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QTLs for response to low temperature stress during seedling growth in rice

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Abstract

QTLs associated with growth of rice seedling under favourable and low temperature affected environments were mapped using a DH population derived from a cross between lowland *indica* variety, IR64 and upland *japonica* variety, Azucena. A dynamic approach to conventional mapping technique was employed in conjunction with a novel mapping technique termed as 'conditional mapping' using age-specific measures for seedling height, seedling weight and root depth. Among 15, 12 and 17 QTLs detected for seedling height, seedling weight and root depth, respectively, only 7, 6 and 2 QTLs for the respective traits were common to favourable temperature and low temperature affected environments indicating that, while a set of QTLs could hold the key to growth irrespective of growing environments, few QTLs with situation-specific expression might determine the differential response of the genotypes to environmental stress. The study also indicated that the difference in time of expression of some of the QTLs detected across growing environments might be additional feature of differential response of genotypes to varied growing environments. The conditional mapping technique allowed detection of 4 QTLs for seedling height and 6 QTLs each for seedling weight and root depth- which remained undetected by the conventional mapping technique indicating temporal pattern of gene expression and suggesting the importance of this technique in QTL analysis for developmental traits.

Key words: Rice, seedling growth, quantitative trait loci (QTL), low temperature stress, conditional mapping

Introduction

Rice is cultivated in a wide range of ecosystems under varying temperature and water regimes. Quick seedling growth and vigorous root system are some of desirable traits for modern varieties of rice for all ecosystems, particularly, for direct-seeded, flooded and upland rice areas. Low temperature is one of the major constraints of rice production. More than 15 million hectares of rice throughout the world suffers from cold damage at one or another stage of growth, and in South and Southeast Asia alone, low temperature limits production of modern rice cultivars on an estimated 7 million hectares [1, 2]. In eastern India and Bangladesh, low temperature-induced inhibition of seedling growth is a serious problem for the winter season rice crop commonly known as *boro* rice. It is thus, important to develop rice varieties with better seedling vigour and greater root depth for both ideal and low temperature affected situations.

Molecular marker-mediated genetic analysis has made it possible to dissect complex quantitative traits into individual Mendelian factors and thus providing a better understanding of the genetics of quantitative traits to formulate appropriate breeding strategy. In rice, volumes of research findings have been reported about QTL mapping for many important traits including seedling growth [3-9]. However, barring a few cases, QTL mapping efforts reported, so far, have focused on a terminal character at a specific or final growth stage. The conventional mapping method 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. Such mapping method can not, therefore, fully reveal the QTLs controlling a trait, as the development of complex morphological structures is assumed to occur through actions and interactions of many genes that act

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differentially during ontogeny [10]. To gain better insight into the genetic control mechanism of complex quantitative traits, a dynamic QTL mapping method is needed, which can detect the actions and interactions of the QTLs during the entire path of trait development, and also can 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 [11]. Based on such statistical method, a mapping technique called '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 map the QTLs associated with early vegetative growth under favorable temperature, and also in response to low temperature stress during growth following dynamic approach to conventional mapping in conjunction with 'conditional mapping' technique.

Materials and methods

Plant material

A population of 105 doubled-haploid (DH) lines derived from a cross between a lowland *indica* variety, IR64 and an upland *japonica* variety, Azucena [12] 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 [13, 14].

Hydroponic evaluation for seedling growth

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 favourable 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 [15] and allowed to grow under favourable temperature condition (daily mean temperature varied from 22°-33°C). 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 x 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. Sixteen days after sowing of seeds (7 days after transferring the seedlings to a nutrient culture system), seedlings of each of the DH lines were divided into two sets. One set of seedlings was subjected to 15°C/10°C day/night temperature for 6 days, maintained in an incubator, and then returned to favourable temperature condition. The other set of seedlings raised similar way but without the low temperature treatment was maintained as a check. Starting from 16 days after sowing of the seeds (d), every 7 days interval, observations were recorded on the seedlings from both the sets for seedling height, seedling weight and root depth.

QTL analysis

QTLs for seedling growth at different stages of observation were detected and mapped on the chromosomes using QTLMAPPER V.I [16], developed on mixed-model based composite interval mapping (MCIM) [17]. According to the MCIM method, the phenotypic mean measured at time t on an individual of DH population can be partitioned as

$$y_{k(t)} = \mu_{(t)} + a_{i(t)} x_{A_{ijk}} + a_{j(t)} x_{A_{jjk}} + aa_{ij(t)} x_{AA_{ijjk}} + \sum_{f} u_{M_{fk}} e_{M_{f(t)}} + \sum_{l} u_{MM_{lk}} e_{MM_{l(t)}} + \varepsilon_{k(t)} \quad \ldots (1)$$

where $y_{k(t)}$ is the phenotypic value of any quantitative trait measured on the k-th individual ($k = 1, 2, \ldots, n$) at time t; $\mu_{(t)}$ 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 x additive epistatic effect (fixed effect) between two loci at time t; $x_{A_{ijk}}$, $x_{A_{jjk}}$ and $x_{AA_{ijjk}}$ are coefficients of QTL effects derived according to the observed genotypes of the markers and the testing points; $e_{M_{f(t)}} \sim N(0, \sigma^2_M)$ is the effect of marker f at time t with coefficient $u_{M_{fk}}$; $e_{MM_{l(t)}} \sim N(0, \sigma^2_{MM})$ is the effect of the l-th marker interaction at time t with coefficient $u_{MM_{lk}}$ and $\varepsilon_k \sim N(0, \sigma^2_\varepsilon)$ is the random residual effect at time t. The factors $e_{M_{f(t)}}$ and $e_{M_{l(t)}}$ 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 $(t-1)$ $[y_{(t|t-1)}]$.

Like that in Equation (1), the conditional phenotypic value $y_{(t|t-1)}$ can be partitioned as

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

where, $y_{k(t|t-1)}$ is the phenotypic value of the k-th individual ($k = 1, 2, \ldots, n$) at time t conditional on the phenotypic value at time $(t-1)$; $\mu_{t(t|t-1)}$ is the conditional population mean; $a_{f(t|t-1)}$ and $a_{j(t|t-1)}$ are the conditional additive effects (fixed effects) of two putative QTLs, respectively; $a_{ij(t|t-1)}$ is the conditional additive x 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; $e_{M_{f(t|t-1)}} \sim N(0, \sigma^2_M)$ the conditional effect of marker f with coefficient $\mu_{M_{fk}}$; $e_{MM_{l(t|t-1)}} \sim N(0, \sigma^2_{MM})$ is the conditional effect of the l-th marker interaction with coefficient $\mu_{MM_{lk}}$ and $\varepsilon_{k(t|t-1)} \sim N(0, \sigma^2_\varepsilon)$ 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 [11]. The QTLs detected by this conditional mapping would reflect the action of genes during the time period from $(t-1)$ to t.

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

Results and discussion

The mean phenotypic values of the DH population for the traits at different dates of observation are presented in Table 1. Presumably, the population distributed normally, as symmetric, bell-shaped curves were obtained for almost all the traits at all the stages of observation under both the temperature situations, suggesting that the data were suitable for QTL analysis.

Altogether 15 QTLs for seedling height, 12 for seedling weight and 17 QTLs for root depth were detected (Tables 2, 3, 4 and Fig. 1) with appearance of many QTLs varying according to age-specific observations and environmental conditions. In the QTLs detected, alleles from either of the parents affected growth of seedling positively depending on the QTLs. However, each of the QTLs, when detected at more than one age-specific observation and environmental conditions, affected the traits in the same direction during the entire seedling growth period irrespective of the growing conditions. When the QTLs were detected over several stages of observation by conventional mapping technique they were generally detected continuously without break in between. However, in few cases like that in case of QTLs Shl-1 and Sw3-I (Tables 2 and 3) there was break in the continuity of appearance of the QTLs. Similar breaks in the continuity of QTL appearance was observed in earlier studies also [18-20]. While not ruling out these to be the cases of random chance, such breaks in appearance of the QTLs may, however, be explained in the light of the concept of developmental genetics. Since the detection of QTLs by conventional mapping is based on the accumulated effects of the QTLs over a period, the variation in the population caused by such cumulative effects can be made insignificant by the mutually opposite effects of the same QTL or closely located QTLs expressed at different time thus rendering the QTL undetectable by conventional mapping. Hints of such possibility were obtained from the present study itself. Two QTLs associated with root-depth, one identified by conventional (Rd8-I) and another identified by conditional mapping (Rd8-2), were located very close to each other and affected the trait in different directions. These two QTLs might represent two tightly linked loci or might be the case of negative pleiotropism of one individual locus at different stages of growth. It was earlier also reported that the individual QTL might have opposite genetic effects on the same trait at different stages of growth [19, 21].

It was expected in the study that the QTLs detected for the seedling growth parameters in the two sets of the same population would be the same before one of the sets was exposed to low temperature stress for a brief period, as the two sets were genetically exact

Table 1. Phenotypic values of the DH population for different seedling traits at five stages of observation

Traits	Growth stages	Favourable temperature situation			Low temperature affected situation		
		Mean	Skew	Kurt	Mean	Skew	Kurt
Seedling height (cm)	16d	24.00±3.75	0.27	-0.26	23.90±3.37	0.29	-0.66
	23d	36.54±5.05	0.32	0.12	24.80±3.56	0.24	-0.71
	30d	51.13±7.33	0.27	-0.05	32.23±6.82	0.33	-0.44
	37d	58.46±9.35	0.23	-0.15	43.45±9.02	-0.10	-0.28
	44d	65.45±11.37	0.16	-0.40	54.00±10.33	0.05	-0.37
Root Length (cm)	16d	12.00±2.19	0.73	0.33	12.22±2.41	0.80	1.31
	23d	14.76±2.15	0.44	0.50	12.12±2.24	1.12	2.80
	30d	18.35±2.57	0.13	0.56	12.86±1.84	0.39	0.85
	37d	19.69±2.91	0.14	-0.04	15.60±2.41	-0.03	0.70
	44d	20.88±2.74	0.28	-0.10	18.64±2.64	0.44	-0.23
Seedling Weight (g)	16d	0.32±0.06	0.42	0.14	0.32±0.06	0.65	0.14
	23d	1.70±0.39	0.47	-0.36	0.37±0.09	0.46	0.19
	30d	4.04±1.04	0.49	-0.38	0.9U0.28	0.31	-0.24
	37d	6.73±1.96	0.53	-0.27	1.9U0.68	0.38	-0.17
	44d	11.35±4.10	0.50	-0.26	4.98±2.22	1.46	5.62

replica of each other and grown initially under exactly the same environmental condition. It was also expected that after subjecting one set of the DH population to low temperature, some QTLs determining the constitutive genetic variation for the traits would be common in both the two sets while some other QTLs would be different, as the two sets, though genetically the same, would take different courses of development due to the influence of varying environmental conditions caused by two different temperature regimes. As expected, on the first day of observation (16d), before one of the two sets of the DH population was subjected to low temperature stress, four QTLs each for seedling height and weight and two QTLs for root depth, respectively were detected and all these QTLs were exactly the same in both the sets of the population with little difference in the estimated effects of the QTLs (Tables 2, 3 and 4). Again as expected, among eleven QTLs and nine QTLs detected for seedling height and weight, respectively in each of the two sets of the population after subjecting one set to low temperature stress, seven QTLs for seedling height and six QTLs for seedling weight were found to be common to both the sets. Though probability of random chance of differential detection of QTLs under different situations cannot be totally ruled out [22], the result was in full agreement with the expectation. The fact that several QTLs detected only in one environmental condition had relatively high contribution (>10 per cent) to the total variation had further diminished the probability of differential appearances being just random chance. Thus, it appears from the study that, while a set of QTLs with relatively large share of contribution to the phenotypic variation holds the key to growth irrespective of growing environments, few QTLs with situation-specific expression may determine the differential response of the genotypes to the environmental variation. Similar studies with consideration of more traits and environmental variations would surely help to better understand the genetics of response to varied environmental conditions.

In the study, only two QTLs (Rd2-2 and Rd8-2) for root depth were detected under both the situations and that too just after the low temperature treatment and these QTLs happened to be those two which were detected at the first observation (Table 4 and Fig. 1). The study thus, tends to suggest that the growth of root depth under significantly different situations may be under control of different gene systems. In earlier studies also, maximum root depth was observed to be highly sensitive to environmental variation [5] even though probability of relatively higher experimental error associated with phenotyping for this trait was not totally ruled out. Periodical phenotyping, which was

Table 2. Estimated additive genetic effects of the QTLs detected for growth of seedling height (cm)

Chr. No.	QTL	Marker interval	Dist. (cM)	Environ.	Conventional mapping					Conditional mapping			
					16d	23d	30d	37d	44d	23d|16d	30d|23d	37d|30d	44d|37d
1	Sh1-1	RG146-RG345	34	Control		-1.46*(10)	-1.75*(05)		-2.47*(03)				
				Low				-2.60*(05)					
1	Sh1-2	RZ730-RZ801	30	Control	-2.01*(29)	-1.39**(09)	-3.81*(22)	-5.48*(23)	-7.83*(29)				
				Low	-2.21*(32)	-2.23*(28)	-4.88*(30)	-4.99*(20)	-4.42*(12)	-0.31*(07)			
2	Sh2-1	RG171-RG157	02	Control							-0.94*(10)		
				Low									-1.81*(32)
2	Sh2-2	Amy1A/C-RG95	06	Control	1.58*(18)	1.29*(08)	2.47*(09)	3.96*(12)	6.34*(19)				
				Low	1.40*(13)	1.01*(06)	2.34*(07)	3.94*(12)	4.56*(13)				
3	Sh3-1	CDO87-RG910	04	Control	-1.33*(10)	-1.41*(09)	-2.29*(08)	-4.57*(16)	-4.11*(08)				
				Low	-1.31*(11)	-1.33*(11)	-3.06*(12)	-4.08*(13)	-4.26*(11)				-0.88*(07)
4	Sh4-I	RG190-RG908	10	Control									
				Low					1.72*(02)				
5	Sh5-I	RZ67-RZ70	04	Control									
				Low					4.73*(13)				
6	Sh6-I	Amy2A-RG433	04	Control			1.59*(04)		2.46*(03)				
				Low									
7	Sh7-I	RG773-RG769	24	Control								1.05*(17)	
				Low									
8	Sh8-I	Amp 2-CDO99	04	Control	1.17**(10)	1.82*(15)	1.82*(05)	3.76*(11)	4.67*(10)				
				Low	0.97** (06)	1.31*(10)	2.63*(09)	4.02*(13)	4.67*(13)			0.91*(11)	
9	Sh9-I	CDO590-C711	02	Control						-2.32*(03)			
				Low					-3.01*(05)			-0.92*(12)	
9	Sh9-2	RG667-RG451	10	Control						1.08**(15)			0.97*(11)
				Low									
11	Shll-1	RZ400-RG118	12	Control									
				Low						-0.31*(12)			
12	Shl2-I	Sdh 1-RG463	04	Control									
				Low				-1.43**(02)					
12	Shl2-2	RG958-RG181	00	Control			-2.66*(11)						
				Low									
H^2(S *a*)				Control	66	49	64	63	76	15	10	17	11
				Low	62	57	58	67	69	19	10	12	39

* and ** represent significance levels of P = 0.005 and 0.001, respectively;'+' and '-' indicate that IR64 and Azucena alleles, respectively affect the trait positively, 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 and first digit in the notation for QTL indicates chromosome no. bearing the QTL; figures within parentheses indicate relative contributions of the QTLs.

Table 3. Estimated additive genetic effects of the QTLs detected for growth of seedling weight (g)

Chr. No.	QTL	Marker interval	Dist. (cM)	Environ.	Conventional mapping					Conditional mapping			
					16d	23d	30d	37d	44d	23d|16d	30d|23d	37d|30d	44d|37d
1	Sw1-1	RG810-RG331	06	Control	-0.024*(14)	-0.184*(22)	-0.408*(14)	-0.642*(13)					
				Low	-0.026*(14)	-0.035*(18)	-0.103*(13)	-0.232*(14)					
2	Sw2-1	RG437-RG544	12	Control	0.016*(06)								
				Low	0.015*(06)							-0.038**(07)	
2	Sw2-2	Pall-RZ58	10	Control							0.194*(11)		
				Low									
3	Sw3-1	CDO87-RG910	00	Control	-0.011**(03)	-0.115*(08)	-0.416*(14)	-1.581**(18)					
				Low	-0.020*(08)							-0.051*(14)	
4	Sw4-1	RG190-RG908	08	Control				0.565*(10)	1.349*(13)				
				Low				0.217*(13)	1.003*(27)				
4	Sw4-2	RG449-RG788	10	Control								0.428*(11)	
				Low								0.041*(09)	
5	Sw5-1	RZ67-RZ70	00	Control	0.024*(14)	0.149*(14)	0.379*(12)	0.748*(17)					
				Low	0.020*(08)	0.030*(13)							
8	Sw7-1	CDO418-RZ978	02	Control									
				Low						-0.011**(09)			
8	Sw8-1	Amy3DE-RZ66	20	Control						0.088*(17)			
				Low									
8	Sw8-2	Amp_2-CDO99	10	Control		0.100*(06)	0.387*(12)						
				Low			0.167*(33)	0.246*(16)			0.051*(18)		
9	Sw9-1	RG667-RG451	10	Control									
				Low									0.043*(10)
10	Sw10-1	RZ625-CDO93	02	Control						-0.045**(04)			
				Low									
$H^2(S\ a_i)$				Control		51	53	40	32	22	0	22	0
				Low		31	49	43	27	9	18	40	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, 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 and first digit in the notation for QTL indicates chromosome no. bearing the QTL; figures within parentheses indicate relative contributions of the QTLs.

Table 4. Estimated additive genetic effects of the QTLs detected for growth of root depth (cm)

Chr. No.	QTL	Marker interval	Dist. (cM)	Environ.	Conventional mapping					Conditional mapping			
					16d	23d	30d	37d	44d	23d|16d	30d|23d	37d|30d	44d|37d
1	Rd1-1	RG345-RG381	02	Control					0.73*(06)				
				Low									
2	Rd2-1	RG171-RG157	00	Control								0.37*(11)	
				Low									
2	Rd2-2	RZ318-Pall	04	Control	-0.89*(16)	-0.98*(19)	-1.32*(19)	-1.65*(30)	-1.63*(30)	-0.68*(08)	-0.66*(17)		
				Low	-0.90*(14)	-0.44**(04)							
2	Rd2-3	RG95-RG654	04	Control		0.69*(09)							
				Low									
3	Rd3-1	Pgi 1-CDO87	06	Control									
				Low		-0.73*(11)		-0.54**(04)					
5	Rd5-1	RG229-RG13	16	Control									
				Low							-0.99*(29)	-0.44**(10)	
5	Rd5-2	RZ70-RZ225	00	Control									
				Low				0.66*(06)			0.46**(06)	0.64*(22)	
6	Rd6-1	Pgi 2-pRD10B	10	Control									
				Low						-0.44*(23)			
6	Rd6-2	RG162-RG172	04	Control			0.53*(03)						
				Low									
7	Rd7-1	PGMS0.7-CDO59	00	Control							0.43*(07)		
				Low									
8	Rd8-1	RG978-RG1	06	Control	-0.83*(14)	-0.82*(13)							
				Low	-0.83*(12)	-0.S9*(07)		-0.89*(12)					
8	Rd8-2	RG1-Amy3D/E	02	Control									0.59*(22)
				Low									
8	Rd8-3	Amp 2-CDO99	14	Control									
				Low			1.01*(22)	0.65**(06)			0.77*(17)		
9	Rd9-1	RG667-RG451	18	Control							0.48*(09)		
				Low									
10	Rd10-1	G1084-RG257	00	Control		0.65*(08)		0.97*(10)		0.99*(18)			
				Low									
11	Rd11-1	RG103-RG1109	10	Control			-1.15*(14)				-0.77*(23)		
				Low									
12	Rd12-1	RG574-RZ816	04	Control									
				Low		0.48**(05)						-0.52*(14)	
H^2(S a_i)				Control	30	46	35	39	36	27	56	11	22
				Low	26	28	22	27	0	23	48	38	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, 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 and first digit in the notation for QTL indicates chromosome no. bearing the QTL; figures within parentheses indicate relative contributions of the QTLs.

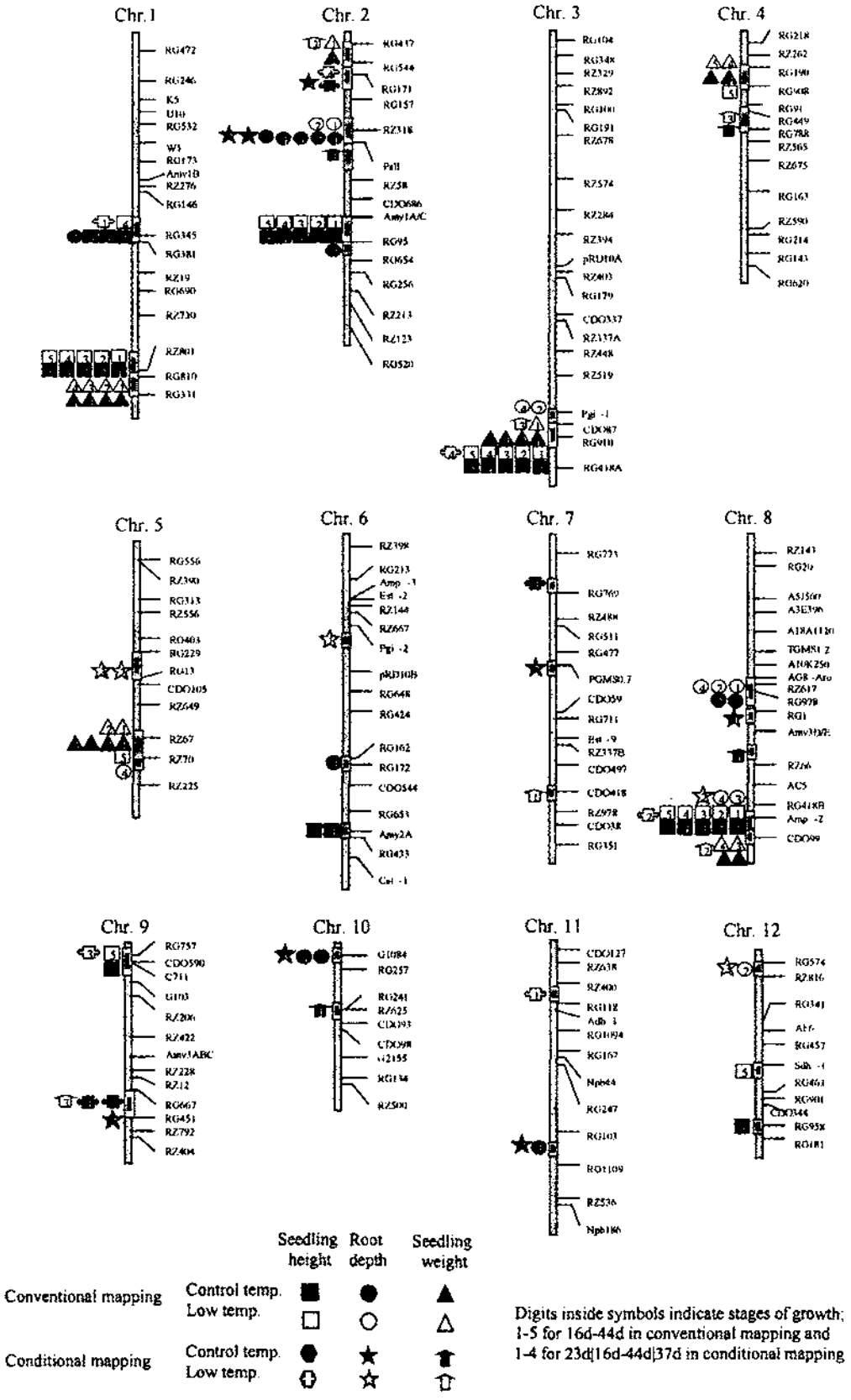

Fig. 1. Linkage map showing estimated genomic positions of the putative QTLs associated with seedling growth parameters under control and low temperature affected situations

unavoidable in the present study, might have caused some degree of shock to the delicate root system thereby disturbing the normal root growth. Moreover, even after utmost care while recording the observations, some amount of damage to the roots affecting the accuracy of the measures is not totally ruled out in the present study. However, identification of almost entirely different sets of QTLs after the same genetic population passed through significantly different environmental conditions is not at all implausible according to the concepts of developmental genetics. Development is sequential and hierarchical in nature that generates integrative networks of interrelationships within and between levels of organization. Developmental genetic theories suggest that, because of its sequential and hierarchical nature, development is epigenetic and involves cascades of interactions among directly and indirectly acting controlling factors and these interactions may vary during ontogeny [23]. Thus, it was highly expected that several QTLs, though not necessarily entirely different set of QTLs, should be detected only in one of the situations differentiated by the temperature treatment.

In the study, conditional mapping allowed to identify several QTLs for all the three growth parameters under both the temperature situations, which would have otherwise remained undetected by conventional mapping technique (Table 2, 3 and 4) suggesting the importance of this technique in QTL analysis for developmental traits. Similar results were reported earlier also with plant height and tiller number [18, 19]. Conditional mapping also revealed an interesting aspect of genetics of response to stress. One QTL for seedling height (Sh2-l) detected by conditional mapping appeared in both the situations but at two different period of growth in different situations. Under control temperature situation, it appeared during the period- 23d to 30d while under low temperature affected situation, it appeared during the period 37d to 44d (Table 2). The dynamic approach to conventional mapping technique also revealed similar delay in expression of at least one QTL for seedling height under low temperature situation. Under favourable temperature situation, the QTL Shl-1 was first detected at 23d but under low temperature affected situation, the same QTL was detected at 37d only (Table 2). Similar delay in expression of QTL (Sw8-2) under low temperature affected situation was observed for seedling weight also (Table 3). Such delay in expression of certain loci under low temperature affected situation might be an additional genetic explanation of retarded growth of the seedlings under low temperature affected situation (Table 1) besides expression of some QTLs under specific environment only.

The study demonstrated the importance of adopting dynamic approach to conventional QTL mapping to understand the genetics of response to stress during the course of development of a trait. The results also illustrated the significance of conditional mapping. A dynamic approach to conventional mapping in conjunction with conditional mapping would allow to clearly reveal the gene system associated with the developmental traits under optimum as well as stressed conditions.

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