QTL ANALYSIS FOR DEVELOPMENTAL BEHAVIOR OF PLANT HEIGHT IN RICE (*ORYZA SATIVA* L.)

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A doubled haploid population of 123 lines from IR64/Azucena was used to analyze the developmental behavior for plant height by conditional and unconditional QTL mapping methods in rice. Results showed that the number of QTLs detected was different at various stages. Some QTLs could be detected at all stages and some QTLs can be identified at only one or several stages. Temporal patterns of gene expressions for plant height changed with the development of trait.

1 Introduction

Since the introduction of molecular markers, quantitative trait loci (QTL) mapping in numerous species and for various traits have been carried out and well documented[3]. Up to now, in most of these reports, the phenotypic values at a given time (usually at the final trait) in plant development were used for QTL analysis. These studies have however ignored the distinct gene actions at different developmental stages which is a very important factor influencing quantitative trait development. In this paper, two kinds of dynamic QTL mapping strategies, refereed to as conditional and unconditional mapping, respectively, were used to explore the developmental behavior of plant height.

2 Materials and Methods

2.1 Materials

A population of 123 double haploid (DH) lines derived from a cross between the indica variety IR64 and japonica variety Azucena was used in the experiment. An RFLP map of the population contains 175 markers covering 2005 cM with an average distance of 11.5 cM between pairs of markers was used in mapping QTLs for plant height development[2].

2.2 Field Experiment

The total of 123 DH lines and their parents, IR64 and Azucena, were evaluated in the field in a randomized complete design with two replications in Hangzhou, China in 1996. The germinated seeds were sown in a seedling bed and seedlings were transferred to the field 30 days later. After 10 days of transplanting, plant

height (from the surface of soil to the tip of plant) was measured every 10 days in 5 plants (fixed throughout all stages) from each plot until all lines had headed. A total of 9 different measures was taken.

2.3 Statistical Analysis

Unconditional QTL mapping was conducted based on phenotypic value at time $t(y_{(t)})$ by the procedure of composite interval mapping[5,6].

$$y_{j(t)} = \beta_{0(t)} + \beta^*_{(t)} X^*_j + \sum_i \beta_{i(t)} X_{ij} + \varepsilon_{j(t)},$$

where $y_{j(t)}$ is the phenotypic value of the jth individual measured at time t; $\beta_{0(t)}$ is the population mean at time t, $\beta^*_{(t)}$ is the accumulated QTL effect at time t; X^*_j is the coefficient for QTL effect; $\beta_{i(t)}$ is the accumulated effect for the ith marker at time t; X_{ij} is the coefficient for the ith marker effect; and, $\varepsilon_{j(t)}$ is the residual error of the jth individual at time t.

Conditional QTL mapping was conducted based on the phenotypic mean at time t conditional on phenotypic mean measured at time $t - 1 (y_{(t|t-1)})$ also by the procedure of composite interval mapping[5,6].

$$y_{j(t|t-1)} = \beta_{0(t|t-1)} + \beta^*_{(t|t-1)} X^*_j + \sum_i \beta_{i(t|t-1)} X_{ij} + \varepsilon_{j(t|t-1)},$$

where $y_{j(t|t-1)}$ is the conditional phenotypic value of the jth individual; $\beta_{0(t|t-1)}$ is the conditional population mean, $\beta^*_{(t|t-1)}$ is the conditional QTL effect; $\beta_{i(t|t-1)}$ is the conditional effect for the ith marker; and $\varepsilon_{j(t|t-1)}$ is the conditional residual error of the jth individual.

Conditional phenotypic mean $(y_{(t|t-1)})$ of plant height in rice were obtained by the mixed model approaches[8,9]. Then both conditional and unconditional values in different stages were used to perform analysis of QTLs linked to molecular markers for plant height by QTL Cartographer v.1.1b[1]. The likelihood ratio (LR) value of 11.5, which equal to LOD score of 2.5[7], was used as threshold to declare the detection of a QTL.

3 Results

3.1 Unconditional QTL Mapping

Quantitative trait loci (QTL) detected for plant height at 9 different stages based on unconditional composite interval mapping were presented in Table 1. Four QTLs (ph1, ph2, ph3 and ph4) for the final plant height were detected

Table 1: Map regions and estimated genetic effects of QTLs for plant height of DH population at different stages based on conditional and unconditional mapping.

QTL	Marker	Dist.	Genetic effect	10D	20D	30D	40D	50D	60D	70D	80D	90D
Ph1	RZ730-	33.1	A_t	-2.23	-3.01	-2.94	-3.85	-6.36	-9.62	-14.2	-15.7	-17.66
	RZ801		$A_{t|t-1}$	-2.23				-1.79	-1.72	-1.65	-1.93	
Ph2	Amy1A/C	12.8	A_t			2.41	2.86	4.19	6.15	6.91	8.28	7.06
	-RG95		$A_{(t|t-1)}$		-0.65			1.24		-1.63		
Ph3	CDO87-	9.2	A_t			-3.45	-3.61	-4.72	-6.94	-7.7	-7.42	-7.12
	RG910		$A_{(t|t-1)}$		-0.81	-1.21	-1.57					
Ph4	RZ590-	2.7	A_t	-1.99	-2.51	-3.29	-4.52	-5.24	-7.46	-9.68	-8.84	-9.22
	RG214		$A_{(t|t-1)}$	-1.99		-0.96	-1.44					
Ph5-1	RG313-	6.6	A_t	2.18	2.36							
	RZ556		$A_{(t|t-1)}$	2.18		-1.06						
Ph5-2	RZ67-	12.8	A_t									
	RZ70		$A_{(t|t-1)}$					-1.87				
Ph6	Amy2A-	4.4	A_t					1.72	3.11		3.82	
	RG433		$A_{(t|t-1)}$					1.72	2.04			
Ph8	Amy3D/E	25.1	A_t				2.08	1.98	2.12			
	-RZ66		$A_{(t|t-1)}$							-2.48		

A_t and $A_{(t|t-1)}$ indicates unconditional and conditional genetic effects, respectively.

on chromosome 1, 2, 3 and 4, respectively. These QTLs were detected at most early stages. They also showed higher significant level with larger genetic effects than the others, and can be treated as major QTLs. Alleles from IR64 increasing plant height at ph2, but decreasing it at the others. Besides those, three other QTLs (ph5-1, ph6 and ph8) were detected at only early stages. Results indicated that more QTLs could be identified with time-specific QTL mapping.

3.2 Conditional QTL Mapping

The conditional QTL mapping results are also presented in Table 1. Unconditional QTLs found at time t have cumulative genetic effects due to genes expressed from the initial time to time t, while conditional QTLs detected for time $(t|t-1)$ will have net genetic effects of genes expressed only through time $(t-1)$ to time t. Unconditional QTLs detected at 10 days indicate the cumulative gene expression from initial time to 10 days of transplanting. Therefor, these QTLs are equivalent to conditioned QTLs at 10 days conditional on initial time. QTLs detected at 20 days conditional on 10 days indicating net effects of gene expression at the period for 10 to 20 days and so on.

A total of 8 genomic regions significantly affecting plant height were de-

tected by conditional mapping. For all these map positions, conditional QTLs were detected only at one to several stages. It indicated that gene or genes at a specific map position expressed at only some stages during the trait development. By comparing the conditional and unconditional mapping results, we found that all map positions of QTLs detected by unconditional mapping were also detectable by conditional mapping. It is interesting to note that a conditional QTL (ph5-2) was identified between markers RZ67 and RZ70 on chromosome 5 for (50D|40D), But no corresponding QTL was identified by unconditional mapping.

4 Discussion

The gene expression for the development of a trait is time-dependent, dynamic QTL mapping is necessary to explore the gene actions for the development of a trait[4]. Two kinds of dynamic QTL mapping strategies were used in the present study. The first is to map QTLs by time-specific measures, which is refereed to as unconditional QTL mapping in our study. These unconditional QTLs will reveal cumulative gene expressions from initial time to time t. The second is to map QTLs by predicted conditional genetic effects for time $(t|t-1)$[8]. This method which is refereed to as conditional QTL mapping, is first proposed in the present study. Conditional QTLs will reveal the gene expression for a specific growth period from time $t-1$ to time t.

In this study, four major QTLs for plant height were detected throughout most stages by unconditional mapping method (Table 1). Prior to the evaluation of conditional mapping results, we assumed that gene expression for plant height might be similar at different development stages. But the conditional mapping results showed that the temporal patterns of gene expressions at these 4 regions were quite different. For example, conditional QTLs were detected for (10D|initial), but undetected from 20 to 40 days, then consecutively detected from 40 to 80 days at ph1. This suggests that the gene or genes at this map region were expressed before 10 days, stopped for 20 days and then re-expressed from 40 to 80 days. The renewed gene expression at this locus from 40 to 80 days corresponded to the stem elongation and heading stages of rice. At ph4, conditional QTLs were detected at 10 days, and from 20 to 40 days. Results showed that at this map position the gene or genes controlling plant height were only expressed at the seedling stage before 40 days, but their cumulative effects could be detected across later developmental stages based on unconditional mapping method.

The gene expression pattern at ph3 is different from those of ph1 and ph4. Unconditional QTL at this map region was firstly detected at 30 days and then

across all later stages. Conditional QTL at the same position was only detected from 20 to 40 days. It shows that conditional mapping method can found gene expression at some stages when it can not be detected as a cumulative effect by unconditional method. Results also imply that the unconditional QTL detected at 30 days was the cumulated gene effects of 20-day period from 10 to 30 days. No conditional QTL was detectable after day 40, which suggests that the gene action at this region might turn off at 40 days. Therefore, the unconditional QTL detected after 40 days might be the cumulative effects of genes expressed at early stages.

At the genomic region of ph2, conditional QTLs were found for (20D|10D), (50D|40D) and (70D|60D), respectively. But opposite genetic effects were observed for these stages. Similar results were also observed for other genomic regions such as ph5-1 and ph8. It was suggested that the individual gene at a specific genomic position might have opposite genetic effects or multiple genes at the similar genomic region might express differently at different stages. These opposite genetic effects expressed at different stages in the similar genomic position might be counteracting with each other and result in failure to detect QTLs of cumulative genetic effects at later stage such as for ph5-1 and ph8.

Acknowledgments

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