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J. D. Ray · L. Yu · S. R. McCouch · M. C. Champoux
G. Wang · H. T. Nguyen

Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.)

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Abstract Root penetration ability is an important factor for rice drought resistance in areas with soils subject to both compaction and periodic water deficits. However, breeding for root penetration ability is inhibited by the difficulties associated with measuring root traits. Our objective was to identify restriction fragment length polymorphisms (RFLPs) associated with root penetration ability. Using wax-petrolatum layers as a proxy for compacted soil, we counted the number of vertical root axes penetrating through the layer, the total number of vertical root axes and the number of tillers per plant of 202 recombinant inbred (RI) lines over three replications. As a measure of root penetration ability, we used a root penetration index defined as the percent of the total number of vertical root axes that penetrated through a wax-petrolatum layer. The RI population exhibited a wide range in the number of penetrating roots axes (10–115 roots), the total number of roots axes (74–226 roots), tillers per plant (6–18), and in the root penetration index (0.11–0.71). Single-marker and interval quantitative trait analyses were conducted to identify RFLP loci associated with the number of penetrating roots, total root number, root penetration index, and tiller number. Four quantitative trait loci (QTLs) were associated with the number of penetrated roots, 19 with the total root number, six QTLs with the root penetration index and ten with tiller number. Individually, these QTLs accounted for a maximum of 8% of the variation in the number of penetrating roots, 19% of the variation in total root number, 13% of the variation in root penetration index and 14% of the variation in tiller number as estimated from regressions. The multi-marker regression model accounting for the greatest proportion of the variation in the root penetration index was a three-marker model that accounted for 34% of the variation. Two-marker models accounted for 13% of the variation in the number of penetrated roots, 25% of the variation in total root number, and 21% of the variation in tiller number. This is the first research paper to apply RFLP quantitative trait analysis to dissect genetic loci associated with the total number of roots, root penetration ability and tiller number.

Key words Rice · QTL analysis · Root number · Root penetration · Recombinant Inbred · Tiller number

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J. D. Ray · L. Yu · H. T. Nguyen (✉)
Department of Plant and Soil Science and Institute for Biotechnology, Mail Stop 2122, Texas Tech University, Lubbock, TX 79409, USA

S. R. McCouch · M. C. Champoux
Plant Breeding Department, Cornell University, Ithaca, NY 14853, USA

G. Wang
Department of Plant Pathology, University of California, Davis, CA 95616, USA

Introduction

About one quarter of the world's rice crop is produced in areas classified as rainfed lowland (Khush 1984; Garrity et al. 1986). The rainfed lowland classification encompasses a great diversity of growing conditions subject to varying amounts and duration of rainfall, varying frequency and duration of flooding, as well as edaphic and topographic differences (Khush 1984). Garrity et al. (1986) stated that in South and Southeast Asia, future increases in production area would come largely from the rainfed lowland ecotype. They estimated that more than 50% of lowland rice production could be further classified as drought-prone or highly drought-prone. These areas may experience frequent and severe water deficits at any time during rice growth and may be subject to uncertain rainfall distribution patterns

(Garrity et al. 1986). Although the effect of water deficits on rice growth and yield is complex, lack of water is recognized as perhaps the most widespread constraint to increased rice yields (IRRI 1982).

O'Toole and De Datta (1986) and Thangaraj et al. (1990) stated that the ability to maintain water uptake during drought appeared to be a major attribute that confers increased drought resistance on traditional rice varieties and speculated that increased rooting depth and density would increase the capacity to extract available water. Yoshida and Hasegawa (1982) and Chang et al. (1982) concluded that a deep root system was beneficial during drought, in avoiding water stress by absorbing water from deep soil horizons. While a deep root system may be beneficial in avoiding drought, water in deeper horizons is often inaccessible due to the prevalence of hard pans (compacted soil layers) below the topsoil in rainfed lowland ecosystems (Chang et al. 1982).

Compacted soil layers present physical and physiological constraints to overall plant growth (Bengough and Mullins 1990; Tu and Tan 1991) and have been shown to reduce leaf area, dry matter accumulation, root elongation rate, transpiration rates, and crop yield (Masle and Passioura 1987; Ludlow et al. 1989; Asseed et al. 1990; Masle 1992). Additionally, by limiting the depth of the root system, compacted soil layers may subject plants to water deficits they might otherwise have avoided. In rice, root penetration and proliferation is sharply (> 50%) reduced at soil strengths of 0.5 to 2 MPa (O'Toole 1982). Genotypic variation in the ability of rice to penetrate compacted soil layers and simulated compacted layers has been shown to exist (Kandasamy 1981; O'Toole 1982; Yu et al. 1995). However, incorporation of root selection criteria, such as root penetration ability, into plant breeding programs is difficult due to the lack of reliable and efficient screening techniques and the time consuming, laborious nature of measuring root characteristics (Mambani and Lal 1983; Ekanayake et al. 1986; Gregory 1989; O'Toole 1989). Hanson et al. (1990) suggested that identifying and mapping molecular markers associated with the ability to penetrate compacted soil layers could be used in breeding programs to develop rice varieties better adapted to water deficit environments. Our research was undertaken to identify restriction fragment length polymorphic (RFLP) molecular markers associated with increased root penetration ability for use in developing rice cultivars with greater productivity in the presence of compacted soil layers.

Materials and methods

A root penetration screening system was designed which used wax-petrolatum barriers as a substitute for compacted soil layers (Yu et al. 1995). This system was utilized to screen the root penetration ability of 202 recombinant inbred (RI) lines. The screening method provided a means of fabricating large numbers of partial barriers to vertical root growth. The wax-petrolatum layers have the advantage of challenging vertical root growth with a barrier of consistent hardness which is not susceptible to changes in hardness due to moisture content as are soil systems.

RI population and RFLP data set

The RI population was developed at IRRI from a cross between CO39 (*indica*, wet lowland adapted) and Moroberekan (*japonica*, dry upland adapted) for the purpose of mapping blast resistance in rice and to establish a permanent mapping population (Wang et al. 1994). The RI population consists of 281 RI lines of which a randomly selected subset of 202 lines were evaluated in the present study. The RFLP data set for this RI population was developed by Wang et al. (1994) and expanded by Champoux et al. (1995) and consisted of 125 RFLP marker allele patterns of 281 RI lines that have been mapped to the 12 linkage groups. Residual heterozygote allele patterns were coded as missing data. Altogether, the marker allele patterns had approximately 6% missing data.

Wax-petrolatum layers

The wax-petrolatum layers used in this study consisted of 60% wax (Reckitt and Colman, Wayne, N.J.) and 40% petrolatum (Spectrum Chemical, Gardena, Calif.) by weight. This wax formulation had a strength (mechanical impedance) of 1.4 MPa at 27 °C as measured with a soil penetrometer (Bush Soil Penetrometer SP 1000, Findlay Irvine Ltd., Midlothian, Scotland) using a 12.83-mm diameter, 30° cone. The wax and petrolatum were combined in beakers in the appropriate weights to give a total weight of 50 g. The mixture was then melted at 80 °C, mixed, poured into molds and allowed to solidify at room temperature. The resulting wax-petrolatum disks were 13.5 cm in diameter and 3 mm thick.

Screening system

The wax-petrolatum disks were placed on a plastic 1-cm grid suspended 2 cm from the bottom of 12-cm diameter by 8-cm tall plastic pots from which the bottom had been removed. Pots were filled to within 1 cm of the top with potting medium (a mixture of 0.5 m^3 of peat moss, 0.08 m^3 of perlite, 0.04 m^3 of vermiculite, 36 kg of calcine clay, and 1 kg of osmocote). Wooden tanks, lined with plastic sheeting, were constructed to hold the pots which were suspended in the tank by plywood sheets with holes cut to slightly less than the diameter of the top of the pots. The bottom of each suspended pot was submerged in water to a level just above the wax-petrolatum disk. The wooden tanks were placed on tables in the greenhouse where the experiment was conducted.

Seeds from the 202 RI lines were soaked in water for 48 h at 20 °C in the dark. The seeds were then germinated in small pots and 7–8 days later transplanted to the pots (one plant per pot) containing the wax-petrolatum disks described above (on either 21 or 22 May 1992). The pots were arranged in the tanks in a randomized complete block design with three replications. Plants were grown until 25 days after transplanting and then harvested by replication. The number of vertical root axes penetrating through the wax-petrolatum disk and the total number of vertical root axes were counted by hand. Hereafter, vertical root axes are referred to as roots. Root penetration ability was estimated by a root penetration index which was defined as the fraction of the total number of roots that penetrated through the wax-petrolatum layer. Tiller number per plant was also determined by hand counting at harvest. Means and descriptive statistics were generated using SAS (SAS Institute, Cary, N.C.). Tests of the fidelity (skewness and kurtosis) of parameter distributions to normality were conducted using the Frequency routine of CoStat (CoHort Software, Berkeley, Calif.).

QTL analysis

Phenotypic data were analyzed for the detection of RFLP markers linked to loci controlling their expression. Analysis for markers

associated with each trait was conducted using the general linear model (GLM) procedure of SAS (SAS Institute, Cary N.C.) for both single and multiple evaluations (multiple regression). Single-marker regressions served as the primary method of detecting associations between markers and the traits (referred to hereafter as single-marker analysis). Groups of two or more closely linked markers that showed significant associations were assumed to identify the same QTL. T-tests were used to compare the means of homozygous marker classes for each trait.

Additional information regarding the location of each putative QTL detected by single-marker analysis was obtained using interval analysis. Interval analysis was conducted using the program MAPMAKER/QTL (Paterson et al. 1988; Lincoln et al. 1992) using interval lengths calculated by Champoux et al. (1995) with the program RIPM/SKEW (Manly 1993).

MAPMAKER/QTL does not have an algorithm specifically designed for analyzing RI lines (the F_2 intercross design was employed for this analysis) and handles missing data differently from the method used in single-marker analysis. In single-marker analysis missing data are eliminated, whereas in interval analysis an inference is made based on data from flanking markers and the missing data point is replaced. For these two reasons, interval analysis may introduce a degree of error not present in single-marker analysis. Therefore, interval analysis was used to confirm the results of single-marker analysis and to provide a more precise location of putative QTLs identified by single-marker analysis. In some cases interval analysis identified putative QTLs in an interval for which the flanking markers did not meet the 0.05 threshold. Because of the limitations imposed on MAPMAKER/QTL by the data (an RI population with missing marker data), putative QTLs identified by MAPMAKER/QTL and not supported by single-marker analysis are not reported.

QTL thresholds

Experiment-wise threshold values for single-marker analysis were generated based on the randomized t-test procedure described by Churchill and Doerge (1994). The procedure involves cycles of breaking the relationship between the phenotypic data and the marker data by randomization and determining the maximum t-statistic from each cycle. Churchill and Doerge (1994) indicate that by randomizing the data and selecting the maximum t-statistic 1000 times, sorting the accumulated maximum t-statistics from lowest to highest and taking the 950th value will give a threshold at the 0.05 probability level. Similarly, sorting the maximum t-statistics from 10000 randomizations and selecting the 9900th value gives a threshold at the 0.01 probability level. This results in a threshold value that is specific for the data set and each trait (Churchill and Doerge 1994). A program was written in Microsoft Visual BASIC (Microsoft Corporation, USA) to generate threshold t-statistics at the 0.05 and 0.01 probability levels for each trait. LOD threshold values for the interval analysis were calculated from the threshold t-statistics determined for the single-marker analysis as outlined by R. W. Doerge in the appendix of Champoux et al. (1995).

Results

Population and marker database description

Although Wang et al. (1994) and Champoux et al. (1995) described both the population and the map, several aspects of the population and corresponding RFLP data set are pertinent to the results presented here. Of the 281 RI lines used to develop the map, 202 lines were evaluated in this study. The allelic distribution of the 125 markers in the data set was strongly skewed toward the CO39 parent, with the markers identifying an average of 76% CO39 alleles and 21% Moroberekan alleles (not including 3% heterozygotes and non-parental alleles) in the subset of 202 RI lines evaluated. This skewness towards CO39 alleles was also evident in the 281 lines used by Wang et al. (1994) and Champoux et al. (1995) during map development and indicates that the allele frequency of the subset of RI lines evaluated in this study reflects the larger mapping population.

The marker skewness observed in the population was probably a result of both genetic and environmental factors. Genetic sterility is commonly observed in *japonica-indica* crosses (Guiderdoni et al. 1989) which can result in skewed segregation of F_2 progenies resulting from complex actions of F_1 gametophyte or sporophyte genes (Ikehashi and Araki 1986; Nakagahra 1986; Oka 1988). In addition, during population development, advancing generations were grown in small pots under flooded conditions that resulted in anaerobic soils to which Moroberekan is not well adapted (Senadhira et al. 1993). Therefore, the selection pressure during population development favored CO39 and may have contributed to a loss of Moroberekan alleles (see Wang et al. 1994 for further details on population development).

Phenotypic screening

Root measurements

Root penetration ability was estimated as the fraction of the total number of roots that penetrated through a wax layer (root penetration index). This index indicates the relative ability of a plant's roots to penetrate a wax layer and serves to reduce the effect of differences in total root number when comparing individuals. The mean and range of values for the total number of roots, number of penetrating roots, and root penetration index observed in the RI population and in the two parents are summarized in Table 1. For all three variables, the 202 RI lines showed a normal distribution (based on tests of skewness = 0 and kurtosis = 0 at the 0.05 probability level).

Of the variables used to derive the penetration index, both the total number of roots and the number of roots penetrating the wax layer demonstrated transgressive variation toward smaller, weaker root systems (Table 1). Approximately 20% of the 202 RI lines had a total root number as high as or greater than the mean of Moroberekan and none had a total root number as high as CO39. Only one RI line had as many penetrating roots as CO39 and none had as many as Moroberekan. In the RI population, the number of penetrating roots and the total number of roots had a positive linear correlation ($r = 0.33$, $P = 0.0001$). However, the low correlation coefficient indicates a weak relationship between the total number of roots and the number of roots penetrating through the wax layer.

Table 1 Descriptive statistics of four variables, measured on 202 recombinant inbred (RI) lines and the two parental lines, in three replications

Parameter	Minimum	Maximum	Mean	SD[a]	CV[b]	Lsd[c]
Number of penetrating roots						
RI Lines	10	115	56	20	32	29
CO39	78	127	105	25	24	
Moroberekan	67	162	124	50	41	
Total number of roots						
RI Lines	74	226	162	33	18	48
CO39	216	354	305	77	25	
Moroberekan	132	232	192	53	28	
Penetration index[d]						
RI Lines	0.11	0.71	0.34	0.12	26.4	0.15
CO39	0.32	0.36	0.35	0.02	7.2	
Moroberekan	0.51	0.70	0.65	0.10	16.5	
Number of tillers						
RI Lines	6	18	12	2.5	17.4	3.25
CO39	14	36	27	11.4	42.6	
Moroberekan	6	13	10	3.6	36.1	

[a] Standard deviation
[b] Coefficient of variation
[c] Least significant difference, $P = 0.05$
[d] Number of penetrating roots divided by the total number of roots

In the RI population, the mean fraction of roots that penetrated the wax layer was 34%, and individual values ranged from 11 to 71%. The mean of the population was similar to the mean penetration index for the weaker-rooted parent, CO39 (35%), and well below the mean of Moroberekan, which was almost twice as high, at 65%. Only one RI line had a penetration index as high as or higher than the mean of the Moroberekan parent, while the individuals with the lowest penetration index were well below the mean of the CO39 parent.

For all three root measurements, comparison of the RI population with the parental lines indicated that there was transgressive variation in the RI population toward weaker root systems that were less able to penetrate the wax layer. These results are in agreement with those presented by Champoux et al. (1995) in which transgressive variation toward smaller, shallower root systems was reported for this same population. Nonetheless, the RI population exhibited a large range of variation for all root parameters examined.

Number of tillers per plant

In the RI population, the number of tillers per plant ranged from 6 to 18 with a mean of 12 (Table 1) and fit a normal distribution. Mean tiller number in the RI population was only slightly higher than the mean for the Moroberekan parent and well below the mean of the CO39 parent (Table 1). There was a positive correlation between tiller number and the total number of roots per plant ($r = 0.52$, $P < 0.0001$) and tiller number was weakly correlated with the number of penetrated roots ($r = 0.31$, $P < 0.0001$). Tiller number was not correlated with the root penetration index ($r = 0.03$, $P < 0.67$).

Identification of markers linked to QTLs

Number of penetrating roots

Single-marker analysis (simple linear regression) identified one marker significantly associated with the number of roots that penetrated the wax layer at the 0.01 threshold level and three more were identified at the 0.05 threshold level as summarized in Table 2. Using interval analysis (MAPMAKER/QTL), three of the four markers were also shown to be associated with putative QTLs for this trait (Table 2). For all markers associated with this trait, the greater number of penetrated roots was associated with the CO39 allele. The location of the markers associated with the number of penetrating roots is shown in Fig. 1.

Individually, these markers accounted for between 6 and 8% of the phenotypic variation observed for number of penetrating roots. Significant two-way interactions were observed between RG869B (chromosome 12) and RG612 (chromosome 1) as well as between RG869B and RZ393 (chromosome 3), suggesting epistatic interactions among these loci. Multiple regressions suggested that the best two-variable model included RG869B and RG172. This model explained 13% of the phenotypic variation with additive gene effects.

Total number of roots

Thirty-four markers, identifying 19 putative QTLs, were significantly associated with total root number based on both single-marker and interval analysis at the 0.01 threshold (Table 3). In all cases, the CO39 allele was identified with a greater number of roots. These markers were distributed over 11 chromosomes as illustrated in Fig. 1. Differences in total root number between RI lines

Table 2 RFLP markers identified as being associated with the number of penetrated roots as indicated by single-marker analysis at the 0.05 threshold ($F \geq 12.0$) and the most likely QTL position in the surrounding interval as indicated by interval analysis. For all markers the CO39 allele was associated with an increase in the number of penetrating roots

QTL[a]	Marker	Chr[b]	Pos[c]	Distance[d]	LOD[e]	CO39[f]	MORO[g]	Pe[h]	F[i]	R^{2j}
1	RG612	1	14	−6	2.5	134	58	10.7	12.6	0.06
2	RZ393	3	12	−10	3.2	124	76	10.0	12.7	0.06
3	RG172	6	7	+3	4.2	142	55	12.4	17.1	0.08
4	RG869B	12	5	−3	3.3	151	51	11.6	14.0	0.07

[a] Quantitative trait loci, closely linked markers are assumed to identify the same QTL
[b] Chromosome on which the marker is located (Fig. 1)
[c] Marker location in the sequence of markers on the chromosome (Fig. 1)
[d] The location in centimorgans (+ or − relative to the corresponding marker) of the most likely position of the QTL constrained by the markers identified by single-marker analysis as determined by MAPMAKER/QTL
[e] The corresponding LOD score for the QTL as determined by MAPMAKER/QTL. 0.05 LOD threshold = 2.6 and 0.01 LOD threshold = 3.4
[f] Number of RI lines homozygous for the CO39 allele
[g] Number of RI lines homozygous for the Moroberekan allele
[h] Phenotypic effect, the difference in the mean number of penetrating roots of all RI lines homozygous for the CO39 allele and those homozygous for the Moroberekan allele. All differences are significant at the 0.0001 level of probability as determined by the PROC TTEST procedure of SAS.
[i] The F-statistic as determined by the PROC GLM procedure of SAS. Threshold values are 0.05 = 12.0 and 0.01 = 15.9
[j] Coefficient of determination, the percent of phenotypic variation explained by individual markers as determined from the PROC GLM procedure of SAS

homozygous for each class of parental alleles ranged from 21 (RZ404) to 41 (RG211) with an average of 28 more roots in those RI lines homozygous for the CO39 allele. Individually, these markers accounted for up to 19% (RG214) of the variation in total root number (Table 3).

Multiple regression was used to identify a two-marker model which explained 25% of the variation in total root number ($P < 0.0001$). This model included RG214 and WAXY, and used 172 RI lines, of which 21 were homozygous at both loci for the Moroberekan allele. There was a significant ($P < 0.0001$) difference between the class means of 37 roots. There was no significant two-way interaction between these loci and therefore the gene action was inferred to be additive.

Root penetration index

Single-marker analysis identified five markers that were significantly associated with the root penetration index at the 0.01 threshold level and four additional markers at the 0.05 threshold as summarized in Table 4. Together, these nine markers defined six chromosomal regions (Fig. 1). Interval analysis supported the locations of these putative QTLs (Table 4). In all cases except one, the allele from Moroberekan was associated with a significant increase in the percentage of the total number of roots that penetrated the wax layer. The one exception was RG360, for which the CO39 allele contributed positively to the root penetration index. RG360 alone explained 12% of the phenotypic variance (Table 4).

Multiple regressions were performed using the nine markers associated with putative QTLs. The best multivariate model included markers RG324, RG476C, and RG360 and accounted for 34% of the variation in the root penetration index. The analysis included 123 RI lines for which there was no missing data and there were 12 individuals in the group containing all three positive alleles. These 12 lines had an average 0.19 increase in root penetration index compared to the alternative group, indicating that 19% more of the total number of roots present on these plants penetrated the wax layer compared to the rest of the population. There were no significant interactions among the loci examined ($P < 0.05$), indicating additive rather than epistatic effects for these putative QTLs.

Number of tillers per plant

Single-marker analysis and interval analysis identified 19 markers significantly associated with the number of tillers per plant at the 0.01 threshold (Table 5) comprising ten putative QTLs (Fig. 1). For all 19 markers, the CO39 allele was associated with increased numbers of tillers per plant. Over all, the variation in number of tillers per plant accounted for by these markers ranged from 8 to 14% (Table 5).

Multiple regression identified the best two-marker model (RG211 and RZ740) which accounted for 21% of the variation in tiller number. In this model, eight of 194 RI lines were homozygous for the Moroberekan allele and the difference between class means was four tillers ($P < 0.0001$). There were no significant ($P > 0.01$) two-way interactions between these loci and therefore the gene action was inferred to be additive.

Table 3 RFLP markers identified as being associated with total root number as indicated by single-marker analysis at the 0.01 threshold ($F \geq 16.1$) and the most likely QTL position in the surrounding interval as indicated by interval analysis. For all markers the CO39 allele was associated with an increase in number of roots

QTL[a]	Marker	Chr[b]	Pos[c]	Distance[d]	LOD[e]	CO39[f]	MORO[g]	Pe[h]	F[i]	R^{2j}
1	RG350	1	1			157	42	27.9	26.5	0.12
	RG77	1	2	+3	5.9	149	52	23.4	21.4	0.10
2	RG811	1	12	+6	10.1	164	34	24.1	16.2	0.08
	RG140	1	13			162	36	29.3	26.2	0.12
3	RG139	2	6	−4	10.6	171	28	35.7	32.6	0.14
	RZ103	2	7			157	44	24.9	21.5	0.10
4	RG745	3	6	−5	4.7	166	35	26.1	19.8	0.09
5	RZ394	3	8	+5	4.6	157	43	22.1	16.2	0.08
6	RG214	4	2	+1	10.2	130	45	32.0	40.8	0.19
	RG476C	4	3			159	43	32.1	38.1	0.16
	RG329	4	4			157	43	27.5	26.3	0.12
7	RG163	4	6	+7	5.5	139	47	22.0	17.5	0.09
8	RG13	5	4	−2	5.5	170	30	31.9	26.9	0.12
9	WAXY	6	1			154	42	30.9	34.4	0.15
	CDO475	6	2			162	32	25.7	17.9	0.09
	RZ516	6	2			158	35	28.0	23.7	0.11
	RZ144	6	4	−6	6.4	149	50	24.0	22.5	0.10
10	RG162	6	8	−1	7.2	170	24	29.4	18.0	0.09
	RG653	6	9			161	38	28.5	26.0	0.12
11	CDO533	7	2	−4	5.3	141	59	21.7	20.0	0.09
12	RZ272	7	9	+8	7.9	153	37	23.2	16.4	0.08
	RG528	7	10			147	33	26.9	19.8	0.10
13	RG1	8	5			160	36	27.8	23.5	0.11
	RZ66	8	6	+2	7.2	95	29	30.7	21.6	0.15
	RG136	8	7			175	27	28.5	19.1	0.09
14	RG553	9	2	−3	4.2	166	34	25.9	18.8	0.09
15	RZ404	9	8	−1	4.1	138	55	21.0	17.8	0.09
16	RG1109	11	2	+2	7.6	164	27	36.0	32.4	0.15
17	CDO365	11	5	−3	8.9	167	31	34.2	32.2	0.14
	RG211	11	6			181	18	40.8	28.6	0.13
	RG167	11	7			169	30	26.4	17.9	0.08
18	RZ53	11	9			177	20	39.2	28.4	0.13
	RG118	11	10	+2	7.5	176	21	37.2	27.6	0.12
19	RZ397	12	8	+3	4.6	160	27	29.5	19.8	0.10

[a] Quantitative trait loci, closely linked markers are assumed to identify the same QTL

[b] Chromosome on which the marker is located (Fig. 1)

[c] Marker location in the sequence of markers on the chromosome (Fig. 1)

[d] The location in centimorgans (+ or − relative to the corresponding marker) of the most likely position of the QTL constrained by the markers identified by single-marker analysis as determined by MAPMAKER/QTL

[e] The corresponding LOD score for the QTL as determined by MAPMAKER/QTL. 0.05 LOD threshold = 2.7 and 0.01 LOD threshold = 3.4

[f] Number of RI lines homozygous for the CO39 allele

[g] Number of RI lines homozygous for the Moroberekan allele

[h] Phenotypic effect, the difference in the mean total number of roots of all RI lines homozygous for the CO39 allele and those homozygous for the Moroberekan allele. All differences are significant at the 0.0001 level of probability as determined by the PROC TTEST procedure of SAS

[i] The F-statistic as determined by the PROC GLM procedure of SAS. Threshold values are 0.05 = 12.8 and 0.01 = 16.1

[j] Coefficient of determination, the percent of phenotypic variation explained by individual markers as determined from the PROC GLM procedure of SAS

Discussion

Although 19 putative QTLs were identified with increased total root number, a two-marker regression model did account for 25% of the variation in total root number. Drought resistance in Japanese lowland cultivars has been associated with root number (Minabe 1951 as reported by Ekanayake et al. 1986). Our results suggest that more research should be conducted on the relationship between root number and drought resistance and that molecular markers may be useful in breeding programs incorporating root number as a criterion.

All four of the QTLs identified with an increased number of penetrating roots and one of the six QTLs identified with the root penetration index were associated with the CO39 allele. This result suggests that though the CO39 parent is phenotypically poorer in root penetration ability, it contains genes that are capable of contributing positively to root penetration. Additionally, the skewness of the RI population towards CO39 alleles may have confounded the detection of Moroberekan alleles associated with the number of penetrated roots. The mechanisms by which some roots are able to penetrate compacted soils is not well understood but has been associated with physiological and morphological changes such as reduced branching, a

Table 4 RFLP markers identified as being associated with root penetration index as indicated by single-marker analysis at the 0.05 threshold ($F \geqslant 12.8$) and the most likely QTL position in the surrounding interval as indicated by interval analysis. For all markers except RG360 the Moroberekan allele was associated with an increase in the penetration index

QTL[a]	Marker	Chr[b]	Pos[c]	Distance[d]	LOD[e]	CO39[f]	MORO[g]	Pe[h]	F[i]	R^{2j}
Moroberekan allele associated with increase										
1	RG324	2	2	+5	6.2	147	38	0.10	23.6	0.11
	RG73	2	3			155	43	0.10	27.3	0.12
2	RG620	4	1			127	39	0.08	14.7	0.08
	RG476C	4	3	−1	6.1	159	43	0.11	29.6	0.13
	RG329	4	4			157	43	0.08	14.1	0.07
3	RG653	6	9	−3	4.4	161	38	0.08	15.2	0.07
4	CDO365	11	5	−4	5.1	167	31	0.09	14.6	0.07
5	RG118	11	10	−1	3.9	176	21	0.11	16.4	0.08
CO39 allele associated with increase										
6	RG360	5	1	0	4.7	112	20	0.12	17.6	0.12

[a] Quantitative trait loci, closely linked markers are assumed to identify the same QTL
[b] Chromosome on which the marker is located (Fig. 1)
[c] Marker location in the sequence of markers on the chromosome (Fig. 1)
[d] The location in centimorgans (+ or − relative to the corresponding marker) of the most likely position of the QTL constrained by the markers identified by single-marker analysis as determined by MAPMAKER/QTL
[e] The corresponding LOD score for the QTL as determined by MAPMAKER/QTL. 0.05 LOD threshold = 2.7 and 0.01 LOD threshold = 3.4
[f] Number of RI lines homozygous for the CO39 allele
[g] Number of RI lines homozygous for the Moroberekan allele
[h] Phenotypic effect, the difference in the mean penetration index of all RI lines homozygous for the CO39 allele and those homozygous for the Moroberekan allele. All differences are significant at the 0.0001 level of probability as determined by the PROC TTEST procedure of SAS
[i] The F-statistic as determined by the PROC GLM procedure of SAS. Threshold values are 0.05 = 12.8 and 0.01 = 16.1
[j] Coefficient of determination, the percent of phenotypic variation explained by individual markers as determined from the PROC GLM procedure of SAS

Table 5 RFLP markers identified as being associated with tiller number as indicated by single-marker analysis at the 0.01 threshold ($F \geqslant 15.8$) and the most likely QTL position in the surrounding interval as indicated by interval analysis. For all markers except CO39 allele is associated with an increase in tiller number

QTL[a]	Marker	Chr[b]	Pos[c]	Distance[d]	LOD[e]	CO39[f]	MORO[g]	Pe[h]	F[i]	R^{2j}
1	RG140	1	13	−3	5.4	162	36	2.0	20.9	0.10
2	RG139	2	6	+1	5.8	171	28	2.6	31.8	0.14
	RZ103	2	7			157	44	1.6	16.1	0.07
3	RG476C	4	3			159	43	1.8	20.1	0.09
	RG329	4	4	+8	6.8	157	43	1.6	16.4	0.08
	RZ740	4	5			156	41	2.2	28.2	0.13
4	RG788	4	9	+3	5.2	137	57	1.7	21.1	0.10
	RG449	4	10			121	41	1.7	16.1	0.09
5	WAXY	6	1	0	3.7	154	42	1.9	20.9	0.10
	RZ516	6	2			158	35	1.9	19.0	0.09
6	RG1	8	5			160	36	1.8	15.8	0.08
	RG136	8	7	−5	5.0	175	27	2.1	17.4	0.08
7	CDO365	11	5	+3	5.6	167	31	2.0	17.6	0.08
	RG211	11	6			181	18	2.9	24.1	0.11
8	RZ53	11	9	+1	5.2	177	20	2.7	24.3	0.11
	RG118	11	10			176	21	2.4	19.9	0.09
9	RG323	12	1	+4	4.3	151	50	1.6	17.0	0.08
	RG181	12	2			171	30	2.0	16.9	0.08
10	RG9	12	6	−1	3.7	166	26	2.1	16.4	0.08

[a] Quantitative trait loci, closely linked markers are assumed to identify the same QTL
[b] Chromosome on which the marker is located (Fig. 1)
[c] Marker location in the sequence of markers on the chromosome (Fig. 1)
[d] The location in centimorgans (+ or − relative to the corresponding marker) of the most likely position of the QTL constrained by the markers identified by single-marker analysis as determined by MAPMAKER/QTL
[e] The corresponding LOD score for the QTL as determined by MAPMAKER/QTL. 0.05 LOD threshold = 2.6 and 0.01 LOD threshold = 3.3
[f] Number of RI lines homozygous for the CO39 allele
[g] Number of RI lines homozygous for the Moroberekan allele
[h] Phenotypic effect, the difference in the mean tiller number per plant of all RI lines homozygous for the CO39 allele and those homozygous for the Moroberekan allele. All differences are significant at the 0.0001 level of probability as determined by the PROC TTEST procedure of SAS
[i] The F-statistic as determined by the PROC GLM procedure of SAS. Threshold values are 0.05 = 12.0 and 0.01 = 15.8
[j] Coefficient of determination, the percent of phenotypic variation explained by individual markers as determined from the PROC GLM procedure of SAS

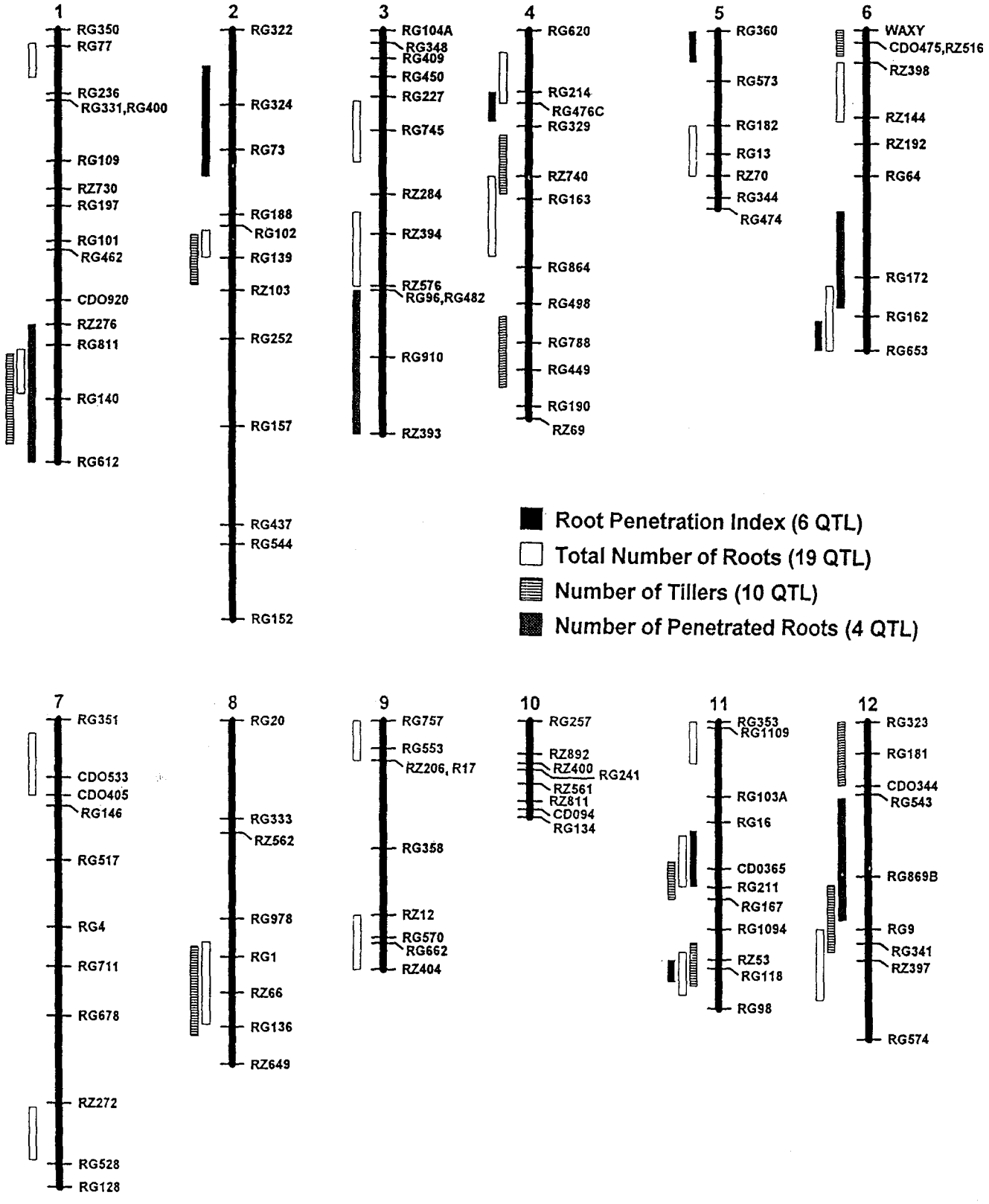

Fig. 1 RFLP linkage map of the CO39/Moroberekan recombinant inbred population showing the location of quantitative trait loci associated with the number of penetrating roots, the total root number, root penetration ability, and tiller number. *Boxes* to the left of each chromosome identify the respective loci over a two-LOD confidence interval as determined by MAPMAKER\QTL. The map was redrawn from Wang et al. (1994) using interval distances from Champoux et al. (1995)

radial thickening of the root, changes in plant growth regulator levels including ethylene and auxin, and changes in root carbohydrate levels and metabolism in impeded roots (see review by Atwell 1993). These changes suggest an underlying complexity of root penetration which may not allow the direct identification

of a few highly associated QTLs with root penetration ability. An analogous situation is the identification of molecular markers associated with yield for which the strategy has been to tag yield components.

Figure 1 shows the most likely QTL positions associated with the number of penetrated roots (4 loci), the total number of roots (19 loci), and root penetration index (6 loci) identified by single-marker analysis and confirmed by interval analysis. All six of the root penetration index loci mapped to within 30 cM of a locus associated with total root number (Fig. 1). Clustering of QTLs associated with root traits was observed by Champoux et al. (1995) who measured morphological root traits such as thickness, length, and weight in a different subset of the same RI population examined in the present study. They suggested that there may be specific regions of the rice genome containing genes that determine root morphology. These regions may contain clusters of genes or, in some cases, genes with pleiotropic effect. Champoux et al. (1995) identified 18 markers associated with root thickness of which 12 mapped in the same regions as the QTLs we identified for total root number. Interestingly, the markers that accounted for the largest variation in Champoux's study (33% and 22%, respectively) were also found to be associated with the root penetration index (RG476C on chromosome 4 and CDO365 on chromosome 11). Though these associations are quite preliminary, it has been shown that genotypes and species with thicker roots tend to have a greater ability to penetrate compacted soil (Materechera et al. 1992).

Positive correlations between total root number and tiller number have often been observed, as it was in this study ($r = 0.52$, $P = 0.0001$). Of the ten QTLs associated with tiller number, all but two, mapped closely to regions identified as associated with total root number (Fig. 1). This may indicate genes having a pleiotropic effect on tiller and root numbers. More detailed studies are required, but our results suggest that molecular markers could play a significant role in studying the relationship of shoot and root characteristics such as tiller and root number.

Most genetic studies of tiller numbers have concentrated on terminal characteristics whereas the tiller numbers in this study reflect a much earlier growth stage. Xu and Shen (1991) in a diallel analysis of tiller number at different growth stages in rice concluded that an identical polygenic system controls tiller number at the different stages. However, they do caution against making generalizations based on measurements from one point in time and suggested that selection would be more effective at later stages. Molecular markers associated with tiller number, such as those identified in this study, may be very useful in dissecting the control of tiller number over time by observing how the relationship between markers and tiller number changes with the growth stage.

The results presented here indicate the feasibility of mapping QTLs associated with these complex traits and represent the first research report on the location of genetic loci involved in root penetration ability as well as the total number of roots and the number of tillers per plant. Currently, in conjunction with scientists at the International Rice Research Institute, we are developing mapping populations specifically designed for fine mapping of root penetration ability in rice. Using a population more suited to studying root penetration ability will allow the identification of more useful markers which can be utilized in marker-assisted selection programs for the improvement of drought resistance in rice.

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