

21. Markers linked to grain yield using bulked segregant analysis approach in rice (*Oryza sativa* L.)

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Bulked segregant analysis (BSA) (Michelmore et al. 1991) was employed in an earlier study to identify markers linked to maximum root length in rice. This study resulted in the identification and development of a sequence-characterized amplified region (SCAR) marker BH14 from a randomly amplified polymorphic DNA (RAPD) primer OPBH-14 (Sharma et al. 2003) and a microsatellite marker RM201 (Shashidhar et al. 2001). In the current study a similar approach was used to identify RAPD markers linked to grain yield in rice. BSA approach was utilized because of the quantitative nature of grain yield.

Eighty-nine doubled haploid lines derived from an IR64/Azucena mapping population were utilized for this study. DNA from these lines was extracted using a modified CTAB method (Caw and Oard 1997). Two bulks were prepared by pooling equal amounts of DNA from top ten genotypes segregating for higher and lower grain yield. Phenotyping for grain yield was carried out in the test plots in a randomized complete block design under well-watered conditions. Two hundred random decamer primers (Operon Technologies Inc, Alabama, USA) were screened (William et al. 1990) on eighty-nine genotypes of the mapping population. The polymerase chain reaction (PCR) was performed in a total volume of 20µl containing 10X PCR buffer (10mM TrisCl, 50mM KCl, 1.5mM MgCl₂), 12 pmol of each primer, 50ng of rice genomic DNA, 80µM of each of the four dNTPs and 1 unit of *Taq* polymerase (Bangalore Genei, India) with mineral oil overlay. Thermal conditions were 5min for 94° C followed by 35 cycles of 94° C for 1min, 54° C for 1min, 74° C for 1min and a final extension of 72° C for 5min. Amplified products were resolved on 3% agarose gels with ethidium bromide staining.

Of the two hundred RAPD primers screened, two RAPD primers OPAE-09 (5' TGCCACGAGG 3') and OPAE-14 (5' GAGAGGCTCC 3') showed consistent banding pattern after repeated amplifications. RAPD primer OPAE-09 amplified an 1800bp band (Fig. 1) in the high yielding parent IR64 and eight individuals of the high yield bulk, whereas no band was observed in the other parent Azucena and eight individuals of the low yield bulk at this region. The reason for the two individuals in both the bulks not amplifying the required band could be due to grain yield being a quantitative trait and this particular locus being one of them. This proved that OPAE-09 was a positive marker for higher grain

yield. On the contrary, RAPD primer OPAE-14 amplified a 1600bp band (Fig. 2) in the low yielding parent Azucena and nine individuals of the low yield bulk, but absent in the high yielding parent IR64 and all the individuals of the high yield bulk. Thus OPAE-14 was used as a marker to eliminate plants with low grain yield. As selection was being practiced based on absence of the band, care should be taken to see that the reaction has worked. Single marker analysis (ANOVA) established significant association between grain yield for both OPAE-09 ($P < 0.0001$, $R^2 = 12.19\%$) and OPAE-14 ($P < 0.0015$, $R^2 = 25.32\%$).

In addition to the above genotypes, both these RAPD primers were screened on several other diverse genotypes and the results were consistent with their respective yield potential (Fig. 3). Hence, both OPAE-09 and OPAE-14 can be utilized in a marker-assisted selection (MAS) programme for grain yield in rice. Efforts are underway to convert the two RAPD primers into SCAR markers and subsequently map them.

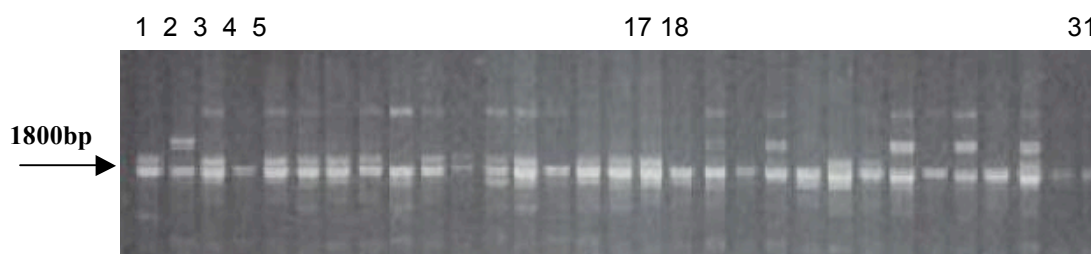


Fig. 1. RAPD profile of primer OPAE-09 in parents, bulks and individuals of the bulks linked to high grain yield in rice. Lanes 1: IR64, 2: Azucena, 3: high yielding bulk, 4: low yielding bulk, 5-17: individual genotypes of high yielding bulk, 18-31: individual genotypes of low yielding bulk.

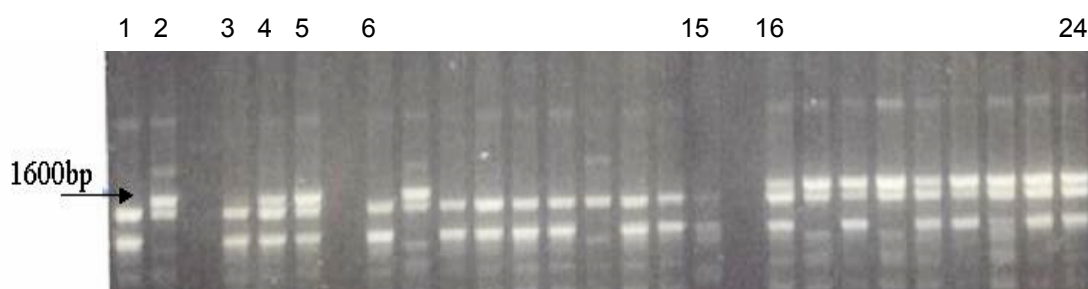


Fig. 2. RAPD profile of primer OPAE-14 in parents, bulks and individuals of the bulks linked to low grain yield in rice. Lanes 1; IR64, 2; Azucena, 3; high yielding bulk, 4; medium yielding bulk, 5; low yielding bulk, 6-15; individual genotypes of high yielding bulk, 16-24; individual genotypes of low yielding bulk.

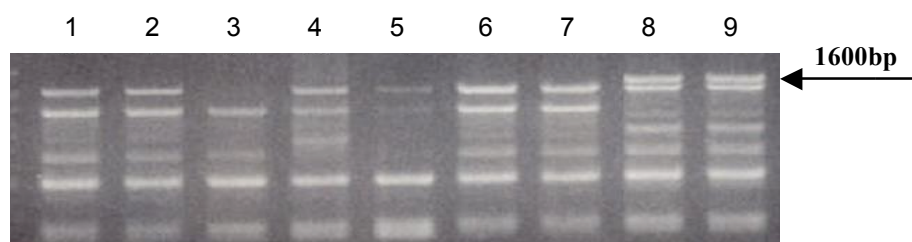


Fig. 3. RAPD profile of primer OP4E-14 linked to low grain yield in diverse varieties of rice. Lanes 1-9: Doddi, TK107, Doddabyanellu, Jaya, Budda, IR20, IR64, Azucena, Moroberekan.

References

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