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Comparative studies on QTL mapping by simple interval mapping and composite interval mapping models for selected growth and yield traits in rice (*Oryza sativa* L.)

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Abstract

QTL mapping was performed for five yield determining traits including grain yield in rice using a double haploid population of IR64 and Azucena cross. A comparison of QTLs detected by two different models simple interval mapping (SIM) using MAPMAKER/QTL and composite interval mapping (CIM) using QTL Cartographer was made, and common QTLs across the models were identified. The SIM detected 5 significant QTLs whereas CIM revealed 9 QTLs and only CIM could dissect flowering and maturity with QTLs, indicating its power and precision in QTL detection. Clustering of QTLs controlling correlated traits was common in both models. Apart from the QTLs detected by SIM, extra five QTLs were observed in CIM model with much higher LOD scores than SIM. The position of QTLs on different chromosomes was identical in both models except in case of new QTLs identified by CIM on chromosome 3 and 10. Congregation of multiple QTLs in the same marker interval concurred with the results of trait phenotypic association with each other indicated sharing of common regions of chromosomes by the correlated traits. These chromosomal regions may be focused for further saturation with additional markers to confirm the nature of QTL affecting the trait. The MAPMAKER/QTL and QTL Cartographer have different approaches in detecting the QTL and because of the more precision of the latter model, additional QTLs that had significant effect on traits were detected. However the common QTL detected by both the approaches, could be considered as stable QTL and be of use in Marker Assisted Selection.

Key Words: Rice, grain yield, simple interval mapping, composite interval mapping

Introduction

Rice is an important crop which feeds nearly 50 % of the world population. Because of its importance as a food crop and its smaller genome size, rice has been extensively used for molecular genetic studies. The genetics of rice facilitates the study of complex traits that are controlled by several genes with minor effects. It has also been important in comparative mapping in graminae species such as wheat, barley, rye, oat and maize. Hence, mapping of genes/QTLs of agronomic importance in rice can be expected to have positive impact on several of the most important cereal crops in the world (McCouch and Doerge, 1995). Most of the economically important traits are polygenic in nature and environment dependent, making the study more difficult. Molecular marker technology has facilitated in dissecting the complex nature of these traits by QTL analysis (Wang *et al.* 1999). An important goal in genetics and breeding is to identify and characterize QTL for the target traits. QTL mapping and the number of QTLs detected, which mainly depends on the accuracy of the experimental data (both phenotypic and marker data), also depends on other important factors including the model used for its analysis. Thus, locating significant and common QTLs detected across models proves to be an important work in this aspect.

Powerful statistical methods are available for dissecting genes controlling quantitative traits and this process is enhanced by the increased availability of molecular markers. Generation of high-resolution genetic maps has added advantage for this process (Doerge, 2002). Most QTL analysis techniques in plants have been developed to associate QTL with molecular markers using mapping population. Among the commonly used models in QTL mapping, Simple interval mapping (SIM) and composite interval mapping (CIM) are known to be widely used models. The principle behind SIM is to test a model for the presence of a QTL at many positions between two marker loci (Lander and Botstein, 1989). Whereas CIM performs SIM in the usual way, except that the variance from other QTL was accounted for by including partial regression coefficients from markers in other regions of the genome (Basten *et al.* 2000). The advantage of CIM methods is that they accommodate multiple QTL. It gives more power and precision than SIM because the effects of other QTL are not present as residual variance and it can eliminate the bias that would normally be caused by QTL that are linked to the position being tested.

In rice, QTL for various quantitative traits of agronomic importance have been mapped by different approaches using different populations. A comparison of regression interval mapping and multiple interval mapping for a linked QTL was carried out using simulated data of different F_2 populations, and distinct

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differences were noticed between the two methods (Mayer, 2005). Cai and Morishima (1998) carried out QTL analysis for yield contributing traits by both SIM and CIM methods. SIM could detect only three QTLs for days to heading and basic vegetative phase, where as CIM revealed other significant QTLs that controlled both traits, and in addition photoperiod sensitive phase was also dissected with QTLs. The differences between maximum-likelihood and regression interval mapping in QTL analysis were investigated analytically and numerically by simulation, and the results showed that maximum-likelihood method is more powerful than the latter in estimating mean squared errors and likelihood ratio test statistics (Cao, 2000).

The use of several marker-trait association analysis techniques such as simple linear regression, simple interval mapping and composite interval mapping provides the means to assess the robustness of QTL detection in a single experimental design. QTL mapping was carried out for a number of agronomically important traits of perennial ryegrass (*Lolium perenne* L.) by the above three models and the efficiency of CIM in detecting QTLs was compared with other models (Yamada *et al.* 2004). Marsan *et al.* (2001) made a comparison of SIM and CIM models while characterizing QTLs for grain yield and grain-related traits in a segregating population of maize. The results indicated that the CIM method employed had greater power in the detection of QTLs, and provided more precise and accurate estimates of QTL positions and effects than SIM. Though, both SIM and CIM models have been utilized for QTL mapping, but the comparison of both models in a single experiment are rare in case of rice. We carried out QTL analysis of grain yield and selected plant traits in a double haploid mapping population of rice using interval and composite interval mapping models, and a comparison of the same is discussed.

Material and Methods

Experimental details: The plant material consisted of a subset of 80 doubled haploid (DH) lines of IR64 x Azucena cross. The experiment was carried out in an augmented randomized complete block design, with five blocks; each block consisted of parents and the checks, during summer 2002 at Hebbal, Bangalore. Observations on five plants were taken at the time of harvesting and the mean values were used for the QTL analysis.

Linkage map and QTL analysis: The linkage map of rice consisting of 175 polymorphic markers (146 RFLPs, 3 isozymes, 14 RAPDs and 12 cloned genes) for IR64/Azucena DH population was previously developed (Huang *et al.* 1994) and recently 85 new markers were added to this map (Vaishali M.G. 2005, unpublished). The new map with 260 markers covering 2457 cM with an average distance of 9.45 cM between adjacent markers was used in the study. QTL mapping was carried out by performing simple interval and composite interval analysis using Mapmaker/QTL V 1.1b and QTL Cartographer V2.00 with threshold LOD scores of 2.0 and 2.50 respectively.

Results and Discussion

The number of QTL, detected by both SIM and CIM, along with the chromosome number, flanking markers, peak LOD value, % variation explained and the additive effect are presented in Table 1 and the chromosomal location of these QTLs on linkage map of rice is represented in Figure 1.

Simple Interval Mapping: Interval mapping detected a total of 5 QTLs for plant height, number of tillers and grain yield. No QTL for days to first flowering and days to maturity could be detected. A significant QTL for Plant height was detected on chromosome 8 (RG20-RZ143) which explained 7.3 % of total phenotypic variation at 2.16 LOD. This could be a new QTL for plant height as no QTL has previously been reported in that region on chromosome 8. Two QTLs, which together explained 18 % of phenotypic variation, were detected for number of tillers; one on chromosome 6 in between RM225 and RZ398 markers explaining 10.2 % of phenotypic variation at 3.09 LOD and the other QTL was found on chromosome 8 (2.2 LOD) in between markers RM38 and RG20 which contributed for 7.8 % of variation. Grain yield, an economically important trait was associated with two significant QTLs, which explained 18.5 % variation. The QTL detected on chromosome 6 had high LOD value (3.51) in between RM225 and RZ398 markers and it explained 11.5 % of the phenotypic variation. The other QTL located on chromosome 8 (RM38-RG20) explained 7 % of variation (Table 1).

Composite Interval Mapping: In total, nine QTLs controlling 5 traits were identified by composite interval mapping. The maximum number of QTLs was detected for grain yield (three), whereas days to first flowering accounted for only one QTL. Plant height was associated with two QTLs, which were located on chromosomes 3 and 8. The QTL on chromosome 3 (RZ284- RG179) had a significant LOD of 3.50 while the other on chromosome 8 (RM38-RZ143) had 3.36 LOD, both of which contributed for 18% of total variation and both possessed positive additive value of more than 13.0. Two QTLs were detected for number of tillers per plant. Of these, one was found on chromosome 6 in between marker interval RM225 and RM253 (3.88 LOD) explaining 11% of the trait. The other QTL mapped on chromosome 10 (RM239-G1084) controlled 7% of the trait phenotype. In contrast to SIM, the CIM could detect one significant QTL (9% variation) with an additive effect of 15.97 for days to first flowering on chromosome 3, indicating its increased power in detecting significant QTLs even at higher threshold values than that of SIM. Similarly, QTL for days to maturity was detected on chromosome 3 (RZ284-RG179) at 3.74 LOD with a positive additive value of 21.74, and the same chromosomal region was known to harbor QTLs for plant height and days to first flowering (Table 1). Grain yield was found to be associated with three significant QTLs on chromosome 3 (RZ 284-pRD10A), on chromosome 6 (RM225-RM253) and on chromosome 8 (RM38-RZ143). All these three QTLs contributed for 26 % of grain yield, and shared similar genomic regions with other traits (Figure 1).

Table 1: List of QTLs identified for selected plant traits and yield in rice using IR64xAzucena double haploid population by simple and composite interval mapping models

Trait	Model	No. of QTLs	Chromosome	Flanking markers	LOD	% Variance	QTL effect
Plant height	SIM	1	8	RG20 - RZ143	2.16	7.3	-11.931
	CIM	2	3	RZ284 - RG179	3.50	9.0	13.48
			8	RM38 - RZ143	3.36	9.0	13.40
Number of tillers	SIM	2	6	RM225 - RZ398	3.09	10.2	-1.6589
			8	RM38 - RG20	2.2	7.8	-1.4895
	CIM	2	6	RM225 - RM253	3.88	11.0	1.74
			10	RM239 - G1084	2.57	7.0	1.40
Days to flowering	SIM	-	-	- -	-	-	
	CIM	1	3	RZ284 - Prd10A	3.30	9.0	15.97
Days to maturity	SIM	-	-	- -	-	-	
	CIM	1	3	RZ284 - RG179	3.74	10.0	21.74
Grain Yield	SIM	2	6	RM225 - RZ398	3.51	11.5	-2.9551
			8	RM38 - RG20	2.04	7	-2.3393
	CIM	3	3	RZ284 - Prd10A	3.72	9.0	2.68
			6	RM225 - RM253	3.87	10.0	2.84
			8	RM38 - RZ143	2.64	7.0	2.36

Comparison of SIM and CIM: Two models used in this study detected QTLs that were common as well as different and which mainly depended on their approaches. The MAPMAKER/QTL and QTL Cartographer differ in their approaches in detecting QTLs. The number of QTLs detected for selected five traits were almost double in CIM (9 QTLs) as compared to SIM (5 QTLs). In addition, CIM detected QTLs for days to flowering and days to maturity which were not tagged by SIM. Though the threshold LOD fixed for SIM was less than that of CIM, SIM could not dissect QTLs for these two traits. Maximum number of QTLs was tagged by CIM at comparatively higher LOD than single marker analysis and SIM models for grain protein content in bread wheat (Gupta *et al.* 2003). From the comparison of QTL mapping results for grain yield and yield related traits in maize using SIM and CIM, Marsan *et al.* (2001) found out greater power in the detection of QTLs, and precise and accurate estimates of QTL positions and effects by CIM than SIM. However, in the present study, some common QTLs could be detected by both the approaches. Out of 14 QTLs detected together by SIM and CIM, 6 were common across both models with respect to their position on chromosomes (Figure 1). Concurrent QTLs for the same trait were detected mapped for tiller number and grain yield on chromosome 6, and for plant height and grain yield on chromosome 8. The concurrent QTLs identified by both models could be considered as stable QTLs detected and can be targeted in future for Marker Assisted Selection. Further, using large population and high density linkage map, we need to find out the similarities and the differences with these two models with respect to the QTL position, effect and interval-size of QTLs for more number of traits.

Clustering of QTLs for Correlated Traits: Correlation coefficients were calculated to find out the association among the traits. Correlation was negative between plant height and number of tillers whereas positive associations for tiller number and days to first flowering with grain yield per plant (Table 2). Trait correlation and congruence of multiple QTL in the same region can be either due to pleiotropic effects of a single gene or due to tight linkage of genes affecting different traits. Correlated traits share a common region of the chromosome (Hittalmani *et al.* 2002). Therefore, further saturation of these regions with more markers helps fine map the QTL and dissect the QTL further in this aspect. Both simple interval and composite interval mapping techniques identified QTL clusters on different

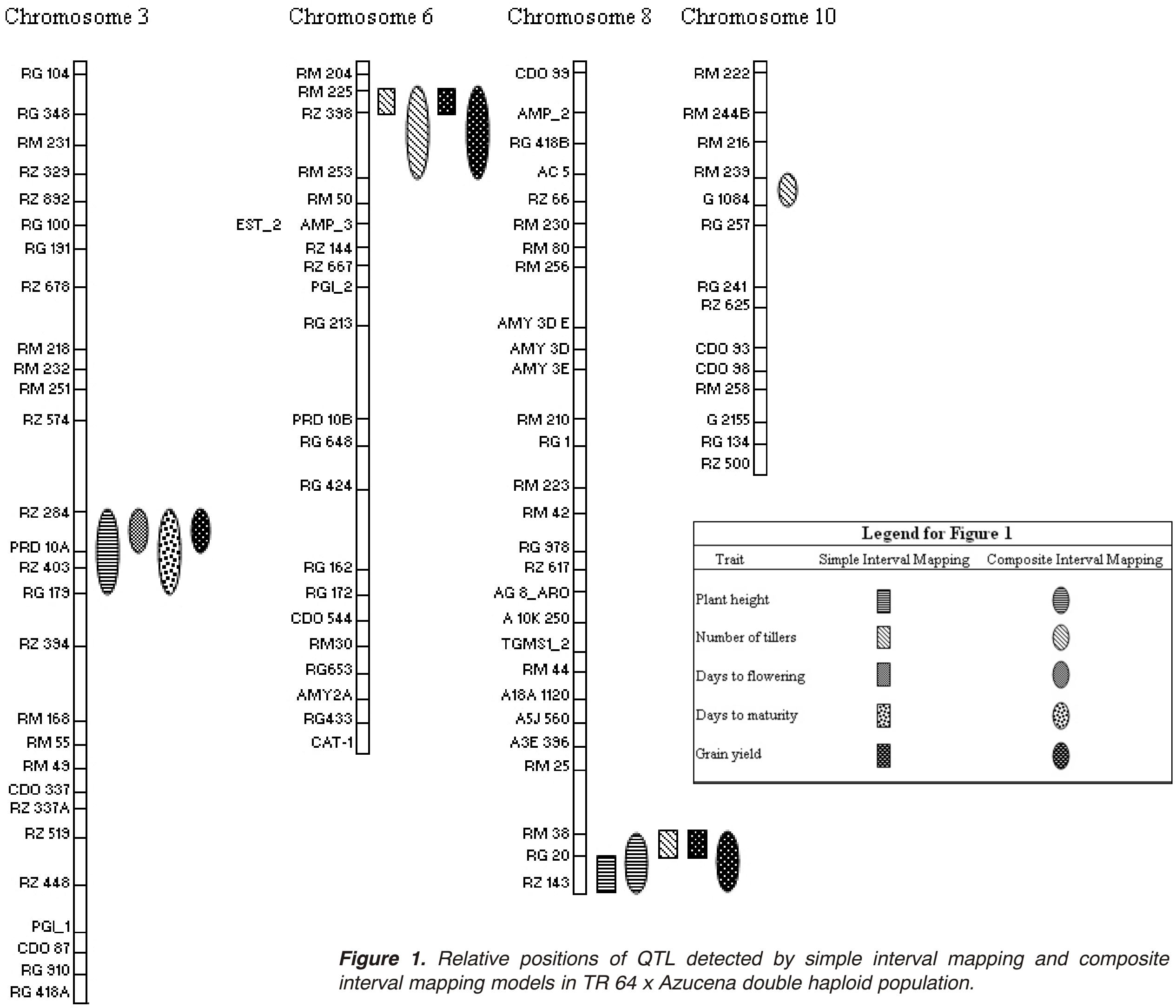

Figure 1. *Relative positions of QTL detected by simple interval mapping and composite interval mapping models in TR 64 x Azucena double haploid population.*

chromosomes wherein QTLs associated with the traits that showed strong positive association were mapped onto the same genomic region. In case of SIM, QTLs for number of tillers and grain yield were detected in the region flanked by RM225 and RZ398 markers on chromosome 6 and RM38 and RG20 markers on chromosome 8. Similarly, CIM revealed overlapping QTLs on chromosome 3 (RZ284-RG179) for all the traits except tiller number, on chromosome 6 (RM225-RM253) for tiller number and grain yield and on chromosome 8 (RM38-RZ143) for plant height and grain yield (Figure 1). The correlated QTLs for grain yield and tiller number have been reported by Xing *et al.* (2002). Zhuang *et al.* (1997) reported clustering of QTL for yield related traits, on chromosome 1, 2, 3, 4, 5, and 8.]

Table 2: Phenotypic correlation coefficient among five characters studied in double haploid lines of IR64 X Azucena cross.

Traits	Number of tillers	Days to first flowering	Days to maturity	Grain yield
Plant height	-0.401**	-0.128	-0.011	-0.121
Number of tillers		-0.199	-0.214	0.411**
Days to first flowering			0.776**	0.066
Days to maturity				0.211

**=Significant at 1%

The study conducted here provides insight into genetic control of grain yield and its related traits. With the same number and set of markers and genotypes used, CIM detected more number of loci for all five traits at higher threshold level than that of SIM. Hence, CIM model proves to be more efficient and sensitive in detecting QTLs. However, considerable resemblance between SIM and CIM was observed for the common QTLs detected by both the models with respect to the position of QTL on chromosomes. The detection of loci for correlated traits and coincident QTL helps in co-selection for multiple traits by targeting such QTLs while designing strategies for the trait improvement through marker-assisted selection.

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