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Mapping QTLs for root morphology of a rice population adapted to rainfed lowland conditions

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Abstract In the rainfed lowlands, rice (*Oryza sativa* L.) develops roots under anaerobic soil conditions with ponded water, prior to exposure to water stress and aerobic soil conditions that arise later in the season. Constitutive root system development in anaerobic soil conditions has been reported to have a positive effect on subsequent expression of adaptive root traits and water extraction during progressive water stress in aerobic soil conditions. We examined quantitative trait loci (QTLs) for constitutive root morphology traits using a mapping population derived from a cross between two rice lines which were well-adapted to rainfed lowland conditions. The effects of phenotyping environment and genetic background on QTLs identification were examined by comparing the experimental data with published results from four other populations. One hundred and eighty-four recombinant inbred lines (RILs) from a lowland *indica* cross (IR58821/IR52561) were grown under anaerobic conditions in two experiments. Seven traits, categorized into three groups (shoot biomass, deep root morphology, root thickness) were measured during the tillering stage. Though parental lines showed consistent differences in shoot biomass and root morphology traits across the two seasons, genotype-by-environment interaction (G×E) and QTL-by-environment interaction were significant among the progeny. Two, twelve, and eight QTLs for shoot biomass, deep root morphology, and root thickness, respectively, were identified, with LOD scores ranging from 2.0 to 12.8. Phenotypic variation explained by a single QTL ranged from 6% to 30%. Only two QTLs for deep root morphology, in RG256-RG151 in chromosome 2 and in PC75M3-PC11M4 in chromosome 4, were identified in both experiments. Comparison of positions of QTLs across five mapping populations (the current population plus populations from four other studies) revealed that these two QTLs for deep root morphology were only identified in populations that were phenotyped under anaerobic conditions. Fourteen and nine chromosome regions overlapped across different populations as putative QTLs for deep root morphology and root thickness, respectively. PC41M2-PC173M5 in chromosome 2 was identified as an interval that had QTLs for deep root morphology in four mapping populations. The PC75M3-PC11M4 interval in chromosome 4 was identified as a QTL for root thickness in three mapping populations with phenotypic variation explained by a single QTL consistently as large as 20–30%. Three QTLs for deep root morphology were found only in *japonica/indica* populations but not in IR58821/IR52561. The results identifying chromosome regions that had putative QTLs for deep root morphology and root thickness over different mapping populations indicate potential for marker-assisted selection for these traits.

Keywords Rainfed lowland rice · QTL · Root morphology · DNA markers

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Introduction

Rainfed lowland rice is grown in bunded fields, where soil conditions range from flooded and anaerobic to droughted and aerobic (Wade et al. 1998). Rice plants in rainfed lowlands generally develop their root system un-

der anaerobic flooded conditions, prior to encountering a water deficit later in the season. It is important to discriminate between constitutive root traits developed before the onset of water stress, and adaptive root traits developed in response to water stress. Kamoshita et al. (2002) defined constitutive traits as those which are expressed under anaerobic, non-water-stressed conditions, do not require water stress for their expression, and may demonstrate variation that is subsequently modified by adaptive traits. Adaptive traits are those which are expressed in response to a water deficit or soil physical/chemical barriers. Less research attention has been devoted to constitutive traits in anaerobic soil conditions.

A deep and thick root system has been thought to be advantageous for improved drought tolerance in the rainfed lowland ecosystem, based on extrapolation from experience with upland rice (O'Toole 1982; Fukai and Cooper 1995). Under anaerobic, well-watered conditions, root system development was observed to have a positive effect on subsequent plant growth during progressive water stress (Hoque and Kobata 1998; Azhiri-Sigari et al. 2000; Kamoshita et al. 2000). Azhiri-Sigari et al. (2000) and Kamoshita et al. (2000) demonstrated genotypic variation in constitutive root traits, and subsequent responses of adaptive root traits, especially in deeper soil layers. Greater root elongation to depth resulted in improved water extraction. Improved seedling vigor was also valuable to growth afterward (Mitchell et al. 1998). In the field, roots are generally shallow in rainfed lowlands (Pantuwan et al. 1997), but genotypes differ with respect to root growth in deeper layers (Samson and Wade 1998). Despite having fewer roots in deeper layers, rainfed lowland rice can extract water from below 15-cm soil depth in subsequent drought periods (Wade et al. 1999). Consequently, both constitutive and adaptive root traits are implicated in an improved performance of rice in these fluctuating water environments.

Modification of the rice root system by conventional breeding is not easy because of tedious screening techniques and the plastic nature of the root system. DNA marker-assisted selection could be an alternative to conventional screening. For this to succeed, basic information, such as genotype-by-environment interaction (G×E), QTL-by-environment interaction, consistency, and differences due to genetic background and epistasis need to be obtained.

QTL-by-environment interaction has been extensively studied for agronomic traits such as flowering time (Jansen et al. 1995) and yield components (Hayes et al. 1993; Lu et al. 1997; Ribaut et al. 1997), but only one study in root systems has been undertaken (Kamoshita et al. 2002). Kamoshita et al. (2002) reported G×E and QTL-by-environment interactions for constitutive root morphology traits where the phenotyping environment was defined by temperature and solar radiation. They also found crossover interaction for deep root mass between the parental lines, one upland adapted and one lowland adapted, which they suggested could be associated with the significant G×E and QTL-by-environment interaction among their progeny.

Only four rice populations have been tagged for QTLs associated with the expression of root morphology traits under hydroponic (Price and Tomos 1997), aerobic (Champoux et al. 1995; Yadav et al. 1997), or anaerobic (Kamoshita et al. 2002) conditions. The small number of populations may limit extrapolation of the results from experimental populations to other breeding populations. All of the populations studied to date have been upland *japonica*/lowland *indica* populations, because of the ease in creating polymorphism and the extent of genetic variation from germplasm including both *japonica* and *indica* types. However, evaluation of upland *japonica*/lowland *indica* populations under anaerobic lowland conditions may be confounded by the difference in adaptation to lowland conditions. Wade et al. (2000), Azhiri-Sigari et al. (2000), and Kamoshita et al. (2000) showed that the upland line CT9993 had smaller biomass production and slower development of a deep root system than the lowland-adapted line IR62266 during early vegetative growth. To improve the root system of rainfed lowland rice, mapping populations from crosses between parental lines that are equally well adapted to lowland conditions should be evaluated.

The investigation reported in this paper examined phenotypic variation and QTLs for gross root morphology under anaerobic lowland conditions in contrasting solar radiation regimes in a population from a cross of lowland-adapted *indica* rice lines. The results were compared with those of previous studies on QTLs for root morphology traits in other mapping populations phenotyped under both anaerobic and aerobic conditions. The objectives of this investigation were threefold: first, to identify QTLs for root morphology in a population derived from lowland-adapted *indica* lines under anaerobic lowland conditions; second, to evaluate the effect of phenotyping environment on identification of QTLs; third, to examine the effects of genetic background.

Materials and methods

Plant population

A population of 184 recombinant inbred lines (RILs) from the cross between IR58821-2-3-B-1-2-1 (IR58821) and IR52561-UBN-1-1-2 (IR52561) (both lowland-adapted *indica* genotypes) was developed at the International Rice Research Institute (IRRI), Los Baños, Philippines (14°11′N, 121°15′E, 23 m altitude), by single-seed descent to the F_7 generation. The gross root morphology of the two parental lines was characterized under both stress and nonstress conditions in the greenhouse (Azhiri-Sigari et al. 2000) and in the field (Sarkarung et al. 1997; Samson and Wade 1998). IR58821 had consistently more deep root mass than IR52561 under anaerobic flooded conditions (Azhiri-Sigari et al. 2000) and a greater hardpan penetration capacity at Rajshahi in northwest Bangladesh (Samson and Wade 1998).

Pot experiments

Root morphology was evaluated in two pot experiments with different sowing dates in the IRRI greenhouse (Table 1). Experimental designs were a 14×14 row-column alpha design for experiment

Table 1 Characteristics of the two screening environments

Experiment code	Lines phenotyped	Lines for QTL analysis	Sowing date	Harvest (days)	Heatsum[a] (°C day)	Radiation (MJ m^{-2} day^{-1})	Soil temperature[b] (°C)
RI1	183	166	8 Aug 97	46	1,031	17.6	28–33
RI2	182	164	3 Feb 98	46	987	21.5	27–34

[a] Base temperature was taken as 9 °C (Kropff et al. 1994)
[b] Average soil temperature measured at 5 cm soil depth at 800 h and 1500 h

1 (RI1) and a 12×16 alpha design for experiment 2 (RI2), with three replicates. An evaporative gradient from the center to the side of the greenhouse was observed in RI1, so replicates were subsequently arranged perpendicular to this gradient in RI2, even though the observed effects on plant growth were small. The alpha design permitted within-replicate trends to be evaluated and adjusted.

Four to five pre-germinated seeds of each RIL were sown on the wet soil and thinned to one healthy seedling per pot at about 10 days after sowing (DAS). The sowing dates were 8 August, 1997 in RI1 and 3 February, 1998 in RI2. Only one line in RI1 and two lines in RI2 were missing due to germination failure (Table 1).

The details on pot preparation, soil characteristics, fertilizer application, and maintenance of standing water were similar to those of Kamoshita et al. (2002). Briefly, a cylindrical pot of 20-cm internal diameter and 55-cm depth with a plastic bag insert was filled with 20 kg of air-dried Maahas clay soil (28% clay, 44% silt, and 28% sand, pH 5.2). Sufficient levels of fertilizer (1.26 g/pot of N as urea 46-0-0; 0.33 g/pot of P as solophos 0-18-0; 0.62 g/pot of K as muriate potash 0-0-60) were applied at puddling and mixed thoroughly into the puddled soil. The level of standing water was maintained at about 2–4 cm by watering daily. The exterior of the pot was covered with aluminum foil at 23 DAS in RI1 and from sowing in RI2 to minimize any rise in soil temperature in pots in the greenhouse, so that high-temperature effects on root growth were minimized (Nagai and Matsushita 1963). Covering the pots with aluminium foil reduced mean soil water temperature by 2.5 °C during 20–25 DAS in RI1. Because of lower maximum temperatures and low solar radiation prior to 23 DAS in RI1 (Fig. 1), any effects of increased soil water temperature in reducing root growth prior to foil covering would be small. No disease or insect damage occurred.

Data collection

The daily maximum and minimum air temperature and the soil temperature at a 5-cm depth from the soil surface at 800 h and 1500 h were recorded in the greenhouse. Solar radiation data came from the IRRI wetland meteorological station about 500 m away. Within the greenhouse, incident radiation was 57% of values at the meteorological station, with an R^2 of 0.93. The heat sum with a base temperature of 9 °C and average daily solar radiation during the experimental period were calculated (Table 1). RI1 had a lower solar radiation than RI2, especially from 10 to 20 DAS and after 40 DAS (Fig. 1). The average daily solar radiation was 17.6 MJ m^{-2} d^{-1} in RI1 and 21.5 MJ m^{-2} d^{-1} in RI2. RI1 had a slightly higher minimum temperature than RI2.

The plants were sampled at 45–47 DAS, prior to panicle initiation, by taking one replicate per day, in both experiments. Measurement and plant sampling procedures were similar to those used in previous experiments of Kamoshita et al. (2002). Briefly, plants were cut at the soil surface. The soil mass inside the plastic sleeve was slowly pulled out of the pots and the soil divided into layers of 0–10, 10–20, 20–25, 25–30, 30–35, 35–40, 40–45, and 45–50 cm from the soil surface. Roots were carefully separated from the soil on a 1-mm sieve screen. The dry weight of each plant component was measured after drying at 70 °C for 4–5 days. Shoot biomass was determined as the sum of above-ground biomass and stem base below the soil surface. Total root mass and deep root mass below a soil depth of 30 cm were obtained, and the deep root ratio, the proportion of the latter to the former, was calculated. Deep root per tiller was calculated by dividing deep root mass by the total number of tillers. Maximum rooting depth was calculated from the deepest soil layer where roots were present and the longest root measured in the layer. Root thickness was measured by microcaliper at soil depths of 0–10 cm and 20–25 cm for seven to ten randomly chosen primary roots. A total of seven traits were analyzed. These included shoot biomass, deep root mass, deep root ratio, deep root per tiller, rooting depth, and root thickness at either the 0–10-cm or 20–25-cm soil depth.

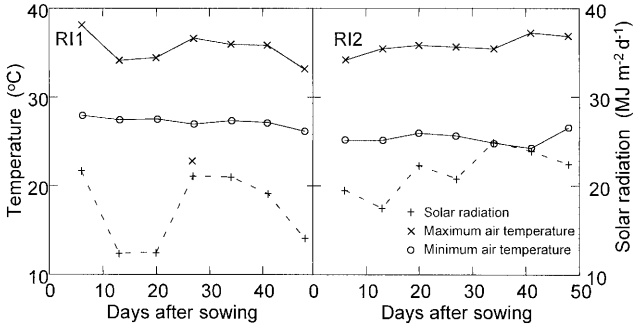

Fig. 1 Daily maximum and minimum temperature and solar radiation during plant growth in RI1 and RI2 experiments

Statistical analysis

Analysis of variance and calculation of means were conducted for the seven traits between the parents using SYSTAT 7.0 (SPSS 1996, 1997) and among all of the progeny used in phenotyping by the SAS package (SAS Institute 1990). The alpha design was used to accommodate variation within blocks, including the effects of evaporative gradient in RI1, and adjusted mean values were calculated. Broad-sense heritability (h^2) was calculated from the estimates of genetic (σ^2_G) and residual (σ^2_E) variances derived from the expected mean squares of the analysis of variance,

$$h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_E/k),$$

where k was the number of replications. Those lines used for QTL analysis were tested in combined analysis of variance using the data from both experiments to compare the mean square of G×E with that of genotypic variation. The Pearson correlation was calculated between shoot and root traits for each experiment using SYSTAT 7.0.

Map construction and QTL analysis

One hundred and sixty-six RILs were used to construct the map that consisted of 96 restriction fragment length polymorphisms (RFLPs) and 303 amplified fragment length polymorphisms (AFLPs) at Texas Tech University (Ali et al. 2000). The level of polymorphism for the two parents was 26% and 17% for RFLPs and AFLPs respectively. The linkage map was constructed with

MAPMAKER Macintosh Version 2.0. Putative QTLs (main-effect QTLs assuming no epistasis) for the traits were identified in both separate analysis for each experiment and combined analysis of two experiments by employing composite interval mapping based on QTLMAPPER (version 1.0) (Wang et al. 1999a, b). The procedure of the analysis was similar to that used by Kamoshita et al. (2002). Only 166 lines in RI1 and 164 lines in RI2 were used in QTL analysis (Table 1) because not all RILs were genotyped. Combined analysis with 166 lines was conducted, with the matrix filled 99%, to estimate epistasis and to calculate the relative and general contributions of additive effect, epistasis, and QTL-by-environment interaction (additive-by-environment and epistasis-by-environment interactions). Main-effect QTLs and epistasis QTLs were declared significant at the thresholds of 0.005 and 0.001, respectively, with the LOD score set higher than 2.0 and 4.0, respectively. A relative contribution was calculated as the proportion of variance caused by a specific genetic source in the total phenotypic variance, taken as a heritability contributed by that genetic source. The general contribution (coefficient of determination) for each genetic source was calculated from the relative contributions of all the putative QTLs involved.

Comparison across mapping populations

QTLs positions for deep root morphology traits were compared across backgrounds, using deep root mass, deep root ratio, deep root per tiller, and rooting depth from IR58821/IR52561 and CT9993/IR62266, and using deep root mass per unit of shoot biomass and maximum root length from IR64/Azucena and Bala/Azucena. Root thickness was measured only at a soil depth of 0–10 cm in populations other than IR58821/IR52561 and CT9993/IR62266. The QTLs taken into account for IR58821/IR52561 and CT9993/IR62266 (Kamoshita et al. 2002) were all putative QTLs present in more than one experiment. For IR64/Azucena, QTLs identified by single marker analysis by Yadav et al. (1997) were employed; for Bala/Azucena, those identified by Price and Tomos (1997) and Price et al. (1999); for Co39/Moroberekan, QTLs identified by Champoux et al. (1995). The set of polymorphic markers was different across the five populations. The maps developed by Causse et al. (1994), Cho et al. (1998), and Harushima et al. (1998) were used as a bridge among the populations compared. Since inconsistent map distance between markers in different maps hampered precision, the comparisons should be considered as indicative.

Results

Phenotypic variation

Shoot biomass was 1.5 times larger in RI2 than in RI1, but more biomass was partitioned to deep roots in RI1 (Table 2). Root thickness at a soil depth of 20–25 cm was larger in RI1. IR58821 had consistently larger values than IR52561 for shoot biomass, deep root morphology traits, and root thickness traits in both experiments. Genotypic variation for all traits was significant among the progeny, with transgressive variation small to medium in size for deep root mass, deep root ratio, and deep root per tiller.

Broad-sense heritability was highest for deep root mass in both RI1 (0.56) and RI2 (0.61), but was low for root thickness traits in RI1 (Table 2). Genotype-by-environment interaction (G×E) was significant for all traits among the progeny, but its mean square was smaller than that of genotypic variation (G) (Table 3). The ratios of G×E to G mean squares were over 0.5 except for deep root mass (0.4), and they tended to be higher for root thickness traits.

The phenotypic correlation coefficients between the RI1 and RI2 experiments ranged from 0.20 (not signifi-

Table 2 Mean values for IR58821 and IR52561, ranges in RILs, and broad-sense heritability (h^2) of the seven traits in RI1 and RI2 experiments, respectively

Trait	IR58821	IR52561	P_P	RIL	P_{RIL}	h^2
RI1						
Shoot biomass						
Shoot biomass (g plant^{-1})	32.5	23.6	***	18.5–41.3	***	0.51
Deep root morphology						
Deep root mass (g plant^{-1})	0.25	0.05	**	0–0.34	***	0.56
Deep root ratio (%)	5.0	1.6	**	0.0–8.0	***	0.50
Deep root per tiller (mg tiller^{-1})	6.9	1.5	***	0.0–10.4	***	0.42
Rooting depth (cm)	47	43	*	37–50	***	0.38
Root thickness						
Root thickness 0–10 cm (mm)	1.4	1.3	*	1.2–1.5	**	0.26
Root thickness 20–25 cm (mm)	1.2	1.1	*	0.9–1.3	*	0.23
RI2						
Shoot biomass						
Shoot biomass (g plant^{-1})	44.7	39.9	*	31.3–53.8	***	0.49
Deep root morphology						
Deep root mass (g plant^{-1})	0.15	0.02	**	0.0–0.22	***	0.61
Deep root ratio (%)	2.3	0.4	***	0.0–3.0	***	0.56
Deep root per tiller (mg tiller^{-1})	3.5	0.4	***	0.0–5.2	***	0.48
Rooting depth (cm)	43	38	***	34–48	***	0.53
Root thickness						
Root thickness 0–10 cm (mm)	1.4	1.2	**	1.2–1.6	***	0.60
Root thickness 20–25 cm (mm)	0.9	0.8	**	0.7–1.0	***	0.40

*,**,*** Significant differences between the two parents (P_P) or among RILs (P_{RIL}) at P=0.05, 0.01 and 0.001, respectively. *ns* Not significant at P=0.10

cant at P=0.10) to 0.51 (P=0.001) depending upon the traits (data not shown). Total root mass and shoot biomass had strong phenotypic correlations in both experiments, but the correlations between deep root mass or root thickness and shoot traits (shoot biomass and plant height) were small or insignificant (Table 4).

Table 3 Mean square of genotype-by-environment interaction (G×E) compared with that of genotypic variation (G) among 166 RILs from IR58821/IR52561

Trait	Mean square of G×E	Mean square of G	Ratio
Shoot biomass	16.2	29.0	0.56
Deep root mass	0.00154	0.00389	0.40
Deep root ratio	0.92	1.71	0.54
Deep root per tiller	1.83	3.24	0.56
Rooting depth	5.66	9.16	0.62
Root thickness 0–10 cm	0.00296	0.00395	0.75
Root thickness 20–25 cm	0.00395	0.00616	0.64

Table 4 Phenotypic correlation (r) among some of the root and shoot traits in each of the two experiments with 166 RILs from IR58221/IR52561

Traits	RI1	RI2
Total root mass and shoot biomass	0.54***	0.52***
Deep root mass and shoot biomass	0.06^{+}	0.18***
Root thickness 0–10 cm and shoot biomass	0.03 ns	0.00 ns
Root thickness 0–10 cm and plant height	0.00 ns	0.00 ns
Root thickness 20–25 cm and shoot biomass	0.05 ns	0.15***

$^{+}$,***Significant at P=0.10 and 0.001, respectively. ns, Not significant

QTL analysis

Putative QTLs from the separate analysis of each experiment are presented in Table 5. For the four deep root traits, 12 QTLs were identified, with the phenotypic variation explained by a single QTL ranging from 5.7%

Table 5 Chromosome and marker intervals that were likely to contain QTLs (P<0.005), distance of the marker interval from the marker of the top arm (in centimorgan), approximate positions of QTLs (Pos), LOD score, and effect (A) and relative contributions (r^2) of QTLs for the seven traits evaluated in IR58821/IR52561 in RI1 and RI2 experiments. Superscript c in the chromosome column shows that these intervals are identified as being significant in combined analysis

Chromosome	Interval	Distance	RI1				RI2			
			Pos.[a]	LOD[b]	A[c]	r^2[d]	Pos.	LOD	A	r^2
	Shoot biomass									
4[c]	RZ536-PC11M4	165.3					0.00	4.78	1.15	13.8
8[c]	PC75M13-G1073	50.7	0.00	3.88	1.16	12.6				
	Deep root mass									
2[c]	PC32M10-RG151	296.2	0.00	7.73	0.02	17.0				
3	PC20M11-PC20M12	55.7					0.00	4.75	0.01	12.7
4[c]	PC75M3-RZ536	161.1	0.00	11.8	0.02	21.4				
9[c]	PC33M1-PC32M8	134.0					0.05	3.66	0.01	10.6
11	PC48M15-PC31M8	107.0					0.00	3.15	0.01	7.3
	Deep root ratio									
2[c]	RG256-RG151	295.9	0.05	6.15	0.55	17.3	0.05	3.13	0.14	5.8
3	PC20M11-PC20M12	55.7					0.00	4.24	0.16	8.2
4[c]	PC75M3-PC11M4	161.1	0.00	11.8	0.69	27.4	0.04	5.04	0.20	12.6
9[c]	PC32M8-RZ596	137.4					0.00	4.93	0.19	10.7
11	PC41M14-PC48M15	105.1					0.00	4.31	0.17	8.7
	Deep root per tiller									
2	PC47M3-PC173M5	82.7	0.00	3.47	–0.56	8.1				
2	PC32M10-RG151	301.2	0.05	7.65	0.91	21.6				
4[c]	PC11M12-PC28M1	44.8					0.00	9.07	–7.40	17.3
4	PC73M4-PC180M10	152.1	0.00	4.61	0.61	9.6				
6[c]	PC48M13-PC32M6	5.0					0.05	5.59	5.47	9.4
7[c]	PC41M6-CDO385	32.4					0.00	10.5	8.04	20.4
	Rooting depth									
1	PC31M10-PC34M6	244.6	0.05	2.46	0.54	6.0				
4[c]	CDO456-PC79M8	10.0					0.00	2.96	–0.58	5.7
4[c]	RZ467-PC184M13	138.9					0.00	12.8	1.32	29.9
4	PC75M3-RZ536	161.1	0.00	8.59	1.14	27.6				
4	RG214-C1016	203.8	0.00	5.35	–0.81	–[e]				
	Root thickness 0–10 cm									
1	PC32M5-PC31M10	218.7					0.00	4.12	0.02	8.7
3[c]	R1925-RG1356	240.1					0.00	6.30	–0.03	13.8
8[c]	PC27M15-C1121	3.8					0.00	3.26	0.03	13.1
8	PC75M12-PC32M7	32.8	0.00	2.02	0.01	6.9				
8	PC75M13-G1073	50.7					0.00	2.31	–0.02	6.2
9[c]	PC32M8-RZ596	147.4	0.10	5.30	0.02	15.1				
	Root thickness 20–25 cm									
4[c]	RZ467-PC184M13	138.9	0.00	4.81	0.02	12.4				
4	RZ536-PC11M4	165.3					0.00	8.36	0.02	23.2

[a] Genetic distance (centimorgan) from the left marker of each interval where the QTL was identified with the highest probability in interval mapping
[b] Likelihood ODds ratio
[c] Effects of substituting a single allele from one parent to another. Positive values show that allelic contribution is from IR58821 and negative values from IR52561
[d] Phenotypic variation explained by a single QTL
[e] –, Analysis unavailable

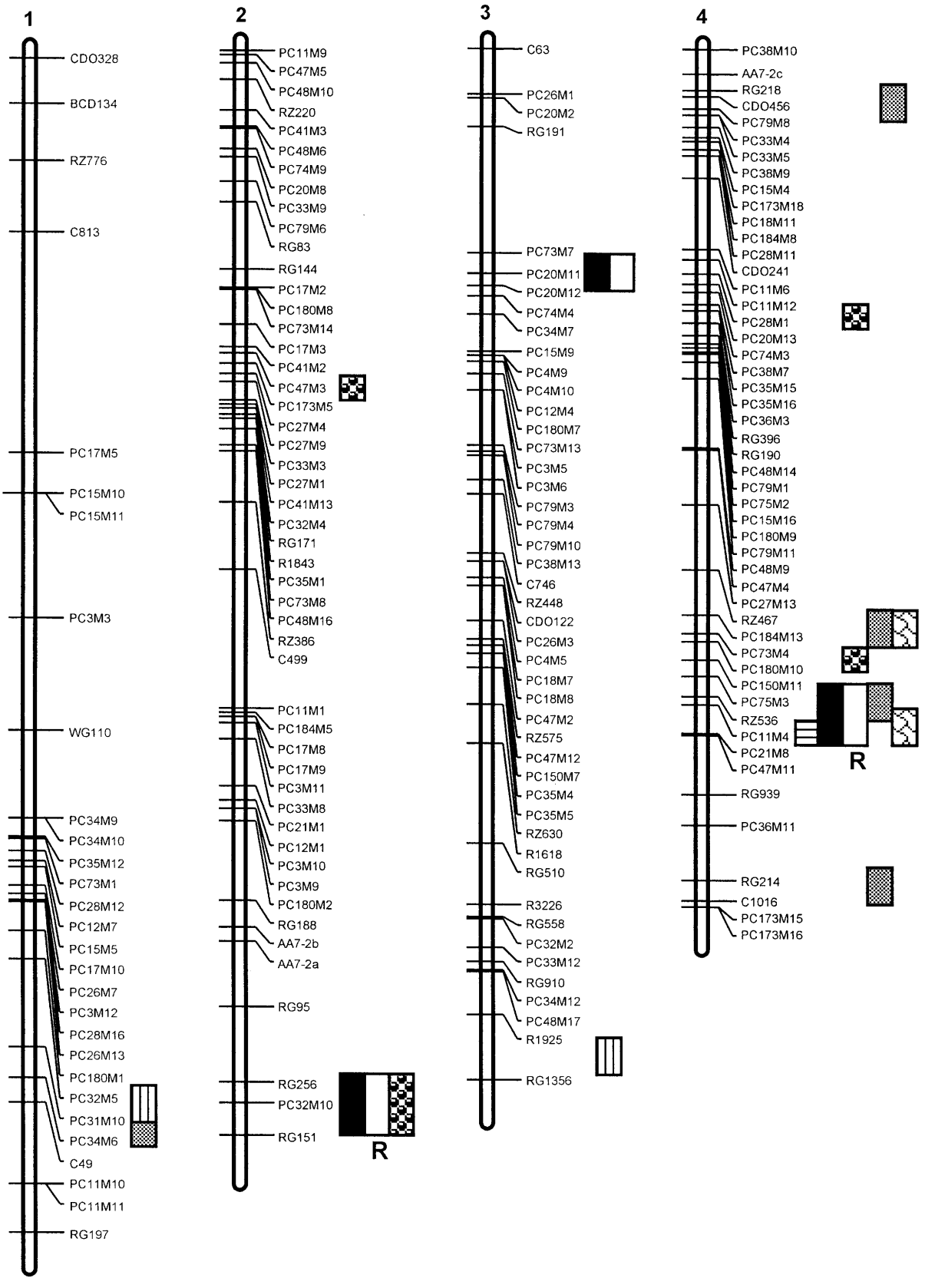

Fig. 2 Approximate position of putative QTLs for shoot biomass, deep root mass below a soil depth of 30 cm, deep root ratio, deep root per tiller, maximum rooting depth, root thickness at a soil depth of 0–10 cm, and root thickness at a soil depth of 20–25 cm in IR58821/IR52561. QTLs with an LOD>2.0 are presented, and those common to both experiments are marked with the letter *R*

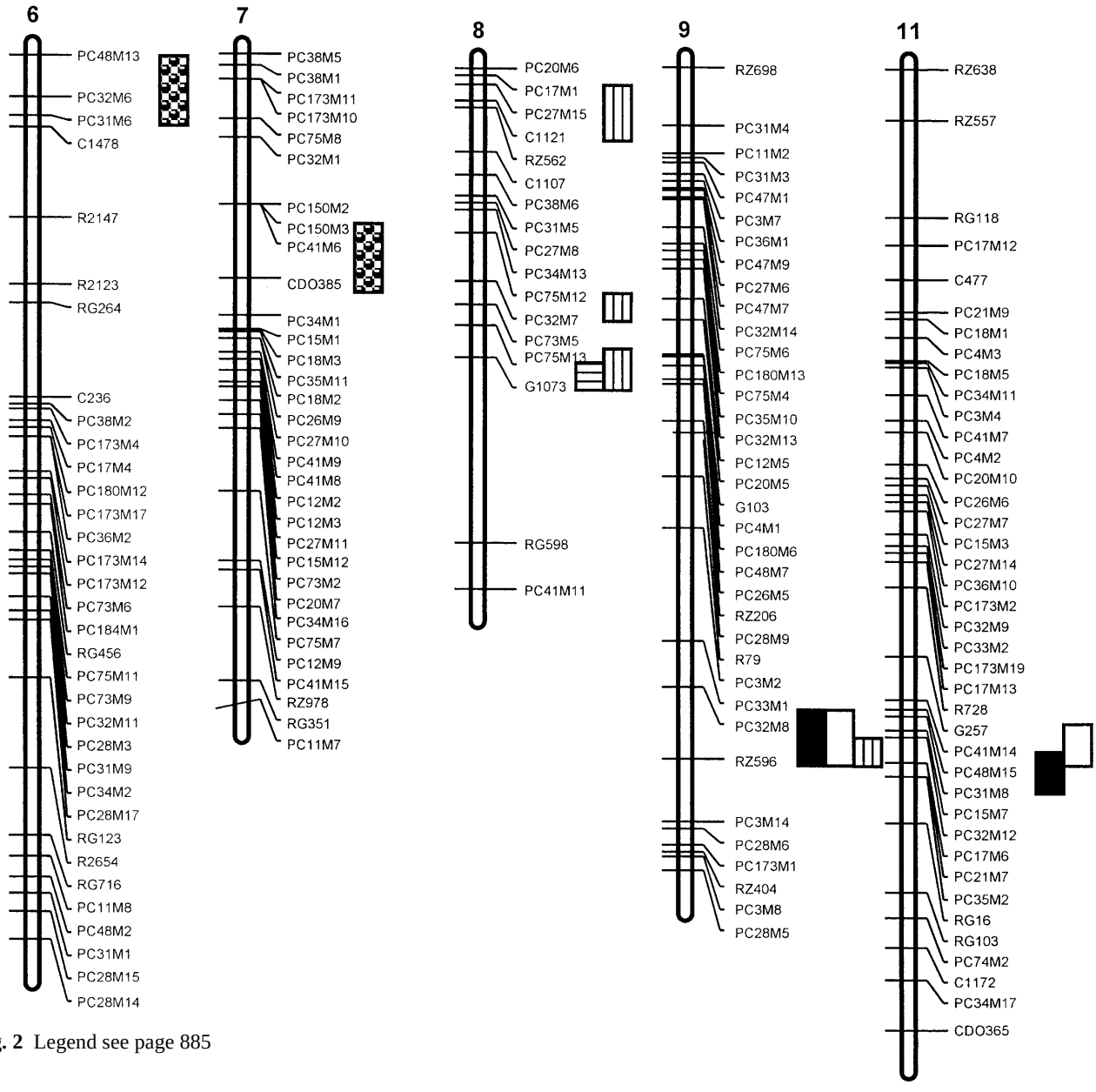

Fig. 2 Legend see page 885

to 30.0%. Only two of them, RG256-RG151 in chromosome 2 and PC75M3-PC11M4 in chromosome 4, were found in both experiments. In the combined analysis, 8 of the 12 QTLs were significant. For root thickness, eight QTLs were identified, with the phenotypic variation explained by a single QTL ranging from 6.2% to 23.2%. All of them were identified in only one of the two experiments (no common QTLs). In combined analysis, four of the eight QTLs were significant. Two QTLs for shoot biomass were in similar chromosome regions, either with QTLs for deep root traits in chromosome 4 or with QTLs for root thickness in chromosome 8. The approximate positions of putative QTLs that were significant in both separate and combined analysis are shown in Fig. 2.

All of the QTLs associated with shoot biomass, deep root traits, or root thickness in Fig. 2 interacted with other

Table 6 Number and general contribution (G_c) of epistasis QTLs for each trait identified in combined analysis. Both epistasis involving main effect QTLs and those consisting of only non-main effect QTLs are presented. Marker loci of epistasis of non-main effect QTLs are listed

Trait	Epistasis with main-effect QTLs[a]		Epistasis with only non-main effect QTLs[b]		
	Number	[c]G_c	Number	G_c	
Shoot biomass	1	0.009	2	0.025	AA72a-RG95 (2)[d] and PC33M10-PC74M7 (12) PC41M8-PC12M2 (7) and PC41M7-PC4M2 (11)
Deep root mass	4	0.099	3	0.057	PC32M3-RG171 (2) and PC38M8-PC35M14 (5) PC184M8-PC28M11 (4) and PC173M16-R1427 (4) PC173M7-PC34M5 (5) and PC73M3-PC36M5 (12)
Deep root ratio	3	0.054	2	0.038	PC41M3-PC48M6 (2) and RG901-PC33M11 (12) PC35M8-C499 (2) and RG351-PC11M7 (7)
Deep root per tiller	1	0.001	5	0.004	CDO328-BCD134 (1) and PC3M11-PC33M8 (2) PC12M1-PC3M10 (2) and PC48M8-PC17M6 (11) RG910-PC34M12 (3) and R728-G257 (11) PC31M6-C1478 (6) and PC4M4-PC21M6 (11) RG4-PC75M7 (7) and PC79M7-PC21M4 (12)
Rooting depth	3	0.025	1	0.019	RZ557-RG118 (11) and PC184M7-R3375 (12)
Root thickness 0–10 cm	2	0.119	0	0.000	–
Root thickness 20–25 cm	1	0.003	3	0.016	CDO328-BCD134 (1) and PC26M7-PC3M13 (1) RG83-RG144 (2) and PC79M8-PC33M4 (4) PC173M9-PC180M3 (5) and PC184M12-PC36M8 (11)

[a] Putative QTLs identified as significant in Fig. 2
[b] QTLs not identified as significant in Fig. 2, but identified as significant in epistasis analysis
[c] G_c, coefficient of determination; 1=100%
[d] The number in parenthesis is the chromosome number

QTLs or marker intervals that were not linked with any of the traits examined in this study, indicating significant epistasis. In total, 3, 22, and 6 pairs of epistasis were found for shoot biomass, deep root morphology, and root thickness traits, respectively (Table 6). Of these, one, ten, and four pairs, respectively involved putative QTLs identified in Table 5. One, seven, and three putative QTLs for shoot biomass, deep root morphology, and root thickness traits, respectively, were involved in epistasis either for the same traits or for other traits. The contribution of epistasis consisting of pairs of QTLs that were not linked with traits here (non-main-effect QTLs) was generally small (Table 6). Epistasis was relatively large for deep root mass (epistasis both with main-effect QTLs and with only non-main-effect QTLs), for deep root ratio (epistasis with main-effect QTLs), and for root thickness at a soil depth of 0-10 cm (epistasis with main-effect QTLs).

Combined analysis of the two experiments showed the general contributions of main additive effect, epistasis, additive-by-environment interaction, and epistasis-by-environment interaction (Table 7). Additive effect was high for rooting depth (0.268), root thickness at a soil depth of 0–10 cm (0.187), deep root mass (0.159), and deep root ratio (0.082). A comparable size of epistasis with additive effect was recorded for deep root mass (0.156), deep root ratio (0.086), and root thickness at a soil depth of 0–10 cm (0.149). QTL-by-environment interaction was generally small except for additive-by-environment interaction for deep root mass and for epistasis-by-environment interaction for deep root ratio.

Table 7 General contributions (coefficient of determination; 1=100%) of additive effect, epistasis, additive-by-environment (E) interaction and epistasis-by-environment (E) interaction for shoot biomass, deep root, and thick root traits from the combined analysis of two experiments

Trait	Additive	Epistasis	QTL×E	
			Additive × E	Epistasis × E
Shoot biomass				
Shoot biomass	0.017	0.034	0.003	0.000
Deep root morphology				
Deep root mass	0.159	0.156	0.083	0.000
Deep root ratio	0.082	0.086	0.046	0.158
Deep root per tiller	0.007	0.005	0.013	0.005
Rooting depth	0.268	0.050	0.063	0.000
Root thickness				
Root thickness 0–10 cm	0.187	0.149	0.033	0.066
Root thickness 20–25 cm	0.047	0.019	0.000	0.013

Discussion

Identification of QTLs

This study mapped, for the first time, QTLs for root morphology traits in recombinant inbred lines derived from a cross between two *indica* lowland rice lines (IR58821/IR52561). This population was also used by Ali et al. (2000) to map root penetration ability through wax-petrolatum layers. The number of marker intervals that were

linked with QTLs for deep root morphology and root thickness were 12 and 8, respectively, which was almost comparable to that observed with a *japonica/indica* population examined in a similar phenotyping system (Kamoshita et al. 2002), though the maximum effects caused by a single QTL to increased deep root mass or root thickness were slightly smaller. Unlike previous studies using populations from *japonica/indica* crosses (Yadav et al. 1997; Kamoshita et al. 2002), both parents were well adapted to anaerobic lowland conditions (Wade et al. 2000), possessing either a deep root system (IR58821) or a high capacity for osmotic adjustment (IR52561) (Kamoshita et al. 2000), and no skewness for the traits was observed among their progeny. Therefore, information on QTLs for root morphology from this mapping population could be highly relevant for improving the root system of rainfed lowland rice.

For example, one marker interval, RG256-PC32M10 in chromosome 2, contained QTLs for deep root morphology in both experiments in this study and also had QTLs for root penetration ability in the study of Ali et al. (2000). Another example is the interval on chromosome 4 (PC150M11-PC11M4), which not only contained QTLs for deep root morphology in both experiments, but also had QTLs for root thickness and shoot biomass. More detailed physiological analysis of this region in the future may provide a greater understanding of the relationship between these root traits and shoot biomass, and possibly grain yield.

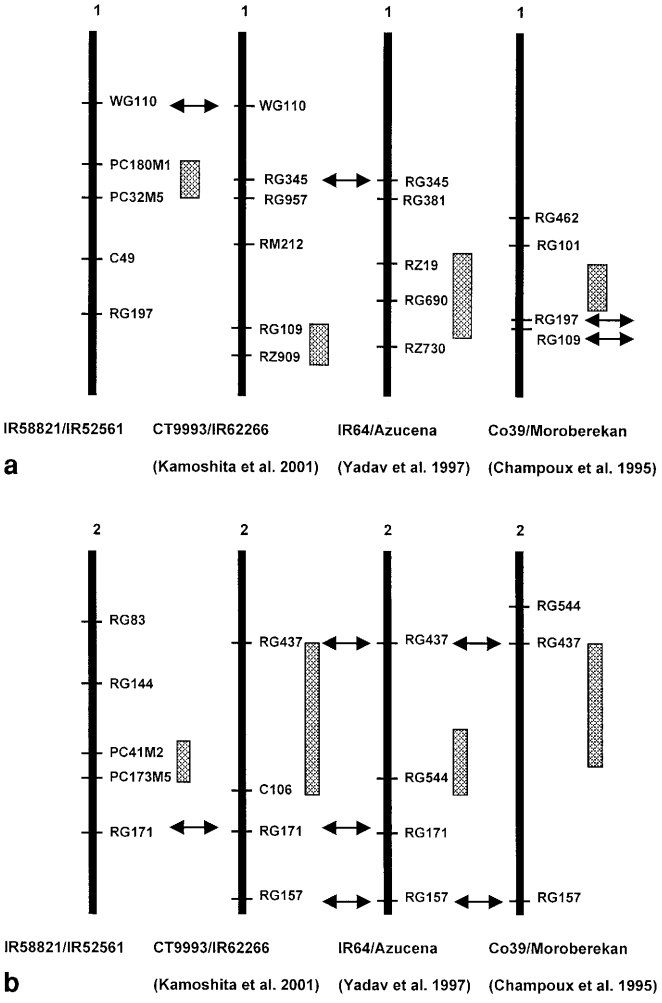

Fig. 3 Comparison of common QTLs for root thickness at a soil depth of 0–10 cm on chromosome 1 (**a**) and deep root per tiller on chromosome 2 (**b**) across mapping populations. Chromosome numbers are indicated *above* each segment of chromosome. The *vertical bars beside* the markers are the genomic regions associated with the traits. *Arrows* indicate common markers across mapping populations

Effect of phenotyping environment

Though the expression of shoot biomass, deep root morphology, and thick root traits was consistent between the two parental lines across the two environments, significant G×E interaction was observed among their progeny and many putative QTLs were identified in only one environment. This confirmed the previous study by Kamoshita et al. (2002) which showed a large effect of the phenotyping environment as defined by temperature and solar radiation on QTL identification for root morphology traits. With respect to agronomic traits such as heading date, plant height, and yield components, Lu et al. (1997) reported that 14 out of 22 QTLs identified in a total of three environments were detected in two or three environments. Compared with agronomic traits, the expression of root morphology traits and their QTLs is more likely to be affected by phenotyping environment, even without any water stress. These results suggest that it is critical to phenotype the QTLs in well-characterized target environments.

Comparison across mapping populations

Three common chromosome regions were found for root thickness traits, and eight common chromosome regions were found for deep root morphology traits across mapping populations in CT9993/IR62266 and IR58821/IR52561 under anaerobic conditions, IR64/Azucena and CO39/Moroberekan under aerobic conditions, and Bala/Azucena under aeroponic conditions (Table 8). Putative QTLs for root thickness were identified near WG110-RG109 in chromosome 1 in four different populations (Fig. 3a) and near PC75M3-PC11M4 in chromosome 4 in three different populations, which explained relatively large amounts of phenotypic variation (approx. 20–30%) in each of the populations. Putative QTLs for deep root mass per tiller were identified near RG437 and RG171 in chromosome 2 in four different populations (Fig. 3b). Segments near PC73M7-PC20M12 in chromosome 3 and near PC33M1-C570 in chromosome 9 were also linked with putative QTLs for deep root morphology traits in three different populations. In R3393-RG158 in chromosome 2 and in RG650-CDO38 in chromosome 7

Table 8 Common QTLs for deep root morphology and root thickness traits across different genetic backgrounds. The data of Kamoshita et al. (2002) for CT9993/IR62266, Yadav et al. (1997) for IR64/Azucena, Price and Tomos (1997) for Bala/Azucena, and Champoux et al. (1995) for Co39/Moroberekan were used

Chromosome	IR58821/IR52561			CT9993/IR62266			IR64/Azucena			Bala/Azucena			Co39/Moroberekan		
	Position	Trait[a]	%	Position	Trait	%	Position	Trait	%	Position	Trait	%	Position	Trait	%
Deep root morphology															
1	PC31M10-PC34M6	RD	6	RG957-C813	DRW DRW/RW DRW/TN	9 8 10	RG381-RZ730	DRW DRW/TN RD	8 11 9						
2	PC41M2-PC173M5	DRW/TN	8	RG437-ME1018	DRW DRW/RW DRW/TN RD	25 52 40 15	RG171-RG157	DRW/TN RD	5 10	R683-RG171	MRL	–[c]	RG437	DRW/TN	17
2				R3393-RG158	RD	17	PALI-RZ58	RD	9				? RG139	DRW/TN	12
2	?[b] RG256-RG151	DRW DRW/RW DRW/TN	17 17 22	? EM1813-ME97	RD	16									
3	PC73M7-PC20M12	DRW DRW/RW	13 8	EM119-RG409	DRW	15	RZ329-RZ892	DRW	–	C643	MRL	–			
3				RZ474-ME82	DRW	16	? RZ519	RD	10	C746	MRL	5			
4	RG218-PC79M8	RD	6				RG190	DRW DRW/SW	– –	RG620-RG190	MRL				
4	PC150M11-PC11M4	DRW DRW/RW RD	21 27 28	RG939-RG214	DRW/TN	5									
	RG214-C1016	RD	–												
5				ME513-RG403	DRW/RW DRW/TN	9 9	RZ390-RG403	DRW/SW RD	9 9	?R3166-R2232	DRW				
7				RG650-EM184	RD	11	RG711-RG351	DRW DRW/SW	15 22				RG351	DRW/TN	6
				RG404-CDO38	RD	16		DRW/TN RD	19 18						
9				G103-ME58	RD	5	RZ206-Rz422	DRW	4						
9	PC33M1-C570	DRW DRW/RW	11 11				Amy3ABC-RG667	DRW DRW/SW DRW/TN RD	6 5 7 9				RZ12	DRW/TN	5
11	G257-PC31M8	DRW DRW/RW	7 9	G320-ME26	DRW DRW/RW DRW/TN	36 42 –									
11				CDO365-EM1819	DRW DRW/RW DRW/TN	6 8 11				RG2	MRL	7			
				ME107-RG1109	RD	9									

Table 8 (continued)

Chromosome	IR58821/IR52561			CT9993/IR62266			IR64/Azucena			Bala/Azucena			Co39/Moroberekan		
	Position	Trait[a]	%	Position	Trait	%	Position	Trait	%	Position	Trait	%	Position	Trait	%
Root thickness															
1	? PC180M1-PC32M5	RT10	9	ME1014-RZ909	RT10	9	RZ730-RZ801	RT10	5				RG197	RT10	25
2				RG437-C106	RT25	22							RG437	RT10	15
2				R3393-RG158	RT10 RT25	16 18	PALI-RZ58	RT10	5						
3										? RG191	RT10	21	? RG104A	RT10	17
3				? EM194-RZ672	RT25 RT10	– –							? RZ394-RZ576	RT10	19
4	PC75M3-PC11M4	RT25	23	RG939-RG214	RT10 RT25	30 18							RG214	RT10	33
8				ME21-EM141	RT10	18	RZ66-RG418	RT10	8				RZ66	RT10	22
9	PC32M8-C570	RT	15										RZ12-RG662	RT10	19
11				ME67-EM1819	RT10	7							CDO365	RT10	21

[a] *DRW* deep root mass below 30 cm; *RW* total root mass; *TN* tiller number; *RD* maximum rooting depth; *SW* shoot biomass; *MRL* maximum root length; *RT10* root thickness measured at a soil depth 0–10 cm; *RT25* root thickness measured at 20–25 cm

[b] ?, Identification of commonness of the position not clear

[c] –, Data not available

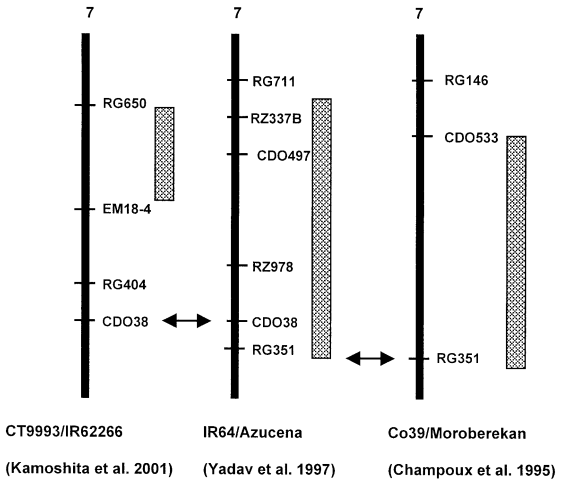

Fig. 4 Comparison of common QTLs for deep root morphology traits on chromosome 7 across *japonica/indica* mapping populations. Chromosome numbers are indicated *above* each segment of chromosome. The *vertical bars beside* the markers are the genomic regions associated with the traits. *Arrows* indicate common markers across mapping populations. The traits used to examine deep root morphology are maximum rooting depth in CT9993/IR62266 and deep root per tiller in IR64/Azucena and Co39/Moroberekan

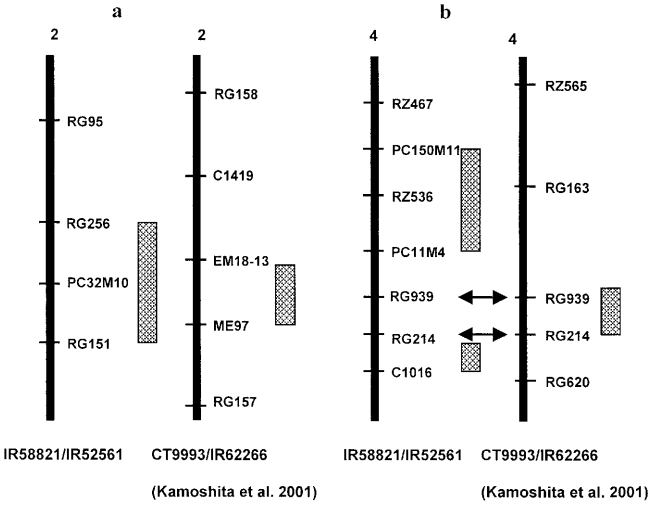

Fig. 5 Comparison of common QTLs for deep root morphology traits in IR58821/IR52561 and CT9993/IR62266 phenotyped under anaerobic conditions on chromosome 2 (**a**) and chromosome 4 (**b**). Chromosome numbers are indicated *above* each segment of chromosome. The *vertical bars beside* the markers are the genomic regions associated with the traits. *Arrows* indicate common markers across mapping populations. The traits used to examine deep root morphology under anaerobic conditions are deep root mass in IR58821/IR52561 and maximum rooting depth in CT9993/IR62266 on chromosome 2, and maximum rooting depth in IR58821/IR52561 and deep root per tiller in CT9993/IR62266 on chromosome 4

(Fig. 4), QTLs for deep root morphology were found only in the three *japonica/indica* populations but not in the *indica/indica* population (IR58821/IR52561). Three chromosome regions, RG256-RG151 in chromosome 2, PC150M11-PC11M4 in chromosome 4 (Fig. 5), and G257-PC31M8 in chromosome 11 were identified as regions with QTLs for deep root morphology traits only in the IR58821/IR52561 and CT9993/IR62266 populations. These two populations were phenotyped under anaerobic conditions and, hence, these QTLs may be involved in trait expression only under anaerobic conditions. Epistasis was significant in previous studies (Yadav et al. 1997; Kamoshita et al. 2002). Epistasis for deep root morphology in IR58821/IR52561 was comparable with that in CT9993/IR62266, but epistasis for root thickness tended to be larger.

Three common chromosome regions for deep roots (around RG158 in chromosome 2, RZ474 in chromosome 3, and RG650 in chromosome 7) and one common chromosome region for thick roots (around ME21 in chromosome 8) were identified across three *japonica/indica* populations but not identified in IR58821/IR52561. There are three possible reasons for this. First, the absence of the QTLs may have been due to a lack of polymorphism in that particular region of IR58821/IR52561. Second, phenotypic variation in root morphology traits was slightly less in IR58821/IR52561 than in the *japonica/indica* populations (Champoux et al. 1995; Yadav et al. 1997; Kamoshita et al. 2002), which may have contributed to the absence of the QTLs in IR58821/IR52561. However, the number of QTLs for root morphology traits in IR58821/IR52561 was comparable with or greater than the number observed in each of the four *japonica/indica* populations, suggesting that the second reason may not be valid. Third, the position of QTLs for root morphology traits may be different between *japonica/indica* and *indica/indica* populations because of different genetic backgrounds. Though only a single population from a cross between *indica* lines (IR58821/IR52561) was studied, which limits generalization, our study indicated that some of the QTLs for root traits identified in *japonica/indica* populations (Champoux et al. 1995; Price and Tomos 1997; Yadav et al. 1997; Kamoshita et al. 2002) may not be found at the same marker intervals if the target populations for selection are from an *indica/indica* cross.

It is of interest to note that the QTL for deep root ratio near RG256 was found in both experiments in IR58821/IR52561 and that the QTLs for root thickness near RG214 were identified in three out of the four experiments in CT9993/IR62266 (Kamoshita et al. 2002). Ali et al. (2000) also reported that RG256-PC32M10 in chromosome 2 consistently located QTLs for root penetration index in two experiments in IR58821/IR52561. Shen et al. (1999) targeted four segments for single QTL selection for root traits in IR64/Azucena (around RZ730 in chromosome 1, RG157 in chromosome 2, RG351 in chromosome 7, and RG667 in chromosome 9). All of these segments were identified in our study to contain QTLs for deep or thick root morphology in at least three different populations. These four segments and RG214 were also located with QTLs for leaf rolling as a measure of drought avoidance during the seedling or vegeta-

tive stages in multiple field experiments (Champoux et al. 1995).

Implications for selection

The effects of environment (defined by solar radiation and temperature) on the identification of QTLs for constitutive root morphology traits were complex, as QTL-by-environment interactions were significant, even in the absence of water stress. This result emphasizes the importance of defining conditions for phenotyping which relate closely to the target environment where the traits are to be expressed, and in reproducing those conditions consistently. Even plant age and size can influence the expression of such quantitative traits (Kamoshita et al. 2002). For rainfed lowland, the plants will also encounter water stress, so identification of QTLs for adaptive root morphology traits is also important. Since the types of drought likely to be encountered will vary with location and season, it is critical to phenotype the QTLs in a wide range of target environments. This work also highlights the need to characterize environments adequately. For rainfed lowland rice, both the magnitude and the frequency of drought need to be characterized. QTLs can then be phenotyped within these characterized target environments. Large-scale field phenotyping for constitutive and adaptive root traits is now in progress in India and in Thailand, as are efforts to better characterize these environments (McLaren and Wade 2000).

Although there is evidence from greenhouse experiments simulating rainfed lowland conditions that improved constitutive root traits prior to the onset of water stress were beneficial in subsequent rainfed lowland drought conditions (Azhiri-Sigari et al. 2000; Kamoshita et al. 2000; Wade et al. 2000), only minimal evidence is presently available from field conditions (Samson and Wade 1998). Research is now in progress to quantify the relationships between improved constitutive and adaptive root traits, water extraction, nutrient uptake, and grain yield in rainfed lowland conditions in the field, using progenies from QTL mapping (Salekdeh et al. 2002). Near-isogenic lines (NILs) are also being developed to determine QTL functions and to evaluate their contributions to improved root growth, water extraction, and grain yield.

When suitable target chromosome segments are identified for use in introgression, sets of lines with single desirable QTLs for root traits in different chromosome regions can be produced (Shen et al. 1999). It should be possible to pyramid such desirable QTLs for root traits in one line and quantify epistasis effects among these candidate genes (Charmet et al. 1999). Marker-aided selection could then be instigated for QTLs of proven contribution, leading to the production of lines with improved root traits and improved drought tolerance. But since this study has indicated that some QTLs for root traits may not be found at the same marker intervals if the target populations for selection are from a *japonica/indica* cross as compared with an *indica/indica* cross, separate markers may need to be developed for different breeding populations. As research proceeds to examine QTL functions with NILs and QTL contributions to grain yield and drought tolerance and as further mapping populations are phenotyped in a range of field conditions, the basis for proceeding with marker-aided selection for drought tolerance will become more clear.

Conclusions

This study identified QTLs for constitutive root morphology traits under anaerobic conditions by studying a mapping population derived from a cross between two *indica* rice lines adapted to rainfed lowland conditions. As phenotyping environment, defined by solar radiation and temperature, had large effects on trait expression and the identification of QTLs, even in the absence of water stress, QTL-by-environment interaction was significant. Nevertheless, several marker intervals (e.g., WG110-RG109 in chromosome 1, RG437-RG171 in chromosome 2, and PC75M3-PC11M4 in chromosome 4) were identified as QTLs for root morphology traits across populations with different genetic backgrounds. Some of them were found only in *japonica/indica* populations, whereas others were found in populations that were phenotyped under anaerobic conditions. The QTL for deep root morphology in RG256-PC32M10 in chromosome 2 was found in both experiments with IR58821/IR52561 but was found only in populations that were phenotyped under anaerobic conditions. The PC75M3-PC11M4 interval in chromosome 4 contained QTLs for shoot biomass together with deep and thick root traits. These results identifying chromosome regions that had QTLs for deep root morphology and root thickness traits over different mapping populations indicate a potential for marker-aided selection for these traits.

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