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Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice

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Abstract A deep thick root system has been demonstrated to have a positive effect on yield of upland rice under water stress conditions. Molecular-marker-aided selection could be helpful for the improvement of root morphological traits, which are otherwise difficult to score. We studied a doubled-haploid population of 105 lines derived from an *indica* × *japonica* cross and mapped the genes controlling root morphology and distribution (root thickness, maximum root length, total root weight, deep root weight, deep root weight per tiller, and deep root to shoot ratio). Most putative QTL activity was concentrated in fairly compact regions on chromosomes 1, 2, 3, 6, 7, 8 and 9, but was widely spread on chromosome 5 and largely absent on chromosomes 4, 10, 11 and 12. Between three and six QTLs were identified on different chromosomes for each trait. Individual QTLs accounted for between 4 and 22% of the variation in the traits. Multiple QTL models accounted for between 14 and 49%. The main QTLs were common between traits, showing that it should be possible to modify several aspects of root morphology simultaneously. There was evidence of interaction between marker locations in determining QTL expression. Interacting locations were mostly on different chromosomes and showed antagonistic effects with magnitudes large enough to mask QTL detection. The comparison of QTL locations with another population showed that one to three common QTLs per trait were recovered, among which the most significant was in one or other population. These results will allow the derivation of isogenic lines introgressed with these common segments, separately in the *indica* and *japonica* backgrounds.

Key words Upland rice · Doubled-haploid lines · Root morphology · Molecular markers · QTL analysis

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Introduction

Upland rice (*Oryza sativa* L.) is grown on about 19 million ha worldwide (IRRI 1995). It is the dominant rice ecosystem in Africa and Latin America, and can be locally important in countries of South and Southeast Asia. Upland rice is grown under aerobic conditions and relies strictly on rainfall as the source of its water supply. Drought constitutes a major source of yield reduction and instability, and increasing drought resistance is one of the main objectives of upland rice breeding programs.

A deep and thick root system, with a high ratio of deep root weight to shoot weight and high deep root length density, are factors contributing to resistance to intermittent drought stress in upland rice (Ahmadi 1982; Passioura 1982; Yoshida and Hasegawa 1982; Fukai and Cooper 1995). Moreover, the interest of an improved root system goes beyond the problem of drought as it would also improve competitiveness with weeds and nutrient acquisition, both traits essential for upland rice in the Asian systems. A deeper root system should colonize a larger soil volume and improve the water uptake from the lower layers where water is expected to be available. This would help to maintain a good plant water potential which has a demonstrated positive effect on yield under stress (Mambani and Lal 1983). Thick roots are known to have a wider xylem diameter and consequently less axial resistance to water flow along the xylem, thereby enhancing water uptake through suction (Passioura 1982), though some

results suggest that radial resistance (through cortex and stele to xylem) may be more limiting to water movement than axial resistance (Yambao et al. 1992).

Root thickness is relatively easy to measure, even under field conditions, but this is not true for other characteristics of the mature root system. All the available analytical techniques for evaluating root traits are tedious and time-consuming (Robertson et al. 1985). Hence, these traits have been little used in plant breeding programs. For traits as difficult to evaluate as these, marker-aided selection is likely to enhance the efficiency of selection. If certain alleles at particular marker loci are consistently associated with effects on traits of interest, they could be used as the means of indirect selection, complementing or even replacing phenotypic selection.

The final configuration of a root system under field conditions is largely determined by factors such as chemical gradients, soil moisture content, mechanical impedance, and aeration (Lynch 1995), which are in turn affected by soil, climate, and cropping system. Although the plasticity of the rice root system is huge, large genetic variation in root morphology has been reported in germplasm adapted to different agro-ecological conditions (O'Toole and Bland 1987). *Indica* and tropical *japonica* subspecies of rice, adapted to lowland and upland agro-ecological conditions respectively, differ generally in their root morphology and rooting patterns: cultivars of upland origin are more deeply rooted and have a larger diameter of main axes compared with cultivars of lowland origin (Yoshida and Hasegawa 1982; Courtois et al. 1996).

In a recent study, restriction fragment length polymorphism (RFLP) markers associated with quantitative trait loci (QTLs) controlling some aspects of root morphology and drought avoidance were identified in a population derived from an *indica* × *japonica* cross (Champoux et al. 1995). However, it is not yet known whether QTLs would be found at the same positions in different genetic backgrounds or if they would show the same magnitude of effect. Not much is known about the QTL × environment interaction in rice. In other crops, several authors have reported that the majority of the QTLs were localized in similar genomic regions across environments (Stuber et al. 1992) and mapping populations (Albert et al. 1991; Helentjaris 1992; Doebley and Stec 1993), while others have demonstrated that only some of the QTLs were recovered in different environments (Paterson et al. 1991; Bubeck et al. 1993) and in different populations (Beavis et al. 1991; Huang et al. 1996). The utility of the QTLs will depend on the magnitude of their effect across populations and on their degree of independence from the genetic background. The strategy for their utilization will depend on the breeding goal: stability (accumulation of all compatible alleles found in different environments) or specific adaptation (introgression of alleles highly favorable in a given environment).

The goal of the present study was to identify QTLs controlling root morphology in a doubled-haploid (DH) population derived from an *indica* × *japonica* cross between parents with contrasting behavior, and to compare them with the QTLs identified by Champoux et al. (1995). The results will be used to derive introgression lines carrying the most interesting QTLs.

Materials and methods

Material

A population of 105 DH lines derived by Guiderdoni et al. (1992) from a cross between IR64, an irrigated *indica* variety, and Azucena, an upland tropical *japonica* variety, was used in this study. The parents of this population were demonstrated to have contrasting root morphology, notably in the capacity of nodal roots to reach deep layers, under upland field conditions in Cavinti, The Philippines, as well as under controlled greenhouse conditions at IRRI, Los Baños (B. Courtois, unpublished). The population itself had been mapped for several important agronomic traits which made it particularly valuable for the analysis of favorable and unfavorable associations between traits.

Map construction

A RFLP map of this population was established by Huang et al. (1994) from an initial population of 135 lines. Parents were evaluated for RFLP polymorphism using six restriction enzymes (*Dra*I, *Eco*RV, *Hin*dIII, *Sca*I, *Xba*I and *Eco*RI). A total of 135 polymorphic RFLP markers identified in the parental survey were used to construct a linkage map using Mapmaker (Lander et al. 1987). This map, with an average distance of 13.4 cM between markers, was recently updated by adding 40 new isozyme and/or RAPD markers (Huang et al., in preparation) and presently contains 175 markers with an average distance of 10.3 cM between markers. However, on all chromosomes, there are still a few segments of roughly 20 to 30 cM without markers. In comparison with the first map, the orientation of several chromosomes was modified to take into account the recent localization of the centromeres in rice, the markers on short arms being placed on the top of the figure (Khush et al. 1996). The new map was utilized in this study for the determination and localization of the QTLs.

Methods

Growth and measurement of the plants

The DH lines along with the parents were evaluated in a greenhouse experiment conducted at IRRI in 1995 in a completely randomized design. Four replications of one plant per line were distributed over two runs of two replications each, staggered in time because of space constraints. The plants were grown under aerobic conditions in well-drained plastic bags sleeved into polyvinyl chloride cylinders, 1.0-m long and 0.2-m in diameter and filled with uniform sandy loam soil (12% clay, 21% silt, 67% sand; $pH_{water\ 1:1}$ of 6.6). The soil strength in the tubes was determined using a penetrometer. The cone penetrometer resistance was relatively low, ranging from 6.0 kg/cm^2 on the surface to 4.0 kg/cm^2 at the 0.5-m depth, and uniform across different tubes, signifying limited constraints to root growth. The average daily minimum and maximum temperatures in the

greenhouse were 25°C and 38°C during the experiment. The plants were watered three times a week with 500-ml of culture solution (Yoshida et al. 1976).

Thirty five days after sowing, when differences in root depth between parents were clearly expressed on sacrificed check plants, the number of tillers per plant (NBT) was counted. Shoots and roots were separated and the shoot dry weight (SDW) determined after oven-drying the shoots at 65°C for 72 h. The soil column was cut in three sections of 30 cm each (0 to 30 cm, 30 to 60 cm, and 60 to 90 cm). The maximum root depth (MRL) reached by the plant was then determined by searching sequentially the longest nodal root in the column beginning from the lowest section. The roots from each section were then carefully washed. The average thickness of five nodal roots (THK) was measured 2 cm below the tillering plateau using a micrometer. Washed roots from different sections were stored in 70% ethanol. The root lengths in the 30 to 60 cm and 60 to 90 cm sections were determined after staining the roots with methyl violet blue (0.5% w : v for 10 min), using a scanner connected to an image-analysis system. The root length densities in the two lower sections were averaged to obtain the deep root length density (DRL). The root length in the 0 to 30 cm section was not measured, except on the parents, due to the volume of roots in this layer and the time necessary to spread the roots on the scanning table. Root samples were then oven-dried for 72 h at 65°C and weighed to determine the root dry weight in the three sections. The total root weight (TRW) was obtained by summing the weight of the three sections, and the deep root weight (DRW) by summing the weight of the 30 to 60 cm and 60 to 90 cm sections. Deep root dry weight per tiller (DR/T) and deep root to shoot dry weight ratio (DR/S) were derived from these parameters as a measure of the balance between water absorption and transpiration. The parameters linked with root distribution at depth being our main focus of interest, the total root weight per tiller (R/T) and total root per shoot dry weight ratio (R/S) were only computed to allow comparisons with data from Champoux et al. (1995).

Statistical analysis

Analyses of variance were performed to determine the genetic variation between lines of the DH population for all the measured traits. Broad-sense heritabilities at the genotypic mean level were computed as $h^2_F = \sigma^2_G/(\sigma^2_G + \sigma^2_e/n)$ where σ^2_G and σ^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. Genotypic correlations between traits were determined as $r = \sigma_{ij}/\sigma_i\sigma_j$ where σ_{ij} is the genetic co-variance between traits i and j and σ_i and σ_j the genetic standard deviation for traits i and j, respectively.

QTL analysis

QTL analysis was performed on the mean of the four replications. Chromosome regions likely to contain QTLs controlling the six main traits were identified using single-marker analysis. The location and magnitude of effects for putative QTLs for all six morphological traits were refined using flanking marker regression analysis (Martinez and Curnow 1992). A model assuming complete interference was used. There were few missing marker values for almost all markers; hence, for each marker interval, data records with missing values for either marker were simply excluded from the analysis. More than 50% of records had missing values for marker RG229 on chromosome 5, however, so that this marker was excluded from the flanking marker analysis. Chromosome segments showing significant QTLs at the 1% level for any trait were identified as active regions. All tests in these segments that were significant at 5% or better were considered to indicate possible QTL positions. Estimates of the effects of a single allele substitution (assuming additivity between alleles) and R^2 values for each of these putative QTLs were computed.

For each trait, all putative QTLs in the active chromosome regions were fitted together in a stepwise linear model to estimate the proportion of variability of each trait accounted for by the most promising combination of QTLs. For this analysis, the probabilities of Azucena alleles in given marker classes were estimated from the nearest non-missing markers in cases where flanking marker classes were missing (Martinez and Curnow 1994).

Finally, interaction between putative QTLs was investigated by testing the interaction between trait means in the four categories defined by the marker genotypes for all pairs of markers in the active chromosome regions. Significant interaction indicates that the effect of an allele substitution at a marker nearest to a QTL is affected by the second marker genotype, or another QTL near the second marker. All analyses were carried out with the BSTAT statistical software developed at IRRI and available from the author (McLaren 1996).

Comparison of QTLs in IR64 × Azucena and Co39 × Moroberekan

The sets of polymorphic markers were different in the two populations. For the five common traits, the comparison was performed using the linkage map established by Causse et al. (1994) as a bridge to determine the relative order of the two sets of markers. The QTLs taken into account for IR64 × Azucena were all putative QTLs present in the most active segments; for Co39 × Moroberekan, those identified by Champoux et al. (1995) were employed.

Results

Statistical parameters of the population

The phenotypic values of the parents and of the DH population are presented in Table 1. The two parents were different, with Azucena having a higher phenotypic value than IR64 for most examined traits except total root weight and total root to shoot ratio. The comparison of root weight in different soil sections revealed that, despite the similarity in aboveground and underground biomass of the two parents at the stage we harvested the experiment, their rooting patterns were different. The roots of IR64 were proportionally more concentrated in the upper soil layer with 91% of the total root dry weight in the 0 to 30 cm layer while Azucena had more root weight in the deeper soil layer with 73% in the 0 to 30 cm layer. For root weight per tiller and deep root weight per tiller, the differences between the parents are even more acute as a result of the low tillering ability of Azucena compared with that of IR64.

The mean frequency histograms were monomodal though not normally distributed for all traits except root thickness, suggesting oligogenic or polygenic control. The analyses of variance revealed highly significant genotypic differences among the lines for all the root traits examined.

Broad-sense heritabilities were observed to be moderate to high (Table 1). Among the six main root traits

Table 1 Phenotypic values of the parents and the DH population

Trait	Abbr.	IR64	Azucena	DH[a] Mean	DH sd[b]	DH Range	Broad sense h^2[c]
Root thickness (mm)	THK	1.06	1.20	1.08	0.09	0.90–1.36	0.84
Maximum root length (cm)	MRL	67.2	99.3	81.8	12.1	47.3–103.5	0.77
Root dry weight 0–30 cm (mg)	SRW	1177	819	984	294	478–2111	0.72
Root dry weight below 30 cm (mg)	DRW	122	299	171	91	20–478	0.60
Total root dry weight (mg)	TRW	1299	1118	1156	357	562–2440	0.71
Root length density below 30 cm (cm/cm^3)	DRL	0.133	0.205	0.112	0.049	0.026–0.292	0.49
Total root dry weight per tiller (mg)	R/T	72	141	114	35	54–252	0.69
Root dry weight below 30 cm per tiller (mg)	DR/T	7	36	17	10	3–49	0.61
Root dry weight/shoot dry weight ratio (%)	R/S	16.8	16.8	20.1	3.5	13.5–31.6	0.55
Deep root dry weight/shoot dry weight ratio (%)	DR/S	1.2	4.3	2.8	1.1	0.5–5.7	0.62

[a] DH = Doubled-haploid lines
[b] sd = standard deviation
[c] h^2 = heritability

Table 2 Genotypic correlations between main root morphological traits

Trait	Abbr.	THK	MRL	TRW	DRW	DR/T
Root thickness	THK					
Maximum root length	MRL	0.55				
Total root weight	TRW	0.32	0.33			
Deep root weight	DRW	0.51	0.77	0.77		
Deep root weight/tiller	DR/T	0.52	0.74	0.50	0.88	
Deep root/shoot ratio	DR/S	0.44	0.83	0.43	0.90	0.84

evaluated, root thickness had the highest heritability (0.84) while deep root length density (DRL) had the lowest heritability (0.49).

The genetic correlations between traits are presented in Table 2. All the root traits studied were positively correlated with some high values due to the redundancy of the measured traits. We examined the genetic correlations of root length density in a given soil layer and root weight density in the same layer (Table 3). They were extremely high in all soil sections (ranging from 0.95 to 0.98), indicating that root weight is an excellent predictor of root length under our experimental conditions. For the following analyses, only root-weight parameters were used since the precision in their measurement was higher than for root length.

Table 3 Genotypic correlations between root density and root length density

Trait	Root length density 30–60 cm	Root length density 60–90 cm	Root length density 30–90 cm
Root density 0–30 cm	0.66	0.40	0.59
Root density 30–60 cm	0.97	0.86	0.95
Root density 60–90 cm	0.91	0.98	0.94
Root density 30–90 cm	0.98	0.91	0.97
Average root density	0.79	0.55	0.73

Identification of QTLs associated with root morphological traits

The results of the single-marker analysis are presented in Fig. 1. The threshold was fixed at 0.05. This meant a high risk of spurious QTLs but was useful for allowing comparisons with other populations. Table 4 contains the results of the flanking-marker-regression analysis with estimates of location and magnitude for all putative QTLs in the most active regions of the genome. The percentage of phenotypic variance accounted for by each putative QTL and the significance level are indicated in Table 4 for the six morphological traits analyzed. The segments shown in Table 4 contain all tests significant at 1% or higher over the whole genome. In addition all tests significant at the 5% level in the selected regions are indicated. A total of 264 test positions (44 tests per trait) are shown. Of these, 123 (47%) were significant, in most cases at a level higher

Fig. 1 QTLs controlling six traits determining root morphology and distribution in a IR64 × Azucena doubled-haploid population (single-marker analysis with $P < 0.05$). The distances between markers on the figure are approximate

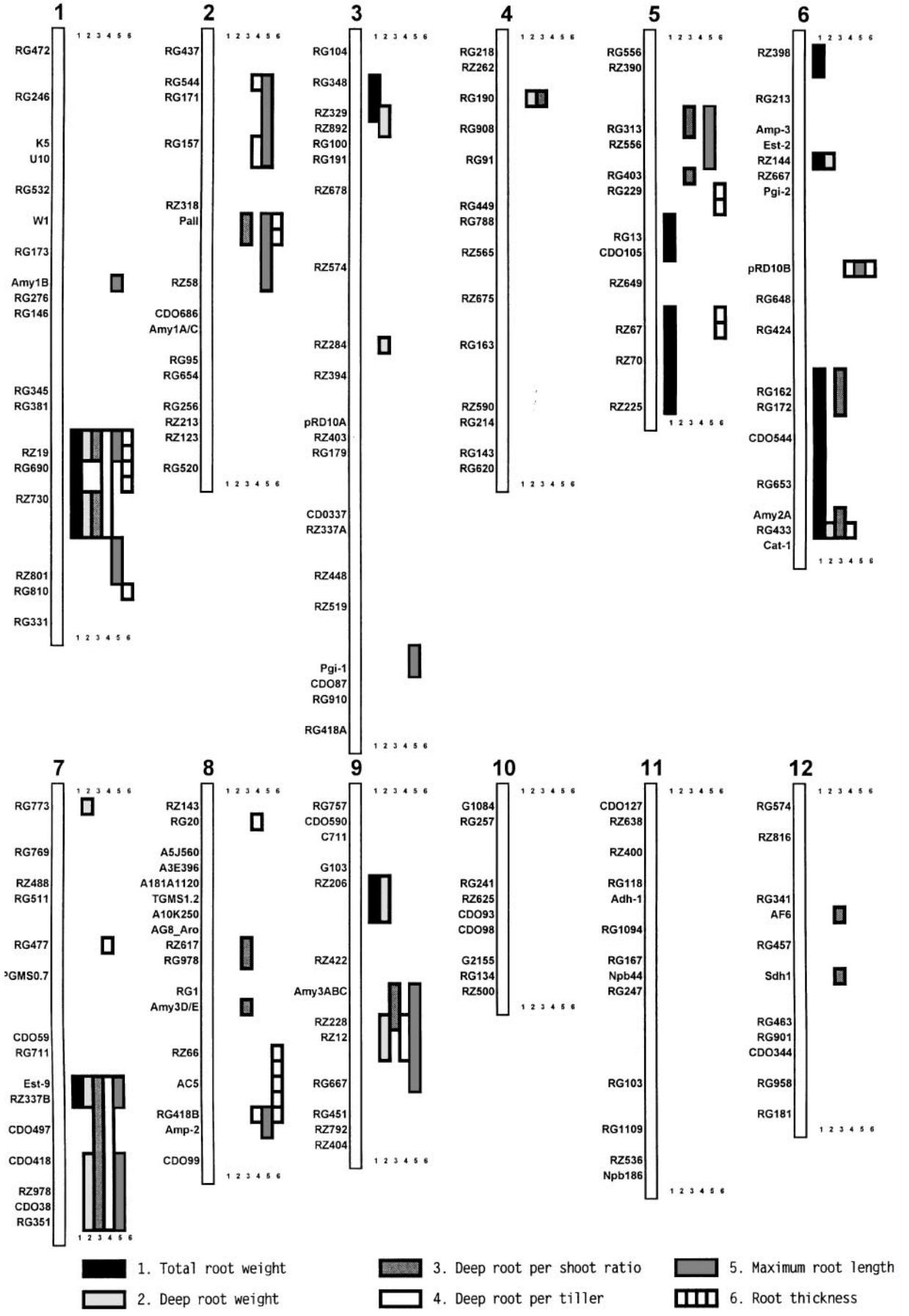
1
RG472
RG246
K5
U10
RG532
W1
RG173
Amy1B
RG276
RG146
RG345
RG381
RZ19
RG690
RZ730
RZ801
RG810
RG331
2
RG437
RG544
RG171
RG157
RZ318
Pall
RZ58
CDO686
Amy1A/C
RG95
RG654
RG256
RZ213
RZ123
RG520
3
RG104
RG348
RZ329
RZ892
RG100
RG191
RZ678
RZ574
RZ284
RZ394
pRD10A
RZ403
RG179
CD0337
RZ337A
RZ448
RZ519
Pgi-1
CDO87
RG910
RG418A
4
RG218
RZ262
RG190
RG908
RG91
RG449
RG788
RZ565
RZ675
RG163
RZ590
RG214
RG143
RG620
5
RG556
RZ390
RG313
RZ556
RG403
RG229
RG13
CDO105
RZ649
RZ67
RZ70
RZ225
6
RZ398
RG213
Amp-3
Est-2
RZ144
RZ667
Pgi-2
pRD10B
RG648
RG424
RG162
RG172
CDO544
RG653
Amy2A
RG433
Cat-1
7
RG773
RG769
RZ488
RG511
RG477
PGMS0.7
CDO59
RG711
Est-9
RZ337B
CDO497
CDO418
RZ978
CDO38
RG351
8
RZ143
RG20
A5J560
A3E396
A181A1120
TGMS1.2
A10K250
AG8_Aro
RZ617
RG978
RG1
Amy3D/E
RZ66
AC5
RG418B
Amp-2
CDO99
9
RG757
CDO590
C711
G103
RZ206
RZ422
Amy3ABC
RZ228
RZ12
RG667
RG451
RZ792
RZ404
10
G1084
RG257
RG241
RZ625
CDO93
CDO98
G2155
RG134
RZ500
11
CDO127
RZ638
RZ400
RG118
Adh-1
RG1094
RG167
Npb44
RG247
RG103
RG1109
RZ536
Npb186
12
RG574
RZ816
RG341
AF6
RG457
Sdh1
RG463
RG901
CDO344
RG958
RG181
1. Total root weight
2. Deep root weight
3. Deep root per shoot ratio
4. Deep root per tiller
5. Maximum root length
6. Root thickness

Table 4 Results of flanking marker regression tests for QTLs affecting root morphology. POSN = least squares estimate of the distance from the first marker to the putative QTL; W = least squares estimate of the effect of substituting a single Azucena allele into IR64 background at the putative QTL; R^2 = percent of phenotypic variability accounted for by the putative QTL; SIG = significance level of the test for QTLs:

Chr. no.	Flanking markers		Separation (cM)	TRW				DRW				DR/S			
				Posn	W	R2	Sig	Posn	W	R2	Sig	Posn	W	R2	Sig
1	RG381	RZ19	23.5	23.5	.1114	8.6	**	23.5	.0203	5.0	*	.	.	.	
1	RZ19	RG690	8.2	**4.4**	**.1190**	**9.6**	**	5.3	.0208	5.5	*	7.3	.278	5.6	*
1	RG690	RZ730	13.2	8.7	.1232	11.3	**	13.2	.0210	6.0	*	**13.2**	**.257**	**5.1**	*
1	RZ730	RZ801	33.1	7.9	.1383	11.9	**	**7.3**	**.0276**	**7.5**	*	0.0	.291	6.1	*
2	RG171	RG157	22.2	.	.	.		.	.	.		.	.	.	
2	RG157	RZ318	27.4	.	.	.		.	.	.		.	.	.	
2	RG318	PALI	6.3	.	.	.		.	.	.		.	.	.	
2	PALI	RZ58	29.3	.	.	.		.	.	.		15.2	.280	4.5	*
3	RZ519	PG-1	32.1	.	.	.		.	.	.		.	.	.	
3	PGI-1	CDO87	7.1	.	.	.		.	.	.		.	.	.	
5	RZ390	RG313	35.0	.	.	.		.	.	.		29.4	.377	7.8	**
5	RG313	RZ556	6.6	.	.	.		.	.	.		0.0	.300	6.1	*
5	RZ556	RG403	19.4	.	.	.		.	.	.		**19.4**	**.345**	**9.0**	**
5	RG403	RG13	25.2	**25.2**	**−.0944**	**7.2**	**	.	.	.		0.0	.342	8.7	**
5	RG13	CDO105	4.2	0.2	−.0829	5.4	*	.	.	.		.	.	.	
5	CDO105	RZ649	9.5	0.0	−.0787	4.9	*	.	.	.		.	.	.	
5	RZ649	RZ67	21.3	21.3	−.1060	9.4	**	.	.	.		.	.	.	
5	RZ67	RZ70	12.8	2.8	−.1079	9.3	**	.	.	.		.	.	.	
5	RZ70	RZ225	19.7	**3.9**	**−.1247**	**9.9**	**	.	.	.		.	.	.	
6	RG424	RG162	30.4	29.2	−.1059	8.7	**	.	.	.		30.4	−.282	5.9	*
6	RG162	RG172	4.2	0.0	−.0866	6.0	*	.	.	.		4.2	−.261	5.0	*
6	RG172	CDO544	11.8	8.7	−.1070	7.8	**	.	.	.		0.0	−.260	4.9	*
6	CDO544	RG653	19.6	**8.0**	**−.1266**	**10.5**	**	.	.	.		.	.	.	
6	RG653	AMY2A	10.8	0.2	−.1144	10.4	**	.	.	.		.	.	.	
6	AMY2A	RG433	4.4	4.4	−.1227	10.7	**	4.4	−.0250	8.3	**	4.4	−.357	8.4	**
6	RG433	CAT-1	8.6	0.0	−.1202	10.1	**	0.0	−.0250	8.0	**	0.0	−.360	8.3	**
7	RG711	EST-9	14.1	14.1	.0937	6.9	*	14.1	.0262	7.6	*	14.1	.330	7.5	*
7	EST-9	RZ337B	3.2	0.0	.0916	6.5	*	0.0	.0258	7.7	**	3.2	.350	9.2	**
7	RZ337B	CDO497	13.0	.	.	.		4.3	.0230	5.9	*	8.6	.361	8.7	**
7	CDO497	CDO418	15.1	15.1	.0813	5.4	*	**15.1**	**.0336**	**14.7**	***	15.1	.516	20.5	***
7	CDO418	RZ978	11.5	**7.8**	**.0801**	**4.8**	*	1.4	.0341	14.9	***	**0.8**	**.543**	**22.3**	***
7	RZ978	CDO38	5.8	.	.	.		5.8	.0279	9.2	**	1.8	.449	14.4	***
7	CDO38	RG351	9.4	.	.	.		0.0	.0268	8.6	**	0.0	.393	11.9	**
8	AMY3D/E	RZ66	25.1	.	.	.		.	.	.		8.3	.337	4.4	*
8	RZ66	AC5	11.8	.	.	.		.	.	.		.	.	.	
8	AC5	RG418B	11.0	.	.	.		.	.	.		7.0	.283	4.6	*
8	RG418B	AMP-2	6.4	.	.	.		.	.	.		0.0	.275	4.3	*
9	G103	RZ206	7.2	**7.2**	**.1034**	**7.9**	**	.	.	.		.	.	.	
9	RZ206	RZ422	36.1	1.8	.1072	8.2	**	**5.1**	**.0204**	**4.3**	*	.	.	.	
9	RZ422	AMY3ABC	11.2	.	.	.		.	.	.		11.2	.240	4.2	*
9	AMY3ABC	RZ228	9.4	.	.	.		9.4	.0200	5.0	*	**9.4**	**.270**	**5.2**	*
9	RZ228	RZ12	4.9	.	.	.		0.0	.0219	5.6	*	0.0	.260	4.9	*
9	RZ12	RG667	7.4	.	.	.		0.0	.0212	5.2	*	.	.	.	
Average SE for W					.0414				0.104				.135		
R^2 for multiple-QTL model						43.4				22.7				32.6	

* = 5%, ** = 1% and *** = 0.1%. Results shown in bold represent the Multiple-QTL Model selected by stepwise regression from all significant QTLs for each trait. TRW = total root weight; DRW = deep root weight; DR/S = deep root per shoot ratio; DR/T = deep root weight per tiller; MRL = maximum root length; THK = root thickness

DR/T				MRL				THK			
Posn	W	R2	Sig	Posn	W	R2	Sig	Posn	W	R2	Sig
23.5	2.70	9.7	**	**23.5**	**3.661**	**8.9**	**	23.5	.0278	8.5	**
6.6	2.70	11.6	**	2.1	3.869	8.9	**	**3.7**	**.0314**	**10.4**	**
13.2	2.74	10.3	**	.	.	.		.	.	.	
2.3	**3.02**	**11.2**	**	.	.	.		17.5	.0228	4.6	*
16.2	**2.12**	**4.8**	*	**20.4**	**3.812**	**9.9**	**	.	.	.	
.	.	.		6.3	3.793	8.9	**	.	.	.	
.	.	.		6.3	3.059	7.6	*	.	.	.	
.	.	.		12.3	4.123	8.6	**	**0.0**	**.0205**	**5.0**	*
.	.	.		**32.1**	**3.828**	**9.6**	**	.	.	.	
.	.	.		0.0	3.033	6.7	*	.	.	.	
.	.	.		27.6	3.967	8.2	**	.	.	.	
.	.	.		**6.6**	**3.487**	**8.4**	**	.	.	.	
.	.	.		0.6	3.358	7.7	**	.	.	.	
.	.	.		.	.	.		.	.	.	
.	.	.		.	.	.		.	.	.	
.	.	.		.	.	.		.	.	.	
.	.	.		.	.	.		**21.3**	**−.0201**	**5.1**	*
.	.	.		.	.	.		.	.	.	
.	.	.		.	.	.		.	.	.	
.	.	.		.	.	.		.	.	.	
.	.	.		.	.	.		.	.	.	
.	.	.		.	.	.		.	.	.	
.	.	.		.	.	.		.	.	.	
4.4	−1.84	4.9	*	.	.	.		.	.	.	
0.0	−1.77	4.9	*	0.0	−2.673	4.5	*	.	.	.	
13.0	3.21	11.2	**	10.9	2.934	5.4	*	.	.	.	
0.0	2.96	10.8	**	3.2	2.884	5.8	*	.	.	.	
5.6	2.81	9.5	**	0.0	2.427	4.5	*	.	.	.	
15.1	**3.73**	**18.7**	***	**15.1**	**4.896**	**17.7**	***	.	.	.	
0.0	3.75	19.6	***	0.0	5.539	20.9	***	.	.	.	
2.1	3.00	9.9	**	0.0	3.795	9.6	**	.	.	.	
3.0	2.76	8.4	**	9.4	3.796	10.4	**	.	.	.	
.	.	.		.	.	.		25.1	.0286	8.3	**
5.5	2.13	4.5	*	.	.	.		8.5	.0215	4.5	*
11.0	2.69	8.2	**	11.0	3.587	6.9	*	4.1	.0241	5.3	*
0.0	2.57	6.1	*	2.2	4.065	7.9	**	.	.	.	
.	.	.		.	.	.		.	.	.	
.	.	.		.	.	.		.	.	.	
.	.	.		11.2	2.548	4.5	*	.	.	.	
9.4	**2.32**	**6.6**	*	9.4	3.680	9.4	**	.	.	.	
0.0	2.45	7.1	**	2.9	3.527	8.6	**	.	.	.	
0.0	2.07	5.1	*	**0.0**	**3.582**	**8.8**	**	.	.	.	
	1.027				1.374				.0105		
		39.7				49.2				14.4	

than 5%. A total of 119 possible QTL positions outside the segments shown in Table 4 were tested for each trait, i.e. 714 tests, of which 33 (4.6%) were found to be significant only at the 5% level. Hence these are likely to be spurious QTLs and, for the following comments, we concentrate on the regions shown in Table 4.

Total root weight (*TRW*)

Six segments in the most active regions of chromosomes 1, 5, 6, 7 and 9 showed significant association with TRW, with QTLs accounting for between 5 and 11% of phenotypic variation. The multiple-QTL model selected by stepwise regression contained six QTLs (highlighted in Table 4), which together accounted for 43% of the phenotypic variation in TRW. This model selected a single QTL from each active region except for chromosome 5 where two QTLs were postulated.

Deep root weight (*DRW*)

Five segments in the main regions of chromosomes 1, 6, 7, and 9 were observed to be associated with effects on DRW. Individual QTLs in these genomic regions accounted for variation ranging from 4 to 15%. The multiple-QTL model comprised QTLs near markers RZ730, CD0418 and RZ206 (highlighted in Table 4) and accounted for 23% of the variation for DRW.

Deep root weight to shoot ratio (*DR/S*)

Nine segments in the most active regions of chromosomes 1, 2, 5, 6, 7, 8, and 9 were detected. Individual QTLs in these genomic regions accounted for between 4 and 22% of the total phenotypic variation for this trait. The multiple-QTL model accounted for 33% of the variation combining QTLs near markers RZ730, RG403, CD0418 and RZ228 (highlighted in Table 4).

Deep root weight per tiller (*DR/T*)

Six genomic regions on chromosomes 1, 2, 6, 7, 8, and 9 with a significant effect on DR/T were identified. Individual QTLs in the most active regions accounted for between 4 and 20% of the phenotypic variation for the trait. The multiple QTL model accounted for 40% of phenotypic variation and comprised QTLs near markers RZ730, RG157, CDO418, and RZ228 (highlighted in Table 4).

Maximum root length (*MRL*)

Eight genomic segments in the active regions of chromosomes 1, 2, 3, 5, 6, 7, 8, and 9 had a significant influence on MRL. Putative QTLs in these regions accounted for between 4 and 21% of phenotypic variation in this trait. The multiple-QTL model included six QTLs on chromosomes 1, 2, 3, 5, 7 and 9 (highlighted in Table 4) and accounted for 49% of the phenotypic variation.

Root thickness (*THK*)

Five regions on chromosomes 1, 2, 5 and 8 were observed to have a significant effect on root thickness. The variation accounted by the QTLs in these regions ranged from 4 to 10%. The multiple-QTL model included QTLs near markers RZ19, PalI and RZ67, and only explained 14% of the variation for the trait.

Two long segments had major effects on several traits, (interval RG381 to RZ801 on chromosome 1 and interval Est-9 to RZ978 on chromosome 7 spanned five markers each. It is possible that two or more QTLs are present in these regions although the multiple-QTL models selected by stepwise regression only select a single QTL in these regions (Table 4). In both cases, for some of the traits, we observed a drop in the R^2 values for the markers located in the middle of the segments.

Not all alleles coming from the best parent (Azucena) exerted a positive effect, as shown by the sign of the weights (Table 4). However, alleles from a particular parent in specific segments exerted an effect in the same direction for all traits.

Interaction between markers

Interaction was tested between all pairs of markers in the active chromosome regions (all markers shown in Table 4). The results are shown in Table 5, with 23 pairs significant at the 1% level for TRW, 10 for DRW, 14 for DR/S, 12 for DR/T, 16 for MRL and 4 for THK. Three marker pairs showed simultaneous interaction for three or more traits: RZ519*RZ206, RZ225*CAT-1 and RZ225*AMY2A. Eight pairs showed interaction for two traits. Most interaction was between regions on different chromosomes except for three locations, one on chromosome 5 and two on chromosome 7.

Nine of the interactions were significant at the 0.1% level; details of these are given in Table 6. Interaction effects were comparable in magnitude to the main effects shown in Table 4. In all nine cases, the effect of the Azucena alleles near the second marker was opposite in sign when Azucena alleles were present at the first marker compared with when IR 64 alleles were present there. In fact, 70 of the 79 significant interactions in Table 5 showed the same antagonistic interaction, often sufficient to mask the effect of a QTL near one or other of the markers. For example, the substitution of the Azucena alleles near RG667 on chromosome 9 caused

Table 5 Chromosome markers showing significant interaction for six root morphological traits. Positions in the matrix correspond to interaction between the corresponding markers on the horizontal and vertical scales. Dots indicate non- significant interaction, numbers correspond to significant interaction ($P < 0.01$) for the correspondingly numbered traits: 1 = total root weight (TRW); 2 = deep root weight (DRW); 3 = deep root per shoot ratio (DR/S); 4 = deep root weight per tiller (DR/T); 5 = maximum root length (MRL); 6 = root thickness (THK)

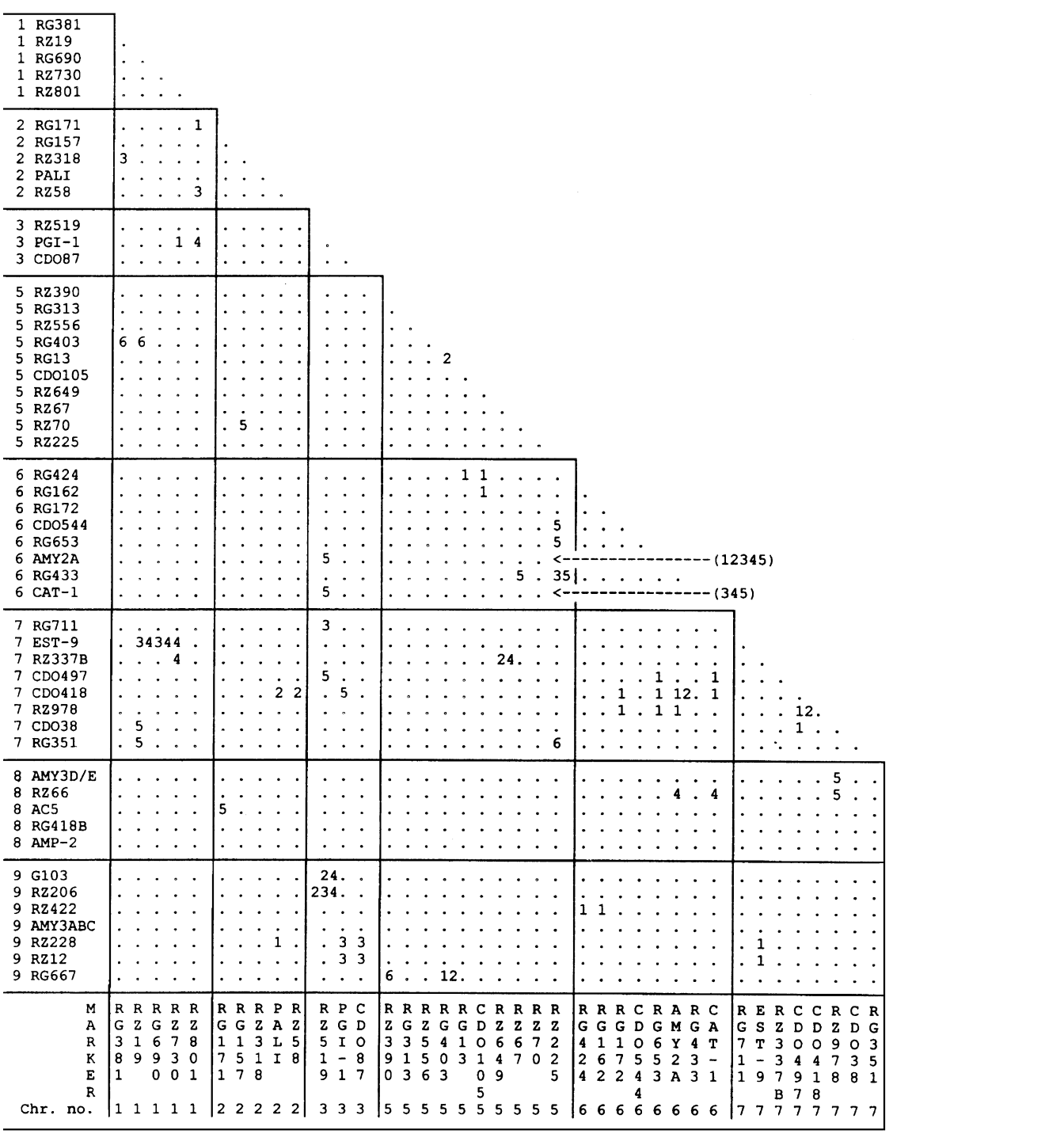

Table 6 Cell means and interaction effects for marker pairs in the active regions of the genome showing significant interaction at the 0.1% level. II = IR 64 allele at the two markers; A = Azucena allele at the two markers; IA = IR 64 allele at the first marker and Azucena allele at the second marker; AI = Azucena allele at the first marker and IR 64 allele at the second marker; Interaction effect = (mean II − IA − AI + AA)/4; SE is the standard error of the interaction effect. ns, ∗, ∗∗ = significance of the single marker test: respectively non-significant, significant at the 5% level and at the 1% level. ↑, ↓ = direction of effect at specified levels of first marker

Trait	First marker	Second marker	Means for marker classes interaction					Effect	S.E.
				II	IA		AI	AA	
TRW	RG403^{ns}	RG667^{ns}	↓	1.272	1.029	↑	0.981	1.273	0.1337	0.03495
TRW	RG162*	RZ422^{ns}	↑	1.115	1.450	↓	1.133	0.988	− 0.1199	0.03388
TRW	RG653**	CD0418*	↓	1.283	1.192	↑	0.878	1.247	0.1147	0.03325
DRW	RZ225^{ns}	AMY2A**	↑	.1459	.2564	↓	.1915	.1389	− 0.0407	0.01138
DR/S	PGI-1^{ns}	RZ228*	↑	2.135	3.514	↓	2.985	2.609	− 0.4387	0.11750
DR/S	RZ225^{ns}	AMY2A^{ns}	↑	2.148	3.277	↓	3.263	2.264	− 0.5317	0.14655
MRL	RG171*	AC5^{ns}	↓	80.91	62.32	↑	80.57	88.79	6.702	1.7947
MRL	RZ519**	CAT-1^{ns}	↑	79.34	83.81	↓	88.47	75.33	− 4.403	1.2723
MRL	RZ225^{ns}	CAT-1^{ns}	↑	79.11	90.62	↓	84.77	76.43	− 4.963	1.2800

a decrease of 0.243 ± 0.100 g TRW when IR 64 alleles were present at marker RG403 on chromosome 5, while the same substitution causes an increase of 0.292 ± 0.100 g when Azucena alleles were present at RG403. Yet there was no evidence of a QTL near RG403 or RG667 for TRW in Table 4.

Comparison of QTLs detected in IR64 × Azucena and Co39 × Moroberekan

For some segments, common markers between the two populations allowed an easy localization of detected QTLs. However, for QTLs linked to markers not present in both populations, it was necessary to use as a bridge the interspecific map developed by Causse et al. (1994), which included the two marker sets. There was some imprecision, as the distance in cM between markers varied from one map to another. Thus, these results should be taken as indicative. We considered as common QTLs those segments which overlapped when common markers were available in the area as well as adjacent chromosomal segments when no common marker was available in the area. Comparison of the results of the two populations showed that the number of common QTLs varied from 1 to 3 per trait (Table 7).

Discussion

Phenotypic variation

The rooting depth of a variety is unequivocally considered as an important trait to improve water uptake in upland rice. The *indica* lowland and *japonica* upland parents of the studied population had contrasting expression of root depth under our experimental conditions. These results are in accordance with field observations (B. Courtois, unpublished) – though IR 64 had a deeper root system under greenhouse conditions than in the field – and with previous reports that roots of upland rice reach deeper soil layers than roots of lowland rice (Yoshida and Hasegawa 1982).

Root length density is the parameter generally favored by agronomists to evaluate the ability of a root- system to extract water, rather than root-weight density said not to be very sensitive to variation in fine root length. We observed very high correlations between root dry weight and root length in the same layers. The same levels of correlation between total root weight and total root length were obtained in experiments conducted under hydroponic conditions (M. Olofsdotter, International Rice Research Institute, personal communication). Nevertheless, both root weight density and root length density are probably relatively imperfect indicators of water uptake as they assume that this function is evenly distributed along the roots (McCully, 1995).

Transgressions

Trangressions in both directions were observed for all traits, indicating that neither parents carried all the positive or all the negative alleles. This was further confirmed by the negative weight of some of the Azucena alleles. The wide range of transgressions is in agreement with the observation of Second and Ghesquiere (1995) that, because of the diphyletic origin of *O. sativa*, *indica* × *japonica* crosses generate for many

Table 7 Comparison between QTLs identified in IR64 × Azucena and Co39 × Moroberekan. Chr = chromosome;% var = % phenotypic variance explained by the markers

Trait	Common QTL	Localization common QTL				
		Chr.	IR64 × Azucena	% Var	Co39 × Moroberekan	% Var
Root thickness	2	1	RZ19-RG690	10	RG197	25
		8	Army3D/E-RZ66	8	RZ66	22
Maximum root length	1	9	RZ12-RG667	9	RZ12	?
Total root weight per tiller	3	1	RG472-RG246	5	RG140	13
		7	CDO497-CDO418	9	RG351	11
		9	G103-RZ206	8	RG553	12
Deep root weight per tiller	3	7	CDO497-CDO418	18	RG351	6
		8	AC5-RG418B	8	RG136	7
		9	RZ22B-RZ12	7	RZ12	8
Root/shoot ratio	3	4	RG908-RG91	5	RG190	13
		7	RZ978-CD0418	6	CDO405	18
		9	G103-RZ206	7	RZ553	22

traits a variability equivalent to that of the whole species.

If the effect of these alleles was confirmed under field conditions, some of the root traits might be improved beyond the best parent value by selecting Azucena or IR64 alleles at chosen loci. QTLs with effect opposite to those predicted by parent phenotype have commonly been detected in QTL-mapping studies (DeVicente and Tanksley 1993 in tomato; Veldboom et al. 1994 in maize; Courtois et al. 1995 in rice).

QTL identification

We detected QTLs for all studied traits and were able to locate them in intervals of two to three markers. The detected QTLs accounted for between 4 and 22% of the phenotypic variation. A few QTLs with large effects were detected as well as several QTLs with smaller effects whose existences are probably less likely since they were inferred from phenotypic data measured in a single environment. For the traits MRL, TRW and DR/T, stepwise regression selected a multiple-QTL model which explained a proportion of the phenotypic variance that was more than 60% of the heritability of the trait. Therefore, as mentioned by Veldboom et al. (1994) under a similar situation, it is likely that undetected QTLs for these traits would account for only a small amount of the variation in this population. For root thickness, however, the gap is still large and there is a need to reassess this trait.

We were not able to determine whether one or two QTLs were present in some chromosomal areas. When multiple QTLs are present in the same segment, flanking marker methods give a poor localization of the QTLs, with "ghost" effects and bias on their magnitude (Martinez and Curnow 1992; Whittaker et al. 1996). Moreover, the DH lines studied here were developed from F_1 hybrids after only one meiosis. A graphical representation showed that their chromosomes were not very fragmented, and that, in some cases, entire chromosomes were inherited from the same parent. It was therefore difficult to go further in terms of resolution with a population of this small size, showing limited recombination events, even with a reasonably good coverage of markers. However, the main objective of this analysis was not the precise location of QTLs but rather was exploratory and preliminary to the development of isogenic lines for detailed study. A resolution of 15–20 cM in QTL location may therefore be acceptable as a first step before dissection of the introgressed fragments into smaller pieces to recover recombinants within these segments (Paterson et al. 1990; Lee 1995).

Most of the detected QTLs were common across traits. For instance, one of the QTLs located on chromosome 7 (flanking markers CDO418–RZ978) had very highly significant effects on four root traits (maximum root length, deep root weight, deep root weight per tiller, and deep root to shoot ratio) and a significant effect on total root weight. Regions on chromosomes 1, 2, 6, 8 and 9 also influenced more than one trait. For the different parameters related to root depth, the correlations were expected. However, some of the genomic regions also controlled deep root pattern and root thickness, two relatively independent traits as also shown by Champoux et al. (1995). It was difficult to say if this resulted from a pleiotropic effect, showing that the traits were causally related, or from a clustering of genes. Clustering of QTLs is a common phenomenon as shown by Paterson et al. (1990) after fine mapping in tomato and Witsenboer et al. (1995) for disease resistance.

The recovery of a common chromosomal segment explained the genetic correlations obtained between root traits. Traits with a high genetic correlation had more genomic regions in common compared to traits

with a low genetic correlation. Correlated traits have been hypothesized to have a common genetic base (Albert et al. 1991; Paterson et al. 1991; Lebreton et al. 1995) and several studies have shown that highly correlated traits have most significant markers in common (e.g. plant height, exertion, and panicle length in this population; Courtois et al. 1995).

Among the areas common between traits, the RZ730–RZ801 segment on chromosome 1 corresponds to the position of *sd-1*, a major gene known to control semi-dwarfism, which has been widely used in the IRRI breeding program. *sd-1* was located near marker RZ730 by Huang et al. (1996) in this population, and near RG220, which is between markers RG810 and RZ19, by Yu et al. (1995) in a different genetic background. IR64 is known to have inherited this gene from one of its remote parents, Dee Geo Woo Gen. This segment exerts an effect not only on plant height but also on tillering and biomass. The QTLs detected in the present study on chromosomes 2, 3, 8, and 9 are also common with QTLs identified for plant height at maturity under irrigated field conditions in the same population (Huang et al. 1996). The linkage between above- and below-ground growth through hormonal as well as nutritional feed-back loops has been abundantly documented (Klepper 1991). Therefore, it is not surprising that *sd-1*, which has a massive effect on plant height and tillering, was found to show effects on the root system. However, studies on the root system of a few plant-height mutants of rice and sorghum (Yoshida and Hasegawa 1982) and wheat (Clarke and Mc Caig 1993) showed that not all height-reducing genes affected root biomass. In our population, the QTL on chromosome 7 that was associated with effects on maximum root depth did not seem to be linked with a QTL for plant height. This suggests that it may be possible to decrease the height of traditional tall upland rice varieties without diminishing the quality of their root system.

Additive × additive interactions

The analysis of additive × additive interactions between pairs of markers indicated a frequency of interactions close to the values expected by chance, as reported in previous studies, in which epistasis was concluded to be unimportant (Paterson et al. 1991 in tomato; Edwards et al. 1987 and Bubeck et al. 1993 in corn; Li et al. 1995 in rice). However, among these interactions, some had a very high probability value. These highly significant interactions reversed the hierarchy of allele effects. Moreover, their magnitude was large enough to mask QTL effects, a phenomenon also observed by Wu et al. (1995) for sterility in rice. This implies that the estimates of individual QTL effects, which are evaluated with the underlying assumption of no interaction, are probably biased and should be considered only as indicative.

As for single-marker analysis, when interaction between pairs of markers was detected, it was often observed for several traits at the same time, though no specific pattern of association between traits could be identified. The marker RZ801 on chromosome 1, which is close to *sd-1*, interacted with other markers for TRW, DR/S, and DR/T, a pattern fitting well with the known effects of *sd-1*.

Comparison of QTLs detected in IR64 × Azucena and Co39 × Moroberekan

In the two sets of experiments, the growing conditions of the plants and the trait evaluation methods were reasonably similar, allowing comparison of the results. We identified between one and three common QTLs depending on the trait. Champoux et al. (1995) found just one QTL for MRL in the Co39 × Moroberekan population, in contrast with the six found in IR64 × Azucena population. This difference explains the low number of common QTLs for this trait. The additive effect of these common QTLs, in terms of the percentage of phenotypic variance they explained, varied strongly in the two populations but the common QTLs were among the ones with the most significant effect in one or the other population, e.g., except for the segment on chromosome 8, those which were identified through the multiple-marker regression models in IR64 × Azucena. The fact that several of the QTLs detected in one population were not found in the other may be attributed to allelic differences at the QTL locations between populations, or to the possibility that, in the population with no detected QTLs, both parents carried the same allele at the QTL location. The gaps in the maps, as segments of 20–30 cM are still without markers, can also limit the chances of detection of QTLs with weak effect in these areas. The choice of the statistical thresholds (power of the tests), the genetic background itself (existence of non-allelic interactions as shown here), or a combination of the above mentioned factors might also affect the recovery of QTLs common between populations.

The development of isogenic lines, starting from the existing DH lines, would help to clarify the proper value of the common QTLs by eliminating the confounding effects of other genomic regions, and to fine-tune their location. Backcross-aided selection will take into account the uncertainty of QTL position by introgressing chromosomal segments rather than individual markers. Following an idea proposed by Second and Ghesquiere (1995), isolines will be developed in both backgrounds, the *indica* variety being introgressed with *japonica* alleles and the *japonica* variety with the *indica* ones.

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