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Temporal expression of QTLs for plant height and tiller number in rice (*Oryza sativa* L.)

K. Nagabhushana, M. G. Vaishali and Shailaja Hittalmani

Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore 560 065

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Abstract

A double haploid population of 80 lines derived from IR 64/Azucena cross was used for identifying quantitative trait loci (QTL) underlying the development of plant height and tiller number in rice (*Oryza sativa* L.). The composite interval mapping revealed a total of nine QTLs for plant height and six QTLs for tiller number on four chromosomes (3, 6, 8 and 10). On chromosome 3, a common QTL flanked by RZ284 and RG179 markers was detected with its peak expression (4.35 LOD) at 75 days after sowing (DAS) indicating varied level of gene expression for plant height. No QTL could be detected for tiller number at 60 days after sowing (DAS), while the remaining stages were found to be associated with six QTLs, of which the QTL detected on chromosome 6 (RM225-RM253) was a common QTL. Thus the QTLs identified at successive growth stages were different while some QTLs were common for all growth stages for both traits explaining the differential patterns of gene expression.

Key words: Rice, QTL analysis, temporal QTLs, plant height, number of tillers

Introduction

Rice (*Oryza sativa* L.) is the major staple food crop in most rice growing countries of the world and significant yield increase has been achieved in rice by conventional breeding strategies. Several agronomically important traits such as yield and its related traits are controlled by polygenes with varying effects. It is rather very difficult to study their nature of gene actions and interactions with other genes and with the environment by conventional approaches. The availability of molecular markers and linkage maps in rice assist to dissect the genetic basis of quantitative traits through QTL analysis [1]. QTL analysis enables to dissect polygenes and their interaction effects for complex traits into individual Mendelian factors by determining the number, locations, gene effects and magnitude involved and their interaction with other loci and with environment [2].

Grain yield is determined by agronomically important traits and among them plant height and number of tillers are important. Identification of chromosomal regions associated with these traits helps to characterize the relative importance of marker regions in terms of the proportion of phenotypic variation each QTL controls and determine common QTL regions [3]. The quantitative nature of these two traits has been extensively dissected by QTL analysis [4-6], but most of these earlier works concentrated on mapping of QTL for different traits observed at only one stage, usually the final stage of the crop growth period. Such QTL analysis helps in estimating the effects of individual QTL accumulated from the beginning of ontogenesis to the time of observation [7]. According to developmental genetics, the development of a trait occurs through the action and interaction of many genes that might behave differentially during different growth periods. In addition, different genes may have different expression dynamics during the crop growth period [8]. Therefore, it is important to analyse the traits measured at successive growth stages that would provide detailed information on genetic function of QTLs and their expression dynamics. It also increases the statistical power of QTL identification, as the repeated observations on the same individual at successive growth stages represent a form of replication [9].

On the basis of methodologies of conventional quantitative genetics, the developmental genetics of quantitative traits has been studied for several decades [10]. There are limited reports on developmental genetics of quantitative traits studies in rice [11-14]. Keeping this in view, in the present study QTL analysis was carried out for both plant height and tiller number measured at successive growth stages by following the method of composite interval mapping (CIM).

Materials and methods

Plant material: Doubled haploid (DH) population of eighty lines, a sub set of the original population consisting of 135 lines derived from a cross between IR 64, an *indica* variety and Azucena, a *japonica* variety [15] constituted the genetic material for this experiment. IR 64 is a semi dwarf and high yielding variety, well suited for irrigated conditions while Azucena is a tall and low yielding, traditional aromatic variety suited for upland conditions.

Experimental details: Doubled haploid lines along with four standard checks including the parents, were evaluated in an augmented randomised complete block design with five blocks and each block represented parents and checks. Seeds were directly sown in the main field with 20 × 15 cm spacing and all cultivation practices were carried out by following the recommended package of practices. Observations of both plant height and tiller number were taken at four successive growths stages *viz.*, 60, 75, 90 days after sowing (DAS) and maturity. Plant height was measured in centimeters from soil surface to tip of the younger leaf and both productive and non-productive tillers were counted while recording number of tillers per plant. Five center plants were selected for recording all observations throughout plant growth 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 IR 64/Azucena DH population was previously developed [16] and recently 85 new markers were added to this map. 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 for both the traits measured at four different growth stages by performing composite interval analysis using QTL Cartographer V2.0 with a threshold LOD score of 2.50.

Results and discussion

The frequency distribution of DH lines for plant height and tiller number traits and the phenotypic values of parents are shown in Fig. 1. Normal distribution was observed among genotypes for both traits at all four stages. IR 64 and Azucena parents showed diverse phenotypic variation for plant height and tiller number at all successive growth stages (Fig. 1). Transgressive segregants were observed for both traits at different growth stages as some DH lines had higher phenotypic values than the higher parent and lower than the lower parent. The QTLs detected for plant height and tiller number based on CIM analysis and its chromosomal locations are presented in Table 1 and Fig. 2, respectively. Temporal expression pattern of QTLs across different growth stages is presented in Table 2.

QTL mapping for plant height: The composite interval analysis with threshold LOD > 2.50 detected a total of nine significant QTLs associated with plant height at different growth stages. The genomic region flanked by RZ284 and RG179 markers on chromosome 3 was found to be significantly associated with plant height throughout the lifecycle. But the phenotypic expression of this common QTL was to its maximum at 75 DAS stage (LOD 4.31) explaining 11.30% phenotypic variation followed by 90 DAS with 9.68 % variation at 3.70 LOD (Table 1). On chromosome 8, the region between RM38 and RZ143 markers was associated with plant height QTL at 75 DAS, 90 DAS and maturity and it had its peak expression at maturity (3.36 LOD). The third genomic region bracketed by RM244B and G1084 markers was found to control plant height at 60 and 90 DAS with its peak LOD (3.12) at 90 DAS. Of all the QTLs mapped for plant height, $qPHT_{75}$-3 on chromosome 3 exhibited highest variation (11.30 %) followed by $qPHT_{90}$-3 contributing 9.68 % variation while a maximum of 27.56 % variation (Table 1) was explained together by three QTLs detected for plant height at 90 DAS. Although plant height was controlled by nine significant QTLs, it was found to be associated with only two QTLs at maturity. Of these, the QTL detected on chromosome 3 was found to be common because of its continuous expression throughout plant's growth and development. A QTL detected on chromosome 10 at early stages of growth could not be revealed at subsequent or final stage, while a QTL detected on chromosome 8 at maturity, was absent at 60 DAS stage (Table 2). This indicates varied kind and level of gene expression at different stages of the plant growth. This is because some genes could be selectively controlling a particular growth stage(s) of a trait while some throughout the ontogeny. Suppose the QTL analysis was carried out for observations measured only at maturity, we would be able to detect only two QTLs for plant height unlike in the present study, where another significant QTL on chromosome 10 has been detected for early growth stages. The QTL on chromosome 3 detected at maturity could also be detected at all previous growth stages, but its peak expression was noticed at 75 DAS with a very high LOD score of 4.31 and this could be due to the peak-activity of plant height QTLs at vegetative growth stages. These findings showed that only one out of nine QTLs expressed throughout the ontology of rice plant in our study.

Similar findings were reported in the same DH mapping population of IR 64/Azucena cross in which the QTLs detected for plant height at the early growth stages were absent at the final stage by both conditional and unconditional QTL mapping methods. Maximum number of QTLs was detected between 30th and 60th day of plant growth [11]. Similar results were observed in maritime pine in which, using a selfed progeny, differentially expressed QTLs were detected for height measured at three developmental stages [17]. The earlier reports on conventional and conditional QTL mapping used for estimating the epistatic QTLs and

Table 1. QTLs detected for plant height and tiller number at four growth stages in rice by composite interval mapping

Trait	Growth stage	QTL name*	Chromosome number	Flanking markers	LOD score	R^2 (%)	QTL effect
Plant height	60 DAS	$qPHT_{60}$-3	3	RZ284-PRD10A	3.58	9.41	6.51
		$qPHT_{60}$-10	10	RM216-RM239	2.63	7.47	5.90
	75 DAS	$qPHT_{75}$-3	3	RZ284-RG179	4.31	11.30	11.03
		$qPHT_{75}$-8	8	RG20-RZ143	2.71	7.18	8.75
	90 DAS	$qPHT_{90}$-3	3	RZ284-RG179	3.70	9.68	13.77
		$qPHT_{90}$-8	8	RM38-RZ143	3.33	9.03	13.23
		$qPHT_{90}$-10	10	RM244B-G1084	3.12	8.85	13.52
Tiller number	At maturity	$qPHT_{mat}$-3	3	RZ284-RG179	3.50	9.13	13.48
		$qPHT_{mat}$-8	8	RM38-RZ143	3.36	9.14	13.40
	75 DAS	qTN_{75}-3	3	RZ284-PRD10A	2.72	7.09	1.18
		qTN_{75}-6	6	RM225-RM253	3.19	8.85	1.32
	90 DAS	qTN_{90}-6	6	RM225-RM253	3.86	10.65	1.69
		qTN_{90}-10	10	RM239-G1084	2.62	7.03	1.38
	At maturity	qTN_{mat}-6	6	RM225-RM253	3.88	10.74	1.74
		qTN_{mat}-10	10	RM239-G1084	2.57	6.90	1.40

(QTLs are named by trait abbreviations, growth stage and chromosome number), R^2 = Percent phenotypic variance explained by the locus.

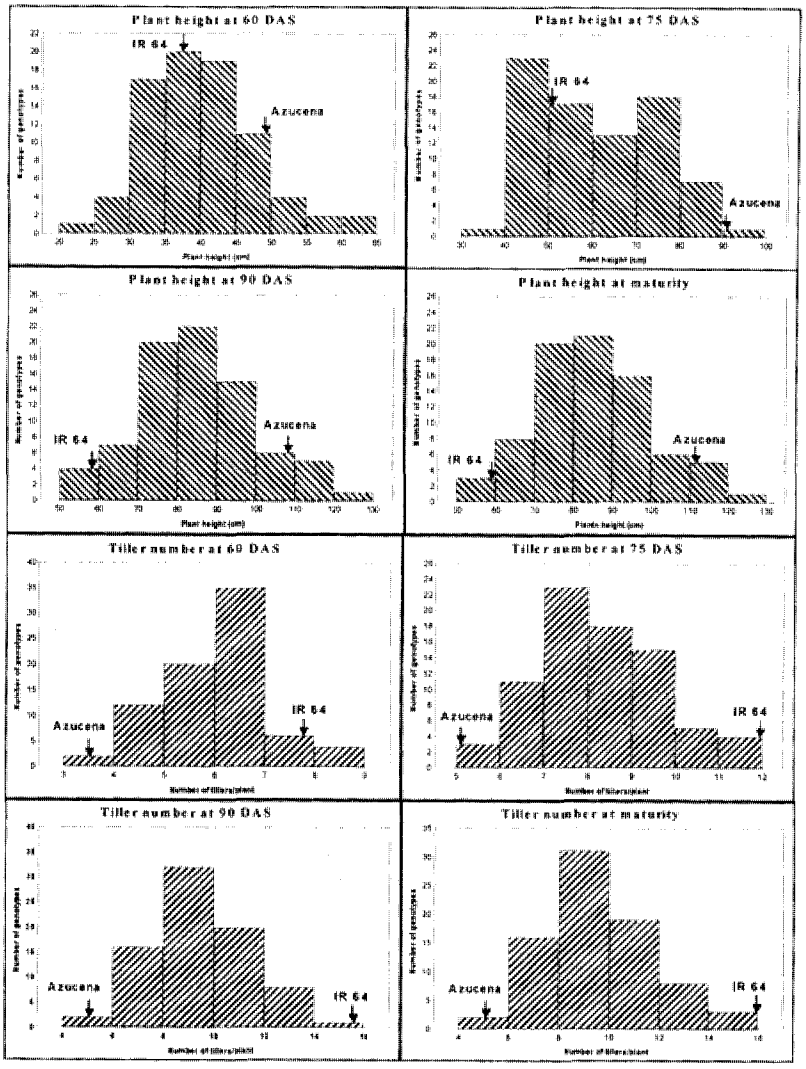

Fig. 1. Frequency distribution of double haploid lines of IR 64/Azucena for plant height and tiller number in rice (Arrows indicate phenotypic values of the parents)

Table 2. Temporal expression pattern of QTLs for plant height and tiller number in rice IR 64/Azucena double haploid population

Trait	Chromosome no.	Marker interval	Temporal expression pattern of QTLs			
			60 DAS	75 DAS	90 DAS	At maturity
Plant height	3	RZ284-RG179	+	+	+	+
	8	RM38-RZ143	-	+	+	+
	10	RM244B-G1084	+	-	+	-
Tiller number	3	RZ284-PRD10A	-	+	-	-
	6	RM225-RM253	-	+	+	+
	10	RM239-G1084	-	-	+	+

+ sign indicates the presence of a QTL
- sign indicates the absence of a QTL

environmental interaction effects for the developmental behaviour of plant height indicated no single QTL that had its effect during the whole of ontogeny [13].

QTL mapping for tiller number. The results of CIM indicated no QTL identified for tiller number at 60 DAS while six QTLs were detected for the subsequent growth stages. The genomic region, where the common QTL for plant height was mapped in between RZ284 and PRD10A markers on chromosome 3, was also found to be associated with tiller number at 75 DAS. This could be due to time specificity in its expression and hence was limited to 75 DAS stage only. On chromosome 6, markers RM225 and RM253 flanked the region that controlled tiller number from 75 DAS onwards. This QTL had its peak LOD score (3.88) at maturity followed by 90 DAS (3.86 LOD) but the variation

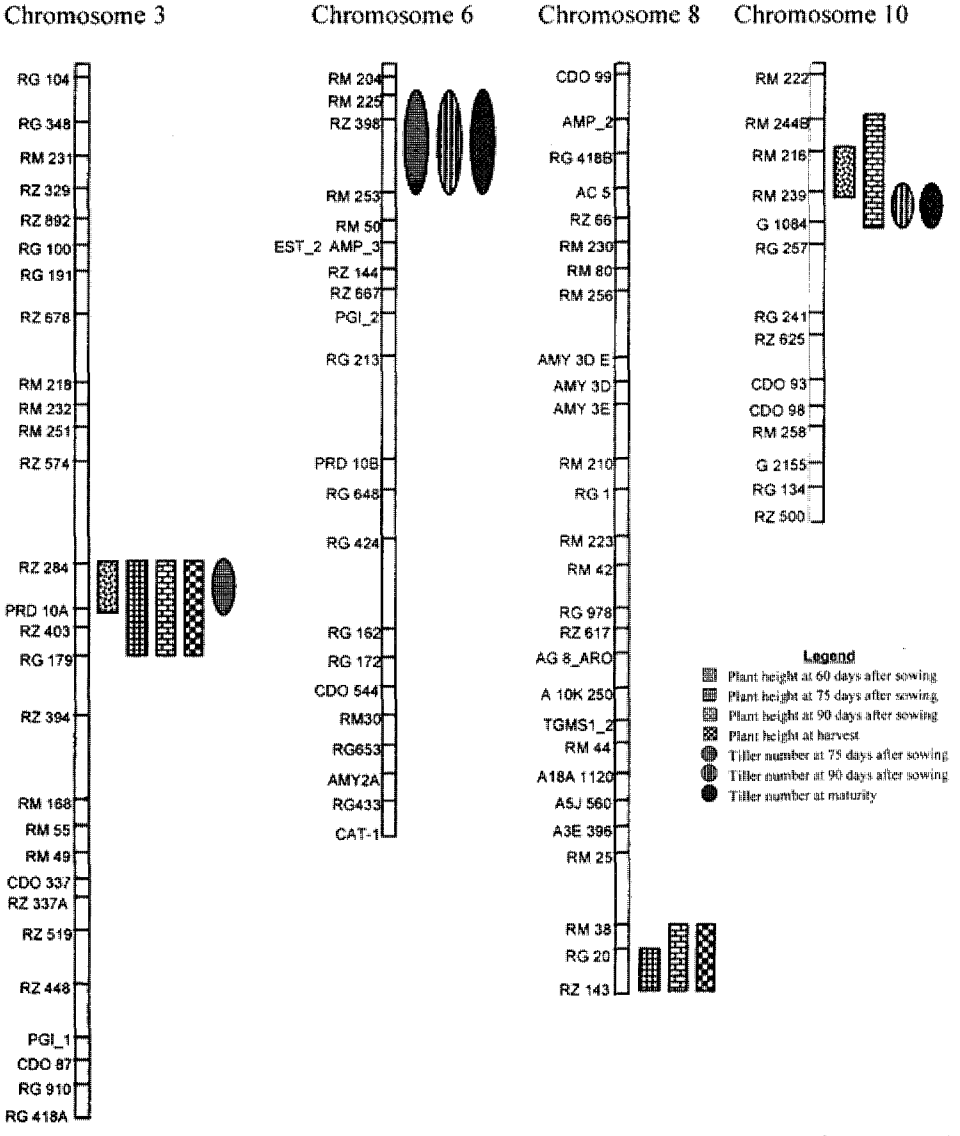

Fig. 2. Chromosomal location of QTLs detected for plant height and tiller number using IR 64/Azucena double haploid population in rice

explained was almost same (10.65 and 10.74% respectively) at both stages of growth. While on chromosome 10, the QTLs *viz.*, qTN_{90}–10 and qTN_{mat}–10 detected in between the markers RM239 and G1084 were specific to tiller number at 90 DAS and maturity (Table 1 and Fig. 2). The differential QTL expression suggested different loci to be controlling tiller number at different stages, thus indicating the differential expression of genes for tiller number also. This information helps the breeder to aim for breeding productive tillers with the aid of marker-aided selection. In this study, a QTL on chromosome 10 expressed at 90 DAS and at maturity can be avoided during MAS because it could lead to the development of non-productive tillers as the tillers developing at later stage in crop growth phase do not contribute to grain yield and would reduce the harvest index.

In studies involving conditional QTL mapping, similar kind of results were obtained [7, 12]. Only three QTLs for tiller number measured at final growth stage were detected in IR64/Azucena DH population of rice. But with time specific measures, fifteen QTLs were detected at successive growth stages and eight of the QTLs could be detected at peak tillering stages from 40 to 50 days. These findings suggest the difficulties of determining the genetic basis of both plant height and tiller number or any other trait by QTL mapping at maturity only [12]. Hence from these results it is also evident that alleles for QTL for plant height and tiller number are contributed by IR64.

The results obtained in the present study help in understanding the expression dynamics of temporal/time specific QTLs and stable QTLs throughout the plant growth period. It also helps in identifying the right stage QTL and stable QTL across various growth stages and eventually aid in designing the ideal genotypes with desirable QTLs. Hence, the QTLs identified across different stages of the plant growth provide an excellent opportunity for selecting stable chromosomal regions contributing to yield and yield components to develop QTL introgression lines that can be deployed in rice breeding program.

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