Theor Appl Genet (2000) 101:756–766

M.L. Ali · M.S. Pathan · J. Zhang · G. Bai
S. Sarkarung · H.T. Nguyen

Mapping QTLs for root traits in a recombinant inbred population from two *indica* ecotypes in rice

Received: 17 November 1999 / Accepted: 19 March 2000

Abstract Evaluation of root traits in rainfed lowland rice is very difficult. Molecular genetic markers could be used as an alternative strategy to phenotypic selection for the improvement of rice root traits. This research was undertaken to map QTLs associated with five root traits using RFLP and AFLP markers. Recombinant inbred lines (RILs) were developed from two *indica* parents, IR58821–23-B-1–2-1 and IR52561-UBN-1–1-2, that were adapted to rainfed lowland production systems. Using wax-petrolatum layers to simulate a hardpan in the soil, 166 RILs were evaluated for total root number (TRN), penetrated root number (PRN), root penetration index (RPI, the ratio of PRN to TRN), penetrated root thickness (PRT) and penetrated root length (PRL) under greenhouse conditions during the summer and the fall of 1997. A genetic linkage map of 2022 cM length was constructed comprising 303 AFLP and 96 RFLP markers with an average marker space of 5.0 cM. QTL analysis via interval mapping detected 28 QTLs for these five root traits, which were located on chromosomes 1, 2, 3, 4, 6, 7, 10 and 11. Individual QTLs accounted for between 6 and 27% of the phenotypic variation. Most of the favorable alleles were derived from the parent IR58821–23-B-1–2-1, which was phenotypically superior in root traits related to drought resistance. Three out of six QTLs for RPI were detected in both summer and fall experiments and they also were associated with PRN in both experiments. Out of eight QTLs for RPT, five were common in both seasons. Two genomic regions on chromosome 2 were associated with three root traits (PRN, PRT and RPI), whereas three genomic regions on chromosomes 2 and 3 were associated with two root traits (PRT and RPI). Two QTLs affecting RPI and two QTLs affecting PRT were also found in similar genomic regions in other rice populations. The consistent QTLs across genetic backgrounds and the common QTLs detected in both experiments should be good candidates for marker-assisted selection toward the incorporation of root traits in a drought resistance breeding program, especially for rainfed lowland rice.

Key words DNA markers · Drought resistance · QTLs · Rice · Root penetration

Communicated by P. Langridge

Equal contribution from M.L. Ali and M.S. Pathan

M.L. Ali · M.S. Pathan · J. Zhang · G. Bai · H.T. Nguyen (✉)
Plant Molecular Genetics Laboratory,
Department of Plant and Soil Science, Texas Tech University,
Lubbock, TX 79409-2122, USA
e-mail: henry.nguyen@ttu.edu
Tel.: 806-742 1622 +1, Fax: 806-742 2888 +1

Present addresses:
G. Bai, Department of Plant and Soil Sciences,
469 Agricultural Hall,Oklahoma State University,
Stillwater, OK 74078-6028, USA

S. Sarkarung, Plant Breeding,
Genetics and Biochemistry Division,
International Rice Research Institute, P.O. Box 933,
1099 Manila, The Philippines

Introduction

Rice (*Oryza sativa* L.) is grown over a wide range of agro-ecological environments with varying temperatures, hydrologies and soil textures. Yields of rainfed lowland rice, which occupies about 25% of the world's rice areas, are drastically reduced by drought due to unpredictable, insufficient and uneven rainfall during the growing period. To reduce yield losses of rice crops in rainfed lowland areas and to increase overall rice production, new rice varieties with greater adaptation to drought are essential. Hence, the development of drought-resistant varieties with a higher yield potential is one of the main objectives of rainfed lowland rice breeding programs.

Plants have different mechanisms to minimize the effects of drought. Adaptive mechanisms involve different root and shoot characteristics that allow plants to maintain high internal water status when available water is less than the evaporative demand (O'Toole and Chang

1979; Nguyen et al. 1997; Zhang et al. 1999). A root system that enables the crop to extract more soil water has the potential to increase yield under drought (Mambani and Lal 1983). Individual root characteristics, such as thickness, depth of rooting and the ability to penetrate through compacted soils, have been associated with drought avoidance (O'Toole and Chang 1979).

Exploitation of water from deeper soil horizons is beneficial for plants to avoid drought. However, water in deeper horizons is often inaccessible due to the presence of compacted soil layers (''hardpans'') in rainfed lowland rice fields. These compacted soil layers result from the cultural practices used in preparing land for planting (O'Toole and De Datta 1986). Mechanical disruption of compacted soil layers is expensive and short-lived, as these layers often re-form in a few years (Busscher et al. 1986). The limited root system due to the presence of compact soil layers may subject plants to increased water deficits. In the presence of hardpans, especially when water-deficit occurs, root penetration ability through compact soil layers is considered as an important characteristic associated with drought tolerance (Nguyen et al. 1997).

Breeding varieties with increased root penetration ability through hardpans and other root traits is very difficult. This is because screening numerous genotypes under field conditions is laborious and time-consuming, and no easy and efficient techniques have yet been developed (O'Toole and Chang 1979; Mambani and Lal 1983; Ekanayeke et al. 1986). Secondly, soil compaction is not uniform and consistent throughout rice fields, which makes the evaluation of root penetration ability and rooting depth difficult. To develop new varieties with improved root traits, molecular marker technology could be used as an alternative strategy (Nguyen et al. 1994, 1997). Root penetration ability and other root characters are quantitative traits, and so dissection of these complex traits into component genetic factors is a prerequisite to manipulate the traits. Genome mapping using molecular genetic markers offers an excellent opportunity to locate genes or QTLs controlling quantitative characters (Paterson et al. 1988; Lander and Botstein 1989; Tanksley 1993). Markers linked with genes controlling a trait of interest could be used in the selection of genotypes in a breeding program.

Genetic variation and QTLs for root traits have been studied in several *japonica/indica* inter-subspecific crosses (Champoux et al. 1995; Ray et al. 1996; Price and Thomas 1997; Yadav et al. 1997; Zheng et al. 2000), but not in *indica*/*indica* crosses. The necessity of QTL identification based on the variation from the crosses between two related varieties belonging to the same subspecies has been emphasized by various authors (Ingram et al. 1994; Redona and Mackill 1996;Yano and Sasaki 1997). It is, therefore, desirable to use a population derived from an *indica*/*indica* cross to identify genes controlling agronomic traits for the improvement of rainfed lowland rice.

Over the past decade, a number of DNA-based genetic marker systems have been developed. Among these markers, RFLPs (restriction fragment length polymorphisms) and AFLPs (amplified fragment length polymorphisms) are being widely used to locate and tag genes of economic importance. In the present study, RFLPs and AFLPs were used with the objectives: (1) to generate a closely spaced genetic linkage map in rice using *indica*/*indica* RILs, (2) to identify QTLs associated with root traits, and (3) to evaluate genetic relationship among the root traits. This is the first report on QTL analysis using an *indica*/*indica* cross for root traits in rice.

Materials and methods

Plant materials

A subset of 166 F_7 RILs, chosen randomly from a population of 475 lines derived from a cross between two advanced breeding lines, IR58821–23-B-1–2-1 and IR52561-UBN-1–1-2, was used in this research. The RILs were developed by single-seed descent to the F_7 generation at the International Rice Research Institute (IRRI), The Philippines. Panicles were bagged for each generation to avoid outcrossing. Both parental lines were *indica* types and suited to grow in rainfed lowland rice fields. IR58821–23-B-1–2-1 has higher root penetration ability and thicker roots than IR52561-UBN-1–1-2.

Phenotypic evaluation of root traits

Phenotypic evaluation of RILs along with the parents for root penetration ability and other root traits was done in a greenhouse at Texas Tech University during the summer (May–July) and the fall (September–December), 1997. The wax-petrolatum layers used to screen the RILs for root penetration were made of 66.7% paraffin wax and 33.3% petrolatum white by weight. This wax-petrolatum layer, which was developed to simulate hardpans, had a strength of 1.7 MPa, as measured as soil mechanical impedance by a soil penetrometer (Bush soil penetrometer-SP1000, Findlay Irwine Ltd, Midlothian, Scotland). Rice plants were grown in PVC pots of 3.75-l capacity with the base diameter being 12 cm. Detailed information on the preparation of wax layers and setup has been described in Ray et al. (1996) and Yu et al. (1995).

The experiment was laid out in a randomized complete block design with five replications. Three to four seeds of each genotype per replication were sown directly into each pot and plants were thinned to one per pot at 15 days after sowing. Plants were kept watered and fertilized (Ray et al. 1996) before sampling. At 50 days (maximum tiller stage) after sowing for the summer experiment, and 65 days (maximum tiller stage) after sowing for the fall experiment, plants were sampled for total root number (TRN), number of roots penetrated through the wax-petrolatum layer (PRN), penetrated root length (PRL) and penetrated root thickness (PRT). The PRT was measured at 5 mm below the wax layer using an ocular-meter equipped in a microscope. Root penetration ability was determined as the root penetration index (RPI), which was calculated as the ratio of PRN to TRN.

RFLP and AFLP analysis

Fresh leaf tissues were harvested from 30-day old plants and lyophilized for 3 days. Lyophilized leaf samples were ground to fine powders, which were then used for genomic DNA isolation based on a potassium acetate method (Tai and Tanksley 1990). DNA (8–10 µg/sample) was digested with ten enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Xba*I, *Apa*I, *Bam*HI, *Bgl*II, *Hind*III, *Kpn*I and *Sca*I). The

enzymes that revealed polymorphisms for the RFLP markers in the parental survey were then used to genotype the RILs. RFLP was done essentially by following the procedure of Causse et al. (1994). RFLP probes were provided by Cornell University, USA (prefixed with RG, RZ, CDO and BCD), and the Rice Genome Project (RGP), Japan (prefixed with C, R and G). In addition, one multilocus probe (AA7–2) converted from a RAPD marker and one drought responsive cDNA probe developed in Nguyen's laboratory were also used.

AFLP analysis was performed as described by Vos et al. (1995) with minor modifications (Bai et al. 1999). One hundred and fifty AFLP primer combinations chosen randomly were screened for parental polymorphisms. Of these, 30 primer combinations (see Table 1) were selected for genotyping the RILs. Polymorphic DNA fragments were scored from X-ray films as AFLP markers for the parents and the RILs. A specific number was assigned to each primer combination (see Table 1). Polymorphic bands for each primer combination were numbered serially in descending order of molecular weight, i.e., from the top to the bottom of the gel. AFLP markers were named based on the particular primer combination number and polymorphic band number under that primer combination. However, each marker name is prefixed by ''PC'', which means *P*rimer *C*ombination, followed by the primer combination number (see Table 1), then ''M'' meaning marker, followed by polymorphic band number.

Map construction

Map construction was done using Mapmaker Macintosh (version 2.0, Lander et al. 1987) with the Kosambi mapping function (Kosambi 1944). Linkage groups were determined using 'Group' command with a LOD score of 8.0–12.0 and a recombination fraction of 0.40. The order of the markers for each linkage group was determined using command 'First order'. Ungrouped/unlinked markers were assigned to the respective linkage groups using 'try' command. Assignment of linkage groups to the respective chromosomes was based on the rice maps developed by Causse et al. (1994) and Harushima et al. (1998). All AFLP and RFLP markers were evaluated individually by the χ^2 test for goodness of fit against a 1:1 segregation ratio at a 0.01 probability level using QGene software (Nelson 1997). Segregation in the whole population was determined by analyzing all the markers together. Chromosomes were oriented with the short arms at the top (Singh et al. 1996; Harushima et al. 1998).

Data analysis

Analysis of variance was done using the general linear model (GLM) procedure of the SAS program (SAS Institute Inc. 1990). The frequency distribution of the RILs for all root traits was performed using the univariate procedure of SAS and normal distributions were determined using the Shapiro-Wilk test. To have primary information on marker-QTL associations for each trait, a single-point (single-marker) analysis was conducted first, using QGene software (Version 2.30, Nelson 1997). However, to determine the precise locations of the putative QTLs and their phenotypic effects, interval mapping was further conducted by MAPMAKER/QTL software (Paterson et al. 1988; Lincoln et al. 1992). A LOD score of 2.0 was used as the threshold to declare the presence of QTLs. The proportion of the phenotypic variation explained by each QTL was calculated as the R^2 value. The total amount of phenotypic variation explained together by all the putative QTLs was estimated by fitting a multiple-QTL model under a Mapmaker/QTL program for each trait. AFLP and RFLP markers associated with QTLs were tested for possible two-locus interactions with all other markers in the genome (Nelson 1997).

Results

Polymorphism of RFLP and AFLP markers

Among 520 RFLP rice probes surveyed, 136 were polymorphic for the two parents, which revealed 26% polymorphism. Among the 2314 fragments amplified by the selected 30 primer pairs, 395 bands were polymorphic for the two parents, which revealed 17% polymorphism (Table 1). This level of polymorphism was lower than that observed in other studies with rice (Maheswaran et al. 1997; Cho et al. 1998). The low level of parental polymorphism was attributable to the narrow genetic variation between the parents as both were *indica* types and adapted to grow in the same rice ecosystem.

Marker segregation

All 399 markers mapped on the genetic linkage map were tested for segregation with a χ^2 test for goodness of fit. Statistical analysis revealed that 22% of the AFLP markers and 21% of RFLP markers deviated from the expected Mendelian 1:1 ratio. Most of the AFLP and RFLP markers on chromosome 8 segregated in favor of the female parent IR52561. Distorted segregation of molecular markers was also observed in other mapping populations (Causse et al. 1994; Ray et al. 1996; Xiao et al. 1996; Xu et al. 1997; Marques et al. 1998). The genetic basis of segregation distortion may be the uneven abortion of male or female gametes, or the selective fertilization of particular gametic genotypes (Xu et al. 1997). However, when all markers were analyzed together, the segregation of markers in the population fitted the Mendelian 1:1 ratio.

Genetic linkage map

The linkage map, composed of 399 markers, had a total map length of 2022 cM with an average distance of 5.1 cM between markers (Fig. 1). This number fell within the length range of maps constructed from inter-subspecific populations in rice, such as 2027 cM by Lu et al. (1997), 1840 cM by Li et al. (1995), 1814 cM by Cho et al. (1998) and 1811 cM by Huang et al. (1994).

Of the 399 mapped markers, 96 were RFLPs and 303 were AFLPs. Chromosome 12 consisted of two linkage groups. Appropriate markers could not be found to link these two fragments. In general, the order of RFLP markers in this study agreed with those reported by Causse et al. (1994), Harushima et al. (1998) and Xiong et al. (1997). AFLP markers were distributed among most of the chromosomes almost proportionally, as expected on the basis of chromosome length. Chromosomes 2 and 4 had the highest number of markers with 50 markers each. Chromosome 4 had the lowest average distance of 3.6 cM. On the other hand, chromosome 10 had the lowest number of markers (four AFLP and five RFLP markers) and the

Table 1 List of the AFLP primer combinations along with the number of fragments and polymorphism

Primer combination (PC) Two selective / three selective[a]	PC #[b]	Total no. of fragments	No. of polymorphic fragments	Polymorphism (%)
*Eco*RI-AA / *Mse*I-CAC	3	65	14	21.5
*Eco*RI-AA / *Mse*I-CAG	4	92	10	10.8
*Eco*RI-AA / *Mse*I-GCG	11	59	12	20.3
*Eco*RI-AA / *Mse*I-GAC	12	81	9	11.1
*Eco*RI-CT / *Mse*I-ACC	15	76	16	21.0
*Eco*RI-CT / *Mse*I-CAC	17	74	14	18.9
*Eco*RI-CT / *Mse*I-CAG	18	56	11	19.6
*Eco*RI-CT / *Mse*I-CTA	20	67	13	19.4
*Eco*RI-CT / *Mse*I-CTC	21	62	8	12.9
*Eco*RI-CT / *Mse*I-GAC	26	61	11	18.0
*Eco*RI-CT / *Mse*I-TGC	27	74	15	20.2
*Eco*RI-CT / *Mse*I-GTG	28	60	18	30.0
*Eco*RI-CA / *Mse*I-CAC	31	72	12	16.6
*Eco*RI-CA / *Mse*I-CAG	32	92	14	15.2
*Eco*RI-CA / *Mse*I-CAT	33	97	12	12.4
*Eco*RI-CA / *Mse*I-CTA	34	93	18	19.3
*Eco*RI-CA / *Mse*I-CTC	35	72	16	22.2
*Eco*RI-CA / *Mse*I-CTG	36	77	10	12.9
*Eco*RI-CA / *Mse*I-AGC	38	70	13	18.6
*Eco*RI-CA / *Mse*I-TGC	41	86	13	15.1
*Eco*RI-AG / *Mse*I-CAT	47	124	12	9.6
*Eco*RI-AG / *Mse*I-CTA	48	84	16	19.0
*Eco*RI-GC / *Mse*I-CAC	73	50	13	26.0
*Eco*RI-GC / *Mse*I-CAG	74	54	10	18.5
*Eco*RI-GC / *Mse*I-CAT	75	55	14	25.4
*Eco*RI-GC / *Mse*I-CTT	79	80	11	13.7
*Mse*I-CT / *Eco*RI-AAT	150	102	12	11.8
*Mse*I-CA / *Eco*RI-ATA	173	95	19	20.0
*Mse*I-TA / *Eco*RI-ACG	180	70	14	20.0
*Mse*I-TA / *Eco*RI-ATA	184	114	15	13.1
Total	**–**	**2314**	**395**	**–**
Mean	**–**	**77**	**13**	**17.1**
Range	**–**	**50–124**	**8–19**	**11–30**

[a]Primer combination comprised of two selective nucleotides (Υ-^{33}P labeled) and three selective nucleotides

[b]Primer combination number assigned arbitrarily

highest average distance of 13.3 cM. Clustering of AFLP markers was observed on most of the chromosomes.

Despite a large number of markers mapped in the linkage map, there were several large gaps on the map. No marker could be mapped in these gap regions because of the monomorphic genetic make-up commonly shared by the closely related *indica* parents. Generally, monomorphic regions are expected in the genome of a population derived from intervarietal crosses or closely related parents. However, AFLP markers in conjunction with RFLP markers have produced a good coverage of the genome with the desired resolution, making the map quite suitable for QTL analysis.

Phenotypic data

The frequency distribution of the RILs for five root traits was normal as determined by Shapiro-Wilk test (data not shown). Values of the mean and range for the five root traits are summarized in Table 2. The RILs exhibited a wide range of variation for the root parameters studied (Table 2). For all the traits except PRT, the RILs had phenotypic values greater than the higher parent and also had values less than the lower parent (i.e., transgressive segregation). Broad-sense heritability ranged from 24 to 92%, with the RPI being the highest and PRL being the smallest in both seasons (Table 2).

Correlation coefficients among the root traits for both experiments are given in Table 3. Penetrated root number was positively correlated with RPI, PRT, TRN and PRL. The root penetration index was also positively correlated with PRT and PRL, but negatively associated with TRN. Among the correlation coefficients, the correlation between PRN and RPI was the highest (r = 0.76–0.85) in the two seasons, as expected. A highly significant correlation was observed between the summer and the fall experiment for PRN, RPI and PRT. However, a weak relationship was observed for the PRL (r = 0.30) and no relationship was found for TRN between the two seasons, suggesting that the expression of PRL and TRN is sensitive to growing environments.

Identification of QTLs for root traits

Since the single-marker analysis and the interval mapping approach gave similar results in identifying QTLs for each trait, only the results from the latter analysis are discussed here. A total of 28 putative QTLs was found to

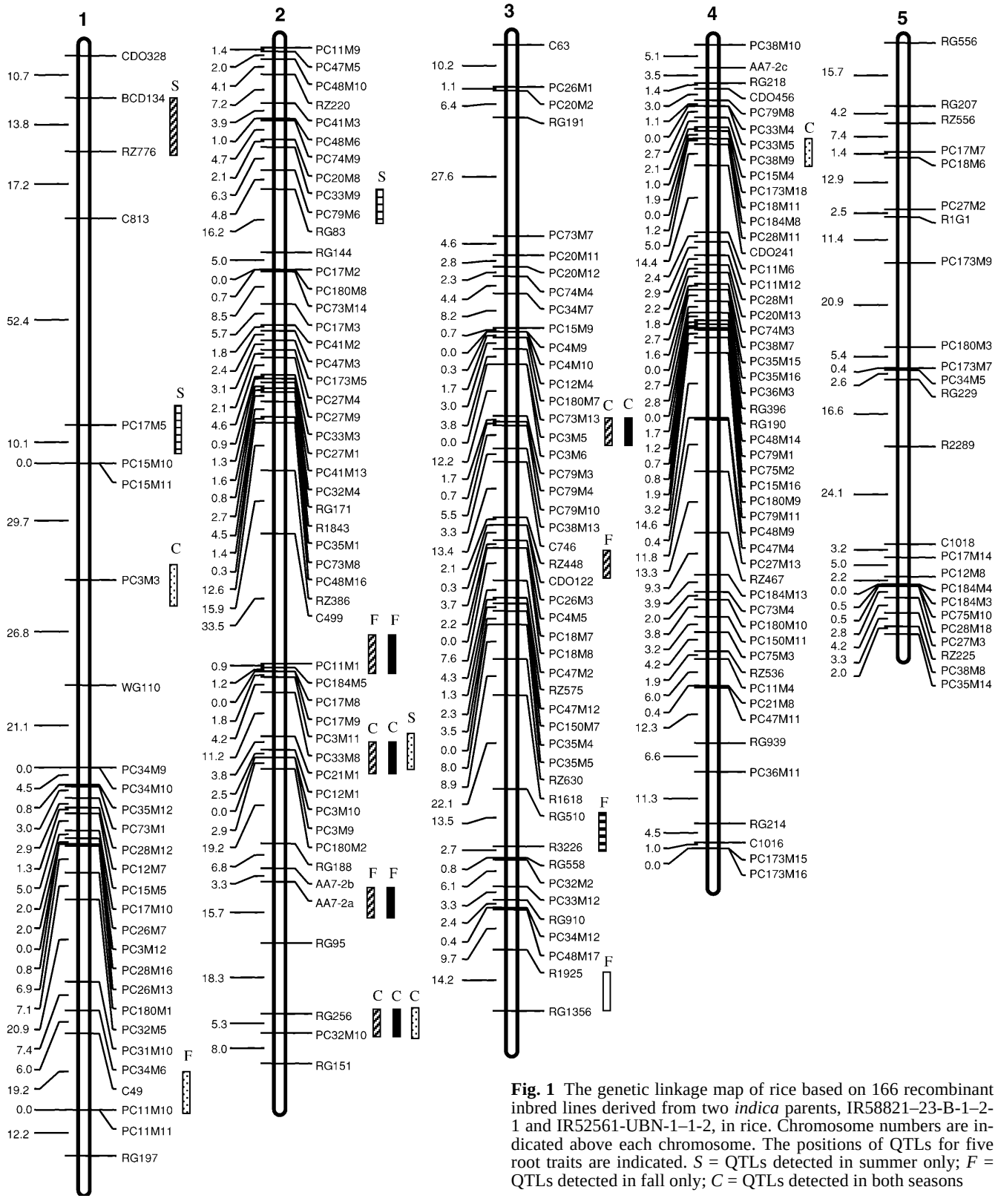

Fig. 1 The genetic linkage map of rice based on 166 recombinant inbred lines derived from two *indica* parents, IR58821–23-B-1–2-1 and IR52561-UBN-1–1-2, in rice. Chromosome numbers are indicated above each chromosome. The positions of QTLs for five root traits are indicated. *S* = QTLs detected in summer only; *F* = QTLs detected in fall only; *C* = QTLs detected in both seasons

be associated with the five root traits when results of the summer and fall experiment were considered together. The detected QTLs individually accounted for 6–27% of the phenotypic variation. Out of 28, 16 QTLs individually accounted for more than 10% of the phenotypic variation. A list of the putative QTLs flanked by the AFLP/RFLP markers along with their phenotypic variance, additive effects and peak LOD scores, are present-

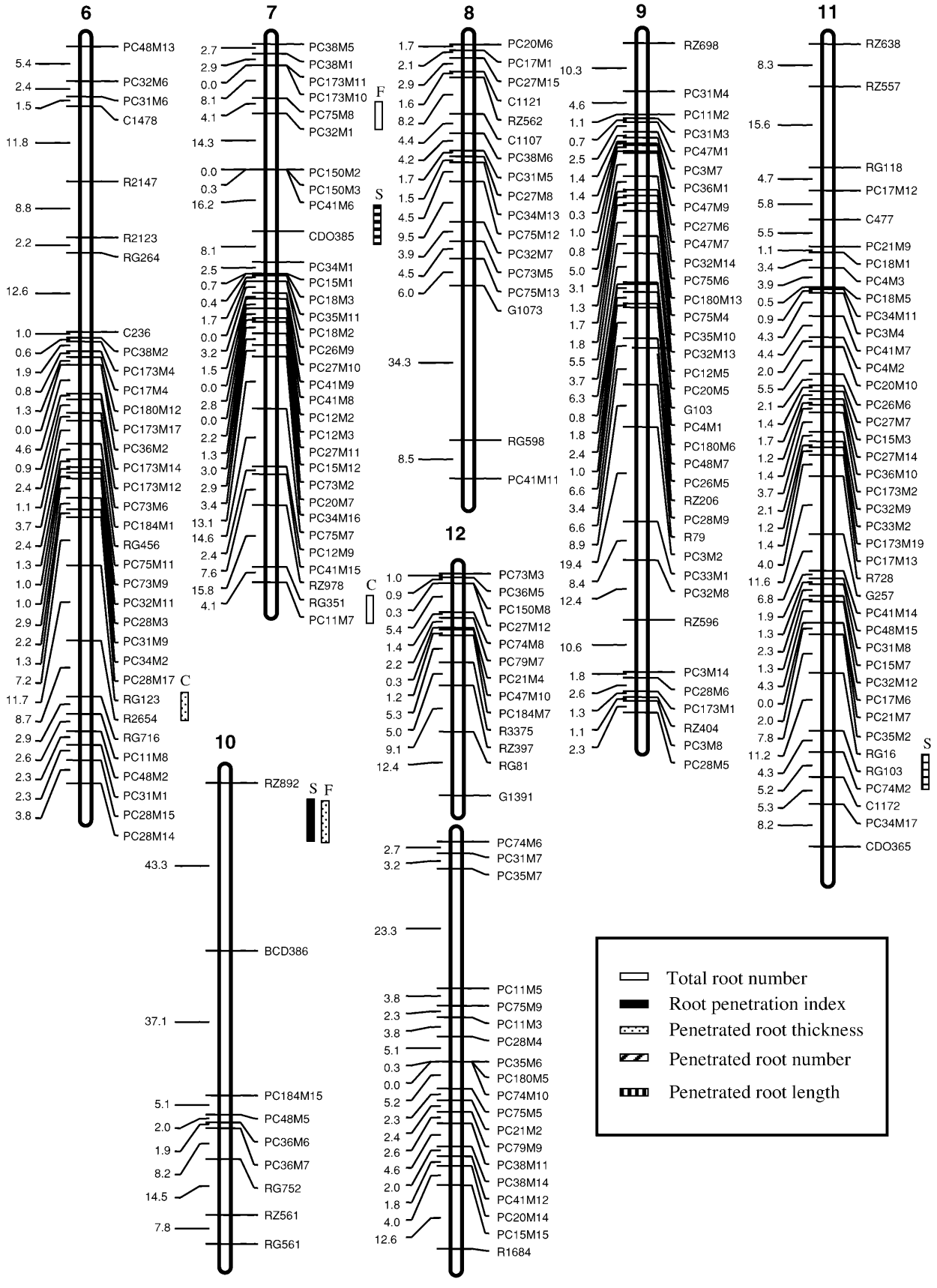

Fig. 1 continued

Table 2 Trait mean values for 166 recombinant inbred lines (RILs) and two parents along with broad-sense heritability (h^2). The heritability was computed as $h^2 = \delta^2_g/(\delta^2_g + \delta^2_e/n)$ where δ^2_g and δ^2_e were the estimates of genetic and residual variances, respectively, derived from the expected mean squares of the analysis of variance and n was the number of replications

Line	Total root number	Penetrated root number	Root penetration index	Penetrated root thickness (mm)	Penetrated root length (cm)
Fall '97					
IR58821 (P_1)	102±1.49[a]	34±1.54	0.34±0.02	0.94±0.05	36.4±2.58
IR52561 (P_2)	93±1.49	10±1.54	0.11±0.02	0.59±0.05	21.2±2.58
P_1 vs P_2[b]	* *	**	**	**	*
RILs (mean)	96±9.50	21±1.96	0.23±0.02	0.78±0.11	33.7±4.13
RILs (range)	45–213	6–57	0.06–0.55	0.4–1.10	19.0–44.0
CV(%)[c]	22.4	20.6	19.1	11.0	27.0
h^2(%)	65.5	91.9	92.0	85.2	24.4
Summer '97					
IR58821 (P_1)	170±10.64	67±4.36	0.39±0.01	1.0±0.06	37.2±2.13
IR52561 (P_2)	185±10.64	26±4.36	0.14±0.01	0.62±0.06	24.0±2.12
P_1 vs P_2	ns[d]	**	**	**	**
RILs (mean)	161±19.16	40±4.71	0.26±0.02	0.82±0.11	32.6±3.36
RILs (range)	71—328	14–122	0.08–0.56	0.40–1.18	20.0–43.0
CV(%)	26.5	26.0	17.9	13.1	23.0
h^2 (%)	59.0	82.1	90.3	77.3	37.7

[a] Mean and standard error of the means
[b] Statistical test for difference between two parents at 5% (*) and 1% (**)levels of probability
[c] Coefficient of variation
[d] Not significant

Table 3 Phenotypic correlation coefficients among different root traits within a season and between summer ('97) and fall ('97) experiments for a given trait

Trait	Season	Total root number	Penetrated root number	Root penetration index	Penetrated root thickness	Penetrated root length
Total root number		0.09[a]				
Penetrated root number	Summer	0.33**	0.56**			
	Fall	0.27**				
Root penetration index	Summer	–0.35**	0.76**	0.68**		
	Fall	–0.21*	0.85**			
Penetrated root thickness	Summer	–0.10	0.52**	0.58**	0.81**	
	Fall	0.07	0.41**	0.40**		
Penetrated root length	Summer	–0.04	0.40**	0.42**	0.27**	0.30**
	Fall	0.15	0.40**	0.31**	0.13*	

* Significant at 5% level of probability
** Significant at 1% level of probability
[a] Underlined values denotes correlation between the two experiments

ed in Table 4. A graphical presentation of QTL locations on the linkage map is shown in Fig. 1.

Eighteen pairs of two-locus interactions for the five root traits were detected ($P < 0.001$). However, the epistatic effects were all smaller than the main effects of detected QTLs. Among the markers which interacted with the QTLs for root traits, only R2654 (on chromosome 6) was linked with a QTL, i.e., R2654 was associated with PRT. These results suggest that two-way interaction in the current mapping population is not important for these five traits.

QTLs for total root number

Two QTLs were detected in the fall experiment. One was flanked by PC75M8-PC32M1 on chromosome 7 and the other one was flanked by R1925-RG1356 on chromosome 3. The QTL on chromosome 7 explained 12.2% of the phenotypic variation with an additive effect of 15.2 roots contributed from the IR52561 allele, whereas the QTL on chromosome 3 explained 9.0% of the phenotypic variation with an additive effect of 12.8 roots contributed from the IR58821 allele. The two QTLs together accounted for 15% of the phenotypic variation and a larger portion of the variation remained unaccountable. Two possible reasons could be postulated for this. Either most of the QTLs affecting TRN had smaller effects which remained undetected at the threshold set for QTL declaration, or the trait was sensitive to the environment. Zheng et al. (2000) also detected only two QTLs explaining only 18% of the phenotypic variation in TRN. In the summer experiment, however, no QTLs were detected for TRN.

QTLs for penetrated root number

For the summer experiment, four QTLs were detected on chromosomes 1, 2 and 3, respectively. For the fall experiment, six QTLs were detected on chromosomes 2 and 3, respectively. Between the two seasons, three common QTLs are found: PC33M8-PC21M1 (on chromosome 2), RG256-PC32M10 (on chromosome 2) and PC73M13-

Table 4 Putative QTLs detected for five root traits by interval mapping via MapMaker/QTL in a recombinant inbred population of 166 lines from IR58821–23-B-1–2-1 and IR52561-UBN-1–1-2 in rice

Trait	Interval	Chrom. #	Length[a]	Position[b]	Variance[c] (%)	Additive effect[d]	LOD[e]
Total root number							
Fall	R1925-RG1356	3	6.3	4.0	9.0	–12.8	2.3
	PC75M8-PC32M1	7	4.3	0.0	12.2	15.2	4.3
					15.4‡		
Penetrated root number							
Sum.	BCD134-RZ776	1	15.8	8.0	9.1	7.9	2.3
	PC33M8-PC21M1*	2	12.6	4.0	16.5	–10.0	5.23
	RG256-PC32M10*	2	5.6	2.0	27.2	–13.6	10.11
	PC73M13-PC3M5*	3	3.9	0.0	16.7	–10.6	6.31
					46.4‡		
Fall	C499-PC11M1	2	41.3	32.0	23.2	–8.6	5.75
	PC33M8-PC21M1*	2	12.6	4.0	16.4	–6.96	4.86
	RG256-PC32M10*	2	5.6	2.0	17.9	–7.3	5.68
	AA7–2b-AA7–2a	2	3.4	0.0	11.4	–5.8	3.84
	C746-RZ448	3	15.3	12.0	7.1	–4.7	2.0
	PC73M13-PC3M5*	3	3.9	0.0	11.1	–5.7	3.86
					40.0‡		
Root penetration index							
Sum.	RG256-PC32M10*	2	5.6	0.0	24.7	–0.08	9.28
	PC33M8-PC21M1*	2	12.6	6.0	12.9	–0.06	3.63
	PC73M13-PC3M5*	3	3.9	0.0	17.7	–0.06	6.81
	RZ892-BCD386	10	54.2	10.0	9.6	–0.05	2.06
					40.8‡		
Fall	C499-PC11M1	2	41.3	32.0	26.2	–0.09	6.37
	PC33M8-PC21M1*	2	12.6	4.0	17.0	–0.07	4.77
	RG256-PC32M10*	2	5.6	2.0	20.5	–0.08	6.56
	AA7–2b-AA7–2a	2	3.4	0.0	7.9	–0.05	2.72
	PC73M3-PC3M5*	3	3.9	0.0	17.9	–0.07	6-54
					46.1‡		
Penetrated root thickness							
Sum.	PC15M11-PC3M3*	1	38.2	38.0	6.8	0.06	2.23
	RG256-PC32M10*	2	5.6	4.0	13.9	–0.09	4.89
	PC3M11-PC33M8	2	4.5	2.0	7.2	–0.06	2.38
	PC33M5-PC38M9*	4	2.8	2.0	7.4	0.06	2.55
	RG123-R2654*	6	13.1	0.0	6.4	–0.06	2.27
	RG351-PC11M7*	7	4.3	0.0	6.3	–0.06	2.08
					28.0‡		
Fall	PC3M3-WG110*	1	34.2	0.0	8.0	0.06	2.49
	C49-PC11M10	1	23.5	6.0	9.2	–0.07	2.21
	RG256-PC32M10*	2	5.6	0.0	12.0	–0.08	3.89
	PC33M5-PC38M9*	4	2.8	2.0	6.4	0.06	2.08
	RG123-R2654*	6	13.1	0.0	7.2	–0.06	2.39
	RZ978-RG351*	7	19.2	18.0	7.1	–0.06	2.22
	RZ892-BCD386	10	54.2	10.0	7.1	–0.06	2.16
					35.2‡		
Penetrated root length							
Sum.	PC17M5-PC15M10	1	11.1	0.0	5.8	2.06	2.13
	PC33M9-PC79M6	2	6.7	4.0	12.8	3.05	4.21
	PC41M6-CDO385	7	18.9	10.0	10.0	–2.69	2.52
	RG103-PC74M2	11	4.4	0.0	11.6	2.93	4.40
					23.8‡		
Fall	RG510-R3226	3	15.6	8.0	10.3	–3.34	2.87

[a] Interval between the two flanking markers (cM) where a QTL is located
[b] QTL position from the first marker (cM)
[c] Phenotypic variation explained by each QTL
[d] Additive genetic effect and a negative sign means that IR52561-UBN-1–1-2 allele reduced the trait value
[e] Maximum likelihood LOD score for the individual QTLs
* Common QTLs for the summer and the fall experiments
‡ Phenotypic variance explained by the QTLs collectively

PC3M5 (on chromosome 3). Total phenotypic variation explained by the detected QTLs in the two seasons was similar (R^2 = 40% for fall and R^2 = 46% for summer). Individual QTLs had R^2 ranging from 9.1 to 27.2% and 7.1 to 23.2% for the summer and fall experiments, respectively. Except for QTL BCD134-RZ776, all alleles were from IR58821.

QTLs for root penetration index

Of the six QTLs associated with RPI, three were found in both summer and fall experiments: PC33M8-PC21M1 and RG256-PC32M10 (on chromosome 2) and PC73M13-PC3M5 (on chromosome 3). Out of the remaining three, one QTL (RZ892-BCD386) on chromosome 10 was de-

tected only in the summer whereas the other two QTLs (C499-PC11M1 and AA7–2b-AA7–2a on chromosome 2) were found only in the fall experiment. In the summer experiment, the phenotypic variation explained by individual QTLs ranged from 9.6 to 24.7%. In the fall experiment, the phenotypic variance explained by individual QTLs ranged from 7.9 to 26.2%. All QTLs had the alleles contributed by IR58821 with additive effects ranging from 0.05 to 0.09. The QTLs affecting RPI together explained 41% and 46% of the phenotypic variation in summer and fall, respectively, suggesting that most of the major QTLs for this trait have been identified. This finding is in good agreement with the high heritability estimates of this trait in both experiments (Table 2).

QTLs for penetrated root thickness

Eight genomic regions were detected to be associated with PRT. Out of the eight, five regions (QTLs), i.e., PC15M11-PC3M3 (PC3M3-WG110) on chromosome 1, RG256-PC32M10 on chromosome 2, PC33M5-PC38M9 on chromosome 4, RG123-R2654 on chromosome 6 and RG351-PC11M7 (RZ978-RG351) on chromosome 7, were common in both summer and fall experiments, indicating their low sensitivity to environmental changes, which is in good agreement with the observed phenotypic correlation (r = 0.81) between the experiments for this trait. In another study by Champoux et al. (1995), QTLs controlling root thickness were also found to be less affected by the environmental differences. Out of the remaining three, one QTL (PC3M11-PC33M8) on chromosome 2 was detected only in the summer experiment whereas the other two (C49-PC11M10 on chromosome 1 and RZ892-BCD386 on chromosome 10) were found only in the fall experiment. In the summer experiment, six QTLs together explained 28% and individually explained 6.3 to 13.9% of the phenotypic variation. In the fall experiment, six QTLs together accounted for 35% and individually accounted for 6.4 to 12.0% of the phenotypic variation. Except for two QTLs (PC15M11-PC3M3 on chromosome 1 and PC33M5-PC38M9 on chromosome 4), all six other QTLs had alleles from IR58821.

QTLs for penetrated root length

Out of the five QTLs associated with PRL, four were detected in the summer experiment while only one was detected in the fall experiment. No common QTLs were found between the two seasons. In the summer experiment, the four QTLs (PC17M5-PC15M10 on chromosome 1, PC33M9-PC79M6 on chromosome 2, PC41M6-CDO385 on chromosome 7 and RG103-PC74M2 on chromosome 11) individually had R^2 ranging from 5.8 to 12.8% and together explained 24% of the total phenotypic variation. In the fall experiment, the QTL flanked by RG510-R3226 was located on chromosome 3 and accounted for 10.3% of the phenotypic variation.

Three of the five QTLs for PRL received positive alleles from IR52561 that generally has poorer root values than IR58821 (Table 2). This fact indicates that although IR52561 is phenotypically poor, it possesses some QTL alleles capable of increasing the trait value. Similarly, Tanksley and Nelson (1996) and Bernacchi et al. (1998) detected QTL alleles enhancing the trait value from a phenotypically inferior parent in tomato.

Genomic regions associated with more than one root trait

Six QTLs were associated with more than one root trait. Two QTLs, PC33M8-PC21M1 and RG256-PC32M10 located on chromosome 2, were associated with three root traits (PRN, RPI and PRT) (Fig. 1). The QTL RG256-PC32M10 was detected in both summer and fall for all the three traits and explained the largest proportion of phenotypic variation. Three QTLs (C499-PC11M1 and AA7–2b-AA7–2a on chromosome 2, and PC73M13-PC3M5 on chromosome 3) were associated with PRN and RPI. The genomic region RZ892-BCD386 on chromosome 10 was associated with RPI and PRT.

Discussion

Phenotypic variation

Significant variation and a normal distribution for the root traits studied made this population suitable for QTL analysis. Except TRN for the summer experiment, the mean values of the RILs were close to the mid-parental values for all traits in both summer and fall experiments (Table 2). Although RILs were distributed normally, transgressive variation was noticed for all traits. Except for PRT, transgressive segregation was observed in both directions for all traits, indicating that neither of the parents carried all the positive or all the negative alleles. Transgressive segregation is commonly observed in segregating populations for quantitative traits (Xiao et al. 1996; Yadav et al. 1997).

Comparison of QTLs across genetic backgrounds

To determine if common QTLs across genetic backgrounds exist, the results of this study were compared to other similar studies on root morphology and root penetration. The map developed by Causse et al. (1994) was used to serve as a bridge among the populations compared. Since inconsistent map distances between markers in different maps hampered precision in the comparison, the comparative results should be considered as indicative.

The QTL in the interval RG256-PC32M10 on chromosome 2 for RPI was found in a similar chromosomal location on the map of IR64/Azucena population (Zheng et al. 2000) (Fig. 2). Similarly, the QTL associated with RPI linked with RFLP markers AA7–2b and AA7–2a on chromosome 2 was located in a similar genomic region of the CO39/Moroberekan population (Ray et al. 1996). For

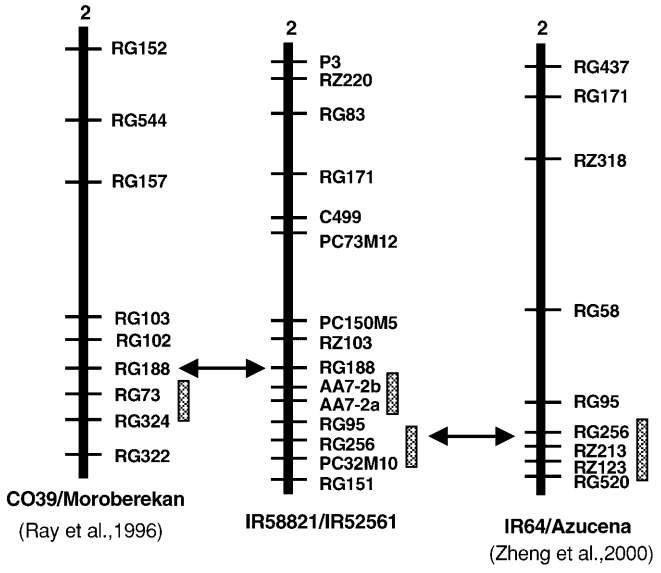

Fig. 2 Comparison of common QTLs for root penetration ability across genetic backgrounds. Chromosome numbers are indicated above each chromosome. The *vertical bars* besides the markers are the genomic regions associated with root penetration ability

root thickness, two QTLs were found to share the similar genomic regions with other populations (Fig. 3). The QTL linked with RFLP marker RZ892 on chromosome 10 was also detected in the CO39/Moroberekan population (Champoux et al. 1995) and the other one linked with PC3M3 on chromosome 1 was found to match a similar QTL in the CT9993/IR62266 population (Zhang et al., in preparation). These results suggest that similarity exists between *japonica*/*indica* crosses and *indica*/*indica* crosses in the genetic control of RPI and PRT. Further research is needed to investigate the nature of these QTLs.

Trait correlation and pleiotropic effects of QTLs

Classical quantitative genetics assumes that trait correlation is a causal effect of pleiotropy or an effect of closely linked genes. Therefore, it is expected that the QTLs for the correlated traits would be mapped in similar genomic regions. A highly positive correlation was observed between PRN and RPI (Table 3), and four QTLs with large effects were found to be associated with these two traits. These two traits were not fully independent as RPI was derived as the ratio of PRN to TRN. Penetrated root thickness showed a significant positive correlation with PRN and RPI (Table 3). In agreement with this, three common QTLs were found to influence these three traits, suggesting that genotypes with higher root penetration ability had relatively thicker roots. This finding is in line with the opinion expressed by Materechera et al. (1992) that genotypes and species with thicker roots tend to have a greater ability to penetrate compacted soil. Yadav et al. (1997) also identified some genomic regions that were involved in the control of deep root pattern and root thickness.

Conclusion

QTLs affecting five root traits were detected based on the variation in a RIL population derived from two *indica* ecotype parents adapted to grow in rainfed lowland conditions. Most of the QTLs associated with PRN, RPI and PRT were detected commonly in both summer and fall experiments (Table 4). QTLs expressing consistently over environments and across genetic backgrounds are most valuable as they could be transferred into desirable genetic backgrounds with the aid of markers and selected in early generations with confidence. These identified RFLP and AFLP markers could also be converted to a simple, rapid and inexpensive PCR-based marker type, like STS, to enhance and economize the breeding programs.

Acknowledgements The authors gratefully acknowledge the financial support by the Rockefeller Foundation and express their appreciation to Dr. J.C. O'Toole for his advice and encouragement for this doctoral research program. The authors also wish to thank Dr. Honggang Zheng and Mr. Bay Nguyen for their help in marker and data analyses. RFLP clones were kindly provided by Drs. S.D. Tanksley, S.R. McCouch and T. Sasaki. We hereby declare that the experiments comply with the current laws of the country in which the experiments were performed. Contribution no. T-4-467 of the College of Agricultural Sciences and Natural Resources, Texas Tech University.

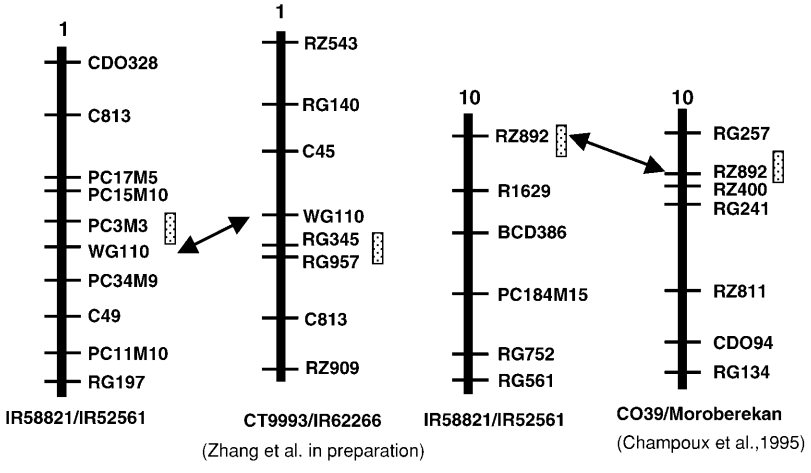

Fig. 3 Comparison of common QTLs for root thickness across genetic backgrounds. Chromosome numbers are indicated above each chromosome. The *vertical bars* besides the markers are the genomic regions associated with penetrated root thickness

References

Bai G, Tefera H, Ayele M, Nguyen HT (1999) A genetic linkage map of tef [*Eragrostis tef* (*Zucc*.) Trotter] based on amplified fragment length polymorphism. Theor Appl Genet 99: 599–604

Bernacchi D, Beck-Bunn T, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley S (1998) Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. Theor Appl Genet 97: 381–397

Busscher WJ, Sojka RE, Doty CW (1986) Residual effects of tillage on coastal plain soil strength. Soil Sci 141: 144–148

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Xu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138: 1251–1274

Champoux MC, Wang G, Sarkarung S, MacKill DJ, O'Toole JC, Huang N, McCouch SR (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor Appl Genet 90: 969–981

Cho YG, McCouch SR, Kuiper M, Kang MR, Pot J, Groenen JTM, Eun MY (1998) Integrated map of AFLP, SSLP and RFLP markers using a recombinant inbred population of rice (*Oryza sativa* L.). Theor Appl Genet 97: 370–380

Ekanayake IJ, Garrity DP, O'Toole JC (1986) Influence of deep root density on root pulling resistance in rice. Crop Sci 26: 1181–1186

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboi Y, Yamamoto T, Lin SY, Antinio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura N, Kurata N, Khush GS, Sasaki T (1998) A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148: 479–494

Huang N, McCouch SR, Mew T, Parco A, Guidersoni E (1994) Development of an RFLP map from a doubled-haploid population in rice. Rice Genet Newslett 11: 134–137

Ingram K, Bueno TFD, Namuco OS, Yambao EB, Beyrouty CA (1994) Rice root traits for drought resistance and their genetic variation. In: Kirk GJD (ed) Rice roots: nutrient and water use. IRRI, Los Banos, The Philippines, pp 67–77

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12: 172–175

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) Mapmaker: an interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1: 174–181

Li Z, Pinson SRM, Stansel JW, Park WD (1995) Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (*Oryza sativa* L.). Theor Appl Genet 91: 374–381

Lincoln S, Daly M, Lander E (1992) Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report, 2nd edition

Lu C, Shen L,Tan Z, Xu Y, He P, Chen Y, Zhu L (1997) Comparative mapping of QTLs for agronomic traits of rice across environments using a doubled-haploid population. Theor Appl Genet 94: 145–150

Maheswaran M, Subudhi PK, Nandi S, Xu JC, Parco A, Yang DC, Huang N (1997) Polymorphism, distribution, and segregation of AFLP markers in a doubled-haploid rice population. Theor Appl Genet 94:39–45

Mambani B, Lal R (1983) Response of upland rice varieties to drought stress. II. Screening rice varieties by means of variable moisture regimes along a toposequence. Plant Soil 73: 73–94

Marques CM, Araujo JA, Ferreira JG, Whetten R, O'Malley DM, Liu BH, Sederoff R (1998) AFLP genetic maps of *Eucalyptus globulus* and *E. tereticornis*. Theor Appl Genet 96: 727–737

Materechera SA, Alston AM, Kirky JM, Dexter AR (1992) Influence of root diameter on the penetration of seminal roots into a compacted subsoil. Plant Soil 144: 297–303

Nelson JC (1997) QGene: software for marker-based genomic analysis and breeding. Mol Breed 3: 239–245

Nguyen HT, Ray JD, Yu LX (1994) Use of molecular markers to exploit rice root traits for drought tolerance. In: Kirk GJD (ed) Rice roots: nutrient and water use. International Rice Research Institute, Los Banos, The Philippines, pp 79–86

Nguyen HT, Babu RC, Blum A (1997) Breeding for drought resistance in rice: physiology and molecular genetics considerations. Crop Sci 37:1426–1434

O'Toole JC, De Datta SK (1986) Drought resistance in rainfed lowland rice. In: Progress in rainfed rice. IRRI, Los Banos. The Philippines, pp 145–158

O'Toole JC, Chang TT (1979) Drought resistance in cereals – rice: a case study. In: Mussell H, Staples RC (eds) Stress physiology of crop plants. Wiley Interscience, New York, pp 374–405

Paterson AH, Lander ES, Hewitt JD, Paterson S, Lincoln S, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335: 721–726

Price AH, Thomas AD (1997) Genetic dissection of root growth in rice (*Oryza sativa* L.). II: Mapping quantitative trait loci using molecular markers. Theor Appl Genet 95:143–152

Ray JD, Yu LX, McCouch SR, Champoux MC, Wang G, Nguyen HT (1996) Mapping quantitative trait loci associated with root penetration ability in rice. Theor Appl Genet 92: 627–636

Redona ED, Mackill DJ (1996) Mapping quantitative trait loci for seedling vigor in rice using RFLPs. Theor Appl Genet 92: 395–402

SAS Institute Inc (1990) SAS/ STAT user's guide, Version 6, 4th edition, vols 1 and 2. SAS Institute Inc., Cary, North Carolina, USA

Singh K, Ishii T, Parco A, Huang N, Brar DS, Khush GS (1996) Centromere mapping and orientation of the molecular linkage map of rice (*Oryza sativa* L.). Proc Natl Acad Sci USA 93: 6163–6168

Tai TH, Tanksley SD (1990) A rapid and inexpensive method of isolation of total DNA from dehydrated plant tissue. Plant Mol Biol Rep 8: 297–303

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27: 205–233

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92: 191–203

Vos P, Hogers R, Bleeker M, Reijans M, Lee TVD, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23: 4407–4414

Xiao J, Li J,Yuan L, Tanksley SD (1996) Identification of QTLs affecting traits of agronomic importance in recombinant inbred population derived from a subspecific cross. Theor Appl Genet 92: 230–244

Xiong L, Liu KD, Dai XK, Wang SW, Xu CG, Zhang DP, Maroof MAS, Sasaki T, Zhang Q (1997). A high-density RFLP map based on the F_2 population of a cross between *Oryza sativa* and *O. rufipogon* using Cornell and RGP markers. Rice Genetic Newslett 14: 110–115

Xu Y, Zhu L, Xiao J, Huang N, McCouch SR (1997) Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, doubled-haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol Gen Genet 253: 535–545

Yadav R, Courtois B, Huang N, Mclaren G (1997) Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. Theor Appl Genet 94: 619–632

Yano M, Sasaki T (1997) Genetic and molecular dissection of quantitative traits in rice. Plant Mol Biol 35: 145–153

Yu LX, Ray JD, O'Toole JC, Nguyen HT (1995) Use of wax-petrolatum layers for screening rice root penetration. Crop Sci 35: 684–687

Zhang J, Nguyen HT, Blum A (1999) Genetic analysis of osmotic adjustment in crop plants. J Exp Bot 50: 291–302

Zheng HG, Babu RC, Pathan MS, Ali ML, Huang N, Courtois B, Nguyen HT (2000) Quantitative trait loci for root penetration ability and root thickness in rice: comparison of genetic backgrounds. Genome 43: 53–61