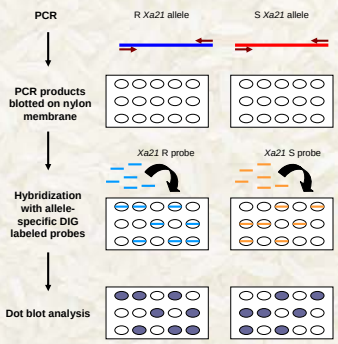
Development of non gel-based, high-throughput, and low-cost technologies for marker-assisted selection in rice

Currently, two obstacles for marker-assisted selection (MAS) are the high cost of genotyping and the limited sample throughput capacity. We are developing new alternative genotyping methods in a collaborative project between IRRI, CIMMYT, and NARES partner institutions. Two methods are being developed as low-cost approaches that can be adopted in laboratories with limited resources. Two other methods are being developed as high-throughput and cost-effective methods that could be utilized in core genotyping institutes. We are validating these methods using previously developed markers and characterized or cloned bacterial blight resistance genes (*xa5, Xa7,* and *Xa21*) in rice (Iyer and McCouch 2004, Porter et al. 2003, Song et al. 1995).

Low-cost, simple genotyping technologies

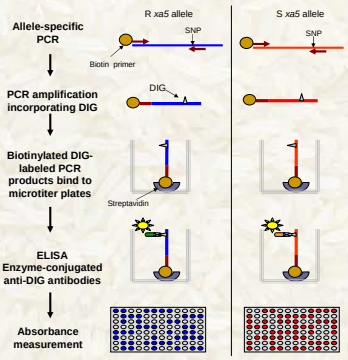
Dot blot genotyping assay

Methods adapted from Shirasawa et al. (2005).



PCR-ELISA detection assay

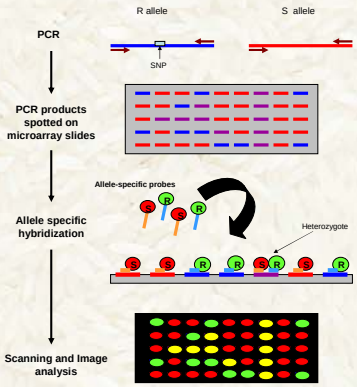
Methods adapted from Luk et al. (1997)



High-throughput genotyping technologies for regional hub laboratories

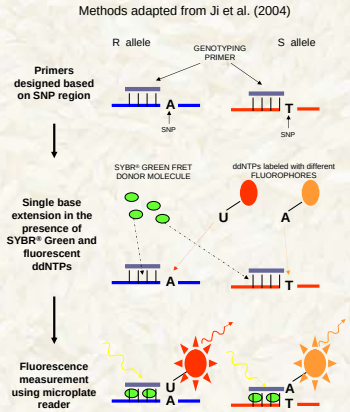
Microarray-based genotyping

Methods adapted from Ji et al. (2004) and Flavell et al. (2003)



Fluorescence resonance energy transfer (fret)

Methods adapted from Ji et al. (2004)



SUMMARY OF RESEARCH PROGRESS

Dot blot assay

Xa21 primers were used to amplify R and S alleles of 96 DNA samples and spotted onto nylon membranes. Results indicated successful hybridization and high specificity. DNA concentration for blotting, hybridization stringency, and different incubation times of substrate detection are currently being optimized. High correlation was observed between the dot blot and gel electrophoresis results.

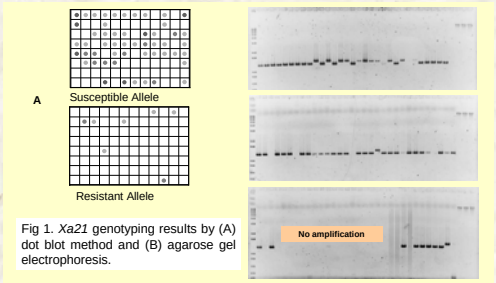


Fig 1. *Xa21* genotyping results by (A) dot blot method and (B) agarose gel electrophoresis.

B: Agarose gel electrophoresis: resistant lines with higher molecular weight; susceptible lines with lower molecular weight

The low and high stringency washes used were homemade, while the washing, blocking, and detection solutions were obtained from Roche. The amount of PCR product, as well as genomic DNA, will be optimized to obtain uniform color development of spots. Other bacterial blight resistance genes such as *xa5* and *Xa7* will also be used to validate the dot blot method.

PCR-ELISA method

PCR primers specific for the *xa5* bacterial blight resistance gene have been designed and amplification conditions have been optimized. The primers clearly discriminated between the R and S alleles at an annealing temperature of 68 °C.

PCR-ELISA was done using a commercially available DIG-DNA detection kit from Roche. Absorbance values were measured using a BMG FLUOStar Optima microplate reader.

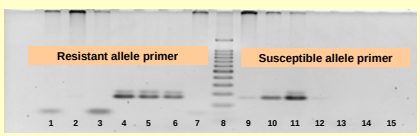


Fig 2. Agarose gel electrophoresis of *xa5* bacterial blight (BB) resistance gene using allele-specific primers. Lanes 1-3 and 9-11: IR24; lanes 4-6 and 12-14: IRBB5; lanes 7 and 15: no template control; lane 8: 100bp ladder.

Although initial results indicate good correlation with gel electrophoresis, further tests will be conducted to determine the threshold value. Conditions to be optimized will be PCR product, anti-DIG-antibody, and substrate concentrations.

Microarray genotyping-based

Purified PCR products (BB NILs, popular breeding varieties, and BB/Basmati lines) and 26- and 50-mer oligonucleotides were spotted on Corning-GAPS slides using a Genomics Solution GENETAC G3 robotics workstation. Alexa Fluor® 647 labeled 12-mer probes specific for the *xa5* resistant (R) allele were hybridized at 37 °C for 14 h using a Gene Machine hybridization chamber. After washing with 2x SSC + 0.1% SDS and 0.2x SSC + 0.1% SDS, the slides were viewed using a Perkin-Elmer Scan Array GX.

The 12-mer probe discriminated between the R and S oligos; no hybridization was observed on the S oligos. Cross-hybridization was observed with the PCR products of the S genotypes.

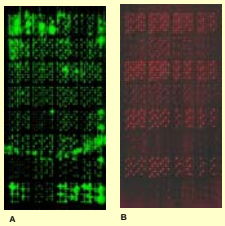


Fig 3. Microarray slides (A) stained with SYBR® green for spot quality check, and (B) hybridized with Alexa Fluor® 647.

FRET-based genotyping

Genomic and single-base extension (SBE) primers have been designed based on the sequence of the *Xa21* resistant line IRBB21 and *Xa21* susceptible line IR24. SBE was performed using purified PCR products and labeled ddNTPs, following the methods described by Takatsu, *et. al* (2004). Fluorescence intensities were quantified on a BMG FLUOstar OPTIMA microplate reader.

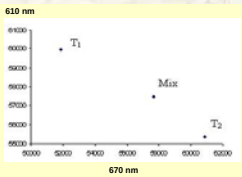


Fig 4. Fluorescence intensity ratios of *Xa21*-resistant (T1), susceptible (T2) and heterozygous lines. ROX®-ddATP and Cy5®-ddUTP were used as labeled ddNTPs for T1 and T2, respectively.

Although results indicate that FRET indeed occurred between SYBR® green and ROX® or Cy5®, the resistant and susceptible lines cannot be distinguished clearly.

Titration experiments involving SYBR® green and labeled ddNTP concentrations will be done. Other parameters, such as template concentration for SBE, will also be optimized.

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