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QTLs for early tillering and leaf development in rice

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ABSTRACT

QTLs associated with early tillering and leaf development in rice were mapped using a doubled-haploid (DH) population derived from a cross between lowland indica variety, IR64 and upland japonica variety, Azucena. Age-specific measures on number of tiller and leaves, recorded seven days interval, starting from 16th day of sowing, were used to detect the QTLs associated with tillering and leaf development during early period of growth following dynamic approach to QTL mapping in conjunction with a novel QTL mapping technique termed as 'conditional mapping'. Altogether five QTLs for early tillering and six QTLs for leaf development were detected. The QTLs varied according to age-specific observations with not a single QTL for both the traits appearing consistently over the growing period. QTLs for both the traits were detected first time at 23 days after sowing. Conditional mapping technique allowed detection of one QTL each for the traits that remained undetected by conventional mapping technique. For all the QTLs, the alleles from the superior parent IR64 had the positive effect on both the traits. The estimated locations of several QTLs detected for tiller and leaf development at the same age-specific observations were nearly the same indicating closer genetic relationship between the traits.

Key words: *Rice early tillering, quantitative trait loci (QTL)*

Quick early growth and tillering of the rice plant are important desirable traits for modern rice varieties. Tillering is one of the most important agronomic traits for rice production as tiller number per plant determines panicle number, a key component of rice yield. Tiller development at early stage of growth is considered particularly important for effective grain bearing. Tiller number plant^{-1} is a quantitative trait with relatively low heritability. Genetic analysis for tiller number has been restricted mainly to final tiller number at the maturity stage (Li, 1977; Murai and Kinoshita, 1986; Ahmed *et al.*, 1986) with very little attention to early tiller development.

Molecular marker mediated genetic analysis has made it possible to dissect complex quantitative traits into individual Mendelian factors providing a better understanding of the genetics of quantitative traits to formulate appropriate breeding strategy. In rice, a host of research findings have been reported with regard to QTL mapping for many important traits including seedling vigor (Redona and Mackill, 1996a,b) and tiller development (Yan *et al.*, 1998). Barring a few cases, QTL mapping efforts reported, so far, have been focused on a terminal character at a specific or final growth stage. Both classical genetic analyses (Xu and Shen, 1991; Wu and Stettler, 1994, 1997) and QTL mapping studies (Cheverud *et al*, 1996; Yan *et al.*, 1998a,b) have clearly demonstrated age-specific gene expression for developmental traits calling for conducting genetic analyses and QTL mapping studies for such traits stretched over the entire growth period. Even, such dynamic approach to conventional mapping method can't fully reveal the QTLs controlling developmental traits as it allows identification of the QTLs based on the cumulative effects of the QTLs from initial time to the specific stage of observation without elucidating the effects of the QTLs during the period between two different growth stages. To gain better insight into the genetic control mechanism of

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complex quantitative traits, a dynamic QTL mapping method is needed, which can also reveal the effect of QTLs within certain period of growth independent of the causal cumulative effects of the QTLs expressed preceding the specific period. Statistical methods have been proposed for analyzing conditional genetic effects and conditional genetic variance components (Zhu, 1995). Based on this statistical method, a mapping technique termed conditional mapping has been proposed. This mapping technique permits detection of the QTLs based on the significant net effects of the QTLs expressed within a period of growth independent of the causal cumulative genetic effects prior to the reference period. The present study was undertaken to dynamically map the QTLs associated with early tillering and leaf development following dynamic approach to conventional mapping in conjunction with conditional mapping technique.

MATERIALS AND METHODS

A population of 105 doubled-haploid (DH) lines derived from a cross between a lowland *indica* variety, IR64 and an upland *japonica* variety, Azucena (Guiderdoni *et al.*, 1992) was used in this study. A molecular map of this population was previously developed from an initial population of 135 DH lines with 175 polymorphic markers covering 2005 cM (Huang et al., 1996; Huang *et al.*, 1997).

Twenty pre-germinated seeds of all the DH lines and the parents were sown in 10 cm-diameter plastic pots half filled with coarse sand and allowed to grow for 9 days under favorable temperature condition (25°-30°C). After 9 days, the seedlings were carefully uprooted, roots were washed without damage and then the seedlings were transferred to hydroponic system (Yoshida *et al.*, 1976) and allowed to grow. The seedlings wrapped at the base above the rooting regions with pieces of spongy material were fitted through the holes on plastic trays with a single seedling per hole. The plastic trays were placed on plastic boxes (65 cm long, 43 cm wide and 12.5 cm deep) allowing the roots to dip into the nutrient solution. Every plastic tray had 10 × 7 equally spaced holes of 25 mm diameter accommodating 10 DH lines, with 7 seedlings each. The nutrient solution was prepared together for all the boxes. Every morning, the tray position in relation to the boxes was changed, nutrient solution was added to maintain a constant level and the pH of the solution was adjusted at 5 to 5.5. At 7 days intervals, the nutrient solution was replaced to maintain the nutrient status at the required levels. Starting from 16 days after sowing the seeds (d) (7 days after transferring the seedlings to hydroponic system), every 7 days interval, observations were recorded on the seedlings for tiller and leaf development.

QTLs for tiller and leaf development at different age-specific observations were detected and mapped on the chromosomes using QTL MAPPER V.1 (Wang *et al.*, 1999), developed on mixed-model based composite interval mapping (MCIM) (Zhu, 1999). According to the MCIM method, the phenotypic mean measured at time *t* on an individual of DH population can be partitioned as

$$y_k(t) = \alpha(t) + a_i(t)x_{A_{ik}} + a_j(t)x_{A_{jk}} + aa_{ij}(t)x_{AA_{ijk}} + \sum_f u_{M_{fk}} e_{M_f(t)} + \sum_l u_{MM_{lk}} e_{MM_l(t)} + \varepsilon_k(t) \quad (1)$$

where $y_k(t)$ is the phenotypic value of any quantitative trait measured on the *k*-th individual ($k = 1, 2, \cdots, n$) at time *t*; $\alpha(t)$ is the population mean at time *t*; $a_i(t)$ and $a_j(t)$ are the additive effects (fixed effects) of the two putative QTLs at time *t*, respectively; $aa_{ij}(t)$ is the additive × additive epistatic effect (fixed effect) between two loci at time *t*; $x_{A_{ik}}$, $x_{A_{jk}}$ and $x_{AA_{ijk}}$ are coefficients of QTL effects derived according to the observed genotypes of the markers and the testing points; $e_{M_f(t)} \sim N(0, \pm^2_M)$ is the effect of marker *f* at time *t* with coefficient $u_{M_{fk}}$; $e_{MM_l(t)} \sim N(0, \pm^2_{MM})$ is the effect of the *l*-th marker interaction at time *t* with coefficient $u_{MM_{lk}}$ and $\varepsilon_k \sim N(0, \pm^2_\varepsilon)$ is the random residual effect at time *t*. The factors $e_{M_f(t)}$ and $e_{MM_l(t)}$ in the model are meant to absorb additive and epistatic effects of background QTLs for controlling the noise caused by these background QTLs. The QTLs, thus identified, were detected based on the

cumulative effect of the QTLs from the initial time to the specific stage at which the observation was made. This is the conventional approach to QTL mapping. Such QTL mapping, however, does not fully reflect the dynamic mode of gene action in agreement with the model of genetic control of developmental traits. To fully dissect the gene system regulating the development of complex traits, action of genes during different phases of development, independent of the gene effects prior to the specified phase, should also be analyzed. To achieve this goal conditional mapping was adopted where, QTL analysis was performed with the phenotypic mean at time *t* conditional on the phenotypic mean at time (*t* J 1) [$y_{(t|t-1)}$]. Like that in Equation (1), the conditional phenotypic value $y_{(t|t-1)}$ can be partitioned as

$$y_{k(t|t-1)} = \alpha_{(t|t-1)} + a_{i(t|t-1)}x_{A_{ik}} + a_{j(t|t-1)}x_{A_{jk}} + aa_{ij(t|t-1)}x_{AA_{ijk}} + \sum_{f} u_{M_{fk}} e_{M_{f(t|t-1)}} + \sum_{l} u_{MM_{lk}} e_{MM_{l(t|t-1)}} + \varepsilon_{k(t|t-1)} \quad (2)$$

where, $y_{k(t|t-1)}$ is the phenotypic value of the *k*-th individual (*k* = 1, 2,⋯, *n*) at time *t* conditional on the phenotypic value at time (*t*−1); $\alpha_{(t|t-1)}$ is the conditional population mean; $a_{i(t|t-1)}$ and $a_{j(t|t-1)}$ are the conditional additive effects (fixed effects) of two putative QTLs, respectively; $aa_{ij(t|t-1)}$ is the conditional additive × additive epistatic effect (fixed effect) between two QTLs; $x_{A_{ik}}$, $x_{A_{jk}}$ and $x_{AA_{ijk}}$ are coefficients of QTL effects derived according to the observed genotypes of the markers and the testing points; $e_{M_{f(t|t-1)}} \sim N(0, \sigma^2_M)$ is the conditional effect of marker *f* with coefficient $u_{M_{fk}}$; $e_{MM_{l(t|t-1)}} \sim N(0, \sigma^2_{MM})$ is the conditional effect of the *l*-th marker interaction with coefficient $u_{MM_{lk}}$, and $\varepsilon_{k(t|t-1)} \sim N(0, \sigma^2_\varepsilon)$ is the conditional residual effect at time *t*. The conditional phenotypic value [$y_{(t|t-1)}$] was obtained by using the statistical method proposed for genetic analysis of developmental traits [8]. The QTLs detected by this conditional mapping would reflect the action of genes during the time period from (*t*J1) to *t*.

The LR thresholds for declaring significance for QTL additive effects were fixed at P = 0.005.

RESULTS AND DISCUSSION

The mean phenotypic values of the parents and the DH population for number of tillers and leaves at different stages of observation are presented in Table 1 and 2. The parents differed significantly from each other for the traits from 23d onwards and IR64 was found to be superior to Azucena for both the traits. The population distributed normally for both the traits at all the stages of observation with skew and kurt values being less than 1 except in case of tiller number at 44d (Table 2) suggesting that the data were suitable for QTL analysis. Transgressive segregants were observed in the population for both tiller and leaf development in all the stages of observation (Table 1 and 2).

Altogether five QTLs for tiller number and six QTLs for leaf number were detected (Tables 3, 4 and Fig. 1). Among these, four and five QTLs were detected by conventional mapping for tiller and leaf development, respectively. The QTLs varied according to age-specific observations with not a single among these many QTLs appearing consistently over the growing period. This clearly indicated age-specific, temporal pattern of gene action in full agreement with the results reported from both classical genetic analyses (Xu and Shen, 1991; Wu and Stettler, 1994, 1997) and QTL

Table 1. Phenotypic mean values of the parents for different seedling traits at five stages of observation

Traits	Stages	IR64	Azucena
Tiller number	16d	1.00±0.00	1.00±0.00
	23d	3.00±0.00	2.00±0.58
	30d	3.57±0.53	2.29±0.49
	37d	3.57±0.53	2.29±0.49
	44d	3.86±0.69	2.29±0.49
Leaf number	16d	3.50±0.55	4.00±0.00
	23d	11.00±1.00	6.57±0.98
	30d	15.43±2.37	9.29±1.50
	37d	16.57±2.82	9.71±0.95
	44d	20.29±3.90	10.71±2.14

Table 2. Phenotypic values of the DH population for different seedling traits at five stages of observation

Traits	Growth stages	Mean	Range	Skew	Kurt
Tiller number	16d	1.00±0.00	1.00-1.00	-	-
	23d	2.65±0.41	1.29-3.71	–0.29	0.30
	30d	2.93±0.31	2.14-3.86	–0.09	1.00
	37d	2.95±0.33	2.14-3.71	-0.45	0.26
	44d	3.23±0.85	2.14-7.29	1.99	5.81
Leaf number	16d	3.44±0.40	2.83-4.00	0.19	–1.20
	23d	8.86±1.08	5.86-11.71	0.05	0.07
	30d	12.13±1.51	8.57-15.86	0.20	–0.47
	37d	12.95±1.71	9.23-17.86	0.15	0.19
	44d	15.93±3.33	9.86-28.29	0.67	1.05

Table 3. Estimated additive genetic effects (and relative contribution) of the QTLs detected for tiller development

Chr	QTL	Marker interval	Dist.(cM)	Conventional mapping					Conditional mapping			
				16d	23d	30d	37d	44d	23d|16d	30d|23d	37d|30d	44d|37d
3	Tn3-1	RG104-RG348	0.04			0.09⬩(06)						
4	Tn4-1	RG788-RZ565	0.06					0.31⬩(11)				
5	Tn5-1	RG13-CDO105	0.02			0.12⬩(12)						
6	Tn6-1	RZ667-Pgi_2	0.02		0.18⬩(16)							
10	Tn10-1	G1084-RG257	0.06							0.16⬩(35)		
$H^2(\sum a_i)$				0	16	18	00	11	-	35	0	0

⬩ represent significance levels of P = 0.001; '+' and '–' indicate that IR64 and Azucena alleles respectively affect the trait positively and the distance indicates the putative position of the QTLs at an estimated distance measured in cM from the left one of the markers bracketing the concerned QTL

Table 4. Estimated additive genetic effects (and relative contribution) of the QTLs detected for development development

Chr	QTL	Marker interval	Dist.(cM)	Conventional mapping					Conditional mapping			
				16d	23d	30d	37d	44d	23d|16d	30d|23d	37d|30d	44d|37d
3	Ln3-1	RG104-RG348	0.02			0.54⬩(10)						
3	Ln3-2	RZ448-RZ519	0.14					1.19⬩(12)				
4	Ln4-1	RG788-RZ565	0.00					1.09⬩(11)				
5	Ln5-1	RG13-CDO105	0.00			0.63⬩(14)		0.48⬩(07)		0.30⬩(10)		
8	Ln8-1	RG418B- Amp_2	0.04		0.62⬩(32)				0.32* (08)			
9	Ln9-1	RZ228-RZ12	0.00								0.29⬩(11)	
$H^2(\sum a_i)$				0	32	25	7	23	8	10	11	0

* and ⬩ represent significance levels of P = 0.005 and 0.001 respectively; '+' and '–' indicate that IR64 and Azucena alleles respectively affect the trait positively and the distance indicates the putative position of the QTLs at an estimated distance measured in cM from the left one of the markers bracketing the concerned QTL

mapping studies (Cheverud et al, 1996; Yan et al., 1998a,b). The results explicitly justify the need to adopt a dynamic approach to mapping QTLs for developmental traits to detect the QTLs expressed at different stages of growth without any QTL escaping identification due to their age-specific action. QTLs for both the traits were detected first time at 23d when one QTL each, located on chromosome 6 and 8, was detected for tiller and leaf development, respectively.

The contributions of the individual QTLs [$H^2(a_i)$] and cumulative contributions of the QTLs *[$H^2(\sum a_i)$]*

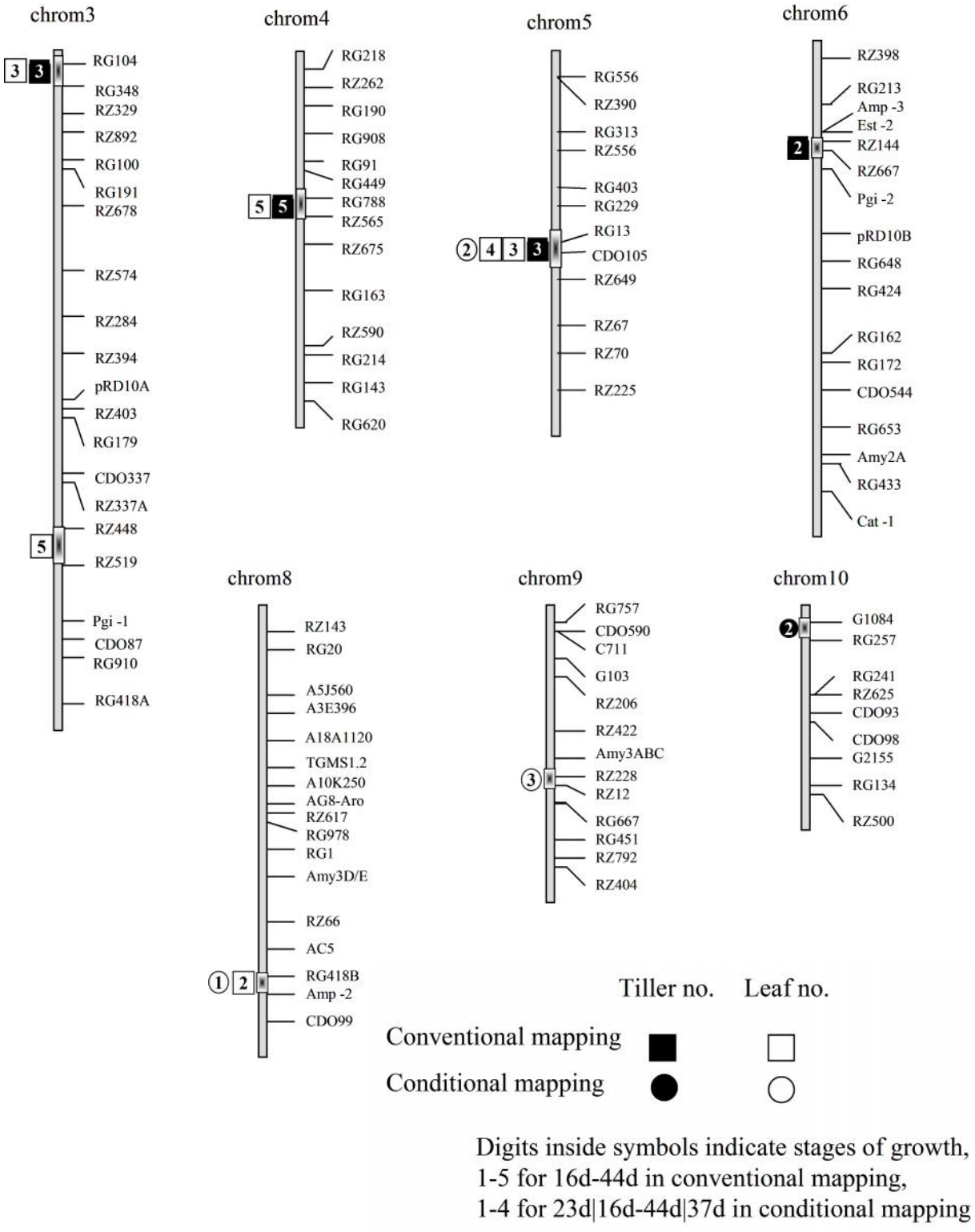

Fig. 1. Linkage map showing putative QTLs associated with early tillering and leaf development

detected by conventional mapping for tiller number at different stages of observation to the total phenotypic variation ranged from 6 to 16 per cent and 11 to 18 per cent, respectively. The contribution of the individual QTLs [H^2] as well as the collective contribution of the QTLs detected by conventional mapping for leaf number at different stages of growth to the total phenotypic variation ranged from 7 to 32 per cent.

Conditional mapping detected only one QTL each for the traits (Tn10-1 and Ln9-1) which were not detected by conventional mapping indicating temporal pattern of gene action and also suggesting the importance of this technique in QTL analysis for developmental traits. Similar results were earlier reported for plant height and tiller number also (Yan et al., 1998a,b).

Interestingly, for all the QTLs, the alleles from the indica parent, IR64 had the positive effect on increasing both tiller and leaf number. It is notable that IR64 is a high tillering lowland variety while japonica parent Azucena is a relatively low tillering upland rice variety.

Tiller and leaf development were observed to be highly positively correlated with genotypic correlation coefficient (r_g) ranging from 0.73 to 0.83 depending on the stages of observation. Several putative QTLs associated with tiller and leaf developments appearing at the same stages of observation were located almost at the same positions of the chromosomes (Table 3, 4 and Fig. 1) affecting the traits in the same direction indicating that the positive association between the two traits could be due to a set of very closely linked genes or positive pleiotropic effects of few genes.

The study has clearly demonstrated the importance of adopting dynamic approach to QTL mapping for developmental traits like tiller and leaf development. The results have also illustrated the significance of conditional mapping. A dynamic approach to conventional mapping in conjunction with conditional mapping would allow to comprehensively identify the gene system associated with the developmental traits for formulation of appropriate breeding strategy to bring about desired improvement.

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