Molecular Dissection of Developmental Behavior of Plant Height in Rice (*Oryza sativa* L.)

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ABSTRACT

A doubled haploid population of 123 lines from IR64/Azucena was used to dissect the developmental behavior and genotype by environment interaction for plant height by conditional and unconditional quantitative trait loci (QTL) mapping methods in rice. It was shown that the number of QTL detected was different at various measuring stages. Some QTL could be detected at all stages and some only at one or several stages. More QTL could be found on the basis of time-dependent measures of different stages. By conditional QTL mapping of time-dependent measures, it is possible to reveal dynamic gene expression for quantitative traits. Mapping QTL for genetic main effects and *GE* interaction effects could help us in understanding the nature of QTL × environment interaction for the development of quantitative traits.

S INCE the end of the 1950s, high-yielding rice varieties of reduced plant height with high lodging resistance, favorable plant type, and high-harvest index have been released in almost all rice-growing countries (Ming 1987). These high-yielding varieties have played a vital role in food sufficiency for countries where rice is a staple food. Genetic studies for plant height in rice have been carried out by many rice researchers (Aquino and Jennings 1966; Ming and Xiong 1983; Ming 1987; Oba *et al.* 1990). Plant height in rice is generally considered to be controlled by both qualitative and quantitative genes (Huang *et al.* 1996). At least 60 dwarfing genes, designated *d*-1 to *d*-60, have been identified in rice by classic genetic analysis (Kinoshita 1995). The recent advent of molecular marker techniques has resulted in the construction of molecular maps to greatly facilitate the study of quantitatively inherited traits and has made it possible to dissect the polygenes for such traits into Mendelian factors and to use them in marker-assisted selection (Paterson 1995; Stuber 1995). In rice, quantitative trait loci (QTL) for various quantitative traits of agronomic importance have been mapped (Yano and Sasaki 1997). Molecular mapping of genes for plant height (Cho *et al.* 1994; Li *et al.* 1995; Xiao *et al.* 1995; Huang *et al.* 1996; Wu *et al.* 1996; Zhuang *et al.* 1997) or its components (Lin *et al.* 1996) in rice has been reported.

According to the theory of developmental genetics, genes are expressed selectively at different growth stages. The development of morphological traits occurs through the actions and interactions of many genes that might behave differentially during growth periods; gene expression can be modified by interaction with other genes and by environmental effects (Atchl ey and Zhu 1997). Conventional statistical genetic analysis results have revealed that gene action was distinct at various developmental stages and the genetic model from the final character could not fully reflect the real gene action during the development of the character (Kheiralla and Whittington 1962; Peat and Whittington 1965; Wu 1987; Xu and Shen 1991; Zhu *et al.* 1993; Zhu 1995). Therefore, to fully understand the genetic expression of the development of quantitative traits, dynamic mapping at different stages is needed. But most QTL mapping efforts reported so far have been focused on a character at a specific or final growth stage (Xu 1997).

Genotype × environment (*GE*) interaction, which is differential genotypic performance across environments, is an important component influencing trait development, especially quantitative traits. Identification of QTL that show consistency in expression across environments, even diverse environments, would be desirable for marker-assisted selection programs (Vel dboom and Lee 1996a). Significant QTL × environment interactions for various traits in a number of crops have been reported recently (Paterson *et al.* 1991; Stuber *et al.* 1992; Schon *et al.* 1994; Lee *et al.* 1996; Lu *et al.* 1996; Romagosa *et al.* 1996; Vel dboom and Lee 1996a,b; Zhuang *et al.* 1997; Yan *et al.* 1998a).

It is necessary, therefore, to understand the dynamics of gene expression and interactions with environments for developmental quantitative traits that will lay down the basis for map-based cloning and for improving the efficiency of marker-assisted selection (Xu 1997). In this article, the dynamic QTL for developmental behavior of plant height in rice was investigated with time-dependent measures evaluated in different locations. Further,

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by combining the newly developed statistical procedures of analyzing conditional genetic effects (Zhu 1995) and the composite interval mapping method (Zeng 1993, 1994), conditional QTL of plant height in rice that account for gene expression at a specific developmental period ($t - 1 \rightarrow t$) were identified. Because the doubled haploid (DH) population was evaluated in two different environments, the genetic main effects and *GE* interaction effects at different stages were predicted and used in mapping of QTL, which will help us explore possible QTL × environment interaction. The temporal gene expressions and the *GE* interaction effects for plant height development are also discussed.

MATERIALS AND METHODS

A population of 123 DH lines derived from a cross between the irrigated indica variety IR64 and the upland japonica variety Azucena (Guiderdoni *et al.* 1992) was used in the experiment. Six restriction enzymes (*Dra*I, *Eco*RV, *Hin*dIII, *Sca*I, *Xba*I, and *Eco*RI) were used for the parental polymorphism survey. An RFLP map of the population was established by Huang *et al.* (1995) from an initial population of 135 DH lines with 135 RFLP markers distributed among the 12 chromosomes. This map was recently updated by adding 40 new isozyme and RAPD markers and presently contains 175 markers covering 2005 cM with an average distance of 11.5 cM between pairs of markers (Huang *et al.* 1997). This new map was used for QTL analysis in the experiment.

Field experiment: The 123 DH lines and their parents, IR64 and Azucena, were grown in a randomized complete design with two replications (plots) in Hangzhou and Hainan Island, China. Hangzhou is located in eastern China at ~30° north latitude. Hainan Island is located in the South China Sea at 18° north latitude. These two places showed great difference in climate, soil conditions, day length, and even rice growing seasons. In Hangzhou, the experiment was carried out from late May to early November 1996. In Hainan, rice can be grown well all year round. Our experiment was done from early December 1995 to late April 1996.

In both locations, the germinated seeds were sown in a seedling bed and seedlings were transferred to a paddy field 30 days later, with a single plant per hill spaced at 15 × 20 cm. Each plot included three to four lines with eight plants per line. After 10 days of transplanting, plant height (from the surface of the soil to the tip of the plant) was measured every 10 days in five central plants (fixed through all measuring stages) from each plot until all lines had headed. A total of nine different measurements were taken during the whole rice growth period. Fertility and cultivation regimes were consistent with optimum rice production for these regions.

Statistical analysis: QTL could be indirectly searched for complicated quantitative traits such as those with *GE* interaction effects or with time-dependent measures of developmental behavior by interval mapping or composite interval mapping methods (Zhu 1998). Because the present experiments were conducted with 123 genotypes in two locations (as different environments) with two blocks, the phenotypic performance of the *j*th genetic entry at time *t* in the *k*th block within *h*th environment can be expressed by

$$y_{hjk(t)} = \mu_{(t)} + E_{h(t)} + G_{j(t)} + GE_{hj(t)} + B_{hk(t)} + e_{hjk(t)}, \quad (1)$$

where $\mu_{(t)}$ is the population mean at time *t*, fixed; $E_{h(t)}$ is environment effect at time *t*, $E_{h(t)} \sim (0, \sigma^2_{E(t)})$; $G_{j(t)}$ is genetic main effect at time *t*, $G_{j(t)} \sim (0, \sigma^2_{G(t)})$; $GE_{hj(t)}$ is genotype × environment interaction effect at time *t*, $GE_{hj(t)} \sim (0, \sigma^2_{GE(t)})$; $B_{hk(t)}$ is block effect in the *h*th environment at time *t*, $B_{hk(t)} \sim (0, \sigma^2_{B(t)})$; and $e_{hjk(t)}$ is residual effect at time *t*, $e_{hjk(t)} \sim (0, \sigma^2_{e(t)})$.

The environment effects (*E*), genetic main effects (*G*) and *GE* were predicted by the adjusted unbiased prediction method (Zhu and Weir 1996). These random effects were then used to predict main effect data $y_{j(G(t))} = \mu_{(t)} + G_{j(t)}$ and *GE* interaction effect data $y_{hj(GE(t))} = \mu_{(t)} + E_{h(t)} + GE_{hj(t)}$, respectively. The composite interval mapping method (Zeng 1993, 1994) was applied to analyze the predicted $y_{j(G(t))}$ for searching QTL with genetic main effects at time *t*,

$$\hat{y}_{j(G(t))} = \beta_{0(G(t))} + \beta^*_{(G(t))}X^*_j + \sum_i \beta_{iG(t)}X_{ij} + \varepsilon_{j(G(t))}, \quad (2)$$

where $\beta_{0(G(t))}$ is the population mean at time *t*; $\beta^*_{(G(t))}$ is the accumulated QTL main effect at time *t*; X^*_j is the coefficient for QTL effect; $\beta_{iG(t)}$ is the accumulated effect at time *t* for the *i*th marker; X_{ij} is the coefficient for the *i*th marker effect; and $\varepsilon_{j(G(t))}$ is the residual error of the *j*th individual at time *t*.

The predicted $\hat{y}_{hj(GE(t))}$ was analyzed also by the composite interval mapping method (Zeng 1993, 1994) for dissecting QTL with *GE* interaction effect in environment *h* at time *t*,

$$\hat{y}_{hj(GE(t))} = \beta_{0(GEh(t))} + \beta^*_{(GEh(t))}X^*_{hj} + \sum_i \beta_{i(GEh(t))}X_{hij} + \varepsilon_{j(GEh(t))}, \quad (3)$$

where $\beta_{0(GEh(t))}$ is the population mean of environment *h* at time *t*; $\beta^*_{(GEh(t))}$ is the accumulated QTL × *E* interaction effect of environment *h* at time *t*; X^*_{hj} is the coefficient for QTL × *E* interaction effect; $\beta_{i(GEh(t))}$ is the accumulated effect for the *i*th marker of environment *h* at time *t*; X_{hij} is the coefficient for the *i*th marker effect; and $\varepsilon_{j(GEh(t))}$ is the residential error of the *j*th individual in environment *h* at time *t*.

Genetic behavior measured at time *t* is the confounded results of genes expressed before time ($t - 1$) and effects within the period from time ($t - 1$) to *t*. These kinds of gene effects are usually not independent. The net genetic main effects and *GE* interaction effects between time ($t - 1$) and *t* can be evaluated by the conditional effects ($G_{(t|t-1)}$ and $GE_{(t|t-1)}$) at time *t* given phenotypic mean measured at time ($t - 1$). The mixed model approaches (Zhu 1995) were used to obtain the conditional genetic main effects ($y_{j(G(t|t-1))} = \mu_{(t|t-1)} + G_{j(t|t-1)}$) and *GE* interaction effects ($y_{hj(GE(t|t-1))} = \mu_{(t|t-1)} + E_{h(t-1)} + GE_{hj(t|t-1)}$) for plant height at different measuring stages in rice. Then the composite interval mapping method (Zeng 1993, 1994) was applied to analyze the conditional data. Conditional QTL with net main effects ($\beta^*_{(G(t|t-1))}$) during time ($t - 1$) to *t* were searched by Equation 2 for predicted $\hat{y}_{j(G(t|t-1))}$. Conditional QTL × *E* interaction effects ($\beta^*_{(GEh(t|t-1))}$) were detected by Equation 3 for predicted $\hat{y}_{hj(GE(t|t-1))}$.

The analysis of QTL with unconditional or conditional effects was conducted by QTL Cartographer v 1.1b (Basten *et al.* 1996). The likelihood ratio value of 11.5, which is equal to a LOD score of 2.5 (Zeng and Weir 1996), was used as a threshold to declare the detection of a QTL.

RESULTS

Phenotypic variation and environmental effects: The phenotypic values of plant height for the DH population and its parents in nine measuring stages are presented in Table 1 for two environments. Azucena showed greater plant height than IR64 at all stages in both locations. Plant height was greater in Hangzhou than in Hainan at all stages for both DH lines and their parents. Highly

TABLE 1

Phenotypic values of plant height (cm) for the DH population and its parents in nine different measuring stages evaluated in two environments

| Location | Stage (day) | Parents | | DH population | | | | | |
		IR64	Azucena	Max.	Min.	Mean	SD	Skew	Kurt
Hangzhou	10	36.7	42.3	49.9	25.3	36.4	5.16	0.28	−0.23
	20	42.4	53.2	63.8	32.6	45.3	6.53	0.42	−0.09
	30	49.3	60.4	72.2	35.6	51.4	7.28	0.43	−0.12
	40	60.5	73.2	82.7	42.9	59.7	8.35	0.35	−0.16
	50	76.4	88.3	102.2	53.3	75.7	11.47	0.15	−0.38
	60	83.2	117.6	137.4	58.9	90.5	16.61	0.26	−0.33
	70	87.7	136.1	148.0	62.6	101.0	19.81	0.07	−0.57
	80	98.1	151.6	159.0	68.8	112.0	20.82	−0.01	−0.70
	90	98.1	151.6	159.0	72.0	113.8	21.65	0.00	−1.02
Hainan	10	18.1	22.3	32.9	12.7	22.31	4.42	−0.53	0.30
	20	33.0	36.5	49.4	24.5	35.76	5.75	−0.36	0.20
	30	36.6	47.9	63.1	29.2	43.22	7.54	−0.35	0.25
	40	42.0	56.3	75.7	32.4	49.23	10.04	−0.47	0.32
	50	43.6	58.9	82.7	33.6	55.21	11.92	−0.72	0.26
	60	47.4	66.8	96.2	39.3	62.17	13.12	−0.76	0.28
	70	50.1	73.6	104.5	39.7	68.43	16.27	−0.97	0.27
	80	64.1	85.2	119.5	46.6	83.00	19.03	−1.19	0.23
	90	69.1	113.6	138.0	58.9	92.78	20.35	−1.08	0.27

significant *GE* interaction was observed at all measuring stages in the combined analysis over the two environments. The *GE* interaction variances accounted for 7.3 to 23.4% of the total genetic variances at different stages (data not shown). The average plant height of DH lines in Hangzhou was 8.2 to 29.0 cm greater than that in Hainan across different stages. The plant height of the DH population segregated continuously and both skew and kurt values were less than 1.0 at most stages (Table 1). It was suggested that plant height segregation of the DH population fit normal distribution for most stages in both locations and was suitable for QTL analysis. Transgressive segregants taller than the tall parent Azucena or lower than the short parent IR64 were observed at all stages.

QTL for plant height growth: *Unconditional mapping:* The chromosomal regions and estimated genetic effects of QTL affecting plant height at different developmental stages evaluated in two environments are presented in Table 2. A total of nine genomic regions significantly affecting plant height growth were detected on 7 out of the 12 chromosomes. Seven genomic regions significantly affecting plant height were detected in the DH population evaluated in Hangzhou. Of these, four identified QTL at the final stage (ph1 between markers RZ730 and RZ801 on chromosome *1*, ph2 between markers Amy1A/C and RG95 on chromosome *2*, ph3 between markers CDO87 and RG910 on chromosome *3*, and ph4 between markers RZ590 and Rg214 on chromosome *4*). Two of these (ph1 and ph4) showed significant QTL at all stages but the other two (ph2 and ph3) were detectable only after 30 days of transplanting. The

other three map regions (ph5-1, ph6, and ph8) showed QTL only at two to three stages. The tall parent Azucena contributed alleles for increasing plant height at ph1, ph3, and ph4, but for decreasing plant height at other map regions.

Six chromosomal regions showing significant association with plant height were identified in Hainan. Among these, three QTL (ph1, ph2, and ph3) that could be detected at all measuring stages were also detectable in Hangzhou. The other three QTL (ph5-2, ph7, and ph10-2), which were significant only at middle stages, were not identified in Hangzhou. Of these six chromosomal regions, alleles for Azucena increased plant height only at ph1 and ph3 but decreased the trait for the other four loci, even though Azucena is much taller than IR64. Results showed that alleles for plant height were dispersed among the two parents (Xu 1997) and revealed the genetic basis for the production of transgressive segregation for plant height in the DH population.

Because of the significant interactions between genotype and environment, QTL mapping was also conducted using both genetic main effects and *GE* interaction effects. QTL detected with genetic main effects indicated that genes at these genomic regions would express the same way across different environments. QTL detected with *GE* interaction effects suggested that the gene expression at these loci was environment dependent. When the genetic main effects were used in composite interval mapping, seven chromosomal regions significantly influencing plant height development were found. Of these, three QTL (ph1, ph2, and ph3) were significant in both environments. Two QTL

TABLE 2

Chromosomal regions and estimated genetic effects of QTL for plant height at different stages in two environments

QTL	Marker interval	Distance (cM)	Days	Hangzhou		Hainan		Main effect		GE^b in Hangzhou		GE in Hainan	
				t	$t/t-1^a$	t	$t/t-1$	t	$t/t-1$	t	$t/t-1$	t	$t/t-1$
						Chromosome *1*							
Ph1	RZ730–RZ801	33.1	10	−2.23	−2.23	−2.87	−2.87	−2.14	−2.14			−1.08	−1.08
			20	−3.01		−2.81		−2.47				−0.95	
			30	−2.94		−4.53		−3.60				−1.45	−0.52
			40	−3.85		−6.8		−4.6				−3.00	−0.52
			50	−6.36	−1.79	−8.52	−1.36	−5.06			−1.68	−3.12	1.05
			60	−9.62	−1.72	−9.39	−1.14	−9.54	−0.55	−3.09	−1.70	−2.64	1.10
			70	−14.20	−1.65	−12.54		−12.57		−4.39		−3.51	
			80	−15.70	−1.93	−15.49	−1.82	−15.01		−4.47		−4.35	
			90	−17.66		−15.68		−16.98		−4.07		−4.36	
						Chromosome *2*							
Ph2	Amy1A/ C–RG95	12.8	10			1.75	1.75	1.03	1.03			0.63	0.63
			20		−0.65	2.16		1.51				0.76	
			30	2.41		2.22		1.93				0.93	
			40	2.86		3.76		2.91		0.99		1.25	
			50	4.19	1.24	4.31		2.77	0.92		−0.74	1.05	−0.74
			60	6.15		4.03		4.74		2.55			
			70	6.91	−1.63	6.06	1.38	6.07		2.67			
			80	8.28		8.31	1.41	7.73		2.42		1.93	
			90	7.06		8.35		7.00		1.62		1.69	
						Chromosome *3*							
Ph3	CDO87–RG910	9.2	10			−1.56	−1.56	−1.03				−0.55	
			20		−0.81	−2.21		−1.94				−0.69	
			30	−3.45	−1.21	−3.26	−0.77	−3.34	−0.58	−1.12		−0.81	
			40	−3.61	−1.57	−5.01		−3.68		−1.01		−1.71	−0.51
			50	−4.72		−5.65		−3.41				−1.73	
			60	−6.94		−5.09		−5.28		−2.78		−1.83	
			70	−7.70		−6.26		−7.16		−3.04		−1.73	
			80	−7.42		−6.93	1.76	−6.95		−2.47		−1.64	
			90	−7.12		−6.94		−6.79		−1.95		−1.85	
						Chromosome *4*							
Ph4	RZ590–RG214	2.7	10	−1.99	−1.99			−1.20	−1.20	−1.15	−1.15		
			20	−2.51						−1.32			
			30	−3.29	−0.96		−0.67		−0.54	−1.56	−0.39		
			40	−4.52	−1.44			−3.52	−0.62	−1.91			
			50	−5.24				−2.56		−1.21			
			60	−7.46				−5.69		−3.35			
			70	−9.68				−5.47		−3.81			
			80	−8.84				−6.11		−3.62			
			90	−9.22				−7.06		−1.95			

(continued)

TABLE 2

(Continued)

QTL	Marker interval	Distance (cM)	Days	Hangzhou t	Hangzhou t|t − 1[a]	Hainan t	Hainan t|t − 1	Main effect t	Main effect t|t − 1	GE[b] in Hangzhou t	GE in Hangzhou t|t − 1	GE in Hainan t	GE in Hainan t|t − 1
						Chromosome 5							
Ph5-1	RG313–RZ556	6.6	10	2.18	2.18					1.10			
			20	2.36									
Ph5-2	RZ67–RZ10	12.8	10			0.96	0.96					0.74	0.65
			20			1.67	0.89	1.85				0.95	0.37
			30		−1.06	2.4					−0.50	1.32	
			40			2.81						1.63	
			50		−1.87	2.72	−0.6					1.79	
			60									1.97	
			70										
						Chromosome 6							
Ph6	Amy2A–RG433	4.4	40	1.72	1.72								
			50	3.11	2.04								
			60	3.82									
			70							2.07	1.27		
						Chromosome 7							
Ph7	RZ978–CDO38	5.8	10			0.97	0.97	1.05	1.05				
			20			1.32		1.30					
			30			1.86		1.50					
			40			2.34	−0.65						
			50			2.78							
			60			2.63							
			70			3.25							
						Chromosome 8							
Ph8	Amy3D/E–RZ66	25.1	20	2.08	2.08			1.34					
			30	1.98				1.71					
			40	2.12									
			60		−2.48		−0.87		−0.86				
			70										
						Chromosome 10							
Ph10-1	G1081–RG257	6.6	70						2.01		1.03		
Ph10-2	RG134–RZ500	3.8	30			1.96							
			40			2.15							
			50			2.28						0.93	
			60			2.78						1.29	

Plant height is measured in centimeters throughout.

[a] t and t|t − 1 indicate unconditional and conditional QTL, respectively.

[b] Genotype × environment interaction.

(ph4 and ph8) were detected only in Hangzhou and the other two (ph5-2 and ph7) were detected in Hainan. No corresponding QTL with genetic main effects were detected for the other two loci (ph5-1 and ph10) that were only found at middle stages in Hainan. The parental contribution of alleles was similar to those detected by phenotypic data, although their magnitude and significant stages showed some differences.

A total of six map regions significantly affecting plant height were identified by using *GE* interaction effects in each location. Among these, three QTL (ph1, ph2, and ph3) showed significant *GE* interaction in both locations, but with unequal magnitudes of gene effects at different stages. At these three map positions, common QTL were also detected by mapping the phenotypic data in both environments. Besides these, other genomic regions, such as ph4 and ph5-2, showed significant *GE* interaction effects at only one location, and corresponding QTL were also detected by using phenotypic data in the respective environment. Results indicated that most QTL detected by phenotypic data might also have *GE* interaction effects.

Conditional mapping: The conditional genetic effects at time t given the phenotypic values observed at ($t -$ 1) will indicate the net effects of gene expression from time ($t - 1$) to t, which are independent of the casual effects (Zhu 1995). Therefore, conditional QTL will reveal the gene expression at the specific period from time ($t - 1$) to t at that locus. QTL detected at 10 days indicate the cumulative gene expression from the initial time to 10 days after transplanting, when the phenotypic values were first measured. Therefore, these QTL are equivalent to conditional QTL at 10 days conditioned on initial time. QTL detected at 20 days conditioned on 10 days indicate net effects of gene expression at the period from 10 to 20 days and so on. When conditional genetic effects were analyzed by composite interval mapping, a total of eight and seven chromosomal regions showed significant QTL for plant height in Hangzhou and Hainan, respectively (Table 2). Of these, five QTL were detected in both environments, although the significant stages showed some difference between the two environments. All genomic regions revealed by unconditional QTL mapping were also detected by conditional mapping except for ph10-2. Most of the chromosomal regions identified significant conditional QTL only at one or several stages indicating that the gene or genes at a specific genomic region expressed only at some times during the whole period of plant growth.

When conditional genetic main effects were used for QTL mapping, a total of six genomic regions showed significant QTL. No genomic regions showing significant QTL for unconditional genetic main effects were undetected by conditional mapping. It is interesting to note that a significant QTL for conditional main effects on chromosome *10* (ph10-1) at day 70 was detected, but no corresponding QTL was detected for unconditional

mapping. There were six and four map regions found to be significantly associated with plant height by mapping conditional *GE* interaction effects in Hangzhou and Hainan, respectively. Of these, two QTL (ph1 and ph2) were detected for significant conditional *GE* interaction effects in both locations. Most of the QTL for conditional *GE* interaction effects were mapped to similar regions as detected by using genetic main effects or phenotypic data, but their significant stages might be different.

DISCUSSION

Classic statistical geneticists have long recognized that the development of complex traits occurs through the actions and interactions of many genes that might behave differentially during growth periods and interact with the environments (Atchley and Zhu 1997). Although molecular marker techniques have provided powerful tools for us to dissect these complex traits, there have been no studies to date on analysis of QTL for developmental behavior and the *GE* interaction of quantitative traits. The greatest difficulty in molecular dissection of *GE* interaction for quantitative trait development is the shortage of appropriate genetic models and analysis methods. If an integrated genetic model could be developed to directly handle different developmental stages in various environments for QTL mapping, it would be extremely complicated. In this article, we recommend using an indirect method to analyze the developmental behavior and its interaction with environments for QTL mapping. With the suggested indirect approaches, the available QTL mapping software such as MAPMAKER/QTL for interval mapping (Lander and Botstein 1989) and QTL Cartographer (Basten *et al.* 1996) for composite interval mapping can be readily used in searching QTL for developmental traits and *GE* interaction. The indirect method could result in more information about dynamic gene expression and *GE* interaction of QTL for the developmental behavior of quantitative traits.

In this study, there were only four and three significant QTL being detected in Hangzhou and Hainan, respectively, for final plant height. But at least seven and six significant QTL were identified with time-dependent measures in Hangzhou and Hainan (Table 2). The number of significant QTL and their magnitudes were also different at various stages. This confirmed the early statistical analysis results that gene action was distinct at various developmental stages, and genetic models of the final character could not fully reflect the real action of genes during the development of the character (Kheiralla and Whittington 1962; Peat and Whittington 1965; Wu 1987; Xu and Shen 1991; Zhu 1995). By conditional QTL mapping, genes possibly expressed at a specific period could be detected. It was shown that for the chromosomal regions significantly associated

with plant height, conditional QTL were found only at one to several specific periods. It was also shown that no genes for plant height were continually active during the whole period of growth. Results are in agreement with the developmental genetics theory that genes are expressed selectively at different growth stages. Even though at some regions unconditional QTL can be detected at all measuring stages, their real gene expression patterns were also different. For example, three genomic regions detected significant QTL (ph1, ph2, and ph3) at all stages in Hainan, but conditional effects of QTL were different at these three loci. Furthermore, parental contribution of alleles at the same map position also changed along with the development of the trait. For example, at the locus of ph2, conditional QTL with opposite genetic effects were detected for (50 days [D]|40D) and (70D|60D) in Hangzhou. Conditional QTL with opposite genetic effects were also observed at other loci such as ph3 and ph5-2 in Hainan. Similar gene expression patterns have also been observed in conditional QTL mapping for tiller number in rice (Yan *et al.* 1998). It could be suggested that the individual gene at a specific locus might have opposite genetic effects at different growth stages or multiple genes at the similar genomic region might express differently along with the trait development. These opposite genetic effects expressed at different stages in the similar genomic position might be counteracting each other and result in failure to detect QTL for cumulative effects such as for ph5-2 and ph8. Therefore, by combining the conditional and unconditional QTL mapping of time-dependent measures, it is possible to reveal the dynamic gene expressions for the development of quantitative traits.

GE interaction is another important component affecting trait development, especially quantitative traits. QTL detected in one environment but not in another environment may indicate QTL × environment interaction (Veldboom and Lee 1996a,b). If *GE* interaction effects for quantitative traits do exist, analyzing data in a single environment is not appropriate for unbiased estimation of genetic parameters. Furthermore, in the absence of QTL × environment interaction, a QTL can also be detected in one environment but not in another environment because the chance of simultaneous detection in both environments is small (Jansen *et al.* 1995). We have proposed a method to obtain predicted genetic main effects and *GE* interaction effects for exploring QTL by environment interaction (Zhu 1998). In the present study, at least three chromosomal regions showed significant QTL at most stages in both environments by mapping QTL separately in each location. When predicted genetic main effects and *GE* interaction effects were used for QTL mapping, we found that all three chromosomal regions showed significant QTL (ph1, ph2, and ph3) with both genetic main effects and *GE* interaction effects (Table 2). QTL for genetic main

effects were significant at all stages in these three map regions. But the significant stages of QTL for *GE* interaction differed at various loci; even at the same locus *GE* interaction was also different in various environments. For example, significant *GE* interaction of ph1 was detected at all stages in Hainan, but only at later development stages after 60 days in Hangzhou. *GE* interaction of ph2 was detected significantly at most stages except at 10 to 20 days and 50 days in Hangzhou, but not at stages from 60 to 70 days in Hainan. It was shown that there were both genetic main effects and *GE* interaction effects for these loci, although significant QTL could be detected in both environments. Therefore, common QTL detectable at different environments might not be conclusive regarding the absence of QTL × environment interaction. Furthermore, in some other chromosomal regions such as ph4, ph5-2, and ph7, significant genetic main effects are shown, but are only detected in one location by phenotypic data. Significant QTL with *GE* interaction effects were also detected in only one environment. One possible reason for these observations is that QTL detected in one environment could be biased because of the *GE* interaction. Therefore, unbiased detection could not be obtained by only using phenotypic data of a single environment. The second reason might be that the statistical method was not powerful enough to detect some minor QTL. For many of these chromosomal regions, possible QTL might be detected if a lower threshold of LOD score were used. The third reason is that there may be possible artifact results caused by the indirect analysis method used in the present study. For other loci such as ph5-1 and ph10-2, QTL with only *GE* interaction effects were identified. This implies that the gene expression at these loci might be controlled mainly by *GE* interaction effects. Therefore, it is impossible to reveal the real QTL × environment interaction by simply comparing QTL detected in multiple environments.

Conditional mapping for *GE* interaction effects will provide us information for the net effects of QTL × environment interaction expressed at a specific growth period. Conditional QTL × environment interaction effects were detected at ph1 before 60 days in Hainan except for (20D|10D), whereas conditonal QTL × environment interaction effects were only detected for (50D|40D) and (60D|50D) in Hangzhou. After further analysis, we found that *GE* interaction effects of genes expressed at the same locus were also different in various environments. For example, QTL × environment interaction effects were positive for (50D|40D) and (60D|50D) in Hainan, but negative in Hangzhou. At the same map region, *GE* interaction effects could also change at various stages. Conditional *GE* interaction effects of QTL ph1 showed negative effects for (30D|20D) and (40D|30D), but positive effects for (50D|40D) and (60D|50D). A similar result was observed at other map regions such as ph2. Therefore, a combina-

tion of conditional and unconditional mapping of QTL with the concept of genetic main effects and *GE* interaction effects has provided us clues to understanding the complexity of QTL × environment interaction for the development of quantitative traits.

IR64 is a semi-dwarf variety carrying *sd*-1 gene (IRRI 1975) and Azucena is a tall variety. *sd*-1 was reported to be a recessive gene and was located on chromosome *1* by classic genetic analysis (Kinoshita 1995). In the present study, a major QTL for plant height linked to marker RZ730 on chromosome *1* was found in the DH population across all measuring stages in both environments. This QTL was also significantly detected at all stages by using genetic main effects. For this QTL, the alleles for shorter plant height were contributed by IR64. Through comparison with classic genetic results, it is inferred that this locus could be the location of the *sd*-1 gene. This is consistent with previous molecular mapping results of the *sd*-1 gene (Cho *et al.* 1994; Huang *et al.* 1996; Wu *et al.* 1996). Significant *GE* interaction effects were also found at this locus by mapping the *GE* interaction effects in both environments. It was suggested that *GE* interaction effects were also very important for the gene expressions at this map position. With conditional mapping, significant QTL were detected at different stages by using phenotype data, genetic main effects, and *GE* interaction effects, respectively. It was shown that gene expressions were time-dependent for this genomic region. This might also explain the reasons the gene actions are different near the *sd*-1 locus (Tsai 1991; Wu *et al.* 1996). But, whether the actions are the distinct expression of the *sd*-1 gene or expressions of multiple genes at different stages needs further investigation.

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