

Assessing the importance of genotype \times environment interaction for root traits in rice using a mapping population II: conventional QTL analysis

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Abstract Modifying plant root systems is considered a means of crop improvement targeted to low-resource environments, particularly low nutrient and drought-prone agriculture. The identification of quantitative trait loci (QTLs) for root traits has stimulated marker-assisted breeding to this end, but different QTLs have been detected in different populations of the same species, and importantly, in the same population when grown in different experimental environments. The presence of QTL \times environment interaction is implicated, and this must be characterised if the utility of the target QTLs is to be realised. Previous attempts to do this suffer from a lack of control over replicate environments and inadequate statistical rigour. The Bala \times Azucena mapping population was grown in two replicate experiments of four treatment environments, a control, a low light, a low soil nitrogen and a low soil water treatment. After a 4 weeks growth, maximum root length, maximum root thickness, root mass below 50 cm, total plant dry mass, % root mass and shoot length were measured. A summary of the overall results is presented in an accompanying paper. Here, QTL analysis by composite interval mapping is presented. A total of 145 QTLs were detected, mapping to

37 discrete loci on all chromosomes. Superficial evidence of QTL \times E (great difference in LOD score) was tested by single-marker analysis which confirmed QTL \times E for five loci representing only five individual trait-loci interactions. Some loci appeared to be stable across environments. Some QTLs were clearly more or less active under low light, low nitrogen or drought. A few notable loci on chromosomes 1, 2, 3, 5, 7 and 9 are briefly discussed. Also discussed are some remaining statistical shortcomings that will be addressed in another companion paper.

Introduction

The way that resources are partitioning to roots, and the below-ground morphology that results, is important in determining the ability of a plant to capture resources and its subsequent productivity. With the advent of molecular markers it has been possible to study the genetic basis of intra-species or intra-genus variation in these traits and identify quantitative trait loci (QTL) of potential benefit in conferring drought resistance or improved nutrient uptake (e.g. for rice, Champoux et al. 1995; for maize, Lebreton et al. 1995). However, both partitioning and morphology of roots are affected by the environment (see recent reviews Lopez-Bucio et al. 2003; Malamy 2005), such that a plant can change its rooting behaviour in order to maximise resource capture (e.g. Ho et al. 2004). QTL analysis of root traits has generally shown that different mapping populations and different experiments on the same population reveal different QTLs (e.g. Kamoshita et al. 2002b; Price et al. 2002), although some notable consistency is maintained (e.g. Tuberosa et al. 2003;

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Zhang et al. 2001). This kind of analysis has been most thoroughly explored in rice, for which root QTLs have been reported in nine different populations and the same population deliberately subjected to different environments in six reports (Zheng et al. 2003; Venuprasad et al. 2002; Kamoshita et al. 2002a, b; Price et al. 2002; Hemamalini et al. 2000). A good description of QTL \times environment interaction was provided by Kamoshita et al. (2002b) who identified root morphological QTLs in a mapping population of rice grown anaerobically in four different experiments differing in the date of sowing and the length of the experiment. The authors found substantial evidence of genotype \times environment interaction which was also reflected in QTL \times environment interaction. The marked differences in the QTLs detected (no root morphology QTLs were detected in more than two experiments except a root thickness QTL detected in three) was considered by the authors to mostly reflect differences in the radiation received and hence on an interaction between overall growth and root distribution. Price et al. (2002) grew a different mapping population in conditions of irrigation and a water deficit. They were able to identify QTLs which appeared to be relatively insensitive to the two environments and some that were specific to only one environment.

There are two major limitations in all six of the reports described or listed above, which mean that the conclusions about QTL \times E must be considered speculative. Firstly, differences between the contrasting environments are not well characterised. In Kamoshita et al. (2002b), for example, differences in radiation between experiments are difficult to dissociate from differences in temperature, whereas in Price et al. (2002), even replications of the two treatments (which were conducted in different years) appeared to be markedly different for largely unknown reasons. Secondly, the criterion for declaring the presence of QTL \times E is not tested statistically. Most commonly, QTL \times E is reported when a QTL is detected in one environment and not another. Yet this is not a statistical test, and could be misleading if, for example, a QTL is present but at just below the operational significance threshold in one environment and above it in another. Software for QTL analysis can take mean results from different environments and test if they are statistically different and this method has been used by Kamoshita et al. (2002a, b). However, this approach does not take into account the variation between replicates within the same treatment environment. Since it has been demonstrated that different results can be obtained even when attempts are made to make replicate experiments as closely as possible (Price et al. 2002), it seems

probable that individual experiments may differ for reasons other than those imposed by the experimenter. The inclusion of the replicated nature of experimental design (where replication means a replicated experiment for each environment rather than replication of genotypes within a single experiment for each environment) can overcome this problem, but cannot be used statistically in the presently available QTL analysis software. While several papers have considered the problem of QTL mapping in a series of experiments (Jansen 1994; Jansen et al. 1995; Piepho 2000; Malo-setti et al. 2004; Broman et al. 2003; Wang et al. 1999; Li et al. 2003; Piepho 2005), the inclusion of complex experimental design structures is not currently implemented in existing QTL software. At this point, we accommodate this problem by a single-marker analysis when QTL \times environment is suggested by the marked difference in QTLs obtained in different environments. By conducting replicated experiments in highly controlled environment conditions and changing one environmental variable only, it should be possible to begin to understand the nature of QTL \times environment interaction that has clearly been suggested to greatly influence root trait development. Here, we grew a mapping population under highly controlled conditions and measured root traits of plants grown in high light, high soil nitrogen and ample soil water and compared these to three environments when each of those variables was reduced; i.e. low light or low soil nitrogen or low soil water. The overall results have been described in a companion paper to this (MacMillan et al. 2006), that revealed the presence of genotype \times environment interaction. Here, we report the detection of QTLs by composite interval mapping and the detection of QTL \times environment interaction by single-marker analysis.

Materials and methods

Full details of the experiments reported here are given in MacMillan et al. (2006). Briefly, a mapping population of 205 bulked F_6 recombinant inbred lines (RILs) was produced from a cross of varieties Bala and Azucena (original parental seed obtained from the International Rice Research Institute) by single-seed descent as described in Price et al. (2000). A total of 168 randomly selected lines were used in this study. A series of eight experiments representing two replications of four treatments (including a control) were conducted using a 90-cm deep box system, each of which allowed 26 plants to be grown in 0.125 m³ of sub-soil. The sub-soil was chosen for its packing qualities, low nutrient levels

and ease of washing from roots. Water release characteristics were determined for the soil and a uniform packing system was developed to achieve a dry bulk density of 1.225 Mg m^{-3} throughout each box, giving a penetration resistance of below 0.5 MPa (i.e. low impedance). The experiments were conducted in controlled growth room facilities in the Macaulay Land Use Research Institute under a high light intensity ($809 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR at soil surface, $1,290 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR at 50 cm above soil). The control experiment was $110 \text{ mg kg dry soil}^{-1}$ of added nitrogen (as NH_4NO_3), $1,290 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR at 50 cm and 21% volumetric water content (field capacity). Guided by preliminary experiments (not presented), the three separate treatments chosen were (1) low nitrogen of zero added N, (2) low light of $562 \mu\text{mol m}^{-2} \text{ s}^{-2}$ at height 50 cm and (3) low soil water where the soil started at 12% volumetric water content and no irrigation water was added after sowing. Each experiment contained seven boxes, each having an Azucena and Bala genotype plus 24 unique RILs, giving a total of 168 RILs out of 182 plants. Experiments were commenced on the following occasions. Control 1, 03/10/01; Nitrogen 1, 19/11/01; Nitrogen 2, 18/02/02; Light 1, 01/04/02; Control 2, 04/11/02; Drought 1, 24/03/03; Light 2, 27/05/03; Drought 2, 21/07/03. Plant height was measured weekly during the 4-week experiment. After harvest, maximum root length (MRL), root diameter, root dry mass above and below 50 cm depth, number of nodal root axis and shoot mass were measured. We have decided to concentrate on only six traits: MRL, maximum root thickness (MRT), root mass below 50 cm (RML), total plant mass (TPM), shoot height at 28 days (SL28) and % root mass (%RM).

The data were subjected to a Box–Cox transformation using a mixed model approach (formally described in MacMillan et al. 2006) in which design effects [replicate experiment, box within experiment (1–7), the frame within box (1–3)], a margin effect (whether individual positioned at a margin of the experiment), a generation effect [Azucena (P1), Bala (P2) or F_6 (RIL)] and a treatment effect were included in the model if appropriate.

Detection of main-effect QTLs was conducted as described in Price et al. (2002). The molecular map includes 102 restriction fragment length polymorphisms, 32 amplified fragment length polymorphisms (markers starting with “e,” e.g. e12m45.1 or e12m36.1) and 17 microsatellite markers (markers with prefix RM, L or MRG) on 13 linkage groups covering a total length of 1,916 cM. QTL identification was conducted by composite interval mapping using the programme

QTLCartographer version 1.15 (C.J. Basten, B.S. Weir and Z-B. Zeng, Department of Statistics, North Carolina State University). Background markers for composite interval mapping were selected by “forward stepwise regression with backward elimination” using the default threshold. The default window size of 10 cM was used. Permutation testing (using QTLCartographer) on some of this data indicated that a LOD score of 3.0–3.3 is suitable as the genome-wide 5% significance threshold for this set of data.

The loci in which QTLs have been detected have been labelled according to their chromosome and position on it. Hence, the three regions containing QTLs on chromosome 8 are labelled 8.1, 8.2 and 8.3, with 8.1 being the uppermost.

The authors are not aware of software that can conduct an adequate test for environmental specificity, which combines the advantages of composite interval mapping and incorporate replicated environments (i.e. replicated environment experiment) as well as design effects (boxes, frames, etc.). Based on the phenotypic model (1) in MacMillan et al. (2006), but with inclusion of a marker covariable and a marker \times environment interaction, a “single-marker” analysis was calculated for markers neighbouring QTL that were discovered by the composite interval mapping scan. We present the Wald F -tests of type 3 hypotheses for the QTL effect and the QTL \times environment interaction. All treatment effects were significant (with a P -value of approx. zero), but are not reported. Traits analysed in this way were limited to only those where a LOD score of 5 was detected in at least one environment.

Results

The parental and F_6 means of each of the six traits under each treatment are summarised in Table 1. All treatments affected the traits (see MacMillan et al. 2006 for full details). Most notable is the fact that the low nitrogen and low light reduced plant mass by about 50%, while the drought treatment reduced it by nearly 90%. As predicted, both low nitrogen and drought increased relative allocation of dry matter to the roots (%RM), drought quite profoundly, while low light reduced it. Also noticeable is the relative stability of MRL, which did not differ greatly (although still significantly) between treatments. For each treatment mean, the standard deviations of the parental lines were large in comparison with the mapping populations (data not shown), indicating that broad-sense heritability within a treatment for most traits were relatively small. When broad-sense heritability was calculated across all

Table 1 Mean trait data for parental genotypes Azucena and Bala, and the F₆ RILs

Treatment	Generation	MRL (mm)	MRT (mm)	RML (mg)	%RM	TPM (mg)	SL28 (mm)
Control	Az	798	1.147	50**	37.2	1,567	633**
	Bala	791	1.091	15**	35.4	1,655*	558**
	F ₆	809	1.088	24**	35.7	1,384*	591*
Low nitrogen	Az	818	1.155**	19*	45.1	696*	461*
	Bala	746	0.953**	4*	45.4	549	361*
	F ₆	749	0.992**	7*	43.4	540*	416*
Low light	Az	696	1.127*	5	30.1	812*	733**
	Bala	625	0.962*	1	28.5	554	595*
	F ₆	683	1.020*	3	30.2	1,335*	661**
Drought	Az	735*	0.481	11	59.5*	184	247**
	Bala	714	0.454	3	52.6*	115	173**
	F ₆	733*	0.470	7	55.3	165	221
Mean CV of parents		13.7%	10.3%	85.1%	12.2%	28.3%	8.7%
Heritability		64%	64%	69%	57%	73%	86%

*, ** indicates significant differences between means at $P < 0.05$ and $P < 0.01$, respectively, within a treatment using Tukey–Kramer adjustment (for comparison of all means)

treatments, however, it was over 70% for all traits except TPM, showing that by increasing replication, heritability is increased as expected. Statistically significant differences between Azucena and Bala were detected for some traits in individual screens but when analysed across all treatments, Azucena had larger values than Bala for all traits (see MacMillan et al. 2006 for full details). There was evidence of genotype \times environment interaction for all traits except MRL either detected in the parents by three-way ANOVA with factors replicate experiment, treatment and parental genotype (Table 5, MacMillan et al. 2006) or in the F₆ population by two-way ANOVA with factors, treatment and genotype (Table 6, MacMillan et al. 2006). This was, however, lower in magnitude than the genotypic effects in all cases, and often much lower.

QTLs for each trait

Details of the QTLs detected for each trait are provided in Tables 2 and 7 and a summary of the QTL positions on the molecular map is given in Fig. 1. A total of 37 loci revealed QTLs overall (Fig. 1), of which 15 loci revealed QTLs for MRL (Table 2), 6 affecting the trait in control, 2 in low nitrogen and low light and 1 in drought. Only three of these loci (1.4, 2.3 and 3.3) contained QTLs in more than one treatment. Eleven QTLs were detected in the overall average MRL and of these only 4 were detected in the analysis of single treatments, loci 1.3, 1.4, 2.3 and 9. A total of 14 loci revealed QTLs for MRT (Table 3), 6 affecting control, 2 in low nitrogen, 11 in low light and 1 in drought. Of these, three were detected in three treatments (1.4, 5.2

and 11.2), while one was detected in two treatments (2.2). Six QTLs were detected for the overall average MRT, all but one being detected in the single treatment analysis. A total of ten loci revealed QTLs for RML (Table 4), with four QTLs in control and low light, three in low nitrogen and two in drought. One QTL (9) was detected in all the treatments, while two QTL (2.3 and 5.2) were detected in two treatments. Six QTLs were detected for the overall average RML, only three of which were detected also in single treatment analysis. A total of 12 loci revealed QTLs for %RM (Table 5), 3 in control and low nitrogen, 2 in low light and 5 in drought. Two loci (2.1 and 4.1) were detected in more than one treatment. Six QTL were detected for the overall average %RM, all but one of which were detected in the single treatment analysis. A total of 17 loci revealed QTLs for TPM (Table 6), 6 in control, 4 in low nitrogen, 11 in low light and 3 in drought, of which 2 (loci 1.4 and 5.3) were detected in three treatments, while 2 (2.2 and 2.3) were detected in two treatments. Eight QTL were detected for the overall average TPM, and each was detected in at least one treatment. A total of 15 loci revealed QTLs for shoot length at 28 days (Table 7), of which there were 8 in control, 3 in low nitrogen, 6 in low light and 7 in drought treatments. Of these, three were detected in three treatments (loci 1.4, 3.5 and 9). These same three QTLs were the only ones detected for overall average SL28.

Sorting the loci by treatments, most QTL were detected in low light (36 QTL), then control (33 QTL), then drought (19 QTL) and least in low nitrogen (17 QTL). There where 40 QTL detected for the overall average.

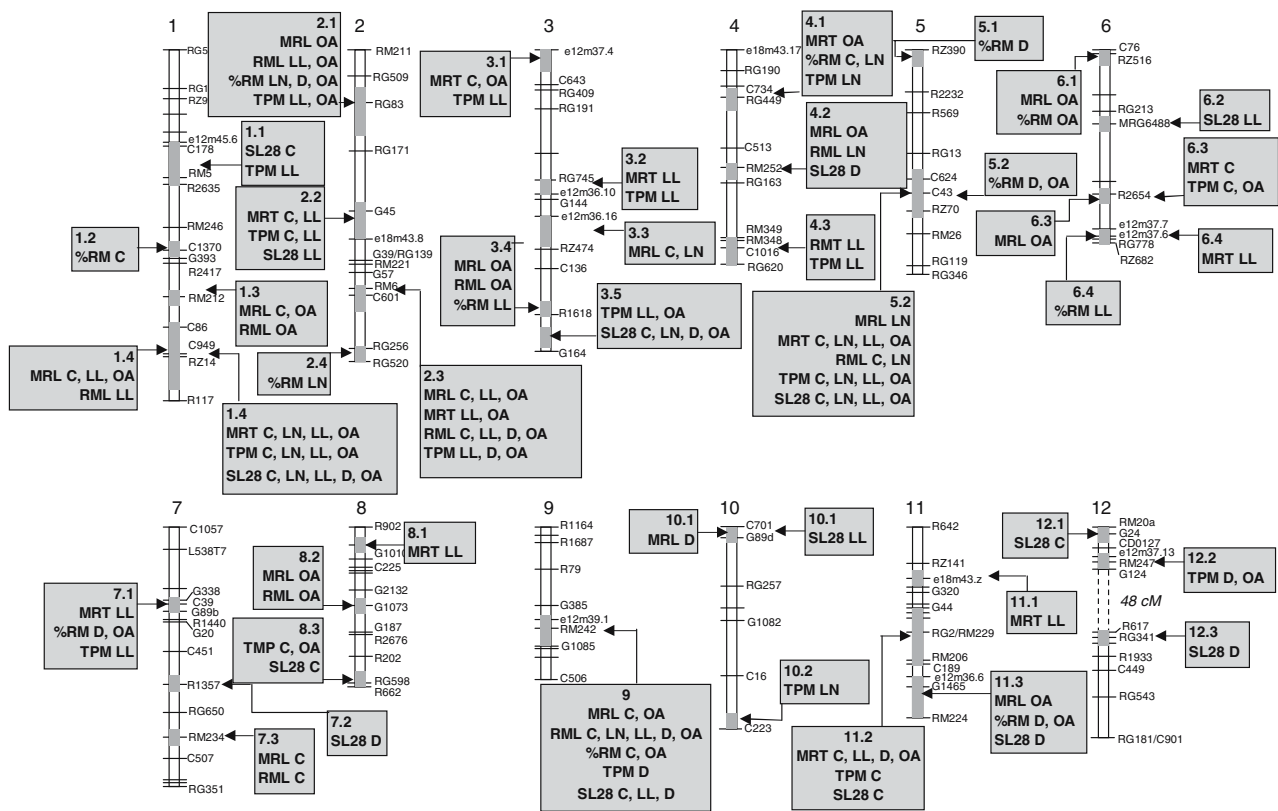


Fig. 1 Linkage map of Bala × Azucena mapping population showing the location of QTLs for the six traits for each of the four treatments (C control, LN low nitrogen, LL low light, D drought) and overall average (OA). Arrows on the right of the chromosome indicate Azucena allele increased the trait, those on the left

indicate that the Bala allele was positive. *MRL* maximum root length, *MRT* maximum root thickness, *RML* root mass below 50 cm, *%RM* percent root mass, *TPM* total plant mass, *SL28* shoot length at 28 days

Pleiotropic effects of detected QTLs

Of the 37 regions revealing QTLs, a total of 15 showed evidence of pleiotropy. That is, QTLs are detected for more than one trait under the same treatment (not including overall average). Most (seven) display pleiotropy in only one treatment, but for one region (QTL 2.2), two treatments displayed pleiotropy, while four loci (QTL 1.4, 2.3, 5.2 and 9) had pleiotropy in three treatments. These four loci are the most notable. The Azucena allele at locus 1.4 increased TPM in three treatments in addition to both MRT and SL28, but also had a negative effect on MRL in the control and low light treatments (no pleiotropy, indeed only one QTLs were detected in the drought treatment). At locus 2.3, the Azucena allele increased MRL, MRT and RML in either control or low light or both, and RML under drought while it increased TPM under low light and drought. At locus 5.2, the Bala allele increased all traits except %RM (which it reduced in the drought treatment), but only in some treatments. At locus 9, the Azucena allele increased RML in all treatments, SL28 in low light and drought, MRL and %RM in control and TPM in drought.

In 13 of the loci showing pleiotropy (including the ones just described), TPM was affected by the locus in addition to root traits, suggesting that plant growth is related to the development of root traits through a directly causal (physiological) link. Some caution must be used in interpreting this relationship, however, since root mass is a component of TPM. Thus, whether better shoot growth leads to better roots, or vice versa, or whether indeed the two are formally linked is not dissectible from the presented data. QTL analysis of shoot dry weight data, which is not presented here, reveals evidence of pleiotropy between shoot mass and TPM QTLs in all treatments except drought. In the control, four out of the six TPM QTLs are also QTLs for shoot dry weight (only exceptions is 2.2 and 6.3), in low nitrogen all three TPM QTLs are shoot mass QTLs, while in low light six out of ten QTLs for TPM are also QTLs for shoot mass. The absence of such pleiotropy in the drought treatment suggests that in that treatment, it is not shoot growth that predominantly controls root traits when plants experience water deficit. Nine of the pleiotropic events did not involve TPM, those being MRL and RML in control at

Table 2 QTLs for traits MRL revealed by composite interval mapping

QTL	QTL ^a position	LOD	R ² (%)
Control			
1.3	RM212 + 4	4.0	10.7 ^A
1.4	RZ14	6.2	10.8 ^B
2.3	RM6	3.7	6.4 ^A
3.3	e12m36.16	3.5	6.2 ^A
7.3	RM234 + 4	3.9	10.4 ^A
9	e12m39.1	4.5	8.5 ^A
Low nitrogen			
3.3	RZ474 – 1	3.5	8.6 ^A
5.2	C624	3.6	6.7 ^B
Low light			
1.4	RZ14	3.2	6.3 ^B
2.3	C601 + 2	3.9	9.5 ^A
Drought			
10	C701 + 2	3.5	8.0 ^B
Overall average			
1.3	RM212 – 3	3.9	7.5 ^A
1.4	RZ14	3.1	3.9 ^B
2.1	RG83 – 3	6.1	10.4 ^B
2.3	C602	7.2	9.4 ^A
3.4	R1618	3.1	4.2 ^B
4.2	RM252 – 4	3.2	5.8 ^A
6.1	C76	3.5	4.4 ^B
6.3	R2654 – 4	3.6	5.0 ^B
8.2	G1073	5.4	7.1 ^B
9	RM242	8.3	13.2 ^A
11.3	e12m36.6	4.3	5.3 ^A

A/B donor of positive allele, A Azucena, B Bala

^a QTL position in cM above (–) or below (+) nearest marker

locus 2.3 and locus 7.3, %RM and SL28 in drought at 11.3 and for several root traits and SL28 under all treatments except drought on locus 9.

Evidence of QTL × environment interaction

From a detailed scrutiny of Tables 2, 3, 4, 5, 6 and 7, it is possible to suggest that as many as 15 of the 37 loci reported here display QTL × environment interaction. These are loci where a QTL is detected with a high LOD value in only one or some of the treatments, but not detected in the others, or where the LOD values for the different treatments are substantially different (by at least 2). The results of the single-point analysis based on the mixed model discussed above and testing such loci for QTL effects and QTL × environment interaction are presented in Table 8. Perhaps the best example is the locus 1.4, which affects MRL, MRT, TPM and SL28. Here, QTLs for SL28 were detected in every treatment, but the LOD value in low light of 8.5 was at least 3.5 higher than in the other treatments. For both MRT and TPM, QTLs were detected in all treatments except drought, but the LOD was very high only

Table 3 QTLs for traits MRT revealed by composite interval mapping

QTL	QTL position ^a	LOD	R ² (%)
Control			
1.4	RZ14	6.1	8.7 ^A
2.2	e18m43.8	4.7	7.1 ^B
3.1	C643 – 6	4.0	8.4 ^B
5.2	C624 – 1	6.6	10.5 ^B
6.3	R2654 + 4	3.7	7.4 ^A
11.2	C189 + 2	6.1	10.0 ^B
Low nitrogen			
1.4	C86 + 8	4.7	11.0 ^A
5.2	RZ70	4.8	7.4 ^B
Low light			
1.4	RZ14	10.1	13.2 ^A
2.2	G45 – 5	3.0	5.7 ^B
2.3	RM6 – 2	4.1	6.0 ^A
3.2	e12m36.10 – 1	5.3	7.3 ^A
4.3	C1016	5.4	7.8 ^A
5.2	RZ70 + 4	3.1	5.5 ^B
6.4	e12m37.6	6.2	7.4 ^A
7.1	C39	7.2	9.2 ^B
8.1	e12m36.7	3.0	3.7 ^A
11.1	e18m43.z – 4	4.2	6.6 ^A
11.2	G44	3.9	4.8 ^B
Drought			
11.2	RM229	3.1	6.2 ^B
Overall average			
1.4	RZ14	9.7	14.3 ^A
2.3	C601 + 12	4.1	11.2 ^A
3.2	e12m37.4 + 10	3.6	8.2 ^B
4.1	RG449 – 2	4.6	8.3 ^A
5.2	RZ70 – 3	9.0	16.0 ^B
11.2	C189 – 1	6.4	9.2 ^B

A/B donor of positive allele, A Azucena, B Bala

^a QTL position in cM above (–) or below (+) nearest marker

in low light. For MRL, QTLs were detected with high LOD in control, at low LOD in low light, but not detected at all in low nitrogen and drought. The single-marker analysis confirms that, for SL28, there is evidence for highly significant QTL × environment interaction, while for the other traits at this locus, the Wald statistic indicates non-significant interaction. The single-marker analysis indicated that the additive effect of the QTL on shoot length ranked (greatest to least) low light > control > low nitrogen > drought. Four other significant QTL × environment interactions were detected: one for SL28 at QTL 3.5, two for TPM at QTLs 2.1 and 5.2 and one for MRT at QTL 11.2. For QTL 2.1, the additive effect on TPM ranked low light > control > low nitrogen > drought. For QTL 3.5, the additive effect on SL28 ranked control > low light ≈ low nitrogen > drought. For QTL 5.2, the additive effect on TPM was ranked low nitrogen ≈ low light > control > drought, and a notable observation here is that the direction of the additive effect in

Table 4 QTLs for traits RML revealed by composite interval mapping

QTL	QTL position ^a	LOD	R ² (%)
Control			
2.3	C601 + 2	3.6	6.3 ^A
5.2	C43 – 4	3.6	6.6 ^B
7.3	RM234 – 2	3.5	6.4 ^A
9	e12m39.1	8.0	13.5 ^A
Low nitrogen			
4.2	RM252 – 4	4.8	10.8 ^A
5.2	RZ70	5.9	10.8 ^B
9	e12m39.1	5.0	11.5 ^A
Low light			
1.4	RZ14	3.2	5.9 ^B
2.1	RG509 + 4	4.3	11.3 ^B
2.3	C601	6.0	11.6 ^A
9	e12m39.1	3.3	7.3 ^A
Drought			
2.3	RM6	3.4	7.8 ^A
9	RM242	4.1	9.7 ^A
Overall average			
1.3	RM212	4.5	6.8 ^A
2.1	RG83 – 5	4.9	8.3 ^B
2.3	C601	13.2	18.3 ^A
3.4	R1618	3.8	5.1 ^B
8.2	G1073	4.9	6.4 ^B
9	RM242 – 2	12.5	19.4 ^A

A/B donor of positive allele, A Azucena, B Bala

^a QTL position in cM above (–) or below (+) nearest marker

drought is opposite to that of the other treatments, giving evidence of crossover interaction. Finally, for QTL 11.2, the additive effect on MRT ranked control \approx drought > low light \approx low nitrogen. For the remaining 19 marker-trait interactions presented in Table 8, the single-marker analysis suggests no evidence of statistically significant QTL \times environment interaction.

Discussion

A number of QTL relevant to root and shoot growth have been detected here. The most notable confirm previous results obtained using this and other populations. For example, the QTL affecting plant height and a number of root traits at locus 1.4 associated with the *sd-1* gene has been previously shown to affect total plant weight and several root traits in this population when grown in soil for 8 weeks (Price et al. 2002). Another QTL with major influence on root traits found by Price et al. (2002) corresponds to locus 9, which affects all traits in the present study. The experiments in Price et al. (2002) attempted to estimate the stability of QTLs in both wet and dry soil environments, but treatment replication across years was problematic, meaning that conclusions (such as that the QTL on chromosome 9

Table 5 QTLs for traits %RM revealed by composite interval mapping

QTL	QTL position ^a	LOD	R ² (%)
Control			
1.2	C1370	3.1	6.1 ^B
4.1	C734 + 2	4.8	10.3 ^A
9	G1085	3.0	5.4 ^A
Low nitrogen			
2.1	RG171 – 9	3.4	12.0 ^B
2.4	RG520 – 2	3.4	6.4 ^B
4.1	RG449 + 10	5.1	17.8 ^A
Low light			
3.4	R1618 – 4	4.6	12.7 ^B
6.4	e12m37.6	3.5	5.6 ^B
Drought			
2.1	RG83 + 2	3.1	7.0 ^B
5.1	RZ390 + 6	3.2	8.0 ^B
5.2	RZ70 – 1	4.6	9.5 ^A
7.1	G338	3.3	10.3 ^B
11.3	e12m36.6	3.8	6.4 ^A
Overall average			
2.1	RG83 + 4	7.2	15.1 ^B
5.2	C624	5.9	8.6 ^A
6.1	C76	3.5	5.1 ^B
7.1	G89b	3.7	5.0 ^B
9	e12m39.1 – 3	4.1	7.2 ^A
11.3	e12m36.6	3.6	5.0 ^A

A/B donor of positive allele, A Azucena, B Bala

^a QTL position in cM above (–) or below (+) nearest marker

affecting root traits in both environments only affected TPM in drought) could only be speculative. The present study was designed to test genotype by environment interaction more rigorously and to use a greater range of environmental variables that would each predictably alter root partitioning and distribution.

In a companion report (MacMillan et al. 2006), the magnitude of genotypic and genotype by environment interactions of this mapping population when grown under the four contrasting but well-characterised environments was reported. It was clearly shown that, while the treatment had very substantial effects on the six traits more or less in line with prediction, genotype effects were important and that genotype by environment interaction was detectable for all traits except MRL. The QTL analysis of the same data reported here confirmed the presence of significant genotype \times environment interaction, since five loci displayed significant QTL \times E as detected by single-marker analysis (Table 8), representing five individual trait-loci interactions involving traits MRT, TPM and SL28.

Noteworthy loci

Four loci contained 10 or more QTLs, and these are considered particularly noteworthy since they account

Table 6 QTLs for TPM revealed by composite interval mapping

QTL	QTL position ^a	LOD	R ² (%)
Control			
1.4	R117 – 7	4.7	14.1 ^A
2.2	e18m43.8	3.3	5.0 ^B
5.2	C624	6.8	10.1 ^B
6.3	R2654	4.4	6.6 ^A
8.3	R662 – 2	6.3	9.1 ^B
11.2	C189	5.0	6.9 ^B
Low nitrogen			
1.4	C86 + 6	6.7	17.3 ^A
4.1	RG449 – 3	3.5	7.1 ^A
5.2	C624	7.0	12.8 ^B
10.2	C223 – 12	3.4	11.1 ^A
Low light			
1.1	R2635	4.8	6.1 ^A
1.4	R117 – 13	8.4	17.9 ^A
2.1	RG509 + 4	5.7	7.6 ^B
2.2	G45	4.1	4.4 ^B
2.3	RM6 + 2	9.5	12.3 ^A
3.1	e12m37.4	3.8	4.4 ^B
3.2	RG745	3.4	4.0 ^A
3.5	G164 – 1	3.0	3.5 ^A
4.3	RM349 – 7	3.4	8.0 ^A
5.2	RZ70 – 4	4.8	6.6 ^B
7.1	C39	3.1	3.4 ^B
Drought			
2.3	RM6	3.6	8.0 ^A
9	e12m39.1	4.7	11.2 ^A
12.2	RM247	3.4	7.0 ^A
Overall average			
1.4	RZ14	6.3	8.9 ^A
2.1	RG83 – 3	4.4	7.7 ^B
2.3	C601 + 8	5.3	12.4 ^A
3.5	G164 – 1	3.5	4.8 ^A
5.2	C624	7.2	9.8 ^B
6.3	R2654 + 2	3.7	5.6 ^A
8.3	R662	3.2	4.2 ^B
12.2	RM247	4.0	5.1 ^A

A/B donor of positive allele, A Azucena, B Bala

^a QTL position in cM above (–) or below (+) nearest marker

for 57 of the 145 QTL detected. The Azucena allele at locus 1.4, which is at the *sd-1* semi-dwarfing locus, increased shoot length in all treatments, most effectively in low light and least effectively in drought as indicated by significant QTL × environment interaction. It also increased both MRT and TPM and reduced MRL. This locus was previously shown by Price et al. (2002) to affect TPM, root shoot ratio, root dry weight, root thickness and MRL in this population. Interestingly, in that report there was evidence of crossover interaction, the Azucena allele increasing these traits under irrigated conditions but decreasing them under drought, confirming the conclusion that the *sd-1* locus interacts with drought.

Locus 2.3 was shown to affect traits MRL, MRT, RML and TPM in the overall average and low light

Table 7 QTLs for SL28 revealed by composite interval mapping

QTL	QTL position ^a	LOD	R ² (%)
Control			
1.1	e12m45.4	3.6	4.5 ^A
1.4	C86 + 4	4.2	7.8 ^A
3.5	G164 – 7	7.9	14.6 ^A
5.2	RZ70	10.0	14.5 ^B
8.3	R662 – 2	3.0	3.8
9	G385 + 6	5.6	9.2 ^A
11.2	C189	3.1	3.8 ^B
12.1	G24 – 1	6.2	9.1 ^B
Low nitrogen			
1.4	C86 + 2	5.0	9.3 ^A
3.5	G164 – 7	6.2	13.6 ^A
5.2	RZ70	7.3	12.5 ^B
Low light			
1.4	C949 – 3	8.5	14.6 ^A
2.2	G45 + 4	3.7	5.9 ^B
5.2	RZ70	6.3	8.3 ^B
6.2	MRG6488	4.3	6.1 ^A
9	e12m39.1	4.2	6.2 ^A
10.1	G89d – 1	5.8	8.6 ^A
Drought			
1.4	C86 – 4	5.0	10.5 ^A
3.5	G164 – 3	3.5	6.4 ^A
4.2	RG163 – 2	6.9	12.2 ^A
7.2	R1357 – 4	3.0	5.8 ^A
9	e12m39.1	4.5	7.6 ^A
11.3	RM224	3.0	4.7 ^A
12.3	R617	3.3	4.4 ^A
Overall average			
1.4	C86 + 4	7.5	15.7 ^A
3.5	G164 – 1	7.8	13.2 ^A
5.2	RZ70	7.7	12.8 ^B

A/B donor of positive allele, A Azucena, B Bala

^a QTL position in cM above (–) or below (+) nearest marker

treatment, and most of these four traits in control and drought, but not in low nitrogen. However, the lack of significant QTL × environment interaction suggests that this QTL affects root traits more or less stably irrespective of environment. It was also detected as a QTL for shoot dry weight in control (LOD 3.0), low light (LOD 7.5) and overall average (LOD 3.9) (data not shown), indicating that this locus affects roots and shoots alike in a pleiotropic manner. The Azucena allele at this QTL has previously been revealed to weakly increase many root traits in soil grown plants (Price et al 2002) and to strongly increase root penetration ability (Price et al. 2000).

The Azucena allele at locus 5.2 reduced MRL in low nitrogen and MRT, shoot length and TPM in all treatments except drought, with the latter two of these traits displaying significant QTL × environment interaction, confirming a lack of effect under drought. The allele reduced lower root mass in control and low nitrogen, but increased %RM in drought and overall average,

Table 8 Evidence of genotype by environment interaction for QTLs showing the Wald *F* statistic obtained for two-way analysis of variance where factors are marker genotype (G) at nearest marker and treatment (T)

QTL	Trait	LOD value for each treatment				Nearest marker	Distance between marker and QTL	Wald statistic	
		C	LN	LL	D			G	G × T
1.4	MRL ^a	6.2	0.2	3.2	0.0	RZ14	0	14.2 ^b	13.2
	MRT	6.1	4.7	10	0.7	RZ14	0–9	7.7 ^b	5.0
	TPM	4.7	6.7	8.4	0.6	RZ14	0–8	7.3 ^b	6.3
	SL28 ^a	4.6	5.0	8.5	5.0	C949	0	4.7	20.3 ^b
2.1	TPM	0.5	0.3	5.7	0.1	RG509	0–4	1.5	11.4 ^b
2.3	TPM	0.4	0.9	9.5	3.6	RM6	0–2	3.0	2.9
	RML	3.6	2.8	6.0	3.4	RM6	0–4	8.2 ^b	1.4
3.2	MRT	0.8	0.4	5.3	0.6	e12m36.10	1	2.7	4.4
3.5	SL28 ^a	7.9	6.2	2.8	3.5	R1618	3–7	19.1 ^b	14.9 ^b
4.1	%RM ^a	4.8	5.1	2.8	1.6	RG449	10	14.5 ^b	0.8
4.2	SL28	2.4	2.0	0.9	6.9	RG163	2	3.6 ^b	1.0
	MRT	6.1	4.7	3.1	1.2	RZ70	0–28	5.8	4.4
	RML	3.6	5.9	0.5	0.1	RZ70	0–22	5.5 ^b	9.0
	TPM ^a	6.8	7.0	4.8	0.4	RZ70	4–22	1.3	14.9 ^b
5.2	SL28	10	7.3	6.3	2.8	RZ70	0	10.0 ^b	7.2
	MRT ^a	0.3	0.7	6.2	0.7	RZ682	0–5	1.6	9.4
7.1	MRT	1.1	1.0	7.2	0.5	C39	0	0.4	9.0
8.3	TPM	6.3	2.0	0.5	0.1	RG598	0–2	8.8 ^b	8.8
9	RML	8.0	5.0	3.3	4.1	RM242	2–4	8.1 ^b	9.5
	SL28	5.6	0.8	4.2	4.5	e12m39.1	0–9	10.1 ^b	5.3
10.1	SL28	1.2	0.4	5.8	0.4	G89d	1	4.1 ^b	9.7
11.2	MRT	6.1	1.5	3.9	3.1	C189	0–22	15.2 ^b	17 ^b
	TPM	5.0	0.4	0.6	0.4	C189	0	2.9	8.2
12.1	SL28	6.2	0.2	0.0	0.1	G124	1	0.3	0.3

^aData is also presented in Fig. 2

^bIndicates significant effect for G and G × T interaction at a *q* level of 20%, based on Storey and Tibshirani (2003), who present the *q*-value as a FDR-based measure of significance for genome-wide studies. Tests at all markers were considered in the computation of *q*-values

although the single-marker test for QTL × environment interaction narrowly failed to detect significant effects for these traits. The QTL did display significant QTL by environment interaction for TPM, indicating that its effect on growth under drought was reduced. Indeed, for plant mass, there was evidence of crossover interaction from the additive effect of the single-marker analysis of QTL × environment interaction, indicating an opposing direction of gene action under drought compared to the other treatments. Interestingly, this QTL did not appear to affect root traits in a previous experiment (Price et al. 2002), although it has been shown to affect root penetration ability (Price et al. 2000) and to strongly affect root length and thickness in this population when grown hydroponically (Price and Tomos 1997 and unpublished data).

The Azucena allele for locus 9 increased TPM detected only under drought (and not in the overall average). This trait-locus combination is not included in the single-marker analysis of QTL × E in Table 8 because the highest LOD score was only 4.7 (Table 6), but analysis of this trait with marker RM242 did show

significant interaction (Wald statistic 11.4), indicating that it was most effective under drought, somewhat effective under low nitrogen but not at all under control or low light conditions. This allele increased all other traits except MRT in most treatments and in the overall averages. There was no other evidence of significant QTL × environment interaction at this locus. This locus was previously shown to affect many root traits, including root thickness in this population, but notably more strongly under drought than irrigation (Price et al. 2002), and it did not affect TPM in the irrigated treatment in that experiment. Therefore, the evidence from this and previous studies suggests that this allele increases root and shoot extension growth, and the allometric partitioning to roots and results in improved overall growth under drought and possibly low nitrogen.

Each of the four QTLs above affect plant height under control conditions and only locus 9 does not affect plant mass in these conditions. Another QTL affecting plant height is 3.5. The only other trait this QTL affects, however, is TPM and the effect is small; it does not affect any root traits. This implies that locus

3.5 only effects shoot extension growth in contrast to locus 1.4 (the *sd-1* locus) which affects many traits in addition to height, and which must have a more pleiotropic gene action underlying it.

Biological importance vs. statistical significance

The single-marker analysis for some of the loci-trait combinations displaying significant QTL \times environment interaction is displayed graphically in Fig. 2, along with some additional noteworthy QTLs. From observing Fig. 2 and Table 1 it is clear that some traits

display much more variation than others and that significant effects (QTL or QTL \times environment interactions) are therefore less easily detected. The diagrams in Fig. 2 illustrate the rather substantial biological effect of locus 2.3 (Fig. 2c) and locus 9 (Fig. 2h) on MRL, which is in contrast to the much smaller effect of locus 6.4 on MRT (Fig. 2g) and locus 1.4 and 3.5 on shoot height (Fig. 2b, d), but the statistical significance of these QTLs was similar. The QTL for MRL on chromosome at marker C601 (QTL 2.3) appears to have a fairly large effect in all treatments (Fig. 2c), yet it is not statistically significant in low nitrogen and drought

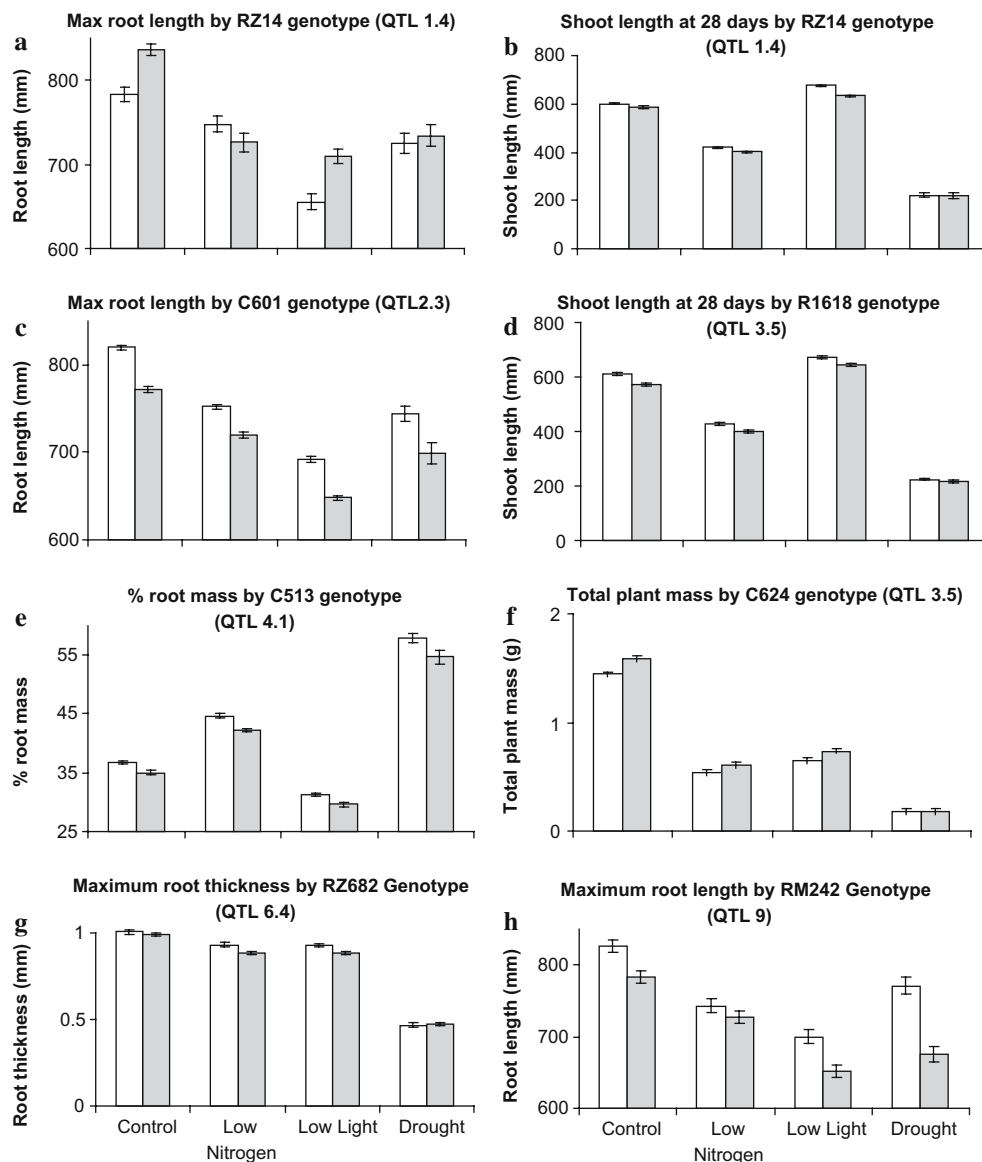


Fig. 2 Single marker analysis of a selection of QTLs. Graphic representation of the effect of a selection of QTLs in each treatment environment as revealed by the trait means of the contrasting genotypes at the nearest marker. The white bar is the mean

value for the genotypes with the Azucena marker allele, the grey those with the Bala allele. The error bar is the standard deviation from the two-way ANOVA (factors; genotype and treatment)

(Table 2), presumably because the large non-genetic variation in the trait (hence large standard deviations). The important message here is that, relative to SL28 at least, the biological importance of QTLs for root traits is under-emphasised by the statistical significance, because of the large error term in the variance of these traits.

Methodology to detect QTL \times E

We have successfully detected evidence of QTL \times environment interaction, but only five (six including TPM at QTL 9) were revealed (Table 8 and Fig. 2b, d, f). In only one case was there evidence of crossover interaction, for QTL 5.2 for TPM, although for QTL 1.4 the statistic for interaction was almost significant for MRL (Table 8), suggesting that the Azucena allele increased MRL in low nitrogen, but decreased it in control and drought (Fig. 2a). Some QTLs revealed here are relatively large (in terms of additive effects) and stable across environments [e.g. QTL 2.3 for MRL (Fig. 2c)], while others are small yet stable [e.g. QTL 4.1 for %RM (Fig. 2e)]. Others appear to be interacting with the environment and yet the interaction term is not statistically significant, for example QTL 9 for MRL (Fig. 2h), which appears to be most effective in drought and non-effective in low nitrogen.

Several approaches have been used to study the phenomenon of QTL \times E. Typical is that used by Price et al. (2002) or Kamoshita et al. (2002a, b). These researchers grew a mapping population in soil containers in different environments (e.g. different sowing dates) and measured root traits. QTL \times environment interaction was indicated if a QTL was detected in one environment but not another. However, there was no formal statistical test of QTL \times E. A more thorough approach is that demonstrated by Li et al. (2003) who grew the Azucena \times IR64 mapping population in 13 different field experiments spread over 7 sites in 4 countries in wet and dry seasons between 1994 and 2000. The means for heading date and plant height were calculated for each experiment as well as the genotype \times environment interaction for each genotype and analysed using the programme QTLMAPPER, which employs a composite interval mapping approach. They found, for example, a plant height QTLs at marker RZ730 which showed remarkable QTL \times environment interaction, this being the *sd-1* locus detected here as QTL 1.4. However, because there was no replication for each experimental environment (rather there was replication within an experiment which was used to produce a mean for each genotype in each environment), it is not possible to conclude whether the environmental interaction was

due to features of the local site, or the climate during that season, or biotic factors which impacted the experiments.

Here, we successfully combine the detection of QTLs in the mean trait data for replicated experiments of four environments with subsequent single-marker analysis of QTL \times environment interaction employing the variation due to replication in order to statistically test the evidence of QTL \times environment interaction. It must be noted that the low number of replicate experiments (two) will limit the ability to detect QTL \times E and hence the importance may be underestimated. In addition, no attempt was made to combine treatments in a full factorial design (e.g. drought and low nitrogen together), which might have been expected to reveal further QTL \times E interaction. Nonetheless, because the environments are well characterised, we can begin to determine which environmental factor is influencing which interacting QTL. The technique is also limited, however, by the separation between QTL and the nearest marker and the effect of background genetic variation on trait values, sources of inaccuracy that composite interval mapping removes. The best analysis would be one that conducts composite interval mapping whilst accommodating the replicated nature of the experiment. An analysis of this data using this approach is presented in an additional paper (that is being prepared for publication).

Conclusion

It is demonstrated that QTL \times environment interaction is an important component of the genetic determination of root growth, but notably less important than QTL main-effects. There is almost no evidence of crossover interaction detected here and many QTLs contribute in several environments. It seems one can be reasonably optimistic that QTLs 2.3, 9 and perhaps 7.2, which have previously been deployed in marker-assisted breeding programmes targeted to root-related improvement of drought resistance (Steele et al. 2006), do appear to offer benefit in the environments most closely related to drought. This is the first attempt to relate root QTLs to specific environmental variables relevant to the field, and although we have not convincingly identified QTLs specific to or absent from one environment, we have demonstrated that some QTL appear to be relatively more or less expressed under different environments, and especially drought.

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