Theor Appl Genet (1996) 92: 395–402 © Springer-Verlag 1996

E. D. Redoña · D. J. Mackill

Mapping quantitative trait loci for seedling vigor in rice using RFLPs

Received: 15 March 1995 / Accepted: 18 August 1995

Abstract Improving seedling vigor is an important objective of modern rice (*Oryza sativa* L.) breeding programs. The purpose of this study was to identify and map quantitative trait loci (QTL) underlying seedling vigor-related traits using restriction fragment length polymorphisms (RFLPs). An F_2 population of 204 plants was developed from a cross between a low-vigor japonica cultivar 'Labelle' (LBL) and a high-vigor indica cultivar 'Black Gora' (BG). A linkage map was constructed of 117 markers spanning 1496 Haldane cM and encompassing the 12 rice chromosomes with an average marker spacing of 14 cM. The length of the shoots, roots, coleoptile and mesocotyl were measured on F_3 families in slantboard tests conducted at two temperatures (18° and 25°C). By means of interval analysis, 13 QTLs, each accounting for 7% to 38% of the phenotypic variance, were identified and mapped in the two temperature regimes at a log-likelihood (LOD) threshold of 2.5. Four QTLs controlled shoot length, 2 each controlled root and coleoptile lengths and 5 influenced mesocotyl length. Single-point analysis confirmed the presence of these QTLs and detected additional loci for shoot, root and coleoptile lengths, these latter usually accounting for less than 5% of the phenotypic variation. Only 3 QTLs detected both by interval and single-point analyses were expressed under both temperature regimes. Additive, dominant and overdominant modes of gene action were observed. Contrary to what was predicted from parental phenotype, the low-vigor LBL contributed 46% of the positive alleles for shoot, root and coleoptile lengths. Positive alleles from the high-vigor parent BG were identified for increased root, coleoptile and mesocotyl lengths. However, BG contributed alleles with only minor effects for shoot length, the most important determinant of seedling vigor in water-seeded rice, suggesting that it would not be an ideal donor parent for introducing faster shoot growth alleles into temperate japonica cultivars.

Key words Quantitative trait locus (QTL) · RFLP · Seedling vigor · Shoot growth · *Oryza sativa*

Communicated by G. E. Hart

E. D. Redoña
Department of Agronomy and Range Science,
University of California, Davis, CA 95616 USA

D. J. Mackill (✉)
USDA-ARS, Department of Agronomy and Range Science,
University of California, Davis, CA 95616 USA

Introduction

Seedling vigor, or the ability of a plant's aerial parts to emerge rapidly from soil or water (Heydecker 1960), is a desirable trait to incorporate into modern rice cultivars. Low temperature-induced retardation of seedling growth is a common problem in temperate rice-growing areas and in tropical and sub-tropical areas at high elevations or with cold irrigation water supply. In California and in parts of the southern United States rice belt, where rice is sown directly into flooded fields, seedling vigor is important for optimum stand establishment. A delayed emergence of the rice seedlings from water greatly increases seedling mortality (Peterson et al. 1978). Good seedling vigor is also important for adequate stand establishment in parts of the southern USA where rice is drill-seeded (Dilday et al. 1990). Vigorous cultivars are likewise needed for large rainfed and upland areas in the tropics where dry-seeding is practiced and competition from weeds is a serious problem. The increasing importance of direct-seeding in many Asian countries (Dingkuhn et al. 1992) has likewise made it critical to improve the seedling vigor of rice cultivars.

Several quantitative traits have been associated with seedling vigor in rice. Seedling characteristics measured in controlled laboratory slantboard tests have been correlated with seedling vigor under field conditions (Jones and Peterson 1976; McKenzie et al. 1980). Long shoots have been associated with seedling vigor in water-seeded rice culture (Peterson et al. 1978). Rapid root growth is important for proper soil anchorage to reduce seedling floatation, a common problem under water-seeding (Williams

and Peterson 1973), and long mesocotyls and coleoptiles promote seedling emergence in drill-seeded rice culture (Turner et al. 1982; Dilday et al. 1990). However, despite the substantial amount of genetic variation for seedling vigor in rice (Jones and Peterson 1976; Mgonja et al. 1993), breeders have had difficulty in improving the level of seedling vigor in modern semi-dwarf cultivars (McKenzie et al. 1994).

The advent of molecular marker technology has led to the development of genetic maps that make it possible to identify and locate genes or quantitative trait loci (QTLs) controlling quantitative characters. At least four restriction fragment length polymorphism (RFLP) maps have been developed in rice (McCouch et al. 1988; Saito et al. 1991; Causse et al. 1994; Kurata et al. 1994). QTLs for yield-related traits (Xiao et al. 1995), blast resistance (Wang et al. 1994) and root morphology (Champoux et al. 1995) have recently been mapped in rice. These QTL maps could assist plant breeders in devising more efficient conventional breeding strategies for these characters as well as in developing marker-assisted selection schemes where selection for the quantitative trait would be based on a set of molecular markers. Determination of the number, location and magnitude of effects through RFLP linkages of QTLs underlying seedling vigor could elucidate the genetic basis of the trait leading to improved breeding and selection efficiency. In the study presented here, we located QTLs for seedling vigor-related traits in a japonica X indica rice cross using RFLPs.

Materials and methods

Plant material

The F_2 mapping population used was derived from a single cross between a USA japonica cultivar 'Labelle' (LBL) and a tropical *aus* cultivar 'Black Gora' (BG). The two parents were selected on the basis of their phenotypic differential for various seedling vigor parameters (Redoña and Mackill 1996), including traits measured in laboratory slantboard tests at the two temperature regimes used in this study (Table 1). The low-vigor LBL belongs to the tropical japonica group grown in the southern United States (Mackill 1995). BG is a vigorous cultivar grown in the direct-seeded culture of India, and was classified in the indica subspecies by RAPD analysis (Mackill 1995). Two hundred and four F_2 plants were used for marker segregation analysis; these plants were grown individually in pots in the greenhouse to produce F_3 seed. One hundred and seventy-two F_3 lines from F_2 plants which produced sufficient seed for replicated tests were used in phenotyping seedling vigor characters.

Seedling vigor screening

Seedling vigor-related traits, namely, length of shoots, roots, coleoptile and mesocotyl, were measured on F_3 families in growth chamber slantboard tests conducted at two temperature regimes (18° and 25°C). In a developing rice seedling, shoot length represents the distance between the seed and the tip of the primary leaf or plumule, root length is the distance between the seed and the primary root tip, mesocotyl length represents the distance between the seed and coleoptile node, and coleoptile length is the distance between the coleoptile node and the tip of the coleoptile, which is the tube-like protective structure enclosing the plumule (Chang and Bardenas 1965).

Table 1 Parental means for the four seedling vigor traits at two temperatures: 18°C for 15 days and 25°C for 10 days

Seedling part measured	Temperature (°C)	Length (mm)		LSD (0.05) (mm)
		Black Gora	Labelle	
Shoot	18	72	38	7
	25	208	145	14
Root	18	122	59	12
	25	213	168	17
Coleoptile	18	22	12	4
	25	14	11	3
Mesocotyl	18	4	3	1
	25	8	3	1

The slantboard test, devised by Jones and Peterson (1976), is a standard laboratory procedure used in California to screen rice breeding materials for seedling vigor. We have recently confirmed its usefulness in seedling vigor evaluation in a study involving growth chamber, greenhouse and field experiments (Redoña and Mackill 1996). Seeds of each F_3 line were sterilized, pre-germinated and attached to slantboards with blotting paper following the procedures described in Jones and Peterson (1976). The seedlings were placed in a Percival incubator in the dark at two constant temperatures: 18°C for 15 days and 25°C 10 days. The shorter duration at 25°C was necessary to avoid seedling overgrowth. The two temperatures were selected to simulate temperate and tropical rice-growing conditions. The optimum temperature of 25°–30°C for early seedling growth of rice (Chapman and Peterson 1962) is common in the tropics, but in California, the water temperature is often below 20°C during seeding (Raney 1963). The slantboard tests were conducted using a randomized complete block (based on position in the incubator) design with two replications for each temperature and vigor traits were measured on 10 F_3 plants per replication.

RFLP analysis

DNA was extracted from the leaf tissue of F_2 plants following procedures described in Mackill (1995). Restriction enzyme digests, probe preparation and ^{32}dCTP-labeling were undertaken as described in McCouch et al. (1988) with some modifications. The alkali Southern blotting, hybridization and membrane stripping (method 1) protocols described in the Hybond N+ membrane kit (Amersham) were followed with minor modifications. Autoradiography was undertaken for 2–7 days depending on probe signal strength. An initial survey for parental RFLP polymorphism was conducted using 268 clones obtained from the laboratory of Dr. Steven D. Tanksley at Cornell University. These clones were selected to provide uniform genomic coverage (about 10–20 cM marker spacing) based on the framework rice molecular map developed at Cornell University (McCouch and Tanksley 1991). Five restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Sca*I, *Xba*I) were used in the parental survey and in assaying the F_2 population.

Data analysis

Analyses of variances (ANOVA), mean comparisons and Pearson correlation analysis for vigor-related traits based on F_3 progeny data all used SAS programs (SAS Institute Inc. 1989). In a combined analysis of variance for the two temperature regimes, the effects of temperature or line X temperature interactions were highly significant ($P<0.01$) for all traits based on F tests. Since tests at each temperature regime were not repeated, the individual ANOVA of each test temperature was used in estimating the variance components and coefficients of variation (CV). F-tests were used to determine the significance of the variance components, and LSD values were com-

puted for comparison of the F_3 line means. Block effects were mostly non-significant based on the F-tests, and means over replications were used in subsequent analyses.

Linkage analysis and map construction were undertaken using MAPMAKER/EXP (Lander et al. 1987). Linkage groups were assigned to rice chromosomes based on the map of Causse et al. (1994). The most likely orders were determined using 'order', 'compare', 'build', 'place' and 'ripple' commands. In regions where other orders were equally likely (LOD difference of <1), order information from previously published maps (McCouch et al. 1988; Causse et al. 1994) was also considered. As required by MAPMAKER/QTL, distances were computed using the Haldane (1919) equation. Chi-square tests, using simple computer macros, were used to check the segregation ratios of individual markers against Mendelian expectation, and in analyzing percent genome composition over marker loci and F_2 plants.

The chromosomal locations of putative QTLs were determined by two methods: single-point analysis using SAS procedures (SAS Institute Inc 1989), and interval mapping (Lander and Botstein 1989) using the MAPMAKER/QTL program (Paterson et al. 1988). The one-way ANOVAs involved testing the significance of the associations at each locus between marker genotypes (three classes for codominant and two for dominant RFLPs) and trait values over all plants using the *F*-tests. To reduce type-II errors (i.e. declaring true associations non-significant), we chose a probability level of 0.05 for the *F*-tests. However, as pointed out by Lander and Botstein (1989), one-way ANOVAs are as efficient as interval mapping, when the information from flanking markers is considered, only if QTL and marker positions exactly coincide. Hence, single-factor ANOVAs were used primarily for confirming the results of interval mapping, and QTLs identified by both interval and single-point analysis are highlighted in this paper. As noted by Darvasi et al. (1993), interval mapping can detect QTLs located in intervals up to 50 cM long with only a slight reduction in power, and Darvasi and Soller (1994) indicated that optimum marker spacing for initial QTL studies can be as wide as 50 cM. In our map, only 4 of 105 intervals were more than 30 cM using the Kosambi (1944) function, with the longest at 41 cM. An LOD threshold of 2.5, based on a free genetics model, was selected for declaring the significance of a putative QTL. This corresponds approximately to a probability of less than 0.05 for declaring one false positive in the entire genome based on sparse-map model (Lander and Botstein 1989). The proportion of trait variation accounted for by each QTL was estimated using MAPMAKER/QTL, which is equivalent to the coefficient of determination or R-square value in linear regression analysis (Paterson et al. 1988).

Results and discussion

Variation for seedling vigor traits

Analyses of variance revealed highly significant differences ($P<0.001$) among F_3 lines for all of the vigor characters at each of the two test temperatures. CV values were all below 18% for shoot, root and coleoptile lengths at each of the two temperature regimes, and they were around 40% for mesocotyl length at both temperatures, which is consistent with previous results for this trait (Redoña and Mackill 1996). Transgressive segregation was observed for all four characters at both test temperatures (Fig. 1). The percentage of F_3 lines with means significantly higher than that of BG and lower than that of LBL based on LSD values at $P<0.05$ was 6% for shoot length at both temperatures, 8% at 18°C and 4% at 25°C for root length, 5% at 18°C and 4% at 25°C for coleoptile length, and 5% at 18°C and 14% at 25°C for mesocotyl length. The correlation coefficient (r) between shoot and coleoptile lengths at 18°C was 0.56 ($P<0.01$, $df=314$). Significant ($P<0.05$) but low ($r<0.4$) correlations were observed between shoot and root lengths, and coleoptile and mesocotyl lengths under both temperatures, and between shoot and coleoptile lengths at 25°C, and root and coleoptile lengths at 18°C.

Marker segregation and genome composition

In the parental survey for RFLP polymorphism, 168, or 63%, of the clones detected RFLPs between LBL and BG for at least one of the five restriction enzymes. Of these, 117 RFLP markers uniformly distributed on the rice genetic map (Causse et al. 1994) were assayed on the F_2 population. Based on chi-square tests, 19 markers (16%) showed highly significant ($P<0.01$) deviations from normal Mendelian segregation, and 33 loci (27%) exhibited significant deviations ($P<0.05$). This level of segregation distortion was less than that previously reported in different rice mapping populations (McCouch et al. 1988; Wang et al. 1994). Distortion was slightly in favor of the japonica parent. Of the loci showing distorted segregation, 11 loci (58%) and 21 loci (64%) had more LBL alleles at $P<0.01$ and $P<0.05$, respectively. Percentage genome composition over RFLP loci and F_2 plants agreed with the expected value (50%, $df=1$) under normal Mendelian segregation except for 4 RFLP loci and 22 F_2 plants at $P<0.01$.

The linkage map

The RFLP linkage map generated consisted of 117 markers, including a gene for black pericarp color mapped on chromosome 4 that segregated in the population (Fig. 2). The total map length was 1496 cM and encompassed the 12 rice chromosomes. The average distance between markers was 14.2 cM. Several gaps greater than 30 cM were detected, notably on chromosomes 1, 4, 9 and 11. Additional markers assayed to fill these gaps failed to detect RFLPs for the two parents. However, the number of gaps in our map is considerably less and/or shorter than those in other maps used for QTL studies in many crop species, including rice (Wang et al. 1994; Xiao et al. 1995). Further, all intervals in our map were within the optimum marker spacing for initial QTL studies based on both theoretical (Darvasi et al. 1993) and practical (Darvasi and Soller 1994) considerations. The marker order generally agreed with published rice genetic maps (McCouch et al. 1988; Causse et al. 1994). However, a few regions were detected where alternate orders were within a ten-fold probability of being as likely as the orders presented.

Number and distribution of QTLs

Sixteen putative QTLs, 8 in each of the two temperature regimes used, were identified using interval mapping at a LOD threshold of 2.5 (Table 2). These represent 13 unique

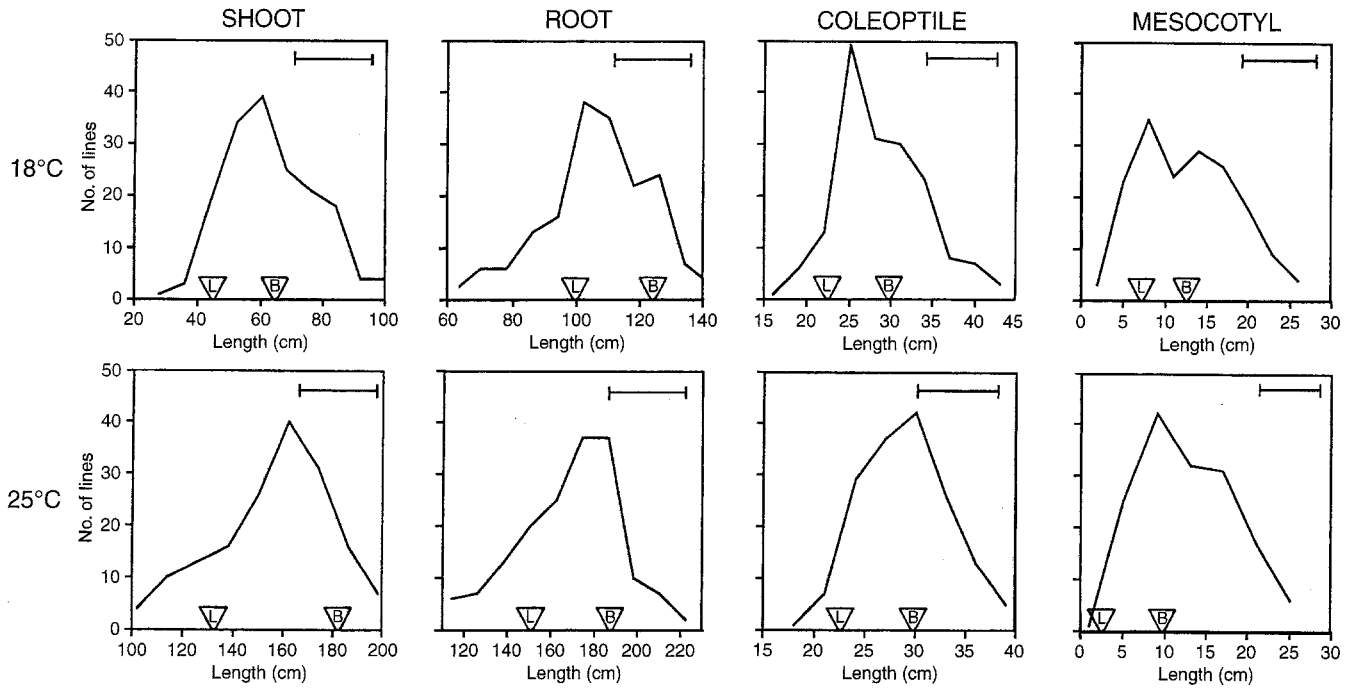

Fig. 1 Distribution of F_3 family means for vigor traits at two temperatures. *L* and *B* LBL and BG means, respectively; *horizontal bars* LSD (0.05)

loci, with 3 QTLs detected at both 18°C and 25°C. One root-length factor on chromosome 1, expressed at both temperatures, accounted for 31% and 38% of the phenotypic variance at 18°C and 25°C, respectively. Of the 10 QTLs detected only at one temperature, 5 each were expressed at 18°C and 25°C. Single-point analysis confirmed the presence of all 16 QTLs identified through interval mapping. Marker genotype-trait associations, usually involving several RFLPs at positions where QTLs were identified by interval analysis, were significant based on *F*-tests at $P<0.05$. Furthermore, single-point analysis detected additional loci for all of the traits not identified by interval analysis including 3 that were detected at both temperature regimes (1 each for shoot, root and mesocotyl lengths). However, the trait variation accounted for by RFLPs detecting additional loci, determined as the ratio of RFLP marker sum of squares and total sum of squares, were mostly below 5%, suggesting that the additional QTLs detected by single-factor ANOVAs had minor effects.

Of the 12 rice chromosomes 7 contained at least 1 of the 13 QTLs identified by interval mapping and confirmed by single-point analysis. Certain regions of the genome influenced more than one trait. For example, QTLs for length of shoot, coleoptile and mesocotyl mapped to the same or adjacent intervals on chromosome 3 (Fig. 2). Similarly, shoot- and root-length QTLs mapped to adjacent intervals on chromosome 1. This phenomenon has also been observed in many QTL-mapping studies in other crops, such as tomato (de Vicente and Tanksley 1993), corn (Schön et al. 1993) and common bean (Nodari et al. 1993). It is not clear whether this is due to linkage or pleiotropy. However, when adjacent QTLs have alleles from different parents, such as the shoot length, mesocotyl, and coleoptile QTLs on chromosome 3 (Fig. 2), linkage is the more plausible explanation. These associations provide a possible explanation for the low but significant correlations observed in this study between shoot and root length, where QTLs mapped to nearby intervals on chromosome 1, and between coleoptile and mesocotyl lengths, where factors mapped to adjacent intervals on chromosome 3. Two of the QTLs identified in this study (the mesocotyl-length QTL on chromosome 1 and the shoot-length QTL on chromosome 9) mapped to locations close to members of the α-amylase gene family, which have been implicated in rice seedling vigor (Ranjhan et al. 1991; Causse et al. 1994; Thomas and Rodriguez 1994).

Gene action and parental contributions at QTLs

Additive gene action as well as dominance or recessive deviations from complete additivity ($d=0$) were observed for the traits under consideration (Table 2). In biometrical studies, additive gene action has been reported to predominate for shoot length (Li and Rutger 1980) and mesocotyl length (Mgonja et al. 1993). A number of the loci observed here, however, showed overdominance effects ($|d/a|>1$–2), of which the most prominent was the shoot-length locus on chromosome 1, where the heterozygote was notably superior to either homozygote at 18°C (Table 2). Single-point analysis also detected 4 RFLP markers in this region (RG469, RZ566, RG350 and RG236) that were significantly associated at $P<0.05$ with shoot length at 18°C and where the heterozygote class was significantly superior to either parent. Possible explanations for this include dominance complementation, as was suggested in a QTL

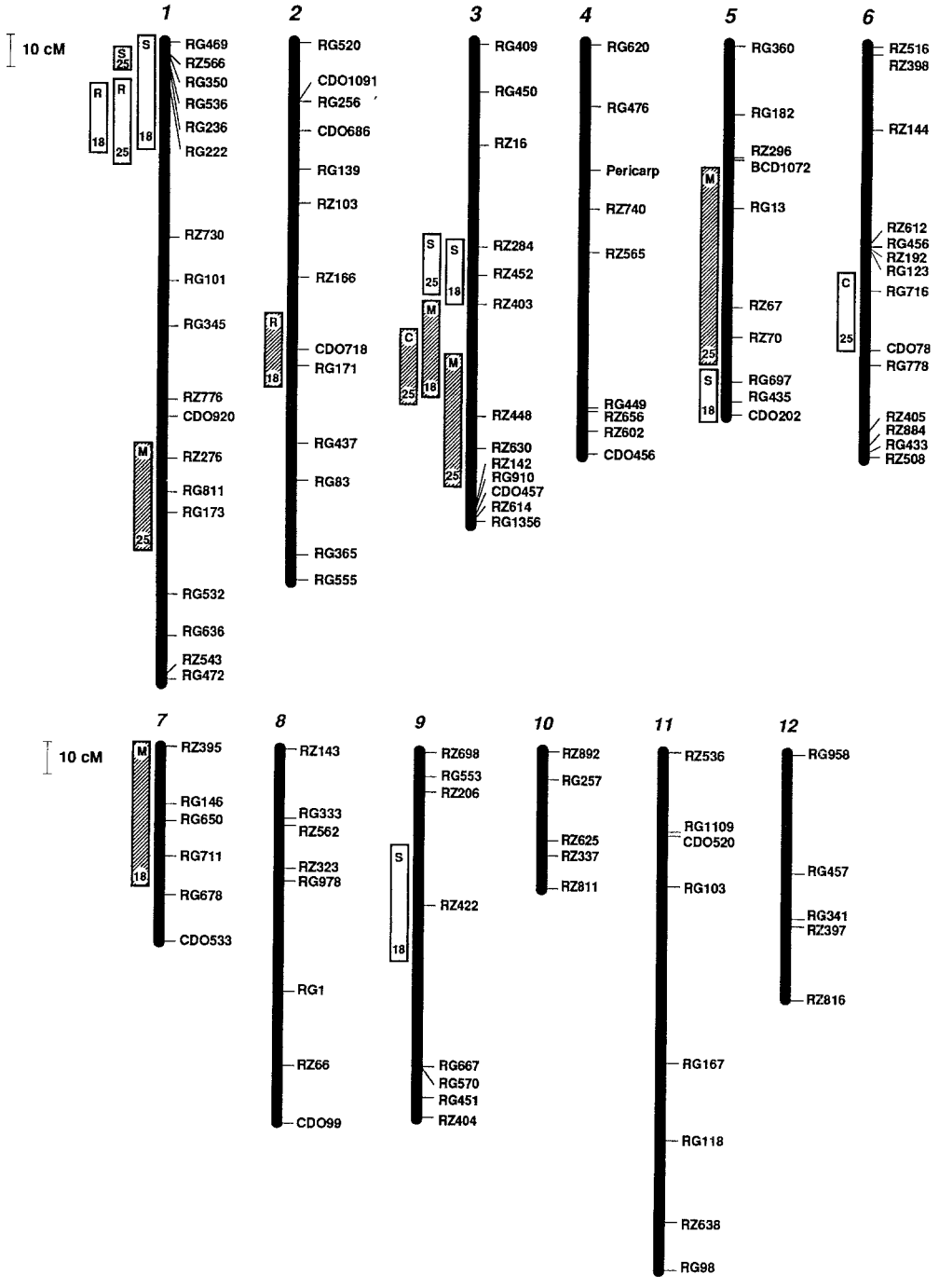

Fig. 2 RFLP linkage map and putative locations of seedling vigor QTLs. *White* and *striped* bars LBL and BG positive alleles, respectively. Traits and test temperature are indicated inside each bar (*S* shoot, *R* root, *C* coleoptile, *M* mesocotyl). Bar length indicates a one-LOD support interval for the most likely QTL position

study for yield-related traits in rice (Xiao et al. 1995), as well as true overdominance, which has been acknowledged as a possible basis for heterosis in corn (Stuber et al. 1992).

Positive alleles from the high-vigor parent BG that increased root, coleoptile and mesocotyl lengths were identified by both interval and single-point analyses. However, BG alleles increasing shoot length were not detected. This suggests that parental phenotypic performance could be a poor indicator of genetic potential for seedling vigor-related traits in rice. The detection of QTLs with effects opposite from those expected from the parental phenotype has also been reported in tomato (de Vicente and Tanksley 1993; Breto et al. 1994) and almond (Asins et al. 1994). In the study of de Vicente and Tanksley (1993), the detection of alleles at QTL (36%) with effects opposite to those predicted from the phenotype of the parents was to some degree associated with transgressive segregation in the F_2. Although transgressive segregation of F_3 family means was observed in all of the traits under both temperature regimes in this study, positive alleles from both parents were detected only in two characters (root and coleoptile length). On the basis of interval analysis, BG had no positive alleles for shoot length, the best vigor indicator in water-seeded rice (Jones and Peterson 1976), which may indicate

Table 2 Peak LOD, percentage of the variation explained, genetic effects and mode of inheritance for four seedling vigor parameters at two temperatures

Vigor trait	Nearest marker	Chromosome number	Temperature (°C)	Peak LOD	Variation (%)	Genetic effects[a]		Mode[b]
						a	d	
Shoot length	RG536	1	18	2.77	7.4	−2.05	19.83	O
			25	2.78	7.5	−10.40	30.33	O
	RZ452	3	18	5.62	17.1	−9.12	−7.52	D
			25	6.00	15.5	−13.29	−9.64	P
	RG435	5	18	3.12	8.0	−6.11	−8.02	O
	RZ422	9	18	2.80	9.9	−4.98	−15.12	O
Root length	RG222	1	18	5.22	31.1	−13.29	27.59	O
			25	8.26	38.0	−22.29	29.56	O
	CDO718	2	18	2.90	9.4	6.03	13.72	O
Coleoptile length	RZ448	3	25	5.31	25.1	3.19	−3.65	D
	RG716	6	25	4.72	15.3	−2.78	−1.03	P
Mesocotyl length	RZ403	3	18	4.73	17.5	3.34	−2.33	P
	RZ630	3	25	3.33	10.3	3.26	0.26	A
	RZ395	7	18	3.36	28.5	3.57	−7.91	O
	RG811	1	25	4.21	12.8	2.49	−5.97	O
	RZ67	5	25	2.67	12.6	3.41	−3.09	D

[a] The additive effect (*a*) is the effect of substituting a LBL allele for a BG allele. Negative values of *a* indicate LBL has the positive allele. The dominance deviation (*d*) is twice that computed by MAPMAKER since traits were measured on F_3 families (Schön et al. 1993). Negative values of *d* indicate recessiveness of the positive alleles. The units of both *a* and *d* are millimeters.

[b] The most likely mode of gene action for each QTL was computed from the absolute value of *d/a* with A= additive (*d/a*=0–0.20), P=partial dominance (*d/a*=0.21–0.80), D=dominance (*d/a*=0.81–1.20) and O=overdominance (*d/a*>1.20) (Stuber et al. 1987)

that the trait is controlled by many loci, most of which have minor effects. This is suggested by the identification through single-point analysis of additional shoot-length alleles (one each on chromosomes 1, 3 and 6) contributed by both BG and LBL that each accounted for about 5% of the phenotypic variation for shoot length and the relatively low proportion of shoot length variation explained by QTLs identified by interval mapping at each temperature (42.4% at 18°C and 23% at 25°C), and it is consistent with conclusions drawn from a biometrical study (Li and Rutger 1980) that at least four to five factors control variation for shoot length. Since no major shoot length alleles were detected from the indica parent BG, it may not be an ideal genetic donor for seedling vigor enhancement of water-seeded japonica cultivars.

Implications for rice breeding

The expression of alleles at QTL over seasons and locations is an important consideration in plant breeding and has been studied in several crop species, such as tomato (Paterson et al. 1991), corn (Schön et al. 1993), barley (Hayes et al. 1993) and almond (Asins et al. 1994). Most of these studies, however, used a fairly limited number of environments and/or, within each environment, a limited number of replications or samples per replication, most probably due to practical considerations. The same limitations and considerations apply to the present study. However, our detection of QTLs expressed at both temperature regimes used in this study and of QTLs expressed only at one test temperature, results similar to those obtained in the above-mentioned studies, suggests that some type of genotype X environment interaction may be involved in the expression of seedling vigor-related traits in rice at the molecular level. This needs further analysis and should be considered, in relation to the target environment, when devising conventional as well as marker-assisted breeding strategies for these traits.

Breeding for increased seedling vigor using conventional strategies in rice has not been very successful in either temperate (McKenzie et al. 1994) or tropical rice-growing regions (Herdt 1991). This may be due partly to the trait's association with undesired characteristics such as tallness, lodging susceptibility, large grain size and earliness (Peterson et al. 1978; Li and Rutger 1980) that are selected against during the breeding process. The results of our study suggest that this could also be due to a lack of QTLs with major effects for seedling vigor-related traits, coupled with the loose relationship between parental phenotype and genotype, making the identification of superior donors and breeding lines based on phenotype alone difficult. QTL analysis to identify superior donors and marker-aided selection (MAS) strategies may, therefore, be useful in breeding for rice seedling vigor. In the LBL X BG cross, alleles for faster coleoptile and mesocotyl elongation from the high-vigor indica parent BG could be introgressed into USA japonica cultivars more precisely and efficiently using MAS strategies. Conversely, QTL alleles contributed by LBL may be useful for improving the seedling vigor of tropical rice cultivars. Our results, however, suggest that indica and japonica cultivars may behave dif-

ferently in crosses with regard to seedling vigor. Although alternate indica sources may possess alleles at QTL with major effects, as far as identifying sources of seedling vigor for japonica breeding programs is concerned, the exploitation of sources within the japonica subspecies may be a more promising approach for improving seedling vigor in temperate rice-growing areas.

Acknowledgments We would like to thank Teresa Erickson, Shelley Nelson and Zhen Zhang for technical support; Theresa Fulton from S. D. Tanksley's lab for providing the clones; C. O. Qualset for valuable advice and assistance; J. N. Rutger, J. J. Oster, B. R. Thomas and G. Wang for comments on the manuscript; and the Rockefeller Foundation for the fellowship awarded to E.D. Redoña.

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