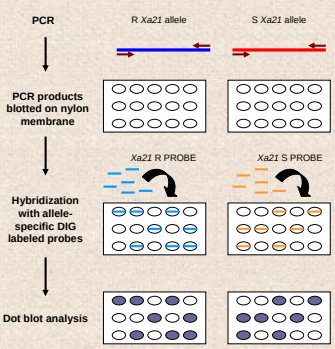
Low cost, non gel-based DNA marker technologies for marker assisted selection in rice

Currently, two obstacles for marker assisted selection (MAS) are the high cost for genotyping and 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, 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 (NON GEL-BASED) SIMPLE GENOTYPING METHODS

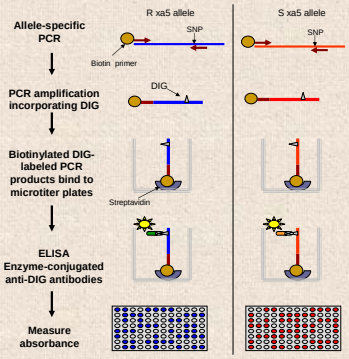
DOT BLOT GENOTYPING ASSAY

Methods adapted from Shirasawa et al. (2005).



PCR-ELISA DETECTION ASSAY

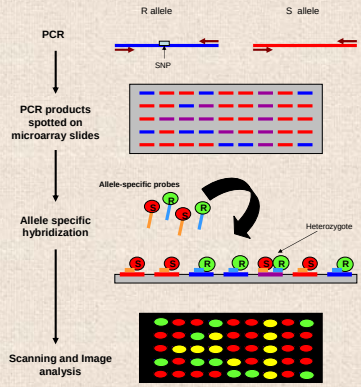
Methods adapted from Luk et al. (1997)



HIGH THROUGHPUT GENOTYPING METHODS FOR REGIONAL HUB LABS

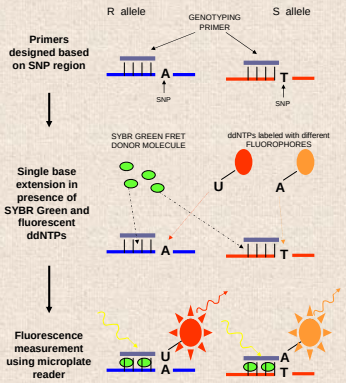
MICROARRAY-BASED GENOTYPING

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



FLUORESCENCE RESONANCE ENERGY TRANFER (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 and spotted on to nylon membranes. Initial results indicated successful hybridization but a lack of specificity. DNA concentration for blotting, hybridization stringency and different incubation times of substrate detection are currently being optimized.

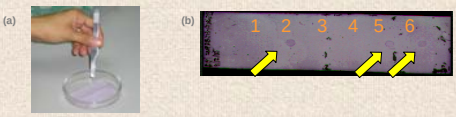


Figure 1. Preliminary dot blot results. (a) DIG-based detection. (b) Analysis of dot blot using *Xa21* allele-specific probes. Arrows indicate lines carrying the susceptible allele.

Gene sequence comparisons

Sequence comparisons have been made for *Xa21* R and S alleles from donor and recipient parents, in order to design diagnostic allele-specific PCR primers and probes. Further sequence analysis of other target genes is also being performed.

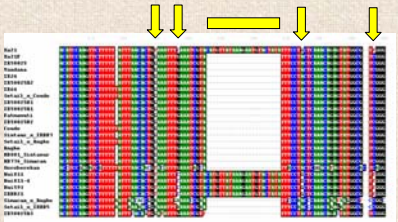


Figure 2. A portion of *Xa21* sequence showing an indel (yellow bar) in the kinase domain of *Xa21* R allele from IRBB21 and S allele from recipient varieties. SNPs discriminating R and S alleles are indicated by arrows.

PCR-ELISA detection assay

Allele-specific PCR primers have been developed for *xa5*. Results indicate that there was a good correlation between agarose gel results and absorbance values. However further optimization of reaction volumes and incubation times and temperatures is needed.

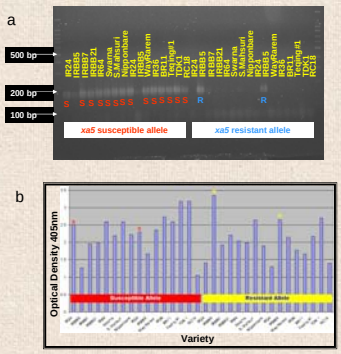


Figure 3. Comparison of agarose gel electrophoresis (a) and enzyme-immunoassay (b) of PCR products amplified using allele-specific *xa5* primers. The enzyme-immuno assay used the anti-DIG–peroxidase system for detecting DIG-labeled amplicons.

Microarray-based genotyping

PCR primers for *xa5* and 12-mer allele-specific R and S *xa5* probes (labeled with Alexa Fluor 546/647 dyes) have been designed. PCR amplification using DNA samples from recipient lines, in preparation for arraying, is currently underway.

FRET

PCR primers have been designed based on SNPs in *xa5* and sequence tagged site (STS) markers linked to *Xa7*. The 3' end of genotyping primers were designed to target regions exactly before SNPs such that fluorescently-labeled ddNTPs will be incorporated by single base extension.

Acknowledgements

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