Theor Appl Genet (1997) 95:143–152 

A. H. Price · A. D. Tomos

# Genetic dissection of root growth in rice (*Oryza sativa* L.). II: mapping quantitative trait loci using molecular markers

Received: 5 May 1996 / Accepted: 14 February 1997

**Abstract** Drought is a major abiotic stress of upland rice, and good root growth has been associated with drought avoidance. We report on the genetic mapping of root growth traits in an $F_2$ population derived from two drought-resistant rice varieties, 'Bala' and 'Azucena'. Restriction fragment length polymorphism (RFLP) between the parents was 32%, and a molecular map with 71 marker loci and 17 linkage groups covering 1280 cM was produced. Quantitative trait loci (QTLs) for eight root growth characteristics were mapped using phenotype data obtained in a hydroponic screen previously described in a companion paper. Using a significance threshold of LOD 2.4, we observed one QTL for maximum root length after 28 days growth on chromosome 11. It had a LOD score of 6.9, explained nearly 30% of the variation and appeared to be largely additive in effect. QTLs for maximum root length after 3, 7, 14 and 21 days of growth were also revealed. Some root-length QTLs, including that on chromosome 11, varied greatly with developmental stage. One QTL for root volume and two QTLs for adventitious root thickness were detected. No QTLs were detected for the length of cells in the mature (fully expanded) zone of adventitious root tips. The results obtained are discussed in the context of previous reports on mapping root growth parameters in rice.

**Key words** Drought resistance · QTLs · RFLPs · Cell division · Root growth

Communicated by G. E. Hart

A. H. Price (✉) · A. D. Tomos
School of Biological Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW, UK

## Introduction

Drought can potentially affect all rice crops that rely only on rainfall for water (some 45% of the world's rice-cropped area (David 1991)). A principal mechanism by which rice has become adapted to water deficiency is through the possession of a pronounced root system which maximises water capture and allows access to water at depth (O'Toole 1982). The overall size and maximum depth of the rice root system and individual root thickness (measured in the field and hydroponics) have been positively related to field drought resistance (Ahmadi 1983; Ekanayake et al. 1985a, b; Loresto et al. 1983; O'Toole and Soemartono 1981; Yoshida and Hasegawa 1982).

Genetic diversity in root growth exists within the rice germplasm (O'Toole and Bland 1987), and traits such as maximum root length, root thickness, root weight and root volume have been shown to have medium to high heritabilities (Ahmadi 1983; Armenta-Soto et al. 1983; Chang et al. 1982; Ekanayake et al. 1985a, b; Loresto et al. 1983). There is, therefore, potential for improving root-related drought resistance in rice through breeding programmes. However, difficulty in selecting root growth characteristics on a large scale in the field has so far hampered progress.

Advances in molecular marker technology have led to the development of detailed genetic maps of many plant species including rice (Causse et al. 1995; Kurata et al. 1994). These markers, usually RFLPs (restriction fragment length polymorphisms), have made it possible to locate and tag genes of major economic importance. For example, genes for aroma (Ahn et al. 1992), photoperiod sensitivity (Mackill et al. 1993), salt tolerance (Zhang et al. 1995), semidwarfism [*sd-1* (Cho et al. 1994) and *sdg* (Liang et al. 1994)] and many agronomically important morphological markers (Yu et al. 1995)

have been mapped to tightly linked RFLP markers. McCouch and Doerge (1995) have reviewed progress in the mapping of quantitative trait loci (QTLs) in rice using similar approaches.

Marker-assisted selection (using a marker associated with a trait as a selection criteria rather than the trait itself) has considerable potential for the rapid breeding of improved varieties (Stuber 1994), and a successful demonstration of its application to rice plant height has been reported by Cho et al. (1994).

QTL mapping of traits theoretically involved in drought resistance has recently been conducted. For example, QTLs controlling the concentration of leaf or xylem absisic acid (ABA) have been mapped in wheat (Quarrie et al. 1994), barley (Sanguineti 1994) and maize (Lebreton et al. 1995). Lebreton et al. (1995) also reported the chromosomal locations of QTLs for many drought resistance-related traits, including stomatal conductance, turgor pressure and water potential under mild drought stress together with root number and root pulling force. In tomato, Martin et al. (1989) used RFLPs and carbon isotope discrimination to map water-use efficiency.

In rice, QTLs for root growth have been reported. Champoux et al. (1995) mapped root morphological QTLs and related these to QTLs for field drought resistance. Ray et al. (1996), using the same mapping population, reported QTLs for root penetration, some, but not all, of which appear to be related to root-growth QTLs reported in Champoux et al. (1995). Also using the same population, Lilley et al. (1996) have mapped QTLs for osmotic adjustment and dehydration tolerance. These reports show that the application of markers to the study of drought stress can greatly advance our understanding of physiologically complex traits. The markers identified should be valuable tools for rice breeders. However, due to the complexity of drought and drought resistance, data from a number of different genetic and physical environments would be desirable to give breeders confidence in particular markers.

In a companion paper (Price et al. 1997), we have shown that traits such as maximum root length and adventitious root thickness of rice varieties grown hydroponically can be related to field drought resistance and to growth in soil. Here, we report on the construction of an RFLP map of 71 markers and its use in mapping the QTLs controlling those root growth traits (maximum root length at various stages of root development, adventitious root thickness, root volume and the length of cells in the mature (fully expanded zone) of the root tip) in an $F_2$ population, 'Bala' × 'Azucena'. The comparison with QTLs previously published for root growth characteristics measured in a different population that was grown in contrasting environments (Champoux et al. 1995 and Ray et al. 1996) is discussed.

## Materials and Methods

### Plant population

An $F_2$ population of 178 plants was obtained by allowing the self-pollination of an $F_1$ hybrid plant produced from a cross between two *Oryza sativa* varieties, a tropical upland variety, 'Azucena', from the *japonica* subspecies, and an improved variety, 'Bala', an *indica* variety derived from a cross of an *indica* TN1 and an *aus* variety, 'N22' (Chaudhary and Rao 1982). These parental lines were selected because 'Azucena' had long and thick roots when grown in hydroponics while 'Bala' had thin and short roots (Price et al. 1997). Both varieties, however, are drought-resistant in the early vegetative stage [based on data from the International Rice Research Institute (IRRI), Los Baños, Philippines].

### Hydroponic screen of root growth

Seeds of the $F_2$, $F_1$ and parental lines and control variety, 'IR36', were surface sterilised in 1% $NaClO_3$ for 1 min, washed with distilled water and allowed to germinate at 36°C for 3 days. Germinated seeds were planted into a hydroponic system (Price et al. 1997). Thirty $F_2$ plants, 1 $F_1$, 2 of each parental variety and 7 'IR36' plants were grown in each of six trays fitting into 25-l hydroponic tubs containing aerated 1/2 strength Yoshida's nutrient solution (Yoshida et al. 1976), pH 5.0, under greenhouse conditions (minimum temp 25°C) with 600 μmol $m^{-2}$ $s^{-1}$ PAR supplementary lighting. The tray positions were rotated daily, and the pH adjusted every other day for the first week, then daily. The nutrient was replaced every week and was made up to full strength after 2 weeks. Maximum root length at various stages of growth and adventitious root thickness and root volume were measured after 4 weeks growth. Root tips were removed from each plant and used to screen for the length of epidermal root cells in the mature (fully expanded) zone following the methods in Price et al. (1997).

### RFLP analysis

DNA extraction followed 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, *Hin*dIII 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. Hybridisation was performed (essentially) as described by McCouch et al. (1988), except that a hybridisation oven (Techne) was used. The filters were washed to 0.4 × SSC stringency.

The majority of the RFLP probes were supplied by Cornell University, USA [prefixed RG, RZ or CDO (see Causse et al. (1995)] or the Rice Genome Project (RGP), Japan [prefixed G, C, R, Y or L (see Kurata et al. (1994))]. The probe inserts were amplified by polymerase chain reaction (PCR) using M13 forward and reverse primers. The amplified product was recovered from a 1% agarose gel by cutting the amplified fragment from the gel, freezing the gel fragment in liquid $N_2$ and spinning the liquid out of the gel at 3,000 RPM in a micro-centrifuge tube. A 10-μl aliquot of the liquid obtained was used as the target for [$^{32}$P]-radio-labelling using the kit supplied by Appligene. Eighteen RFLP probes (designated RAP) were made in Bangor by cutting RAPDs (random amplified polymorphic DNAs) directly from the agarose gels and radio-labelling these. After autoradiography for 5 days, filters were stripped with boiling 0.5% SDS, 0.1% SSC and used up to 20 times. Some polymorphic loci were scored from minor bands, and these are followed by a lowercase letter in Fig. 1.

### Map construction

The linkage map was constructed using MAPMAKER 3 (Lander et al. 1987; Lincoln et al. 1992b) using the Haldane algorithm. 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. Linkage groups were assigned to chromosomes if at least 2 RFLP probes reportedly from that chromosome were present. Chromosomes were oriented (where possible) with the short arm at the top following the data reported by Singh et al. (1996). The orientation of loci detected with Cornell and RPG probes was possible using comparative mapping data presented at Rice Genome III, Manila 1995 (McCouch, personal communication). All raw data were checked for mis-scoring with particular attention to double crossovers.

### QTL analysis

QTL analysis was conducted using the "scan" and "map" functions of MAPMAKER/QTL (Paterson et al. 1988; Lincoln et al. 1992a) and by one-way analysis of variance for all markers. A LOD score of 2.4 was considered significant, based on criteria described by Lander and Bolstein (1989). For all linked markers, single-marker analysis agreed with MAPMAKER/QTL and is not presented. All of the phenotype data were normally distributed except data for maximum root length at days 3 and 7 (which were squared before analysis) and adventitious root thickness (which was log transformed). MAPMAKER/QTL was used to evaluate the most likely genetic models for detected QTLs by comparing LOD scores for dominant, recessive or additive models. If any model gave a LOD score more than 1.0 below the free model, it was considered unlikely.

## Results

### Polymorphism

Of the 135 Cornell and 96 RGP probes analysed, 44 and 31, respectively, detected useful polymorphism. A total of 18 RAPD bands were tested and 4 had useful polymorphism. Of the total of 249 probes tested, 79 gave polymorphic bands, providing a total of 82 loci. The average rate of useful polymorphism with the five enzymes was 32%.

### Linkage map construction

Using a LOD score of 3.0 and a recombination fraction of 0.4, we obtained 17 linkage groups giving a total map distance of 1,280 cM (Fig. 1). Eight markers were unlinked. Fifteen of the linkage groups were assigned to rice chromosomes because they contained at least 2 markers known to be from that chromosome. No linkage group was assigned to chromosome 9. Three linkage groups were assigned to chromosome 2, while 2 were assigned to chromosomes 1 and 5. When a linkage group contained at least 2 markers from the same map (Cornell or RGP) it was possible to orientate it to so that the short arm of the chromosome was at the top (from Singh et al. 1995). It was not possible to orientate the upper linkage group on chromosome 1 since it did not contain 2 markers of known location from the same map. From an analysis of published maps, we had calculated that the gap between the 2 linkage groups on chromosome 1 should have been approximately 37 cM and it was therefore surprising that the 2 groups were not linked. Likewise, the 3 linkage groups on chromosome 2 should have been only 43 and 28 cM apart based on published maps, and the 2 linkage groups on chromosome 5 should have been approximately 32 cM apart.

It was not possible to assign 2 of the linkage groups to chromosomes, and these have been named unassigned linkage groups (UALG) 1 and 2. Three RFLP loci were removed from the map because their inclusion significantly lengthened the linkage group. Those were *RG173* which mapped between *R210* and *RG532* on chromosome 1, *G89b* which mapped between *RG532* and *RG400* on chromosome 1 and *RG470b* which mapped between *G45* and *RG171* on chromosome 2.

Large gaps in the linkage map remained, notably the whole of chromosome 9, the lower portion of chromosomes 3, 4 and 8 and the upper portions of chromosomes 1, 5, 6, 7, 10 and 11. The unassigned linkage groups could fit any one of these missing locations. The 2 comparable markers on UALG1 should have mapped to the bottom of chromosome 8 (*RG598*) and the middle of chromosome 9 (*G385*) while the only comparable marker on UALG2, *G20*, should have mapped to the middle of chromosome 7.

Nine markers were highly skewed in their distribution of classes; *RG400, G1327, RG745, G144, Y1049*, *RG650* and *RG351* in favour of the 'Azucena' allele and *C643* and *G20* in favour of the 'Bala' allele (Fig. 1). However, only *G1327* on chromosome 2 and *G20* on UALG2 did not map to areas of the genome previously identified as displaying distorted segregation by Causse et al. (1995).

### QTL mapping root length

A total of 10 QTLs were observed for maximum root length using the 2.4 LOD threshold (Table 1, Fig. 2), 1 at day 3, 1 at day 7, 4 at day 14, 3 at day 21 and 1 at day 28. Only 2 QTLs (day 14, chromosome 10; day 21, chromosome 5) reflected a positive effect of the 'Bala' allele. The QTL on chromosome 6 was apparent at all but one of the growth stages, although the significance and magnitude of this QTL did vary with time. The QTLs for root growth at different days showed different types of genetic effect. Notably, the QTLs at day 3 and day 7 appeared to be dominant, while the QTL at day 28 on chromosome 11 appeared to be additive. This QTL on chromosome 11 explained 30% of the variation in maximum root length at day 28 . When MAPMAKER/QTL was instructed to fix this QTL and scan for another (which should improve the analysis for

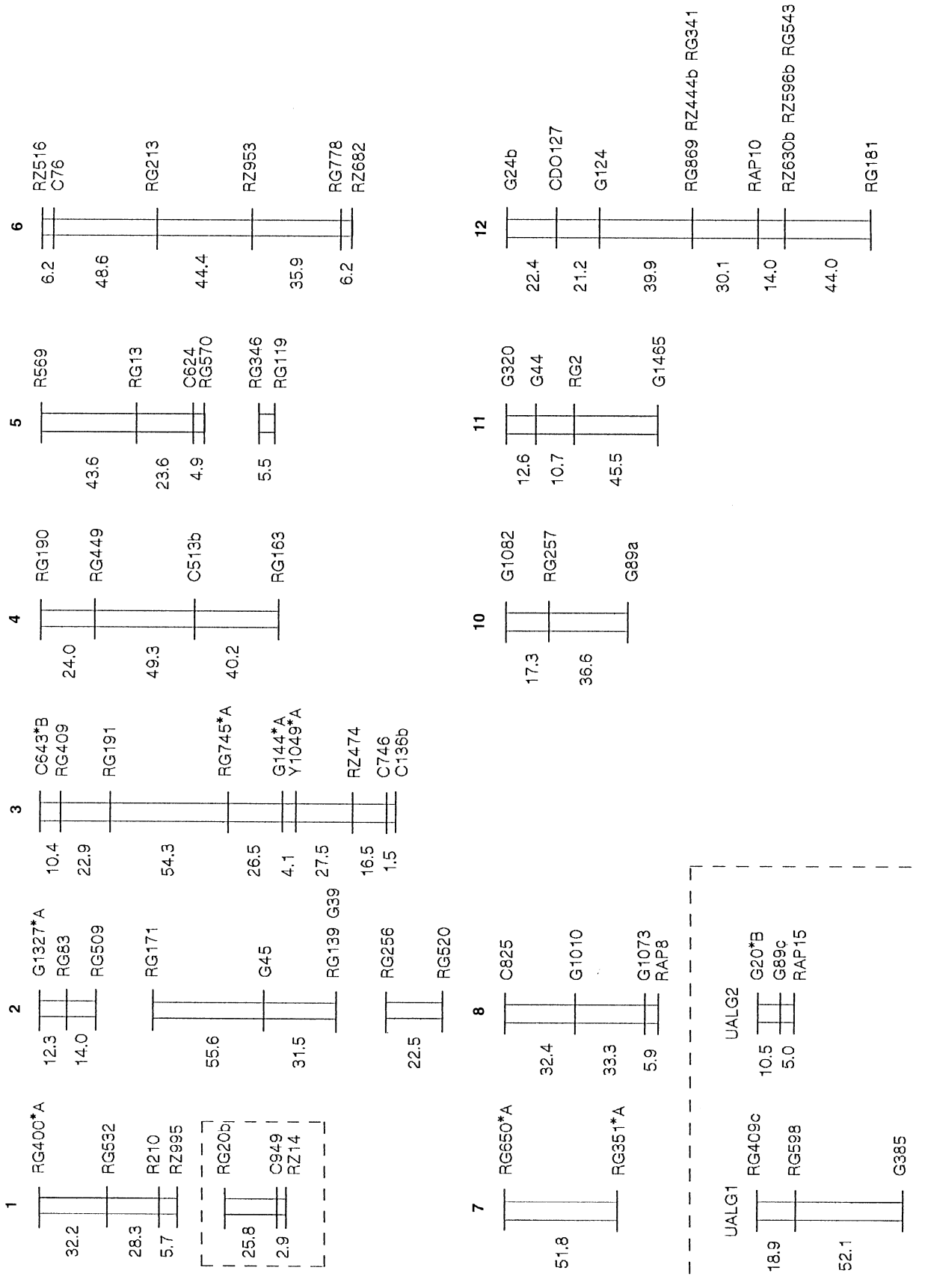
1
RG400*A
32.2
RG532
28.3
R210
5.7
RZ995
RG20b
25.8
C949
2.9
RZ14
2
G1327*A
12.3
RG83
14.0
RG509
RG171
55.6
G45
31.5
RG139 G39
RG256
22.5
RG520
3
C643*B
10.4
RG409
22.9
RG191
54.3
RG745*A
26.5
G144*A
Y1049*A
4.1
RZ474
27.5
C746
16.5
C136b
1.5
4
RG190
24.0
RG449
49.3
C513b
40.2
RG163
5
R569
43.6
RG13
23.6
C624
4.9
RG570
RG346
5.5
RG119
6
RZ516
6.2
C76
48.6
RG213
44.4
RZ953
35.9
RG778
6.2
RZ682
7
RG650*A
51.8
RG351*A
8
C825
32.4
G1010
33.3
G1073
5.9
RAP8
UALG1
RG409c
18.9
RG598
52.1
G385
UALG2
G20*B
10.5
G89c
5.0
RAP15
10
G1082
17.3
RG257
36.6
G89a
11
G320
12.6
G44
10.7
RG2
45.5
G1465
12
G24b
22.4
CDO127
21.2
G124
39.9
RG869 RZ444b RG341
30.1
RAP10
14.0
RZ630b RZ596b RG543
44.0
RG181

**Table 1** QTLs for root growth traits of rice grown in hydroponics; LOD ≥ 2.4 in bold, LOD < 2.4 but ≥ 2.0 in italics

| Trait | Chromosome | Position[a] | LOD[b] | % Var[c] | W[d] | D[e] | Models[f] |
|---|---|---|---|---|---|---|---|
| Maximum root length day 3 | *1* | *RG400 + 18* | *2.3* | *10.0* | *+ 3.3* | *+ 6.8* | |
| | **6** | **RG213+ 33** | **2.9** | **13.3** | **− 5.7** | **− 7.6** | **d** |
| Maximum root length day 7 | **6** | **RZ593+ 13** | **4.8** | **21.9** | **− 11.9** | **− 19.5** | **d** |
| Maximum root length day 14 | **2** | **RG171+24** | **2.5** | **16.1** | **− 14.9** | **− 22.6** | **a d** |
| | **6** | **RZ593+ 15** | **3.2** | **12.9** | **− 18.9** | **− 8.5** | **a d r** |
| | **10** | **G89a** | **2.5** | **37.7** | **− 7.9** | **+ 42.2** | – |
| | **UALG2** | **G89c** | **2.6** | **7.3** | **− 15.7** | **+ 1.6** | **a d** |
| Maximum root length day 21 | *2* | *RG171 + 33* | *2.1* | *13.2* | *− 18.5* | *− 14.0* | |
| | **5** | **RG13+ 14** | **2.6** | **8.4** | **+ 16.0** | **− 2.4** | **a d r** |
| | **6** | **RZ682** | **2.9** | **8.0** | **− 11.6** | **− 17.3** | **d** |
| | *10* | *RG257 + 25* | *2.2* | *13.5* | *+ 5.8* | *+ 30.5* | |
| | **11** | **G44+6** | **2.7** | **7.8** | **− 15.6** | **− 7.4** | **a d** |
| Maximum root length day 28 | *3* | *C746* | *2.0* | *5.3* | *− 14.7* | *+ 11.0* | |
| | *5* | *C624 + 2* | *2.3* | *6.3* | *+ 17.8* | *− 8.4* | |
| | *6* | *RZ593 + 14* | *2.0* | *10.8* | *− 14.9* | *+ 25.6* | |
| | **11** | **RG2+ 24** | **6.9** | **29.8** | **− 41.7** | **− 12.1** | **a** |
| | *UALG1* | *RG598 + 18* | *2.3* | *17.7* | *+ 4.9* | *− 43.5* | |
| Root volume | *1* | *RG20b + 13* | *2.2* | *8.1* | *− 0.75* | *+ 0.15* | |
| | *8* | *G1010* | *2.1* | *6.2* | *+ 0.61* | *+ 0.02* | |
| | **12** | **RG181** | **3.5** | **10.2** | **− 0.78** | **− 0.02** | **a** |
| Adventitious root thickness | **2** | **RG171+ 18** | **2.4** | **14.7** | **+ 0.132** | **+ 0.134** | **d r** |
| | *3* | *RG191 + 31* | *2.1* | *21.3* | *+ 0.131* | *+ 0.138* | |
| | **5** | **C624+ 2** | **2.4** | **7.0** | **+ 0.131** | **− 0.129** | **a r** |
| Root Cell length | *UALG2* | *G20* | *2.1* | *9.5* | *− 0.89* | *− 1.76* | |

[a] Position of the QTL relative to the nearest probe to the left
[b] Maximum-likelihood LOD score for the QTL
[c] Phenotypic variation explained by QTL
[d] Weight calculated by MAPMAKER/QTL. A negative weight meant that the Bala allele reduced the trait
[e] Dominance factor calculated by MAPMAKER/QTL
[f] Genetic models which have a LOD score ≤ LOD score of free model minus 1.0: a, additive; d, dominant; r, recessive

small-effect QTLs by removing some of the variation), the LOD score of the QTL on chromosome 6 rose from 2.0 to 3.0 and the variation this QTL explained rose to 13%.

## QTLs for root volume, adventitious root thickness and root-cell length

One additive QTL was detected for root volume (Fig. 3 and Table 1), on chromosome 12, explaining 10% of the variation. A 3-QTL model using 2 possible low-LOD QTLs (presented in italics in Table 1) explained 30% of the variation in root volume, which was more than the sum of their individual contributions and is indicative of gene interactions.

Two QTLs with LOD score above 2.4 were detected for adventitious root thickness (Fig. 3 and Table 1), on chromosomes 2 and 5, explaining 14% and 7% of the variation. The QTL on chromosome 2 was not additive and represented a gene for thick roots from 'Bala'. The QTL on chromosome 5 is a recessive or additive gene for thick roots from 'Bala'. No QTLs of LOD 2.4 or above were detected for root-cell length.

## Unlinked markers

A total of 8 markers (*RZ390*, *C16*, *G89b*, *G164*, *G393*, *G1085*, *R662* and *RAP12*) were found to be unlinked to any other marker. Only *RZ390* was related to any root growth trait using one-way analysis of variance and a significance level of $P \leq 0.01$. Comparison of *RZ390* and root volume gave an F value of 4.84 ($P = 0.009$); plants with the 'Bala' genotype had the biggest root systems.

←

**Fig. 1** A linkage map of rice based on 178 $F_2$ progeny of a cross between 'Bala' and 'Azucena' with 71 RFLP markers. Linkage groups are assigned to chromosomes (*1–12*) and are orientated with the short arm uppermost where possible. The lower linkage group on chromosome 1 cannot be orientated. Two linkage groups (UALG1 and UALG2) have not been assigned to chromosomes. Markers followed by a *small letter* are scored from minor bands. Markers followed by (**A*) or (**B*) show distorted segregation in favour of the 'Azucena' or 'Bala' alleles, respectively

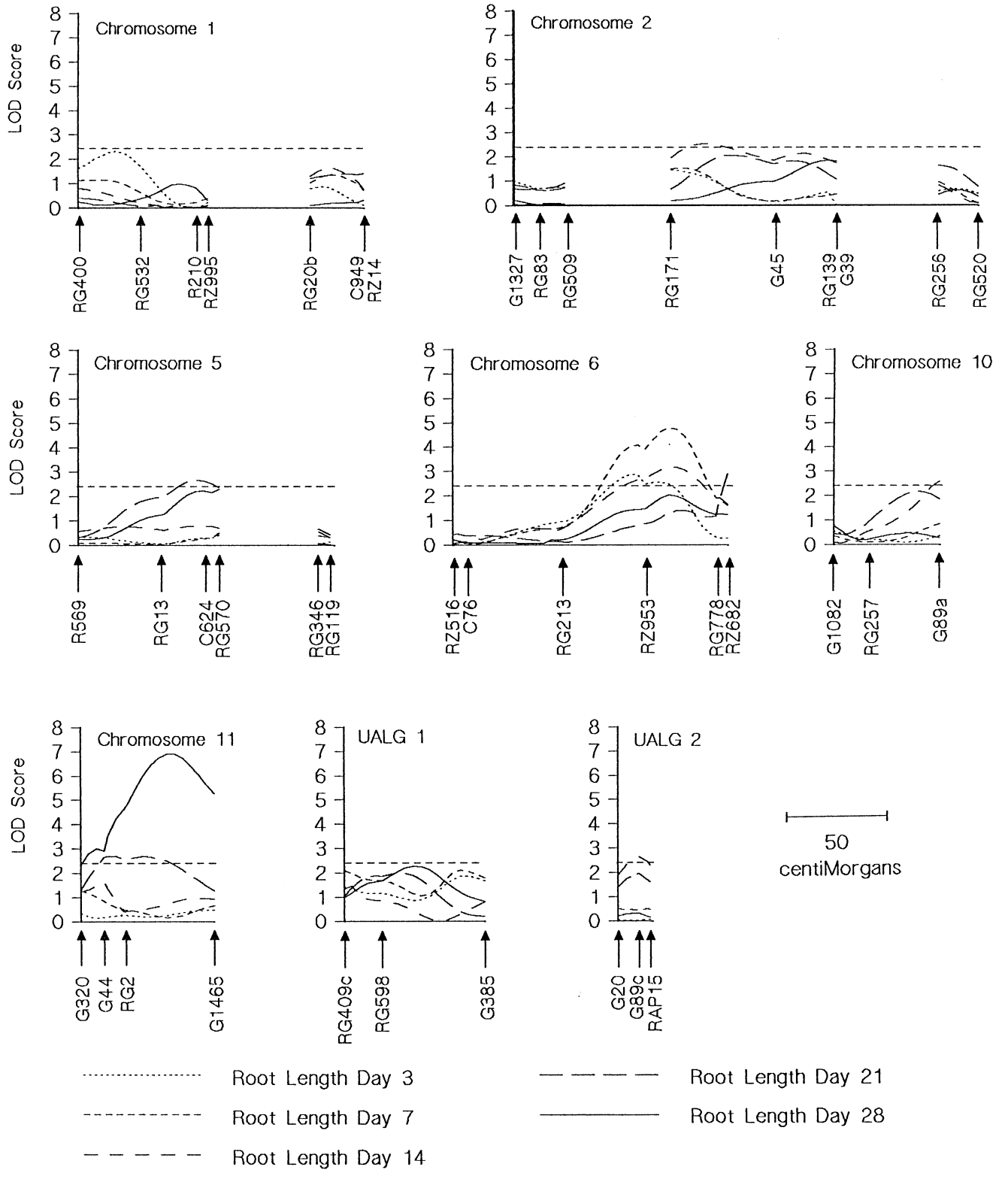


**Fig. 2** QTL likelihood maps showing LOD scores for maximum root length at progressive stages of growth in hydroponics

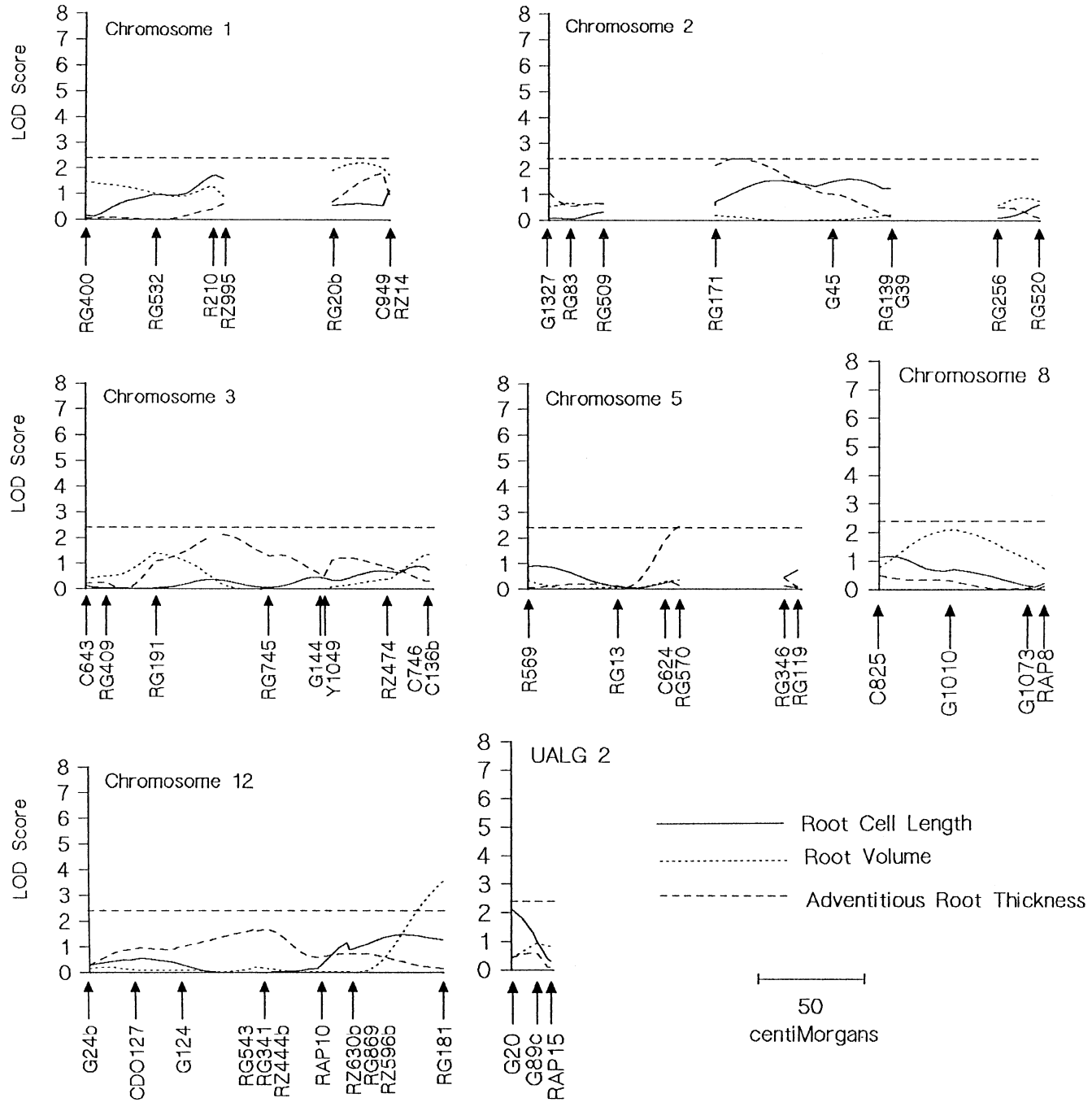


**Fig. 3** QTL likelihood maps showing LOD scores for root volume, adventitious root thickness and root-cell length after 28 days growth in hydroponics

## Discussion

The rate of polymorphism detected (32%) was surprisingly low, considering that polymorphism in *indica/japonica* crosses are reportedly as high as 88% (Abenes et al. 1994). A total of 3 probes detected loci that mapped to locations contradictory to previous reports. This was not due to the marker being scored for a minor (previously unmapped) band. Thus, *Y1049* on chromosome 3 has been previously mapped to chromosome 5, *RG190* on chromosome 4 has been previously mapped to chromosome 12 and *RG570* on chromosome 5 has been previously mapped to chromosome 9. In every case, the size of the DNA fragment amplified by PCR using M13 primers was consistent with the established probe insert size. *RG190* has also been mapped to chromosome 4 by other authors (Champoux et al. 1995; Li et al. 1995).

Several QTLs for maximum root growth were detected. The two most significant, on chromosomes 6 and 11, appeared to display profoundly different developmental influences. The QTL on chromosome 6 was apparent at day 3, became larger on day 7 and then declined to close to insignificance by day 28. The major QTL on chromosome 11, however, was not apparent until day 21, and became pronounced at 28 days. From visual observations made while conducting the experiment, it appeared that the seminal root was the longest in the initial stages of root growth. After 2 weeks of growth in hydroponics, the adventitious roots began to appear and by 21 days these were generally longer than the seminal roots. The QTL on chromosome 11 may, therefore, have been predominantly related to adventitious root growth, with its effects beginning to mask the QTL on chromosome 6.

The rate of root extension growth is a function of both the rate at which cells enter the growing zone (the rate of longitudinal cell division) and the final longitudinal length which those cells achieve. The central region of chromosome 2, which contained QTLs for both maximum root length and adventitious root thickness, did have a high LOD score for cell length (up to 1.6), perhaps suggesting that the gene or genes in this area which influenced root growth did so in part by affecting cell size. However, since most of the QTLs for maximum root length occurred in areas where the LOD score for cell length was very low, it must be concluded that cell expansion was not the major determinant for the rate of root extension growth and that, by default, the rate of cell division must have been.

## Comparison with previously reported QTLs for root growth

The question of whether QTLs are common between different populations and in different environments is of crucial importance. Two of the traits reported here can be directly compared to mapped data for root morphology reported by Champoux et al. (1995) using recombinant inbred lines of a cross between 'CO39' × 'Moroberekan' grown in soil tubes. Since very few of the markers used are common between the two crosses, aligning chromosomes is somewhat speculative in places. Champoux et al. (1995) obtained 4 QTLs for maximum rooting depth from only two of the three screens conducted. One QTL was associated with *RG139* on chromosome 2. In the present study, *RG139* gives a LOD score of 1.9 for maximum root length at 28 days (Fig. 2). Another QTL found by Champoux et al. (1995) associated with *CDO365* in the middle of chromosome 11 was in a very similar, if not identical, location to the QTL for maximum root length at 28 days reported here. A third QTL for maximum rooting depth was associated with *RG*570 on chromosome 9. *RG570* was associated with a QTL for maximum root length at 28 days in this report, but the marker was on chromosome 5 (it has not yet been established if the location of *RG570* to chromosome 5 was due to a chromosomal rearrangement or merely a technical error). The fourth QTL reported by Champoux et al. (1995) for maximum rooting depth was at the top of chromosome 2 and maps in the middle of the gap between 2 linkage groups reported here.

The paper by Champoux et al. (1995) reported 15 QTLs for root thickness, while only two are reported here. The QTL for root thickness associated with *G45*/*RG171* on chromosome 2 reported here is close to a QTL for root thickness associated with *RG437* reported by Champoux et al. (1995). The QTL for root thickness associated with *C624*/*RG570* on chromosome 5 may well be related to the QTL associated with *RZ12* (next to *RG570*) on chromosome 9 reported by Champoux et al. (1995). The lower of the 3 QTLs for root thickness found on the chromosome 3 by Champoux et al. (1995) may be related to a low LOD-QTL for root thickness associated with *RG191*/*RG745* on chromosome 3 reported here. Two of the QTLs reported by Champoux et al. (1995) occur in regions where the LOD score for root thickness measured in this report exceeds 1.5, that in the middle of chromosome 1 (LOD score 1.8 associated with *C949*) and at the bottom of chromosome 12 (LOD score ranges between 1.3–1.7 in the interval between *G124* and *RZ444b*). Of the other QTLs for root thickness reported by Champoux et al. (1995), many do not seem to be revealed by this study. Most notable is the absence of QTLs in the 'Bala' × 'Azucena' population on the bottom of chromosome 6 where Champoux et al. (1995) reported a QTL of large effect on root thickness. The largest QTL for root thickness reported by Champoux et al. (1995) is near the bottom of chromosome 4, which is not covered by markers in this study.

Champoux et al. (1995) also mapped QTLs for root dry weight per tiller, which may be related to root volume. Indeed, *RG181* on chromosome 12, which was associated with a QTL for root volume in this report, was associated with root dry weight per tiller in Champoux et al. (1995). The QTL for root dry weight per tiller reported by Champoux et al. (1995) on chromosome 8 was in a similar location to a low LOD-QTL for root volume found in this report. The location of the other 5 QTLs for root dry weight per tiller do not agree with root volume QTLs in the present study.

Ray et al. (1996), using the same 'CO39' × 'Moroberekan' recombinant inbred lines, found that the lower portion of chromosome 6 contained 2 QTLs for root penetration. This was an area of QTL activity for root length at all stages of development in the 'Bala' × 'Azucena' population. Ray et al. (1996) also found that the central region of chromosome 11, around marker *CDO365*, was associated with strong QTLs for both root penetration index and total number of roots. This was where the major QTL for

hydroponic root growth in 'Bala' × 'Azucena' occurred. However, none of the other QTLs for root penetration reported by Ray et al. (1996) can be related to root growth in the 'Bala' × 'Azucena' population measured here, although the largest effect QTLs for root penetration reported in Ray et al. (1996) (on chromosomes 2 and 4) were in areas not covered in the present map.

In another population, Redoña and Mackill (1996) found 3 QTLs for maximum root length in young (10–15 days old), dark-maintained, rice seedlings. On chromosome 1, in a region not covered in the map presented here, QTLs at both test temperatures (18°C and 25°C) were found. On chromosome 2, around the marker *RG171*, a QTL for root length only at 18°C was detected. This corresponds exactly to a QTL for maximum root length at day 14 found here.

## Conclusions

Some QTLs observed in the 'Bala' × 'Azucena' population are evident in the 'CO39' × 'Moroberekan' population, whilst some are not. In the middle of chromosome 11 there is a locus which influences root growth in three environments and is detectable as a QTL in two populations. Since this QTL appears to be predominantly additive in effect on root length, it may be phenotypically selectable in a breeding programme using hydroponics. Other QTLs for root growth characteristics, some of which appear to be common between crosses and environments, are not all additive and the application of markers to select for these genes should prove invaluable.

Shoot-specific traits related to drought resistance (leaf rolling and stomatal conductance) have also been studied in this $F_2$ population and will be published. Further work to obtain more closely linked markers and investigate associations between field drought resistance and morphological traits in recombinant inbred lines derived from this $F_2$ population are ongoing.

**Acknowledgements** The authors acknowledge the assistance of Beverley Moore, Melisa Clough, Zahid Mahmood, Eirion Owen, Jo Evans, Delyth Jones and Alison Bell. We wish to acknowledge the invaluable advice given by Prof. Gareth Wyn Jones and Drs. J. Witcombe (ODA, Bangor, UK), J. Pritchard (Birmingham, UK), S. Quarrie (John Innes Centre, UK), S. McCouch (Cornell, USA) and M. Arraudeau (IRRI, Philippines). We thank the Rice Genome Project, Japan and the Department of Plant Breeding and Biometry, Cornell University, USA for RFLP probes. The work reported here has been funded by the Plant Sciences Research Programme of the Overseas Development Administration (ODA), UK.

## References

Abenes MLP, Tabien RE, McCouch SR, Ikeda R, Ronald P, Khush GS, Huang N (1994) Orientation and integration of the classical and molecular genetic maps of chromosome 11 of rice. Euphytica 76:81–87

Ahmadi N (1983) Variabilité génétique et hérédité de mécanismes de tolérance à la sécheresse chez le riz *Oryza sativa* L.: I. Dévelopement du système racinaire. Agron Trop 38:110–117

Ahn SN, Bollich CN, Tanksley SD (1992) RFLP tagging of a gene for aroma in rice. Theor Appl Genet 84:825–828

Armenta-Soto J, Chang TT, Loresto GC, O'Toole JC (1983) Genetic analysis of root characters in rice. SABRAO J 15: 103–118

Causse M, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SB, Second GA, McCouch SR, Tanksley SD (1995) Saturated molecular map of the rice genome based on an inter-specific backcross population. Genetics 138:1251–1274

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

Chang TT, Loresto GC, O'Toole JC, Armenta-Soto JL (1982) Strategy and methodology of breeding rice for drought-prone areas. In: Drought resistance in crops with the emphasis on rice. IRRI, Manila, pp 217–244

Chaudhary D, Rao MJBK (1982) Breeding rice varieties for dryland and drought-prone areas if India In: Drought resistance in crops with the emphasis on rice. IRRI, Manila, pp 265–272

Cho YG, Eun MY, McCouch SR, Chae YA (1994) The semidwarf gene, *sd*-1, of rice (*Oryza sativa* L.). II. Molecular mapping and marker-assisted selection. Theor Appl Genet 89:54–59

David CC (1991) The world rice economy: challenges ahead. In: Khush GS, Toenniessen GH (eds) Rice biotechnology. IRRI, Manila, pp 1–18

Ekanayake IJ, Garity DP, Masajo TM, O'Toole JC (1985a) Root pulling resistance in rice: inheritance and association with drought tolerance. Euphytica 34:905–913

Ekanayake IJ, O'Toole JC, Garrity, DP, Masajo TM (1985b) Inheritance of root characters and their relations to drought resistance in rice. Crop Sci 25:927–933

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shoura A, Shimizu T, Lin S-Y, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nature Genetics 8:365–372

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

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

Lebreton C, Lazic-Jancic V, Steed A, Pekic S, Quarrie SA (1995) Identification of QTL for drought responses in maize and their use in testing causal relationships between traits. J Exp Bot 46:853–865

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

Liang CZ, Gu MH, Pan, XB, Liang GH, Zhu LH (1994) RFLP tagging a new semidwarfing gene in rice. Theor Appl Genet 88:898–900

Lilley JM, Ludlow MM, McCouch SR, O'Toole JC (1996) Locating QTL for osmotic adjustment and dehydration tolerance in rice. J Exp Bot 47:1427–1436

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

Lincoln S, Daly M, Lander E (1992b) Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report, 3rd edn, Whitehead Institute, Cambridge, MA, USA

Loresto GC, Zhang WX, Chaudhary D, Chang TT (1983) Aeroponic technique for screening the drought avoidance mechanism of rice genotypes by root characters. Garcia de Orta 10: 77–82

Mackill DJ, Salam MA, Wang ZY, Tanksley SD (1993) A major photoperiod-sensitivity gene tagged with RFLP and isozyme markers in rice. Theor Appl Genet 85: 536–540

Martin B, Nienhuis J, King G, Schaefer A (1989) Restriction fragment length polymophisms associated with water use efficiency in tomato. Science 243: 1725–1728

McCouch SR , Doerge RW (1995) QTL mapping in rice. Trends Genet 11: 482–487

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76: 815–829

O'Toole JC (1982) Adaptation of rice to drought-prone environments. In: Drought resistance in crops with the emphasis on rice. IRRI, Manila, pp 195–213

O'Toole JC, Bland WL (1987) Genotypic variation in crop plant root systems. Adv Agron 41: 91–145

O'Toole JC, Soemartono (1981) Evaluation of a simple technique for characterizing rice root systems in relation to drought resistance. Euphytica 30: 283–290

Paterson A, Lander E, Lincoln S, Hewitt J, Peterson S, Tanksley S (1988) Resolution of quantitative traits into Mendelian factors using a complete RFLP linkage map. Nature 335: 721–726

Price AH, Tomos AD, Virk DS (1997) Genetic dissection of root growth in rice (*Oryza sativa* L.) I: a hydroponic screen. Theor Appl Genet 95: 132–142

Quarrie SA, Lebreton C, Gulli M, Calestani C, Marmiroli N (1994) QTL analysis of ABA production in wheat and maize and associated physiological traits. Russ J Plant Phys 41: 565–571

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

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

Sanguineti MC, Tuberosa R, Stefanelli S, Noli E, Blake TK, Hayes PM (1994) Utilisation of a recombinant inbred population to localise QTLs for abscisic-acid content in leaves of drought-stressed barley (*Hordeum vulgare* L). Russ J Plant Phys 41: 572–576

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

Stuber CW (1994) Breeding multigenic traits. In: Phillips RL, Vasil LK (eds) DNA-based markers in plants. Kluwer Academic Publ, The Netherlands, pp 97–115

Yoshida S, Hasegawa S (1982) The rice root system; its development and function. In: Drought resistance in crops with the emphasis on rice. IRRI, Manila, pp 83–96

Yoshida S, Forno DA, Lock JH, Gomez KA (1976) A laboratory manual for the physiological studies of rice, IRRI, Manila

Yu ZH, McCouch SR, Kinoshita T, Sato S, Tanksley SD (1995) Association of morphological and RFLP markers in rice (*Oryza sativa* L.). Genome 38: 566–574

Zhang G-Y, Guo Y, Chen S-L, Chen S-Y (1995) RFLP tagging of a salt tolerance gene in rice. Plant Sci 110: 227–234