We are developing three alternative genotyping methods for marker-assisted selection (MAS) application in a collaborative project between IRRI, CIMMYT, and NARES partner institutions. Two methods, microarray- and FRET-based genotyping, are being developed as high-throughput and cost-effective techniques that could be utilized in regional laboratory hubs/core genotyping institutes while two other methods, dot blot-based genotyping (see accompanying poster) and PCR-ELISA, are being developed as low-cost approaches that can be adopted in laboratories with limited resources. We are validating these methods using previously developed markers and characterized or cloned bacterial blight resistance genes (xa5, xa7, and xa21) in rice.

Results

Microarray-based genotyping

• Scanning analysis indicated that hybridization did not occur when 40°C was used; a partial hybridization occurred when a hybridization temperature of 35°C, however the signal was extremely weak and effects of uneven hybridization were observed.

• Results with 37°C 1 manual hybridization for 14 hrs with 12-mer R probe showed good discrimination between the R and S complementary oligos spotted on the slide; no hybridization was observed on the S oligos. However, cross hybridization was observed with the PCR products from S genotypes, probably due to length of the probe in relation to the template DNA and DNA purity.

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