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A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTLs for root-penetration ability

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Abstract A combined RFLP and AFLP linkage map of an F_6 recombinant inbred population, which was derived from a previously mapped F_2 of a cross between the two drought resistant upland rice varieties Bala and Azucena, is presented. The map contains 101 RFLP and 34 AFLP markers on 17 linkage groups covering 1680 cM. Also presented is the approximate mapping position of a further four RFLP and 75 AFLP markers, which either could not be given a unique place on the map or for which the available data is not sufficient to allow confident positioning, and the result of quantitative trait locus (QTL) mapping of traits related to root-penetration ability. Root penetration was assessed by counting the number of root axes that penetrated a 3 mm-thick layer consisting of 80% wax and 20% white soft paraffin. Good root penetration would be expected to increase drought resistance where soil strength is high. Single-marker analysis revealed seven QTLs for the number of roots which penetrate the wax layer. In identical locations were seven QTLs for the ratio of penetrated to the total number of roots. Transgressive inheritance of positive alleles from Bala explained four of these QTLs. Comparison of the QTLs identified here with previous reports of QTLs for root morphology suggest that alleles which improve root penetration ability may also either make the roots longer or thicker.

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Introduction

Rainfed rice-growing areas cover about 69 million hectares, or 45% of the area planted to rice world-wide (IRRI 1997). Water stress is considered to be the greatest cause of low yields in upland and lowland rainfed systems (Greenland 1984; Sharma and De Datta 1994), where yields average 1.2 and 2.3 tonnes per hectare respectively (IRRI 1997). Drought has been calculated to cause 1.2 million tons of lost yield per annum in upland rice cultivation in East India alone (Widawsky and O'Toole 1996). Upland rice farmers have traditionally used varieties with deep root systems that can avoid drought by extracting water from deeper in the soil profile (Reyniers et al. 1976; Puckridge and O'Toole 1981). However, ironstone pans or gravely horizons can restrict deep rooting in upland rice (Mambani and Lal 1983; Mutsaers et al. 1997), whereas shallow hardpans are widespread in rainfed lowland rice (Wade 1996). Rice varieties with better root penetration would be expected to be more drought resistant in these environments.

Improving the drought resistance of rice varieties by introducing traits which contribute to drought avoidance or drought tolerance should have considerable potential for increasing rice production in drought-prone areas (Fukai and Cooper 1995; Nguyen et al. 1997; Price and Courtois 1999). Locating parts of the genome which contribute to drought resistance of some varieties by the use of molecular mapping promises to increase our understanding of drought resistance and to produce markers and genomes for the strategic improvement of rice by marker-assisted breeding (Price and Courtois 1999).

Champoux et al. (1995) made a molecular map of quantitative trait loci (QTLs) controlling root morphological characters such as rooting depth and root thickness using a recombinant inbred population of a cross between varieties CO39 and Moroberekan. They also

identified QTLs for visual appearance under field-imposed drought. Ray et al. (1996) used the same population to identify QTLs for root-penetration ability by growing plants in pots in which a layer of 60% paraffin wax/40% white soft paraffin was buried (Yu et al. 1995). Penetration ability was expressed as the number of root axes that had penetrated the wax layer and as the fraction of total root axes that had penetrated the wax layer.

An F_2 population from a cross of two drought-resistant upland rice varieties (Bala and Azucena) was previously used to identify QTLs for root and shoot characteristics potentially related to drought resistance (Price and Tomos 1997; Price et al. 1997a,b). That population has now been advanced to the F_6 by single-seed descent for the purpose of identifying QTLs for traits related both to drought resistance and field performance under drought. We report here the production of a molecular map using restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLPs). We also report the identification of QTLs for root-penetration ability using a modified wax-layer method.

Materials and methods

Plant material

An F_2 population of a cross of upland varieties Bala and Azucena, described in Price et al. (1997 a) and used to locate QTLs for hydroponic root growth (Price and Tomos 1997), was advanced to F_5 by single-seed descent with panicle bagging to prevent outcrossing. Bulked F_6 seed was collected from 205 of these F_5 plants to produce 205 F_6 recombinant inbred lines (RILs, from 310 original F_2 plants). The population was not selected at any stage, but F_6 bulked lines were only obtained from F_5 plants which produced a minimum of 100 seeds.

DNA extraction and molecular-marker analysis

DNA was extracted from bulked leaf samples harvested from at least 14 plants from each line following the methods described by McCouch et al. (1988) except that the phenol concentration of the phenol-urea extraction buffer was halved. DNA (8 µg per sample) was restricted with *Bam*HI, *Dra*I, *Eco*RI, *Hind*III and *Xba*I restriction enzymes. After electrophoresis on 0.8% agarose gels, the DNA was blotted onto Hybond N+ (Amersham, UK) nylon membranes by alkali transfer according to the manufacturer's instructions.

A total of 105 RFLP markers were screened on the population. Those with the prefix RG, RZ or CDO were obtained from Cornell University, USA (see Causse et al. 1995), and those with the prefix C, G or R were from the Rice Genome Project, Japan (see Kurata et al. 1994). Probes were labelled with digoxigenin (Boehringer Mannheim) by PCR using M13 primers. The reaction took place in 100 µl of 10 mM Tris (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 100 µM dNTPs, 125 µM M13 forward and reverse primers, 1µl of digoxigenin-labelled dUTP, 2.5 units of *Taq* DNA polymersase (Promega) and 1 ng of probe plasmid. The temperature profile was 35 cycles of 94°C, 30 s; 48°C, 1 min; 72°C, 2 min. Labelled probes were purified by ethanol-precipitation. Hybridisation was conducted overnight at 65°C according to the manufacturer's instructions, and membranes were washed to a stringency of 0.5 × SSC. Visualisation of the probe was conducted following the manufacturer's instructions using the chemi-luminescent compound CSPD. AFLP analysis was conducted at the John Innes Centre, Norwich, UK, essentially according to Vos et al. (1995) using *Mse*I and *Eco*RI restriction enzymes and adapters. AFLP markers were designated according to the *Eco*R1 primer (e12, e15 or e18) and the *Mse*I primers (m35, m36. m37, m39, m43 or m45) used for amplification. A total of seven primer combinations were used, e12m35, e12m36, e12m37, e12m39 e12m45, e15m35 and e18m43. Each AFLP marker was given a suffix according to the position from the top of the gel (i.e. e12m35.1 was above e12m35.2 on the autoradiograph). Some AFLP markers were suffixed with a letter after the full stop when it was not initially possible to identify which was the Azucena or Bala allele.

Map construction

The linkage map was constructed using MapMaker 3.0 (Lander et al. 1987; Lincoln et al. 1992) using the Haldane algorithm after all heterozygote data had been entered as missing data. Using only the RFLP data, linkage groups were created with a LOD score of 3.0 and a recombination fraction of 0.4 using the "group" command. The order of the linkage groups was determined using the "compare", "try" and "ripple" commands. The "assign" command was then used to identify the linkage group to which each AFLP belonged, and the "order" and "ripple" commands used to order all markers on each linkage group. Where linkage groups contained too few markers for the "order" command, the "compare" and "ripple" commands were used. Some markers could not be uniquely placed by the "order" command and are excluded from the map. Other markers considerably lengthened the map when included, and these have also been excluded. Finally, AFLPs e12m35 or e12m39 were not used to construct the map because they had significant missing data (only 60 individuals scored), but their mapping position is presented. Chromosomes were oriented with the short arm at the top, following the data reported by Singh et al. (1996).

Screening for root-penetration ability

Root-penetration ability was assessed using a modified version of the wax-layer method of Yu et al. (1995). The modified method will be reported in detail elsewhere, but differs essentially in that an 80% wax layer is used to impede the roots as a 60% wax layer was found to offer very little restriction to root penetration (compared with a 3% wax control) in non-flooded conditions. Wax layers were prepared by melting together 10 g of white soft paraffin (J. M. Loveridge plc, Southampton, UK) and 40 g of pastillated paraffin wax (57–60°C solidification point, Merck Ltd, Poole, UK) and pouring the mixture into an aluminium-foil mould made using a Petri dish. The thickness of the wax layers was 3 mm (Yu et al. 1995) and the diameter 145 mm. The aluminium foil was peeled off before using the wax layers.

Wax layers were installed at a 50 mm-depth in a sand growing medium (RH 65 grade silica sand, Hepworth Minerals and Chemicals Ltd, Sandbach, UK) in plastic tubes (152 mm internal diameter, 450 mm length). The tubes were set up in 16 plastic water tanks, eight tubes per tank. The tubes were filled with dry sand to within 55 mm of the tops of the tubes, then watered with nutrient solution until the column of sand was wetted through. The composition of the nutrient solution was 1.5 mM $Ca(NO_3)_2$, 0.15 mM $CaH_4(PO_4)_2$, 1.0 mM KCl, 0.3 mM $MgSO_4$, 50 µM B, 50 µM Fe, 10 µM Mn, 1 µM Zn, 1 µM Cu and 0.5 µM Mo. The sand surface was levelled and the wax layers placed on the sand. The wax layers were then covered with a 50 mm-thick layer of dry sand, which was thoroughly watered with nutrient solution. Nutrient solution was then added to the tanks so that the level in the tanks was 300 mm below the tops of the sand cores. The 3–4-mm gap between the wall of the tube and the wax layer allowed the entire core of sand to be watered by capillary action. Although this gap allowed some roots to avoid the wax layer, low-impedance controls with 3% wax layers showed that this effect did not account for the differences between Azucena, Bala and IR36 in the number of roots penetrating the 80% wax layer (results not shown).

Growth experiments were carried out in a controlled environment room with day/night temperatures of 30 and 26°C respectively, a 16-h day length and a relative humidity of 70%. Lighting was by fluorescent tubes, with supplementary tungsten lighting, and the photosynthetic photon flux density was 300–350 µmol m^{-2} s^{-1}. Seeds of rice (*Oryza sativa* L.) were surface-sterilised in NaOCl (1% available Cl) for 5 min, washed in tap water then distilled water and left to germinate for 3 days between two sheets of wet filter paper in Petri dishes in cardboard boxes covered with aluminium foil to exclude light. A subset of 104 of the RILs was assessed for root-penetration ability. This was compared with the parents Azucena and Bala and with IR36 as a reference. Two runs (experiments) were carried out. In each run, a single plant of each of the RILs was grown but eight plants each of Azucena, Bala and IR36. A completely randomised design was used and the arrangement of the lines was re-randomised between the runs.

The tubes of sand with the wax layers were planted with 3-day-old rice seedlings, one per tube, so that the seed was just below the sand surface. The coleoptiles were covered with a small quantity of moist vermiculite. The nutrient solution level was kept at 300 mm below the tops of the sand cores by topping up with water every 2–3 days. The nutrient solution was changed 10 days and 17 days after planting the seedlings.

Plants were harvested 24 days after planting and tiller numbers and the number of root axes that had penetrated the wax layer were counted. The crowns of the plants were stored at 5°C in water, and the total numbers of root axes subsequently counted. The ratio of penetrated to total root axes was calculated for each plant. Root axes will be referred to as ‘roots’ for short.

Statistical and QTL analyses

For all analyses, mean values from the two runs were used. The trait data from the RILs was transformed by square root before analysis in order to normalise it. In the case of the total number of roots, even after transformation the data were significantly skewed but could not be improved. For declaring significant QTLs, all marker data were used, including heterozygous scores. QTLs were declared significant if single-marker regression gave a significance of 1%. The mean trait values of the two (Azucena or Bala homozygote) marker classes were calculated using analysis of variance (the number of heterozygotes was not sufficient to give reliable estimates of heterozygote means). Positioning of QTLs was achieved by composite interval mapping, conducted using the program QTLCartographer (C.J. Basten, B.S. Weir and Z.-B. Zeng, Department of Statistics, North Carolina State University) with the default settings for model 6 (five background markers and a window size of 10 cM). All skewed markers were removed from the map. Contradictions between composite interval mapping and single-marker analysis are considered in the discussion.

Results

The linkage map

RFLP marker heterozygosity for a bulked F_6 population should theoretically be 6.25%. In practice, for each marker, heterozygosity varied from 2 to 20% but averaged 7.3%, indicating that little unwanted out-crossing had occurred. The seven AFLP primer combinations gave between 11 and 32 polymorphic bands as follows; e12m35, 25; e12m36, 19; e12m37, 15; e12m39, 14; e12m45, 11; e15m35, 16; e18m43, 32. A linkage map of 17 linkage groups covering 1680 cM with a total of 101 RFLPs markers and 34 AFLP markers was constructed (Fig. 1). An additional five RFLP markers and 75 AFLP markers could be assigned to chromosomes, but not to a unique location, or else contained too few data to be reliably placed. A total of 16 additional AFLPs and one RFLP marker could not be assigned due either to no linkage (four markers) or to linkage to two linkage groups.

AFLP markers were less successfully placed by MapMaker than RFLPs and do not appear to be evenly distributed. Thus, chromosomes 5 and 12 contain five and four AFLPs respectively (on average one AFLP for every 35–36 cM) while chromosomes 8 and 11 have 12 and 14 AFLPs respectively (on average one AFLP every 8.5–10 cM).

A small number of the markers were significantly skewed in their distribution. Thus, RG257 on chromosome 10 was skewed towards the Bala alleles (57 Azucena homozygotes, 134 Bala homozygotes and eight heterozygotes). The bottom of chromosome 7 was observed to be highly skewed towards the Azucena alleles. Probe C507 had only nine Bala-genotype and three heterozygous-genotype individuals out of 183 while RG351 had nearly three times as many homozygotes for Azucena compared to Bala.

There are five gaps in the map (two on chromosome 1 and one each on chromosomes 5, 6 and 12). In only one case do markers excluded due to lack of information appear to be located within one of these gaps such that more information would allow the groups to be linked. The marker e12m35.13, which is excluded from the map because only 58 data points are available, lies 25 cM from R2417 and 18 cM from C86 on chromosome 1 linking the two groups. Comparisons with the maps published by Causse et al. (1995) and Kurata et al. (1994) indicate that significant proportions of three chromosomes are missing. The bottom 40% of chromosome 6 and top 30% of chromosome 8 are missing. In addition, the region of chromosome 4 between markers RG449 and RG163 represents only 20% of the chromosome according to Causse et al. (1995), while the region between C513 and C734 represents only 30% of chromosome 4 according to Kurata et al. (1994). RG620 should map below RG163, but in fact maps above RG190. It is difficult, therefore, to be sure what part and what quantity of chromosome 4 is missing from this map. It should be noted that, in addition to these missing segments (and as observed in the F_2), markers reportedly from the upper part of chromosome 11 map to the upper part of chromosome 12, thus making chromosome 11 somewhat smaller than in most other reports.

Root-penetration ability

All phenotypic traits reported here varied considerably between varieties (Table 1). Azucena had the highest number of penetrated roots, the highest ratio of penetrated to total roots and the least tillers. IR36 displayed the highest number of tillers and roots but moderate numbers of penetrated roots and a low ratio of penetrated to total

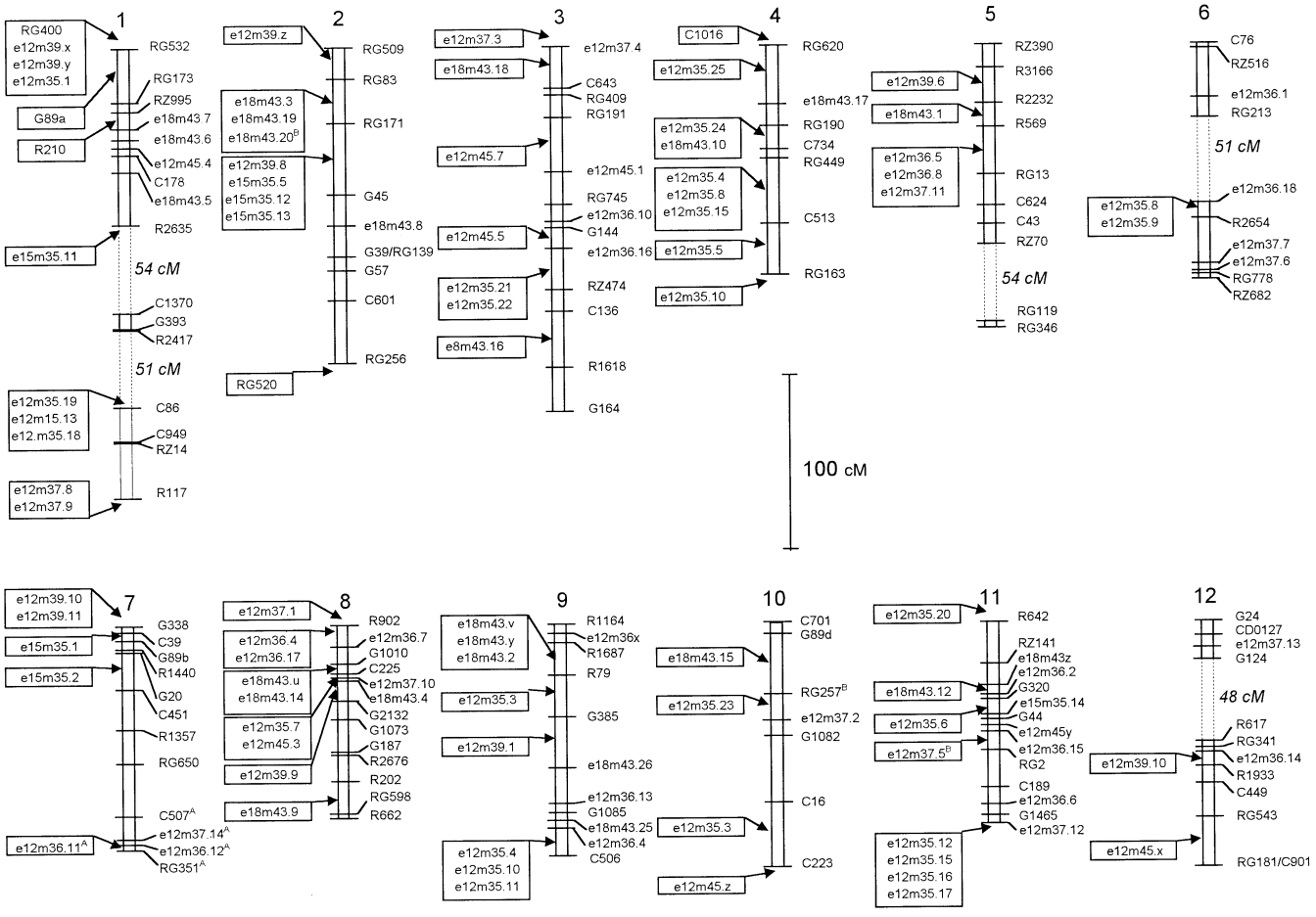

Fig. 1 Combined RFLP and AFLP linkage map of rice obtained from an F_6 population derived from a cross between Bala and Azucena. *Boxes* on the left of the chromosome represent the most likely location of markers excluded from the map because MapMaker could not place them uniquely, because they increased map size, or because they contained insufficient data. *Markers with a suffix A or B* were significantly skewed towards the Azucena or Bala parent respectively

Table 1 Mean (± standard deviation) of parental varieties, segregating population and IR36 for penetration-related traits

Variety/trait	No. of tillers	No. of roots	No. of penetrated roots	Ratio of penetrated: total roots
Azucena (n = 16)	2.56 ± 0.96	31.4 ± 5.9	6.62 ± 2.10	0.214 ± 0.064
Bala (n = 16)	4.25 ± 2.06	55.6 ± 11.7	1.56 ± 1.20	0.026 ± 0.019
F_6 (n =104×2)	3.31 ± 1.70	45.2 ± 14.5	5.17 ± 5.33	0.112 ± 0.098
IR36 (n = 16)	10.75 ± 4.01	96.2 ± 27.6	4.31 ± 3.33	0.042 ± 0.025

Table 2 Correlations of penetration-related traits in the F_6 population. Values presented are r^2 from simple linear regressions

	No. of tillers	No. of roots	No. of pen. roots
No. of roots	0.655***		
No. of pen. roots	0.444***	0.503***	
Ratio pen:total roots	0.280**	0.238*	0.945***

*P<0.05, **P<0.01, ***P<0.001

roots. Bala had moderate tillering and numbers of roots but the lowest number of penetrated roots and the lowest ratio of penetrated to total roots. As expected, the magnitude of the discrimination between these three varieties in root-penetration ability depended on whether this was assessed by the number of penetrated axes or the ratio of penetrated to total roots.

The F_6 population was intermediate between the Azucena and Bala parents for all traits. There was low broad-sense heritability, i.e. 100[1- (mean variance of Azucena + Bala/ variance of F_6)], for the number of tillers (11%), moderate broad-sense heritability for the number of roots (39%), and high broad-sense heritability for the number of penetrated roots (69%) and the ratio of penetrated to total roots (65%).

All traits measured correlated within the F_6 population, the most striking correlation being between the number of penetrated roots and the ratio of penetrated to total roots (Table 2). The number of tillers was strongly correlated with the number of roots and the number of

Table 3 Tillering and root penetration-related QTLs revealed by single-marker regressions significant at $P < 0.01$

Trait	Most significant marker[a]	Chromosome	P[a]	r^2%[a]	Difference between Bala and Azucena marker classes[b]	Donor of Positive Alleles	QTL position in cM above (-ve) or below (+ve) nearest marker[c]
No. of tillers	C949	1	<0.001	12.4	1.03	Azucena	C86 +9
No. of roots	RG173	1	0.008	5.8	6.0	Azucena	RG173
	R117	1	0.004	10.3	7.8	Azucena	R117 –3
	e12m37.2	10	0.006	7.2	6.6	Azucena	e12m37.2 –20
No. of penetrated roots	G45	2	0.002	8.6	2.6	Bala	G45 +1
	C601	2	<0.001	16.7	3.3	Azucena	C601 –5
	e12m37.4	3	<0.001	13.0	3.0	Bala	e12m37.4
	e12m36.16	3	0.004	9.0	2.7	Bala	e12m36.16 +9
	C624	5	0.010	5.2	2.0	Bala	RG569 +10
	e12m37.2	10	0.001	11.3	2.9	Azucena	e12m37.2 –6
	C189	11	0.005	6.9	2.2	Azucena	C189
Ratio penetrated: total roots	G45	2	0.001	9.9	0.049	Bala	G45
	C601	2	<0.001	18.0	0.068	Azucena	C601 –1
	e12m37.4	3	0.001	10.8	0.056	Bala	e12m37.4 +6
	e12m36.16	3	0.003	10.1	0.059	Bala	e12m36.16 +8
	C624	5	0.004	6.8	0.046	Bala	C624 –2
	e12m37.2	10	0.007	7.0	0.047	Azucena	e12m37.2 +5
	C189	11	0.004	7.4	0.047	Azucena	C189

[a] as revealed by regression of trait on marker genotype
[b] as revealed by analysis of variance of trait by marker genotype
[c] as revealed using composite interval mapping

penetrated roots but less strongly with the ratio of penetrated to total roots. The number of roots correlated strongly with the number of penetrated roots and weakly with the ratio of penetrated to total roots.

QTLs for tillering and rooting ability

The results of QTL analysis are presented in Table 3 and Fig. 2. Only one QTL was detected for the number of tillers, at C949 on chromosome 1, controlling 12.4% of the variation (r^2). The plants with an Azucena genotype at this location had more tillers. A QTL affecting plant height (measured in other experiments not described here) in this population is associated with this region and is in the same location as the sd-1 semi-dwarfing locus.

A total of three QTLs were detected for the number of roots, two on chromosome 1 explaining 5.8% and 10.3% of the variation, and one on chromosome 10 explaining 7.2% of the variation. In all cases, the individuals with the Azucena genotype had the higher number of roots (i.e. Azucena alleles had a positive effect).

Although we found previously that the number of roots penetrating the 80% wax layer was the best measure of root-penetration ability, the ratio of penetrated to total root

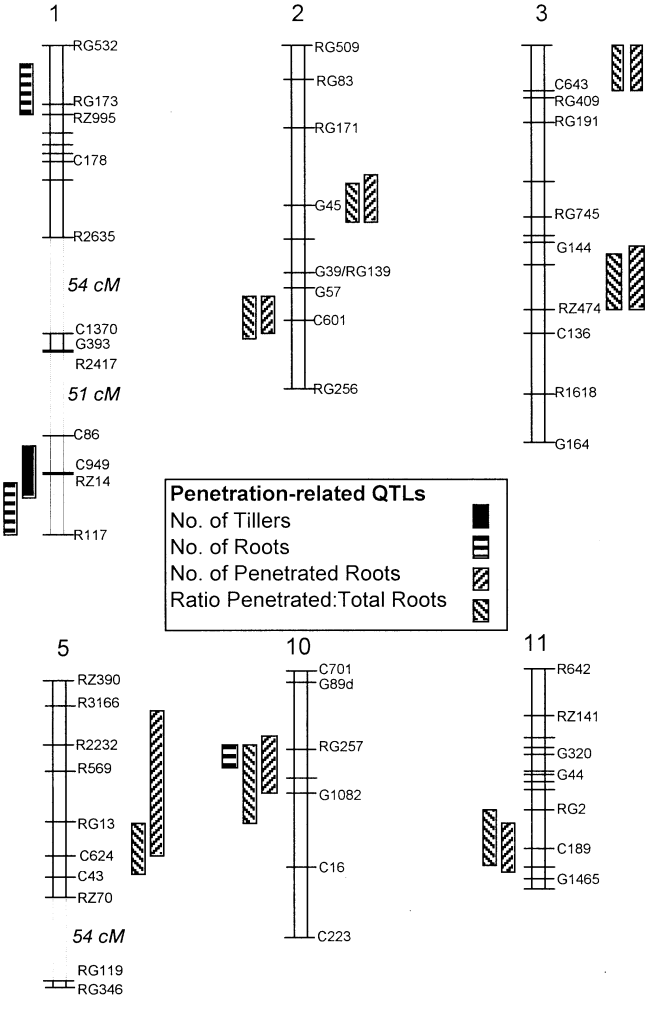

Fig. 2 Quantitative trait loci (QTLs) for characters related to root penetration. QTLs were declared present by single-marker regression ($P < 0.01$). The location of the QTLs was determined by composite interval mapping. *Boxes* represent the one-LOD confidence interval. *Boxes to the left* of each chromosome identify QTLs where Azucena alleles have a positive effect, whereas *boxes to the right* identify QTLs where Bala alleles have a positive effect

data were also subjected to QTL analysis for comparative purposes. Seven QTLs were detected for both the number of penetrated roots and the ratio of penetrated to total roots, all associated with the same markers and explaining a similar amount of the variation for both traits. For each group of seven QTLs, four represent transgressive inheritance since there was a positive effect of the Bala alleles. Most striking is the presence of two QTLs close to each other on chromosome 2 and having opposite effects. Thus a QTL associated with G45 explaining 8.6% and 9.9% of the variation in penetrated roots and the ratio of penetrated to total roots has positive alleles from the Bala parent. Approximately 67 cM away is a QTL associated with C601, which explains 16.7% and 18.0% of the variation for the two traits, and the positive alleles come from the Azucena parent. Two QTLs of positive effect from the Bala parent were detected on chromosome 3, associated with markers e12m37.4 (13.0% and 10.8% of the variation in the two traits) and e12m36.16 (9.0% and 10.1% of the variation). A QTL on chromosome 5 was only just at the threshold level for the number of penetrated roots (5.2% of variation) but was more securely detected for the ratio of penetrated to total roots (6.8% of variation). Again, this is a transgressive QTL (the positive-effect alleles come from the poor-penetrating parent). Two other QTLs were detected on chromosomes 10 and 11, both reflecting a positive effect of Azucena alleles. That on chromosome 10 associated with e12m37.2 explained 11.3% and 7.0% of the variation for the two traits, while that on chromosome 11 associated with C189 explained 6.9% and 7.4% of the variation for the traits.

Discussion

Comparison of F_2 and F_6 maps

The map presented here can be compared to the RFLP map produced for the F_2 population from which this F_6 population is derived (Price and Tomos 1997). On the whole, the order of and the genetic distance between markers is very similar for the two maps. However, it is surprising that, despite having the same markers, the top of chromosome 2 has changed somewhat. The orders of RG83 and RG509 have swapped and RG171 is now linked to those markers when in the F_2 it was not. Another observation is the apparent shrinkage in chromosome 12 in which the markers in the upper linkage group reported here are only half as far apart as in the F_2 (G24–G124=22 cM vs 43 in F_2) and the markers on the bottom of the lower linkage group of chromosome 12 are also closer (RG543–RG181=29 cM vs 44 in F_2). The other notable point is that there is less segregation distortion in the F_6 than the F_2, which indicates that there does not appear to have been any artificial selection during single-seed decent. The lower portion of chromosome 7 is heavily distorted in both F_2 and F_6 maps. A region of chromosome 3 at markers RG745 and G144 which was weakly distorted in the F_2 is not distorted in the F_6.

Agreement between single-marker regression and composite-interval mapping

For 16 of the 18 QTLs reported here, single-marker and composite-interval mapping agreed with each other. However, two QTLs detected by single-marker analysis were not detected by composite-interval mapping using a significance threshold of LOD 2.0 (the *F* statistic can be converted to a LOD score by dividing by 4.6, C.J. Basten, personal communication). In addition, two QTLs for each of the number of tillers and the number of roots were detected by composite-interval mapping that were not detected by single-marker analysis. Thus the composite-interval mapping detected putative QTLs for tillering on chromosomes 7 and 8 (markers G338 + 4 cM, LOD 2.9 and R662, LOD 2.5) and for the total number of roots on chromosomes 4 and 6 (RG620 + 16 cM, LOD 2.2 and RZ682, LOD 2.6) which were not detected by single-marker analysis (interestingly, both QTLs for the number of roots are in regions with QTLs for the number of roots identified by Ray et al. (1996)). The QTLs for the number of penetrated roots and the ratio of penetrated to total roots detected on chromosomes 5 by single-marker analysis do not achieve a LOD of over 2 using composite-interval mapping (LOD 1.2 and 1.3 respectively). This may be because the data used for single-marker regression include heterozygote data, whereas the procedures used to make the map and to locate QTL position scored heterozygotes as missing. If heterozygotes are removed before regression analysis, G338 is significantly related to the number of tillers ($P = 0.008$, $r^2 = 6.9\%$), but removing heterozygoes did not affect the other disagreements indicated above. The effect of epistasis is another possible explanation of disagreement, but two-way analysis of variance with all significant markers in combination did not reveal significant interaction, suggesting that this may not be the cause.

Comparison with previous reports of QTLs related to root-penetration ability

Using similar techniques in a different population, Ray et al. (1996) identified four QTLs for numbers of roots penetrating a 60% wax layer, on chromosomes 1, 3, 6 and 12, all representing positive effects from the CO39 alleles (the alleles from CO39 increase root penetration even though CO39 was the poor-penetrating parent). There is therefore no agreement between the QTLs identified for numbers of penetrating roots by Ray et al. (1996) and those identified here. In contrast to our results, Ray et al. (1996) found that QTLs for the number of penetrated roots and QTLs for the ratio of penetrated roots to total roots were not associated with the same markers. They identified six QTLs for root penetration index, (ratio of penetrated to total roots with a 60% wax layer) on chromosomes 2, 4, 5 and 6, and two on 11, all but that on chromosome 5 representing positive effects

from the Moroberekan alleles (Moroberekan was the high-penetrating parent). The QTL on chromosome 2 associated with C601 reported here is very likely the same as that reported by Ray et al. (1996) between markers RG73 and RG324. This is because C601 is equidistant between markers RG256 and RG139 on this Bala × Azucena map and RG73 and RG324 are also equidistant between RG256 and RG139 according to the map presented in Causse et al. (1995).

Although both Ray et al. (1996) and this report reveal QTLs on chromosome 5, they are not in the same place. It is quite likely, however, that the QTL on chromosome 11 reported here corresponds to that located 4 cM before marker CDO365 in Ray et al. (1996) since, according to Causse et al. (1995), CDO365 is approximately 7–14 cM from RG2 (i.e. RG2 is 11–18 cM from the QTL) and in the present report it is placed 22 cM from RG2. While the region of chromosome 4 in which Ray et al. (1996) identified a QTL is probably not covered by this map, those on chromosomes 6 and the long arm of 11 also revealed as QTLs by Ray et al. (1996) are not revealed as QTLs in this study. Therefore, it appears that two out of the seven QTLs for the ratio of penetrated to total roots that we have identified were also identified as QTLs by Ray et al. (1996).

The differences in the reported locations of QTLs between this study and Ray et al. (1996) are probably due to the different populations studied and to the different methods used for assessing the root-penetration phenotype. We found previously that in non-flooded conditions, a 60% wax layer offered relatively little impedance to root penetration when compared with a 3% wax low-impedance control (root penetration was decreased by only 15% relative to the control in IR36) (Clark, Aphalé and Barraclough, submitted). Differences between varieties in the ratio of penetrated to total roots with the 60% wax layer actually reflected differences in the root depth distribution under unimpeded conditions (using a 60% wax layer, varieties ranked very differently if they were assessed by the number of roots penetrating or the ratio of penetrated to total roots). This may explain why Ray et al. (1996) found that QTLs for the number of penetrated roots were located differently from QTLs for the ratio of penetrated to total roots. We found previously that a much better measure of root-penetration ability was to count the number of roots that penetrated an 80% wax layer (which decreased root penetration by 75% relative to the low impedance control in IR36). With an 80% wax layer, varieties with broadly comparable total root numbers ranked similarly when compared by the number of penetrated axes or the ratio of penetrated to total axes. We suggest that the different screening procedure may explain some of the differences in reported locations of QTLs for root penetration between our study and that of Ray et al. (1996). In effect, we have measured a different phenotype to that measured by Ray et al. (1996). These differences suggest that it will be important to compare screening procedures with root penetration in the field.

Root-penetration QTLs and previous reports of root-morphology QTLs

It is interesting to note that some of the QTLs for root-penetration ability reported here are close to QTLs for root morphology reported in the F_2 (Price and Tomos 1997). Thus, the QTL associated with G45 on chromosome 2 is near a QTL for root thickness in which Bala alleles thicken the root (Price and Tomos 1997). The region of chromosome 2 at RG139 was weakly associated (LOD 1.9) with a putative QTL for longer roots in the F_2, the positive allele being donated by Azucena (Price and Tomos 1997). That region contains a QTL reported by Champoux et al. (1995) in which Moroberekan alleles increase rooting depth. A QTL was also reported in the same place by Yadav et al. (1997) using a population from a cross of Azucena with IR64 in which Azucena alleles both increase rooting depth and increase root thickness. Also, the marker C624 on chromosome 5 was associated with QTLs in the F_2 in which Bala alleles both shortened the root and made it thinner (Price and Tomos 1997). This region was identified as a QTL by Yadav et al. (1997), in which Azucena alleles decrease root thickness. The region of chromosome 11 between RG2 and G1465 contained a QTL for maximum root length in which Azucena alleles increased root length in the F_2 (Price and Tomos 1997). Finally, although no QTL for root morphology was reported significantly close to RG257 on chromosome 10 in the F_2, this is a region in which a QTL for root thickness was detected by Champoux et al. (1995). It is also possible that there is a relationship between the QTL for penetration ability on chromosome 3 reported here and a QTL for root thickness reported by Champoux et al. (1995) in similar positions on chromosome 3. It must be noted that the alleles here from Azucena decrease penetration while those of Moroberekan reported by Champoux et al. (1995) increase root thickness even though both Azucena and Moroberekan are upland japonica varieties with good root-penetration abilities. There is, however, considerable evidence presented here that QTLs for the ability of roots to penetrate a wax layer are associated with QTLs which make the roots either thicker or longer in other studies. It would be expected that thicker roots would have a better ability to penetrate such a wax layer as the root axes would be more resistant to buckling (Cook et al. 1997). This hypothesis agrees with the observations of Materechera et al. (1992) that, when different plant species are compared, those with thicker roots had a greater proportion of roots penetrating from a weak to a strong compacted soil horizon in the field. If the soil impedance is due to a coarse textured sandy or stony horizon, however, it may be that thin roots would penetrate more easily. There is clearly a need to study root penetration in upland rice in more detail in order to establish whether QTLs detected using the wax-layer method confer behaviour that aids penetration in the field.

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