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**TAGGING QTLs FOR MAXIMUM ROOT LENGTH IN RAINFED
LOWLAND RICE (*ORYZA SATIVA* L.) USING MOLECULAR
MARKERS**

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Abstract: A number of morphological, physiological and phenological traits are known to improve the performance of rice challenged by drought. Root morphological traits and stress-induced response form important components of drought tolerance. Enhancing grain yield remains the principal objective of most breeding programs. Interaction between primary traits poses a formidable challenge while dealing with grain yield under stress. The evaluation of root morphology at three different growth stages and grain yield along with related characteristics under contrasting moisture regimes was made using nine backcrosses along with their parent and standard checks. The backcrosses involved transgressant double haploid lines derived from IR64 and Azucena with IR64. Marked genotypic differences were observed for all root morphology as well as grain yield related characteristics across the sampling dates as revealed by individual and combined ANOVA. Among the nine backcrosses studied in this experiment, the BC₁F₂ population of P124 x IR64 were evaluated for forwarding based on their performance with respect to maximum root length and grain yield under both well-watered and low-moisture stress conditions. Sixty-nine plants – ten percent of the backcross population – were selectively genotyped using RAPD primers. Under well-watered conditions two RAPD markers showed strong linkage to QTLs for maximum root length evaluated under ww conditions. Two other markers could explain the considerable amount of variation in MRL under LMS. One of the markers identified under low-moisture stress conditions was also able to explain variability in maximum root length in the mean environment.

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Key Words: Drought Resistance, Maximum Root Length, Molecular Markers, Quantitative Trait Loci (QTLs), Rice.

INTRODUCTION

Rice (*Oryza sativa* L.), a semi-aquatic cereal, is adapted to a variety of climates. A number of morphological, physiological and phenological traits have been proposed to improve the performance of rice challenged by drought. Root system and stress-induced response are important components of drought resistance. Among the root morphological traits, maximum root length, root diameter and the root:shoot dry weight ratio were found to be associated with drought resistance in upland conditions [1,2]. The differential response/sensitivity of root and shoot growth to low-water potential is considered to be a means of avoiding excessive dehydration [3,4]. Despite ample genetic variability for many root morphological traits and other components (primary traits) of drought resistance, the genetic improvement of root characteristics in rice using conventional selection has proven difficult [5-7] because root traits are difficult to measure under field conditions.

The advent of molecular markers has created possibilities to dissect the variation in quantitative traits in a more meaningful way. Selection based on molecular marker genotypes can help breeders to overcome difficulties in the selection of favorable traits. This may yield a correlated response to selection with regard to comapped loci. Such traits often have a low heritability, implying that (conventional) direct selection could be ineffective.

MATERIAL AND METHODS

Five deep-rooted (P107, P192, P210, P331, and P333) and four shallow-rooted (P124, P163, P442, and P467) transgressants for maximum root length from the DH mapping population of IR64/Azucena were backcrossed to IR64. Twenty-four entries including nine BC₁F₁ plants, their parents and standard checks were evaluated in field experiments under different moisture conditions.

The experiment was carried out on the Hebbal campus of the University of Agricultural Sciences, Bangalore, India, during the 1999-2000 period. Two moisture regimes, namely well-watered (WW) and water stress (LMS), were imposed. Under well-watered regime, all the entries were watered daily throughout the cropping period. Under the water stress regime, moisture stress was imposed from 65 days after sowing (DAS) to 80 DAS by withholding irrigation and preventing rainwater access using a rainout shelter. Sampling in both WW and LMS conditions was done at maturity as described by Shashidhar *et al.* [8] and maximum root length (MRL) was measured in cm from the collar region to the tip of the longest root. In the subsequent year, three and four hundred plants from the backcross population involving P124 x IR64 were staggered, 100 plants each under WW and LMS conditions, respectively. The

five deep-rooted and five shallow-rooted plants from each stagger were selected for genotyping in the laboratory using RAPD primers.

As early vegetative stage sampling was involved in the first experiment and because identification of true backcrosses was difficult, RAPD markers were used to identify true crossed plants. Genomic DNA from the parents and all the BC₁F₁ plants was extracted using a miniprep protocol, while for QTL mapping, DNA was extracted as per a modified CTAB method [9]. The RAPD reaction mixture consisted of 2 µl of template DNA, 20 ng of random decamer primer (Operan, USA), 0.1 mM of dNTPs, 1 unit of Taq polymerase (Bangalore Genei, India) and 1X PCR buffer (10 mM Tris pH 8.0, 50 mM KCl, 1.5 mM MgCl₂ and 0.01 mg/ml gelatin) in a volume of 20 µl. The temperature profile was 1 cycle at 94°C for 2 min; 35 cycles at 94°C for 1 min, 40°C for 1 min, 72°C for 2 min; and finally 1 cycle at 72°C for 5 min. The amplified products were resolved on 1.4% Agarose gels, stained with ethidium bromide and visualized using UV transilluminator.

Data was subjected to individual and combined ANOVA based on RCBD over the two moisture regimes to assess the variability among the genotypes. The standard error of the treatment means using PROC GLM on SAS software [10]. The chi-square test was performed to examine the fit between the marker allele contribution of the Azucena and IR64 genomes in the selected DH lines. Cluster analysis of selected DH lines and parents was done by PROC CLUS on SAS software using the average linkage method. QTLs linked to markers for MRL were determined in WW and LMS conditions, as well as for the mean environment using single point analysis.

RESULT AND DISCUSSION

The chi-square test based on the equal contribution of both the IR64 and Azucena marker alleles in the DH lines showed skewedness towards Azucena in three lines, namely P107, P192, and P163. In P192, only 40% of the bands were informative, while in P163, 54% non-informative alleles were observed. A dendrogram based on 175 molecular markers [11] divided the experimental material into two distinct groups. Although grouping was only based on markers, it was fairly similar to the phenotypic classification based on root morphology in this study, with IR64 and Azucena falling into their respective groups.

As early vegetative stage sampling was involved in the experiments, RAPD markers were used to identify the true backcrossed plants. True BC₁F₁ plants were identified based on the presence of a male specific band/male specific bands following amplification with polymorphic RAPD primers. Of the 165 RAPD markers screened, 35 RAPD markers revealed polymorphism between the parents P124 and IR64. These RAPD primers were screened across 69 BC₁F₂ extreme individuals selected from the backcrossed population involving P124 and IR64. Plants were selected from a population of 700 plants evaluated for MRL and other root-related characteristics under either WW or LMS conditions.

Since a selective genotyping approach has been recommended to target a single trait [12, 13], co-segregation of polymorphic markers with phenotypic expression was considered only for MRL. The result of single marker analysis for the tagging of QTLs contributing to the performance of MRL is presented in Table 1.

Tab. 1. RAPD markers linked to QTLs controlling maximum root length under well-watered (WW), low moisture stress (LMS) and mean conditions (MC).

Marker	Condition	R ² (%)	Pr >F
OPBC10	WW	23.1	0.007
OPAK19	WW	13.8	0.047
OPAH13	LMS	12.6	0.026
OPBF02	LMS	9.3	0.062
	MC	5.7	0.05

Among the RAPD markers (Fig.1), OPAH-13 and OPBF-02, linked to a QTL, explained 12.6% and 9.3% of the total variation for MRL under LMS conditions. The statistical significance of these two markers was strong ($P < 0.026$). These two RAPD markers collectively explained 21.8% of the total variation in MRL under LMS conditions, as revealed by the multiple regression approach. Under LMS, a QTL linked to OPBC09 ($P < 0.007$) explained 23.1% of the total MRL variability. In addition, OPAK19 showed co-segregation ($P < 0.047$) with the QTL contributing to MRL under WW conditions. This marker explained 13.8% of the variation in performance for this trait. About 31.7% of the total variation in performance of this characteristic was explained using these two RAPD markers under WW conditions.

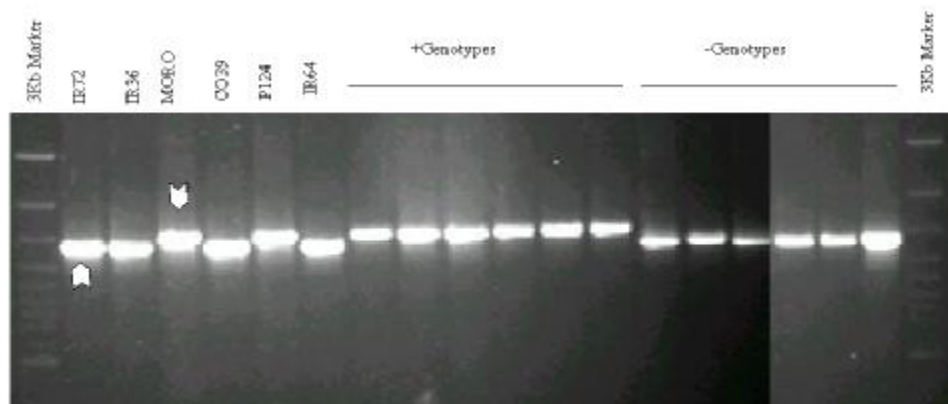


Fig. 1 RAPD amplification pattern of DNA from parent, and selected backcrossed plants and checks using OPAH13 primer

A low statistical threshold was used in this study to avoid false-negatives, although high threshold levels have been used to avoid false positives. The low threshold used in this study was chosen due to the relatively small size of the mapping population used for genotyping, as it is difficult to detect QTLs using a high threshold in a smaller population. Price *et al.* [14] found that 89% of the QTLs detected by composite interval mapping concurred with those detected by single-marker analysis.

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