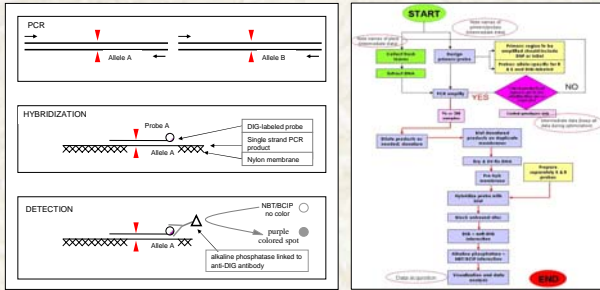


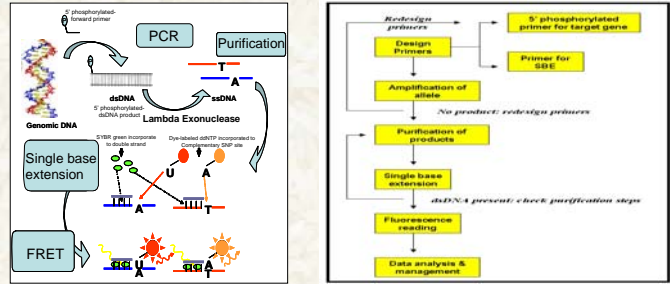
ACCELERATING VARIETAL DEVELOPMENT USING HIGH THROUGHPUT, LOW COST, GENE-BASED MAS TECHNOLOGIES IN CEREALS

High throughput, low cost technologies for marker-assisted selection (MAS) that utilize SNP-based probes were developed using the genes *Xa21* for bacterial blight resistance (rice) and *Opaque2* (maize) for quality protein maize as models, in a collaboration between IRRI, CIMMYT and NARS/SME partners in China, India, Indonesia and the Philippines. The dot blot and FRET methods have been refined and validated using elite lines of Basmati-derived lines in a replicated yield trial and Hui 593-derived population with partners from China, India, Indonesia and the Philippines. Cost reduction experiments, including use of home-made reagents versus commercially available kits, have been investigated and have shown that sensitivity of the assays had been maintained. The dot blot method is now being adapted to detect the allelic state of the submergence tolerance gene (*sub1*) in rice breeding population. Attempts by CIMMYT researchers are currently underway to try to adapt the dot blot assay for use with the wheat dwarfing genes *Rht1-B* and *Rht1-D*. Management of the data generated and accessibility of the same data is being done in collaboration with the IRRI Crop Research Informatics Laboratory (under GCP SP4 2005-25). To transfer and validate these technology platforms to national program partners of the project, two separate workshops were held in Jalna and Hyderabad, India in collaboration with Barwale Foundation.

Low Capital-Based Technology: DOT BLOT ASSAY



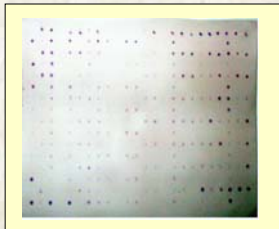
Hub Lab-Based Technology: FLUORESCENCE RESONANCE ENERGY TRANSFER



Development, Refinement and Application

Dot blot genotyping

- **Development & Cost reduction:** Home made washes being used instead of available commercial kits
- **Refinement:** Elimination of dilution step of PCR product prior to spotting on nylon membrane decreased detection time.
- Different methods of spotting of 384 samples or more on a single blot are being investigated
- **Applications:**
 - **Rice:** Elite Basmati-derived lines introgressed with *Xa4*, *xa5*, *xa13* and *Xa21*
 - **Maize:** F₂ plants of CML494x CML492 containing the *Opaque2* allele

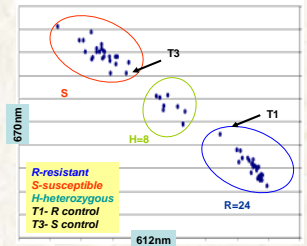


Dot blot result of 91 plants from 15 elite Basmati-derived lines (plus controls) using *Xa21* susceptible allele probe. Samples (0.2nL) were spotted manually using 384 replicating tool (V & P Scientific)

Fluorescence Resonance Energy Transfer (FRET)

- **Refinement & cost reduction:** optimal concentrations of enzymes and fluorescently-labeled ddNTPs have been determined and used in current application.
- **Application:** Hui 593-derived population introgressed with *Xa7* and *Xa21*

Reagent	Concentration Suggested in Literature (cost)	Optimized Concentration (cost)
Cy5-ddUTP	0.2mM (\$0.776)	0.05mM (\$0.194)
ROX-ddATP	0.2mM (\$0.716)	0.05mM (\$0.179)
Lambda Exonuclease	0.4U (\$0.032)	0.125U (\$0.010)
Total Cost	\$1.524	\$0.383



Cost reduction of expensive reagents used. Total cost was reduced four times compared to original recommendation.

Scatter plot of the fluorescence intensity readings of 58 plants from F₂ generation of Hui 593-derived breeding lines containing the *Xa21* resistance gene

Technology Transfer and Capacity Building

- Workshops have been held in Jalna (April 2007) and Hyderabad (August 2007), India, hosted by the **Barwale Foundation**.



Training Materials/Manuals



Jalna and Hyderabad Workshops

Data acquisition and management/LIMS and linking to centralized information system

- **IRRI Crop Research Informatics Laboratory (CRIL)** is establishing a laboratory management information system (LIMS) based on the different outputs of the four genotyping technologies as a case study (under SP4 2005-25).

Gene-based markers in the pipeline

Rice:

- Submergence tolerance: *Sub1*
- Bacterial blight resistance: *xa5*, *xa13*, *Xa7*
- Blast resistance: *Piz*, *Pi2*, *Pi9*, *Pikh*, *Pita2*, *Pik*

Maize:

- Quality Protein Maize: *Opaque2*

Wheat:

- Dwarfing genes: *Rht1-B*, *Rht1-D*

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