsu et al., 2004

# Scatter plot of FRET fluorescence intensities at 610nm and 670nm of 384 F2 lines derived from Hui593/IRBB7.



Dots represent the *Xa21* genotypes of the individual lines: ● = susceptible, ● = heterozygous, ● = resistant, and ● = undefined.

# Overall strategy: Transfer plan to primary users

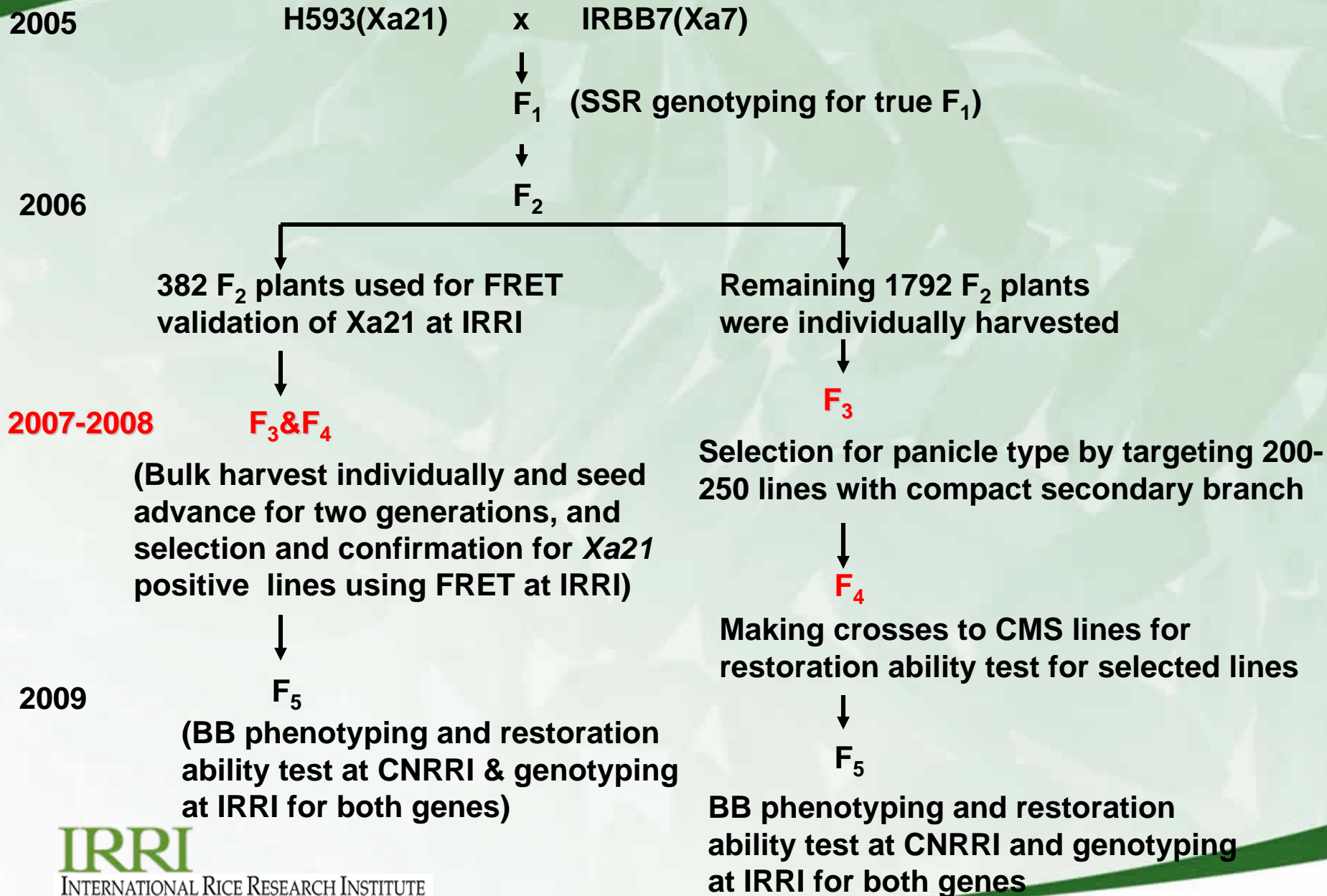


# Improving bacterial blight resistance

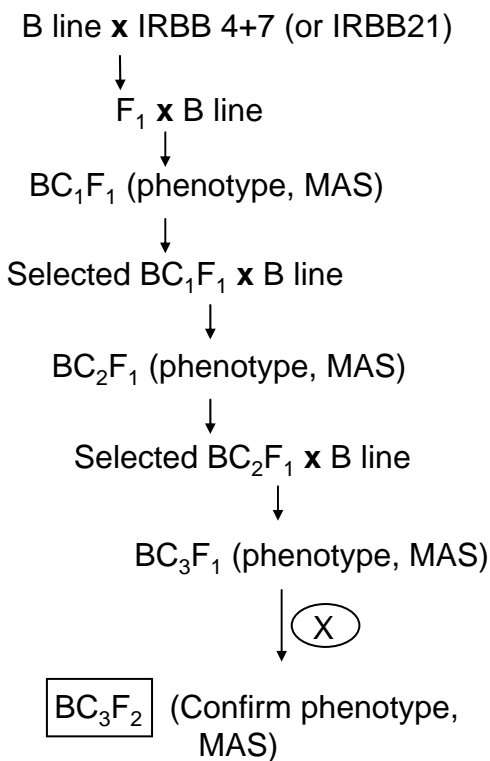
## Restorer lines at CNRRI

- H593 is a three line hybrid restorer line with *Xa21* gene, but R not enough due to a new race that overcomes *Xa21* in China. Further, H593 shows a defect on the panicle format (i.e. dispersed secondary branch that carry to hybrid), an unwanted trait by farmers.
- To improve the resistance, IRBB7 was used as a donor of *Xa7* gene for targeting new products:
  - *Xa21/Xa7* combination,
  - compact secondary branch,
  - restorer genes

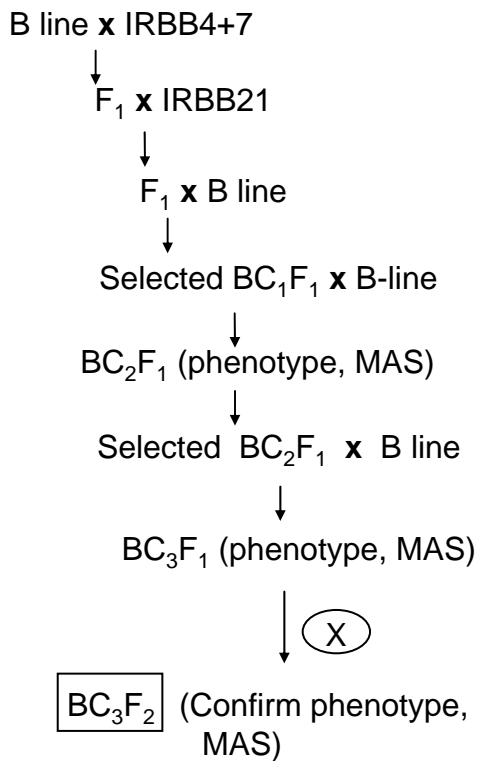
# Breeding scheme for BB R restorer lines at CNRRI, China



### CROSSING SCHEMES 1 & 2



### CROSSING SCHEME 3

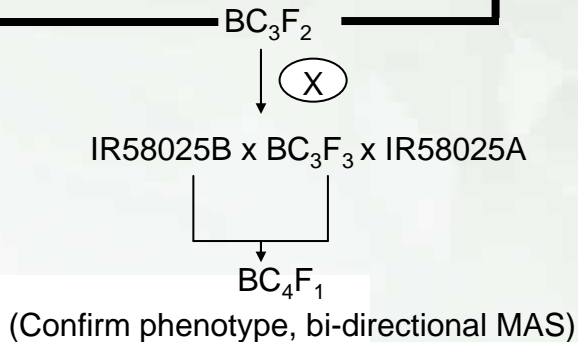


Development of improved hybrid parental lines with BB resistance using bi-directional MAS, PhilRice

**Scheme to pyramid Xa resistance (RR) genes and obtain completely sterile (ff) A lines.**

IR58025B\* = BB-resistant B line;  
 IR58025A\*\* = BB-resistant A line; RR = resistant genotype; rr = susceptible genotype; FF = fertile genotype; ff = sterile genotype; CS = completely sterile.

### CROSSING SCHEME 4



2006 DS

IR58025B\* × IR58025B  
(RR) × (rr)

2006 WS

IR58025A × BC<sub>6</sub>F<sub>1</sub> × IR58025B  
(rr; ff) × (Rr) × (rr)

2007 DS

IR58025A\* × BC<sub>7</sub>F<sub>1</sub> × IR58025B  
(50% Rr; ff) × 50% Rr × (rr)

2007 WS

IR58025A\* × BC<sub>8</sub>F<sub>1</sub>  
(Rr; ff)

# Hybrid parental products

- Promising parental lines introgressed with *Xa* genes were selected:
  - B\* and R\* lines for Mestizo 1, 2 and 3 pyramids of *Xa4+Xa7* and *Xa4+Xa7+Xa21*
  - These lines and AxB\* crosses are morphologically uniform
  - The first set of AxB\* crosses evaluated in 2007DS yielded completely sterile plants that only have *Xa4*
  - Completely sterile AxB\* plants have been observed (2007WS), some carry *Xa4+Xa21*, for backcrossing to B\* lines (LSU)
  - The set of R\* lines were crossed to original A lines (2007WS) for comparison of F1 hybrids with original Mestizo hybrids (LSU)

# Future plans

- Advanced generations of B\* and R\* lines will be compared with their original lines in the field and DNA-fingerprinted in the lab.
- More A×R\* plants (i.e., F1 hybrids) will be produced and tested for heterotic yields.
- More A×B\* plants will be evaluated for male-sterility and morphological uniformity; some of these A×B\* plants that will be tested will have been products of two rounds of crossing to B\* lines (to accumulate *Xa* genes).
- *Xa* and *Rf* genes will continue to be assayed by linked markers, disease resistance will be evaluated in the greenhouse or in the field (whichever is appropriate), and male sterility will be tested by I<sub>2</sub>KI staining of pollen.
- At the end of 2008, we aim to be in a better position to release near isogenic lines of A\*, B\*, and R\* that can be tested on a wider national scale.

# Impact in hybrid rice production

- IR58025A is the female parent of most hybrids released in Asia by both public and private sectors.
  - Good combining ability
  - Heterosis
- With the AxB and AxR seed production technology for IR58025 well established and found to be economically feasible, these BB resistant lines promise to create both national and international impact in hybrid rice production.

# Constraints to plans of transfer

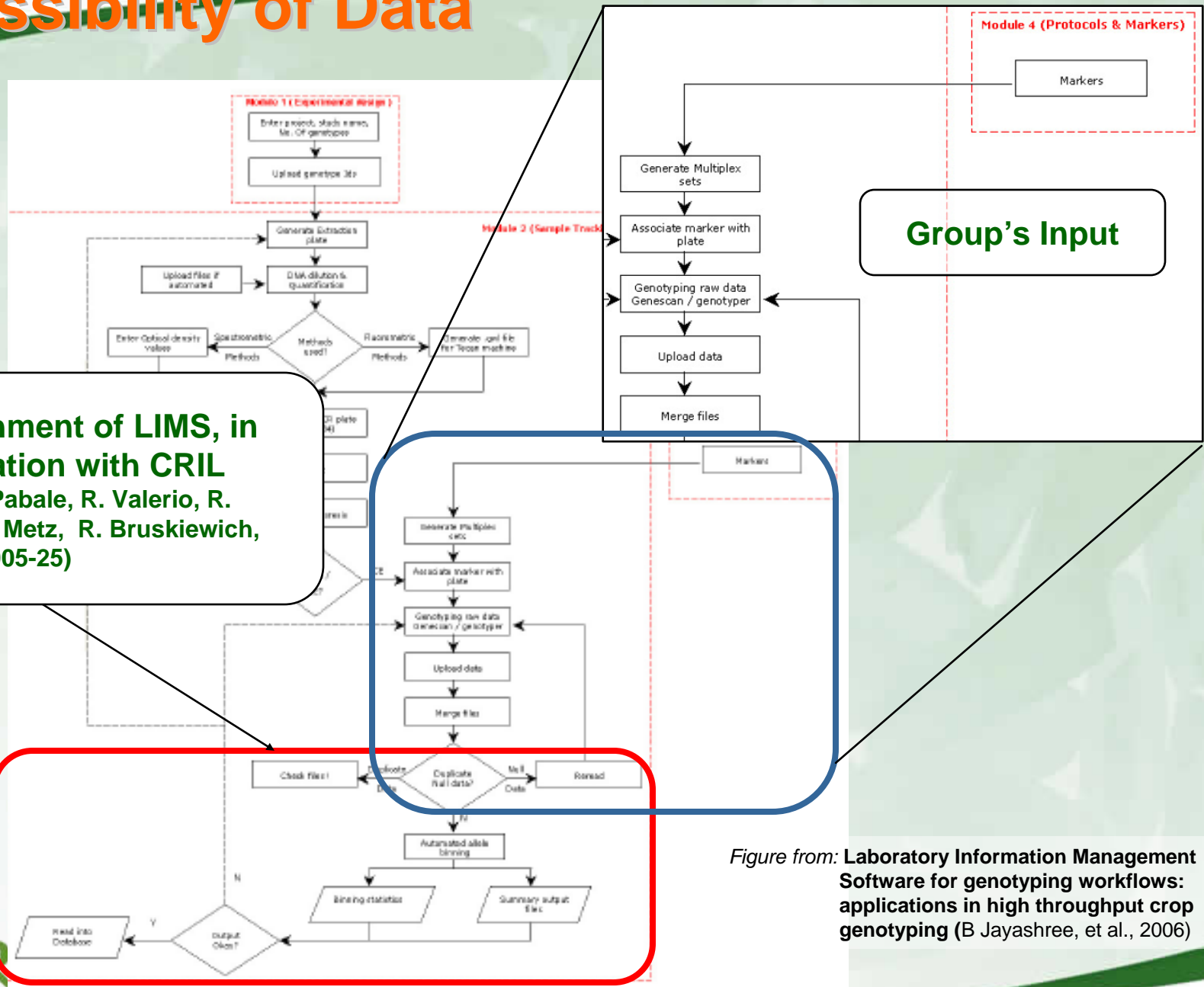
## 'Application gap'

- Cost of using DNA markers and tools
  - PCR costs (consumables)
  - DNA extraction steps (including labor)
- Lack of validation of primers and platform at NARES own lab both at PhilRice and CNRRI (dot-blot)
- Lack of equipment for FRET assay at CNRRI
- Development of marker for *Xa7* (*xa13*, *Sub1*, *Pi*-genes in progress)
- Further development and use of freewares (e.g. ImageJ, etc) in the management and analysis of data
- Sharing of materials among researchers and institutions

# Key elements of a large scale applied marker-assisted breeding program

- High density linkage map
- High throughput genotyping platform
  - Robust and cheap
- Integrated IT systems
- Proven applications
  - Marker-Assisted Selection
  - Marker-Assisted Backcrossing
  - Marker-Assisted Breeding
  - Use of wild rice/exotic germplasm

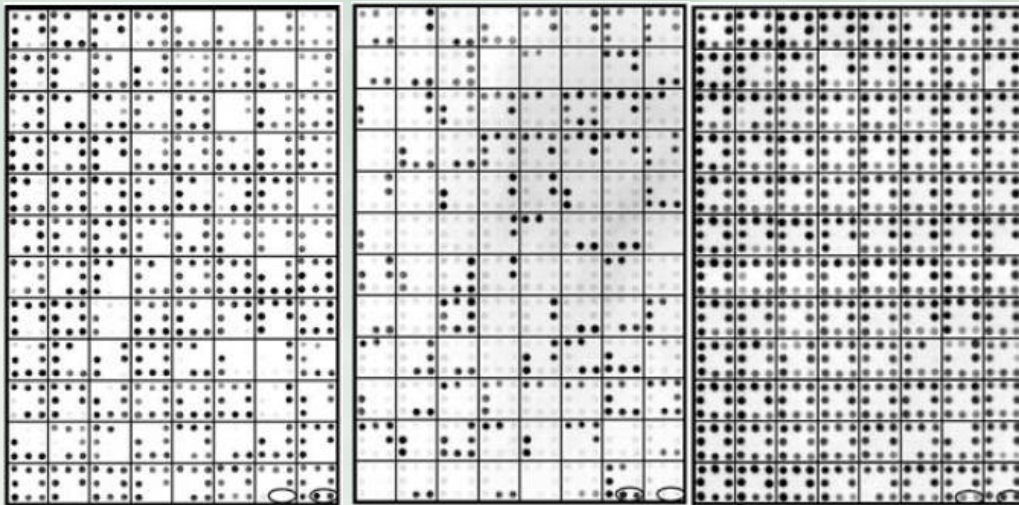
# Accessibility of Data



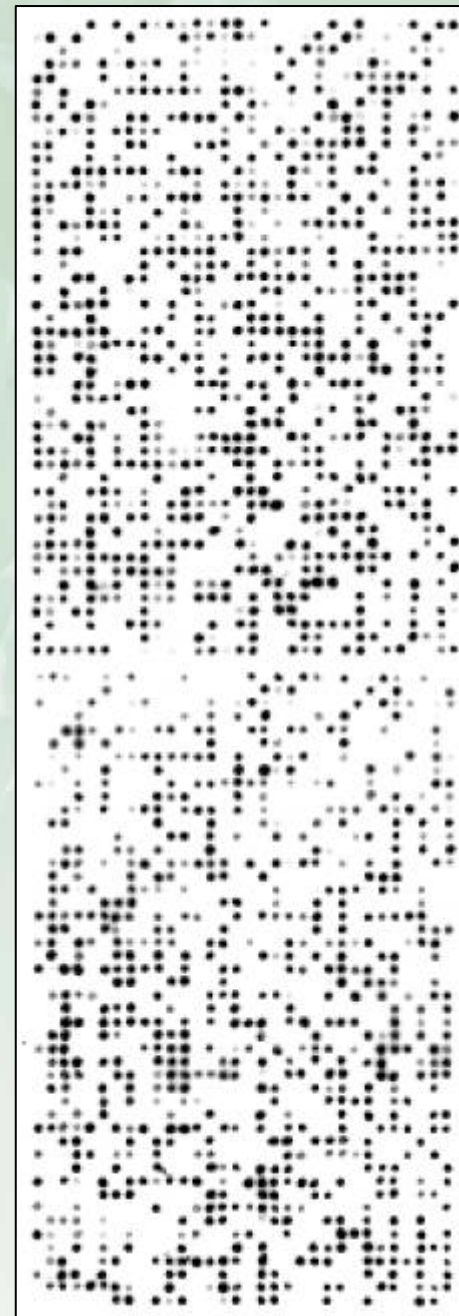
**Establishment of LIMS, in collaboration with CRIL (J. Ulat, D. Pabale, R. Valerio, R. Mauleon, T. Metz, R. Bruskewich, GCP SP4 2005-25)**

Figure from: **Laboratory Information Management Software for genotyping workflows: applications in high throughput crop genotyping** (B Jayashree, et al., 2006)

# Application in breeding programs



**Dot-blot-SNP analysis** of the *Wx* alleles in F4 plants of a crossbreeding program. Two dots were blotted for each plant as shown in the picture at the right. PCR products of 'Koshihikari' (K) having *Wx* and 'Milky Queen' (M) having *Wx-mq* were blotted as positive and negative controls at the upper right corner of each membrane. The pictures show hybridization with the labeled *Wx* probe and the unlabeled *Wx-mq* probe (a), with the labeled *Wx-mq* probe and the unlabeled *Wx* probe (b), and with a mixture of the labeled *Wx* probe and the labeled *Wx-mq* probe (c)

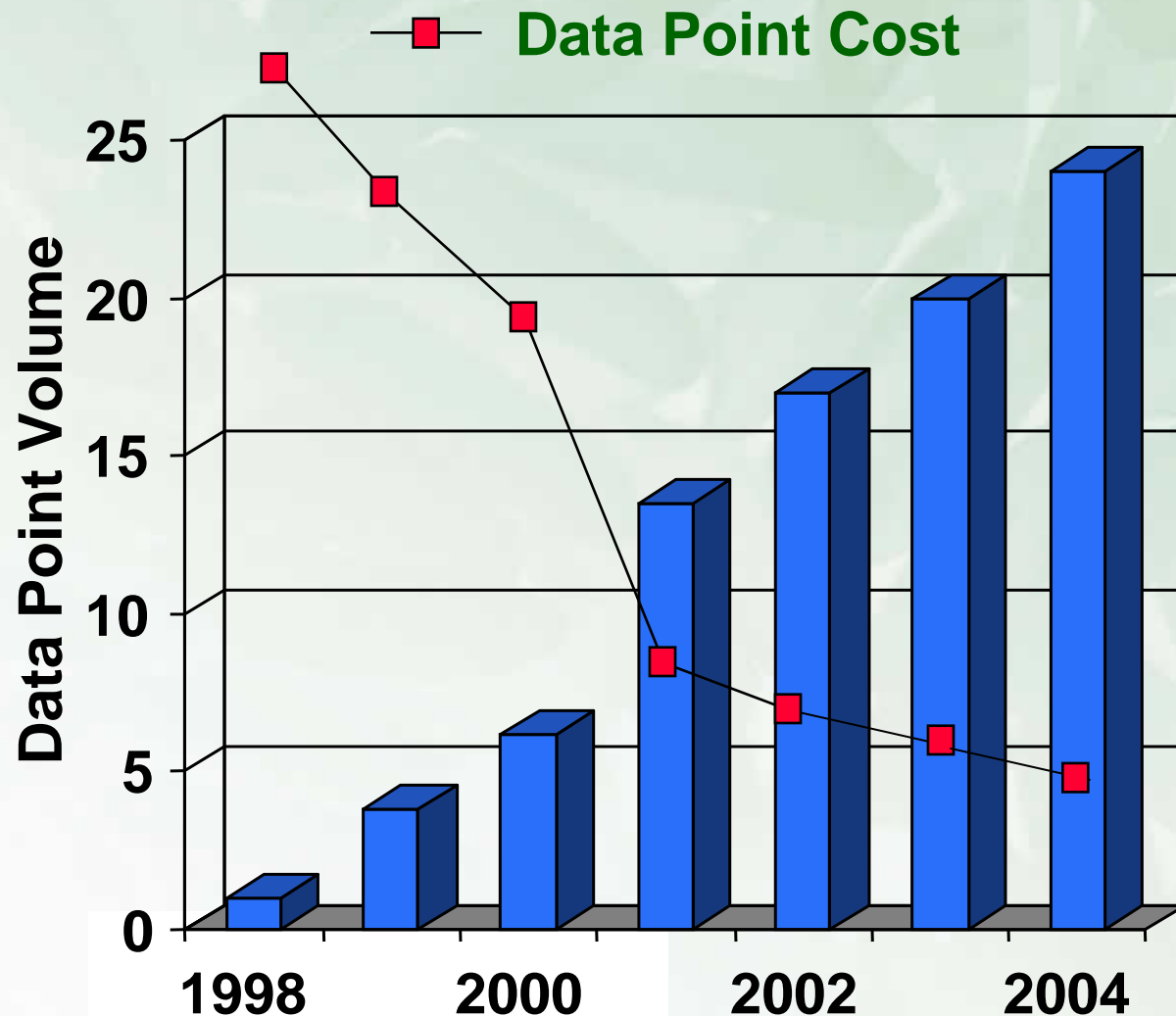


**16X (16 96-well plates) autorad images of complimentary alleles at one locus**

DJ Cahill and DH Schmidt . 2004. Use of marker assisted selection in a product development breeding program. *In* "New directions for a diverse planet". Proceedings of the 4th International Crop Science Congress, 26 Sep – 1 Oct 2004, Brisbane, Australia.

K. Shirasawa, S. Shiokai, M. Yamaguchi, S. Kishitani, T. Nishio. 2006. Dot-blot-SNP analysis for practical plant breeding and cultivar identification in rice. *Theor Appl Genet.* 113: 147–155.

# New genotyping technologies have enabled growth in marker-assisted breeding applications



## *Key Factors Affecting Volume and Cost*

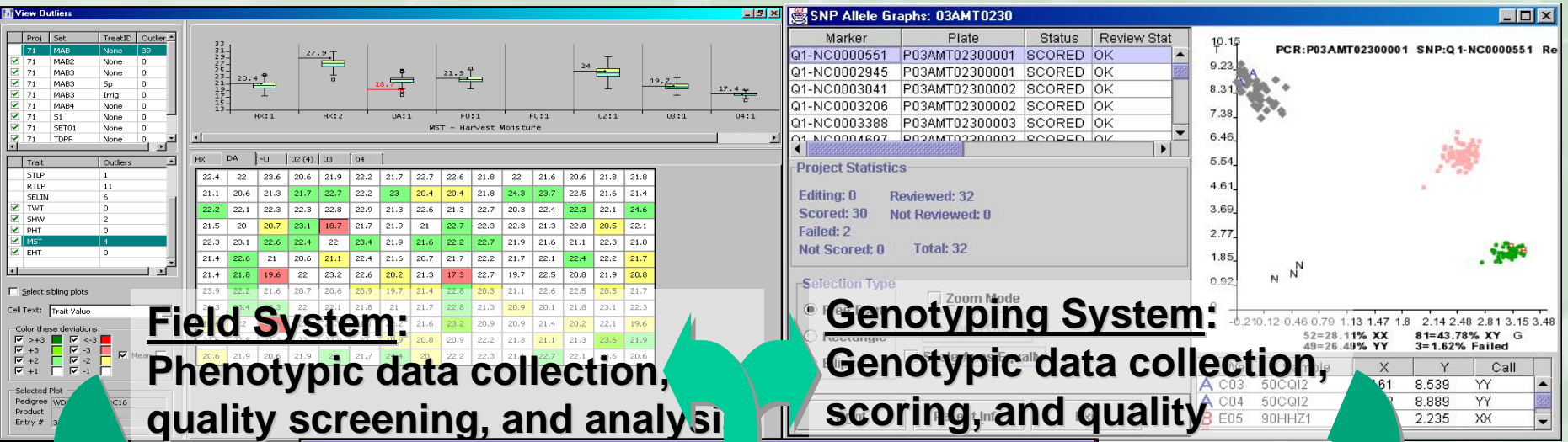
- SSRs to SNPs
- Use of automation
- IT enhancements
- Additional facility
- Additional Staff

V. Consibido

# Global laboratory IT systems facilitate collection of genotypic information

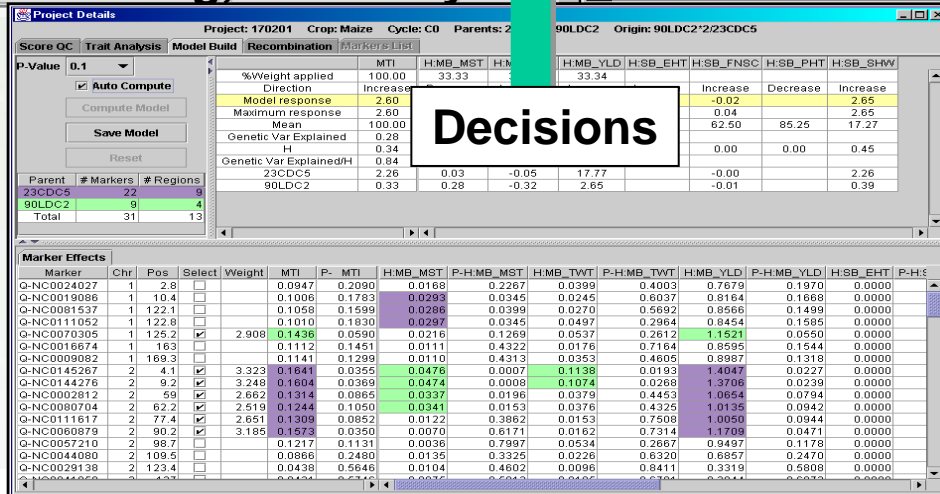
- Key Procedures
  - Manage marker inventory
  - Select markers for projects
  - Track DNA sample through lab procedures
  - Genotypic data QC and analysis
- Global standardization
  - Methodologies
  - Nomenclature
- Centralized database
- Every DNA sample has a unique identification

# Data analysis and decision making systems are connected to IT systems



**Field System:**  
 Phenotypic data collection,  
 quality screening, and analysis

**Genotyping System:**  
 Genotypic data collection,  
 scoring, and quality



**Analysis System:**  
 Integrate and  
 analyze field and  
 marker data

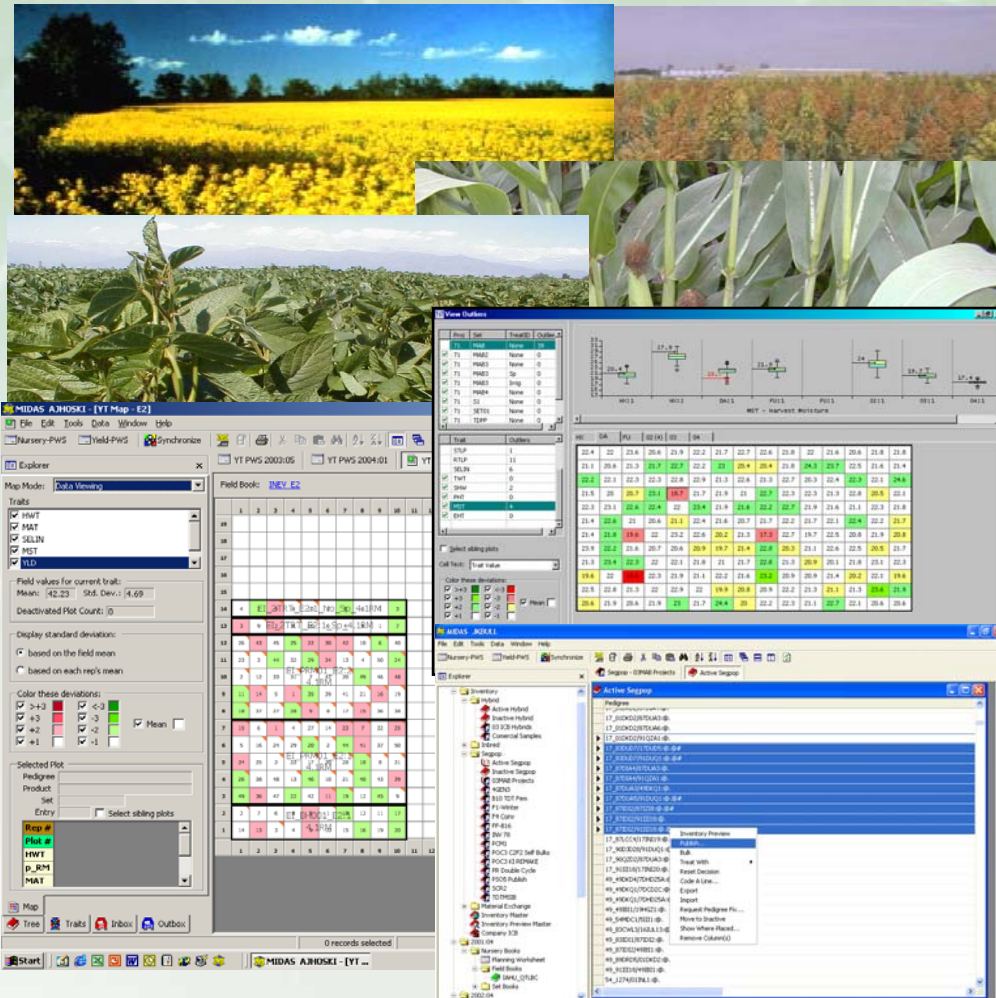
Data

Decisions

Data

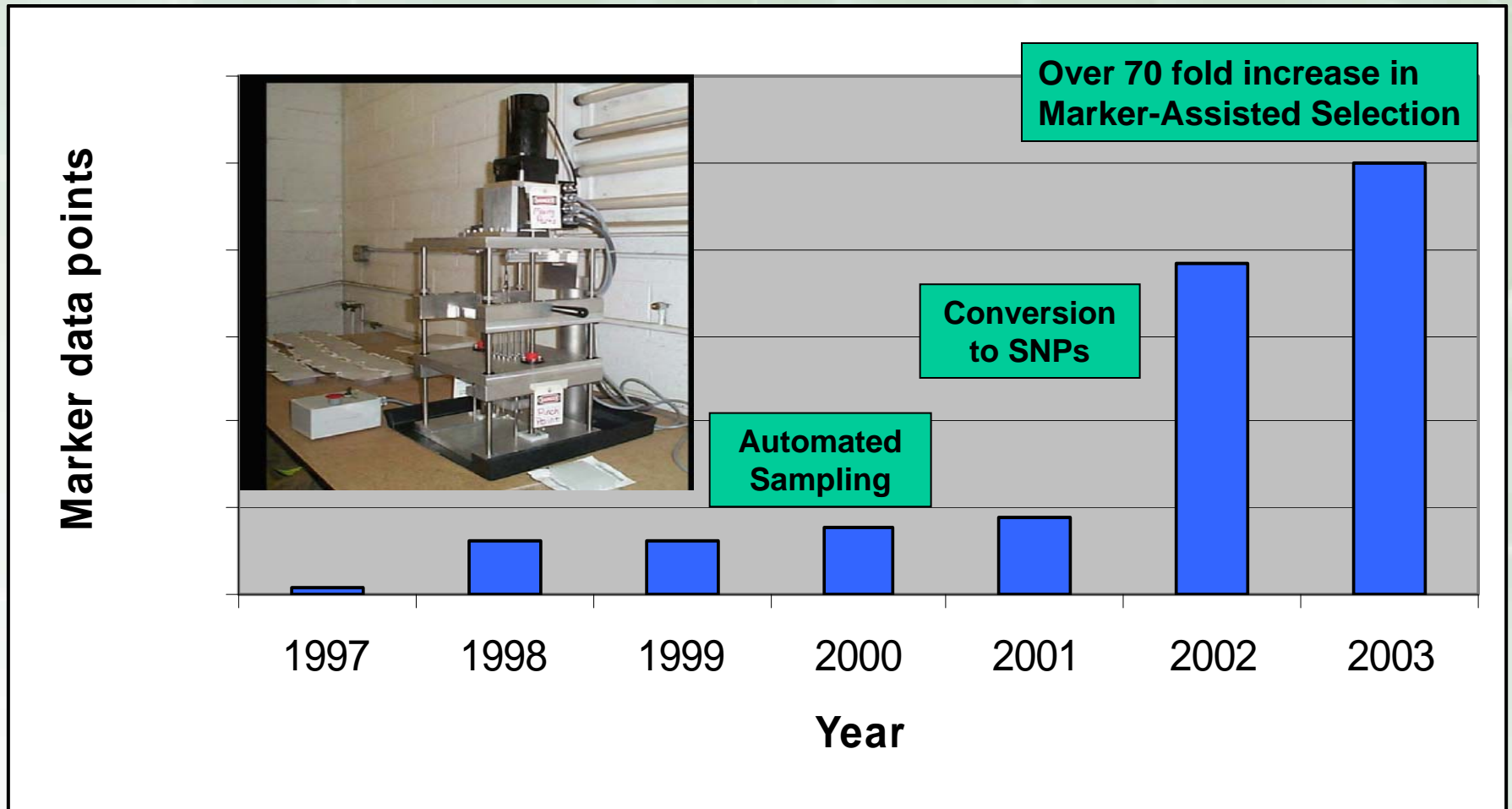
# Global field breeding IT system facilitates collection of phenotypic information

- Key Procedures
  - Create breeding populations
  - Organize and execute all field trials
  - Phenotypic data QC and analysis
- Global standardization
  - One pedigree system
  - One list of traits
  - Standard data attributes
- Centralized database
- Every DNA sample has a unique identification



V. Consibido

# Marker associations combined with new technology have enabled large scale implementation of MAS



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- J. Crouch

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- J.M. Ribaut
- P. Monneveux
- C. De Vicente
- H. Leung
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## Project Partners

- DRR, India – S. Madhav
- IARI, India – F. Hossain
- KARI, Kenya – M. Lagat
- ICERI, Indonesia – M.B. Pabendon

## Participants of the Workshops



J.L. Karihaloo

## Project Partners

- Barwale Foundation, India
  - B.R. Barwale
  - U.B. Zehr
  - D. Joshi
  - M.L. Shanti
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- CNRRI, China
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- WARDA
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