Theor Appl Genet (2006) 113:977–986
DOI 10.1007/s00122-006-0356-5

ORIGINAL PAPER

Assessing the importance of genotype × environment interaction for root traits in rice using a mapping population. I: a soil-filled box screen

K. MacMillan · K. Emrich · H.-P. Piepho · C. E. Mullins · A. H. Price

Received: 27 October 2005 / Accepted: 23 June 2006 / Published online: 28 July 2006

Abstract Altering root system architecture is considered a method of improving crop water and soil nutrient capture. The analysis of quantitative trait loci (QTLs) for root traits has revealed inconsistency in the same population evaluated in different environments. It must be clarified if this is due to genotype × environment interaction or considerations of statistics if the value of QTLs for marker-assisted breeding is to be estimated. A modified split-plot design was used where a main plot corresponded to a separate experiment. The main plot factor had four treatments (environments), which were completely randomized among eight trials, so that each treatment was replicated twice. The sub-plot factor consisted of 168 recombinant inbreed lines of the Bala × Azucena rice mapping population, randomly allocated to the seven soil-filled boxes. The aim of the trial was to quantify QTL × environment interaction. The treatments were chosen to alter partitioning to roots; consisting of a control treatment (high-soil nitrogen, high light and high-water content) and further treatments where light, soil nitrogen or soil water was reduced singly. After 4 weeks growth, maximum root length (MRL), maximum root thickness, root mass below 50 cm, total plant dry mass (%), root mass and shoot length were measured. The treatments affected plant growth as predicted; low nitrogen and drought increased relative root partitioning, low-light decreased it. The parental varieties Bala and Azucena differed significantly for all traits. Broad-sense heritability of most traits was high (57–86%). Variation due to treatment was the most important influence on the variance, while genotype was next. Genotype × environment interaction was detected for all traits except MRL, although the proportion of variation due to this interaction was generally small. It is concluded that genotype × environment interaction is present but less important than genotypic variation. A companion paper presents QTL × environment analysis of data.

Communicated by F. van Eeuwijk

K. MacMillan · C. E. Mullins · A. H. Price (✉)
School of Biological Sciences, University of Aberdeen,
Aberdeen AB24 3UU, UK
e-mail: a.price@abdn.ac.uk

K. Emrich · H.-P. Piepho
Institut für Pflanzenbau und Grünland, Universität
Hohenheim (340), 70593 Stuttgart, Germany

Introduction

Since a plant obtains its water and mineral requirements from its roots and the availability of these resources often imposes a limit to growth, it is difficult to overstate the importance of roots to plant productivity. Root growth is profoundly influenced by the environment. Adverse conditions (chemical or physical) directly inhibit root growth (e.g. low-water potential or high/low temperature). The shoot environment can also indirectly influence root growth either via carbon supply or signaling processes (e.g. light interception, water status and nutrient status). Root development is fundamentally involved in the response to many plant stresses, in particular drought and mineral deficiency. It has been suggested that plants respond to shifts in resource supply by allocating carbon to the organ involved in capturing the limited resource (Thornley

1972; Dewar 1993). When light is limiting, plants invest in shoot biomass. When nitrogen is limiting, they invest in root production. At the mechanistic level, theories implicating sucrose supply (Farrar 1992), hormonal action (Jackson 1993) or a combination of both (Van der Werf and Nagel 1996) have been advanced to explain this phenomenon. Responses to drought or temperature are probably more complex due to a multiplicity of physical and biochemical processes directly affected. At the genetic level the response of roots to the environment is poorly understood although specific genes that regulate root growth responses to some environmental signals are being identified (e.g. Zhang and Forde 1998) and current knowledge on regulation of root growth have been recently reviewed (Lopez-Bucio et al. 2003; Malamy 2005).

One technique that has been used for studying the genetics of root growth is quantitative trait loci (QTL) mapping. Many mapping populations have been screened for root traits and this has been most thoroughly achieved in rice. In this crop, several populations have been evaluated for root traits. It can be concluded that while there is some consistency between mapping populations and between screens of the same population, there are quite marked differences. For example, in the Bala × Azucena population, which was screened in two treatments differing in irrigation and replicated over 2 years, several QTLs were considered screen or treatment specific (Price et al. 2002b). However, in general when trying to compare replicate screens of the same treatment or different treatment it is very difficult to be sure that conditions are exactly the same or predictably different. Thus differences in QTLs obtained could reflect either differences in the environmental conditions used to test populations [and resulting genotype × environment (QTL) interaction] or statistical considerations reflecting the choice of threshold and random error. Understanding which of these possibilities is the most important will enable a better evaluation of the usefulness of different QTLs in breeding for crop plants with altered root systems.

The research reported here is a description of a set of experiments on a mapping population designed to characterize the genetics of root growth in more detail, and specifically estimate the importance of genotype by environment interaction on the root growth. This was attempted by conducting two replicate experiments for each of four treatments designed to alter partitioning. The treatments compared a control (high-soil nitrogen, high light and high-water content) to conditions when one variable was modified on its own; a low-nitrogen treatment, a low-light treatment and a drought treatment. Every attempt was made to ensure that each replicate experiment was as similar as possible, by using a controlled environment growth room, a highly controlled soil packing system and by using a soil of low-nitrogen content that would not become compact on packing or watering and which was easily washed from roots.

Materials and methods

Plant material

Seeds of varieties Bala and Azucena were generated from seeds originally obtained from the International Rice Research Institute (IRRI). A mapping population of 205 recombinant inbred (F_6) lines was produced by single-seed descent as described in Price et al. (2000). One hundred and sixty-eight of these lines were selected at random for inclusion in this study.

Growth boxes

Seven growth boxes constructed from 25 mm thick marine plywood and lined with plastic were utilized in this study. Within each box, three removable hardwood frames were fitted. These possessed three sides of rectangle, with the uppermost side absent. These had a water-permeable polyester material stretched between the opposing faces of each frame. Each box complete with inner frames had internal dimensions of height (depth) 900 mm, width 770 mm and length (thickness) 180 mm giving a total volume of 0.1247 m^3, effectively divided into three equal parts of 60 mm thickness by the sheets of polyester material. The front face of each box was removable, allowing each frame to be removed.

Soil

The soil utilized throughout was Insch subsoil sourced from a cultivated field from Inschfield farm, Aberdeenshire, and NE Scotland (Ordnance Survey Grid Ref: NJ 628 295). Characterized as a freely draining sandy loam, the particle size distribution of the soil was as follows; 2 mm–60 μm = 70%; 60–20 μm = 16%; 20–2 μm = 7%; < 2 μm = 7%. This basic igneous till demonstrated good aggregate stability under irrigation, low-penetration resistance (0.1–0.5 MPa) at dry bulk density 1.27 mg m^{-3}, suitable water release characteristics, and displayed low-adhesion during root washing. The soil was pH 5.2, nitrogen content was 0.08 ± 0.05%, carbon content was 1.11 ± 0.34% and overall organic matter content was 1.94 ± 0.70%. The Insch

subsoil was therefore considered, in both physical and chemical terms, to be a suitable growth medium for the purposes of this study.

The excavated soil was hand sieved to remove stones > 15 mm diameter, before being spread out to air-dry to a gravimetric water content (GWC) of approximately 10%. The air-dried soil was then rewetted, up to a uniform water content with a 25 times concentration of standard nutrient solution (after Yoshida et al. 1976), or standard nutrient solution with the exception of the ammonium nitrate fraction, as dictated by the particular treatment.

Packing

Each box was systematically packed in 40 mm increments with soil in order to achieve a uniform and repeatable dry bulk density of 1.225 mg m^{-3}. This produced a uniform and consistent low (> 0.5 MPa) penetration resistance profile in each box. During the packing process, theta probes (Delta-T Devices, Cambridge, MA, USA) were placed horizontally at a depth of 400 mm in the centre frame of each box in order to monitor changes in soil volumetric water content during each experiment. In the case of the drought treatments, theta probes were positioned at 400 and 500 mm depth in the centre frame of each box.

Growth room

The soil filled boxes were positioned within a large (24 m^3) controlled environment room (Conviron, Canada) at the McCaulay Land Use Research Institute, Aberdeen, which had a vented floor through which a fresh supply of air was continuously circulated. The light in the room was supplied by 20 metal halide and 20 high-pressure sodium lamps (both of 400 W). These provided a mean PAR of 809 μmol m^{-2} s^{-1} (SD 32.9) at soil surface level, and 1,289 μmol m^{-2} s^{-1} (SD 116.7) at height 50 cm. Irrigation water was supplied through a network of piping and drippers, delivering a maximum of 2 l h^{-1} to each plant. A cycle of 12 h lights on at 28°C followed by 12 h lights off at 25°C was adopted. Humidity was maintained at 80% throughout, while irrigation was carried out as dictated by particular treatment.

Seed treatment

Five seeds of each line of the F_6 lines, along with 35 Azucena and 35 Bala, were surface sterilized in 1% sodium hypochlorite and then pre-germinated overnight in an incubator at 37°C. Two seeds of each line, and 14 each of Azucena and Bala, were then selected for transplantation into the soil boxes.

Experimental layout

Each box had 24 (randomly allocated) RILs and one of each parental genotype sown as two seeds per position, which were thinned to one plant after emergence. For each box, the first and last frames (one and three) contained nine lines each, equally spaced at 85 mm intervals commencing at 40 mm from the frame edge. The central frame (2) contained eight lines at 85 mm intervals commencing at 80 mm from the edge. By adopting this precise positioning of lines within the area of the box, each individual plant was exposed to the minimum of competition for light and available rooting space.

Treatments

Two replicates of each treatment were carried out in random order during this study. The treatments were composed of a control treatment, where all environmental factors were maintained at optimum levels for plant growth (soil nitrogen: added at 110 mg kg^{-1} soil (= 168 g per box or 6.5 g per plant), high-light intensity: 1,290 μmol m^{-2} s^{-1} at height 50 cm, and soil volumetric water content of 21%), and three other treatments where, in each case, a single environmental factor was reduced. These three treatments comprised.

Low nitrogen, where the ammonium nitrate fraction was omitted from the nutrient solution used to re-wet the air-dried soil, thus reducing the plant available nitrogen to that contained within the seed, and the minimal background levels within the subsoil itself.

Reduced light, where a white filter was suspended below the light banks, effectively reducing the light intensity levels by 56.4% down to 562 μmol m^{-2} s^{-2} at height 50 cm (but not affecting red:far red ratio).

Reduced water, where the volumetric water content of the soil within the boxes commenced at 12%, with no further irrigation. Measurements of volumetric water content after each experiment (which was calibrated to matric suction via GWC) indicated a matric suction of between –500 and –700 kPa at 15 cm depth, while at 30 cm depth, matric suction was around –100 kPa. Below this depth, matric suction was above –100 kPa.

With the exception of the reduced water treatments, the seven growth boxes were irrigated every 2 days. Sufficient water was supplied to maintain the soil volumetric water content, as monitored with the theta probes within each box, at a constant 21%. Individual experiments were commenced on the following

consecutive dates; Control 1, 03/10/01; Low Nitrogen 1, 19/11/01; Low Nitrogen 2, 18/02/02; Low Light 1, 01/04/02; Control 2, 04/11/02; Drought 1, 24/03/03; Low Light 2, 27/05/03; Drought 2, 21/07/03.

Harvest

After 28 days, the shoots were removed at soil surface level, bagged and dried for 1 week at 70°C. The roots were recovered by tipping each box into a horizontal position, removing the front face of the box and sliding each frame out on to an adjacent platform, where the polyester sheet was removed. A nail board was then forced into the exposed soil and the frame removed. The nail board was subsequently placed in a vertical position and the soil washed off with a supply of water, leaving the intact root structure to be removed from the nail board. The roots were then separated manually, and stored individually in 50% ethanol preservative for later analysis.

Measurements

During the course of each run, volumetric water content within the boxes was recorded daily. Every 7 days maximum shoot length was measured as the distance from soil surface to furthest shoot tip when pulled straight (SL7-21). After the 28 days growth period, the shoots were cut-off and maximum shoot length (SL28), leaf count (NoLe), tiller count (TILL) and shoot dry mass (SDM) recorded.

Measurement of the stored roots comprised; maximum root length (MRL), nodal axis count (NoNa), root thickness, as the mean diameter of the five thickest nodal axes at depth 40 mm (RT), maximum root thickness, as the diameter of the thickest nodal axis at depth 40 mm (MRT). After these measurements, the roots were cut at 50 cm from the shoot and the two separate root fractions dried at 70°C before measuring dry root mass upper (RMU; roots from 0 to 50 cm depth), root mass lower (RML; roots below 50 cm depth), and the sum of these dry mass measurements (RMT). From the resulting data, root: SDM ratios (R:S), total plant dry mass (TPM) and the per cent root mass (%RM; 100 × root dry mass/total dry mass) were calculated. In addition, leaf-rolling was observed in the drought experiments from 23 days and leaf rolling scores were recorded at days 23 and 28.

Experimental design

The trial was planned as a modified split-plot design: eight experiments were conducted, which are considered as main plots, to which four treatments were applied randomly. Thus, at the main-plot level, each treatment was replicated twice in a completely randomized design. The sub-plot factor consisted of 168 RILs and parents of the Azucena × Bala cross, which were randomized into each main plot. A further nested randomization structure is considered, because the genotypes were randomized in the seven growth boxes per experiment and each box consisted of three frames. This randomization scheme is accounted for in the statistical model. Furthermore, a margin effect and a margin × treatment interaction were included if necessary.

Data analysis

The data were subjected to a Box–Cox transformation using a mixed model approach: the fixed part of the mixed model includes a generation effect with levels for Azucena (P1), Bala (P2) and RILs (F_6), a treatment effect, a generation × treatment interaction, a margin effect and the interaction of margin × treatment. To include the randomization scheme of the experimental design, the random effects comprise of the main plot, the boxes nested within main plots and the frames nested within boxes (and main plots). A number of correlation structures to model the genetic correlation between observations of each RIL in different environments were fitted. The mixed model analysis was done by REML estimation, using the software program SAS 8 (SAS Institute Inc, Cary, NC, USA) and the mixed model function SAMM (Butler and Gilmour 2001) for S-Plus (Insightful Corp., Hampshire, UK). Model building was done as discussed in Piepho et al. (2003). The optimal mixed model was chosen by criterion of minimal AIC, analysing the random design structure and the covariance structure sequentially.

The selected model was

$$\mathrm{BC}(y_{ikqrsto}) = \mu+\tau_i+\gamma_k+(\gamma\tau)_{ik}+\delta_\theta+(\delta\tau)_{iq} +d_{rst}+t_o \cdot g_{io(k)}+e_{ikqrsto} \quad (1)$$

where BC() is the optimal Box–Cox transformation, chosen on the basis of residual analysis, $y_{ikqrsto}$ the observation of trait, e.g. MRL of rice, μ the general mean, τ_i the effect for treatments, γ_k the generation effect (RIL, P1, P2), $(\gamma\tau)_{ik}$ the generation × treatment interaction, δ_q the margin effect, $(\delta\tau)_{i\theta}$ the margin × treatment interaction, d_{rst} effect for the rth main plot, sth box in rth main plot, tth frame in sth box and rth main plot. This was modelled as $d_{rst} = a_r + b_{rs} + c_{rst}$, where the effects a_r, b_{rs} and c_{rst} are independently and identically distributed with zero expectations and

variances σ_s^2, σ_b^2 and σ_f^2, respectively, or as $d_{rst} = a_r + b_{rs}$ (for all indices t) or $d_{rst} = a_r$ (for all indices s and t), t_o the dummy variable with $t_o = 1$ for RILs and $t_o = 0$ for P1 and P2, $g_{io(k)}$ the genotype effect of genotype number o under treatment i, nested in generation effect τ_k, whereas parental genotypes are blocked out by regression against dummy t_o, resulting in a correlation structure for RILs with

$$\mathrm{var}\begin{pmatrix} g_{1o(k)} \\ g_{2o(k)} \\ g_{3o(k)} \\ g_{4o(k)} \end{pmatrix} = \Sigma,$$

where Σ is the covariance matrix of the genotypic effects with minimized Akaikes information criterion (AIC), selected from the following covariance matrices:identity (g_{oi} are identically and independently distributed with expectation zero and variance σ^2_{GT}, i.e. $\Sigma = \sigma^2_{GT} \cdot I_4$), diagonal, compound symmetry, factor analytic, heterogenic compound symmetry, unstructured and $e_{ikqrsto}$ is the independently and identically distributed error with expectation zero and variance σ_e^2.

Broad sense heritability was calculated under the compound symmetry model for $\sum = \sigma_{GT \times Env}{}^2 \cdot I_4 + \sigma^2_{GT} J_4$, where σ^2_{GT} is the genetic variance and $\sigma^2_{GT \times Env}$ is the genotype × environment (treatment) interaction variance, I_4 is the four-dimensional identity matrix, J_4 is the matrix with ones everywhere.

$$h^2 = \frac{\sigma^2_{GT}}{\sigma^2_{GT} + \sigma^2_{GT \times Env}/t + \sigma^2_e/(r \cdot t)},$$

where t is the number of treatments (4) and r is the number of replicates (2).

Results

Overview of the experiments

A summary of the means for each trait in each experiment is given in Table 1. This also indicates when replicate experiments differed. It did not prove possible to eliminate variation due to replication in these experiments. Therefore, significant differences between replicates were observed in four traits in the control experiments (RML, %RM, TPM and SL28) and the low-nitrogen experiment (MRT, %RM, TPM and SL28), for two traits in the low-light experiments (MRT and TPM) and the drought experiment (MRL and TPM). However, treatment had a very substantial effect on each trait. The size of the Wald F-statistic for treatment indicates that the traits ranked in order of degree they where affected by treatment as follows; %RM > SL28 ≈ MRT > MRL ≈ TPM ≈ RML. For MRL, all treatments were shorter than the control, with both low nitrogen and drought not differing from each other and the low light being much lower than the others. For MRT, all treatments were thinner than the control, with low light and low nitrogen not differing

Table 1 Trait means for each experiment and statistical information-based mixed model analysis

	MRL (mm)	MRT (mm)	RML[a] (mg)	%RM	TPM[a] (mg)	SL28[a] (mm)
Control 1	798	1.126	34**	34.5**	1924**	641**
Control 2	801	1.092	22**	37.3**	1231**	550**
Low nitrogen 1	768	1.116**	10	43.3**	738**	443**
Low nitrogen 2	775	0.946**	8	45.5**	456**	386**
Low light 1	659	1.072**	3	29.0	756**	674
Low light 2	677	0.998**	2	30.0	599**	657
Drought 1	745**	0.470	9	56.6	174*	217
Drought 2	711**	0.473	7	54.8	141*	207
Wald F-statistics[b]						
Treatment ($df = 3$)	50.9***	143.0***	47.9***	208.5***	50.1***	148.6***
Generation ($df = 2$)	0.5	3.9	21.9***	1.9	15.0***	35.4***
Treatment × generation ($df = 6$)	6.0	17.7***	0.2	9.0	30.5***	218.9***

* and ** indicate the two replicate experiments of the same treatment are significantly different at $P < 0.05$ and $P < 0.01$ level analysis of means was done by mixed model (1) with compound symmetry covariance matrix $\sum$, because least square means in SAS were very similar to optimal mixed model in SAMM, which offers no standard method to compare means. ***$P < 0.01$, other test statistics are not significant

[a]Models for RML, TPM and SL28 contain (fixed) margin and margin × treatment effects (Tests not shown)

[b]Sequential type 3 (i.e. terms adjusted for all others) Wald F-tests of optimal model (in SAMM) for treatment, generation and treatment × generation

from each other and the drought very much reduced in thickness. For RML, all treatments had lower deep root mass than the controls, and while low nitrogen and drought were not different, low-light treatment had a very much-reduced value. For %RM, each treatment and the control were different from each other, with drought having the highest value, followed by low nitrogen followed by the control followed by low light. For TPM, the control value was higher than the others, the low light and nitrogen were about 60% lower (although low nitrogen was lower than low light) and the drought was much smaller (only 11% of the control value). For SL28, all treatments and the control were different, with the low light slightly higher than the control, with the low nitrogen much lower and the drought much lower again.

Correlations between replication, treatments and traits

Correlations between replicate experiments are presented in Table 2. In general, traits correlated between replicate experiments, with the highest for SL28, then TPM, then RML, then MRT or %RM and, finally, the poorest correlations were with MRL. In the drought treatment there were only significant correlations between replicate experiments for TPM and SL28, indicating that the replicate runs of this experiment may have been quite different or that genotypic effects were relatively less important in this treatment.

Correlations between the average values for each treatment are given in Table 3. In general, correlations were highly significant. They were noticeable lower for all traits in comparisons with the drought treatment. The traits can be ranked in order of best to least correlations as follows; SL28 > TPM ≈ RML > %RM > MRT > MRL.

An average value (across all treatments) was calculated for each trait. The correlations between these averages are presented in Table 4. It shows that all traits were correlated with the exception of MRT with MRL, MRT with %RM and %RM with SL28. It also shows that TPM was strongly correlated with all traits.

Table 4 Correlation coefficients between the overall average of each trait

	MRL	MRT	RML	%RM	TPM
MRT	0.131				
RML	0.888***	0.170*			
%RM	0.468***	0.010	0.554***		
TPM	0.402***	0.555***	0.541***	0.265***	
SL28	0.284***	0.418***	0.363***	0.036	0.564***

*, ** and *** indicate significance of Bravais–Pearson correlation coefficient at $P < 0.05$,0.01 and 0.001, respectively

Table 2 Correlation between the two replicate experiments in each treatment and over all treatments based on least squares genotype means of a model with fixed genotypes and a fixed experiment effect

	MRL	MRT	RML	%RM	TPM	SL28	Average
Control	0.277***	0.350***	0.312***	0.260**	0.364***	0.604***	0.361
Low nitrogen	0.170*	0.360***	0.308***	0.300***	0.366***	0.687***	0.365
Low light	0.310***	0.360***	0.474***	0.370***	0.490***	0.705***	0.451
Drought	0.109	0.046*	0.079	0.110	0.219*	0.483***	0.174
Average	0.216	0.279	0.293	0.260	0.360	0.620	

The average for each treatment and for each trait is given in the final column and final row, respectively. n = approximately 160. *,**,***indicate significance of Bravais–Pearson correlation coefficient at $P < 0.05$, 0.01 and 0.001, respectively

Table 3 Correlation coefficients between treatment means and the average of these values for each treatment comparison (left-most column) and for each trait (bottom row)

Treatment comparisons	MRL	MRT	RML	%RM	TPM	SL28	Average
Control × low nitrogen	0.195**	0.408***	0.406***	0.425***	0.380***	0.759***	0.429
Control × low light	0.415***	0.529***	0.497***	0.410***	0.536***	0.756***	0.524
Control × drought	0.231**	0.097	0.229**	0.134	0.258***	0.635***	0.264
Low nitrogen × low light	0.298***	0.475***	0.382***	0.409***	0.488***	0.701***	0.459
Low nitrogen × drought	0.150	0.155*	0.232**	0.187*	0.205**	0.582***	0.252
Low light × drought	0.332***	0.025	0.261***	0.200*	0.21**	0.513***	0.257
Average	0.270	0.281	0.335	0.294	0.346	0.658	

*, ** and *** indicate significance of Bravais–Pearson correlation coefficient at $P < 0.05$, 0.01 and 0.001, respectively

Table 5 Mean trait data for parental genotypes Azucena and Bala, and Wald *F*-statistics (type 3) from optimal mixed model analysis with fixed parental genotypes

	MRL (mm)[a]	MRT (mm)	RML (mg)	%RM	TPM (mg)	SL28 (mm)
Control						
Azucena	798	1.147	50**	37.2	1,567*	633**
Bala	791	1.091	15**	35.4	1,655*	558**
Low nitrogen						
Azucena	818	1.155**	19*	45.1	696*	461*
Bala	746	0.953**	4*	45.4	549*	361*
Low light						
Azucena	696	1.127*	5	30.1	812*	733*
Bala	625	0.962*	1	28.5	554*	595*
Drought						
Azucena	736	0.481	11	59.5*	184*	247**
Bala	714	0.454	3	52.6*	115*	173**
Wald *F*-statistics[a]						
Genotype ($df = 1$)	3.99*	34.8***	39.1***	5.95*	20.37***	230.05***
Treatment ($df = 3$)	6.67***	46.79***	12.94***	55.91***	20.74***	47.33***
Genotype × treatment ($df = 3$)	0.62	4.18**	1.01	1.30	4.66***	5.61***

*, **, ***indicate significant difference at the 5, 1 and 0.1% level between Azucena and Bala (top portion of table) or of the Wald *F*-statistic (lower portion of table)

[a] *F*-tests are calculated on the basis of recoded generation effects to extract parental information. *F*-test for treatment is based on a simple main effect of treatment (in level parents)

Comparison between parental genotypes

A comparison of trait values between the parental genotypes Azucena and Bala is presented in Table 5. Since the parental genotypes are represented seven times in each replicate experiment, it is possible to analyse the variation between replicate experiments. Azucena had trait values significantly higher than Bala for nearly all traits although this was much more significant (i.e. higher *F*-statistic for genotype) for MRT, RML and SL28. Two exceptions are observed, however, for (Control, TPM) and (Low Nitrogen, %RM, difference not significant) the ranking of trait values is exchanged. The analysis reveals treatment effects are larger than genotype effects only for %RM, while for the other traits the genotype effect was of a similar (MRL, MRT and TPM) or larger (RML and SL28) magnitude to the treatment effect. For MRL (although significant) neither treatment nor genotype effect are very large indicating the relative stability of this trait. For three traits (MRT, TPM and SL28) there was evidence of significant genotype × treatment interaction although in nearly every case there was no cross-over interaction (i.e. values for Azucena were nearly always higher than Bala). The only exceptions was for TPM, were Bala was bigger than Azucena in the control treatments but was smaller in all the others treatments.

Variation across all genotypes

In Table 6, the results of analysis of all genotypes (including parents) across all eight experiments are presented. These indicate that variation due to genotype was significant at the 0.01% level, and due to treatment at least at the 5% level. There was evidence of genotype × treatment interaction for all traits except MRL. Broad sense heritability of all traits was high, being lowest for RML (57%) and highest for SL28 (86%).

Discussion

Duration of the experiment

The experiment conducted here was only 4 weeks in duration in order to minimize the competition between plants and allow a box of manageable size. There are two limitations with this. Firstly, it is possible that genotypic differences are not fully expressed in this short time. In a hydroponic experiment using two F_2 populations, including the F_2 from which this population derived, it was demonstrated that heritability in MRL increased with time from 1 to 4 weeks (Price et al. 1997). Kamoshita et al. (2002) found broadly similar heritabilities for root thickness in four experiments on a mapping population harvested between 30 and 49 days but found that heritability for deep rooting traits was highest in the experiment with the biggest plants. It is possible, therefore that heritability would increase further if the experiment was prolonged, but larger spacing between plants (and hence a larger box) would be needed to avoid competition. Secondly, it is possible that root traits do not develop in a linear

Table 6 Wald *F*-statistic and broad sense heritability calculated for the parental genotypes and the 100 RILs for which data is available for all experiments using mixed model with fixed genotype and treatment (and interaction) effects and random design effects

	df	MRL	MRT	RML[a]	%RM	TPM[a]	SL28[a]
Genotype	169	2.87***	3.80***	3.74***	3.08***	4.94***	15.6***
Treatment	3	13.8*	46.9**	12.8**	73.7***	17.0**	46.4**
Genotype × treatment interaction	500–507[c]	1.12	1.36***	1.20*	1.42***	1.51***	2.11***
Broad sense heritability[b]		64%	64%	69%	57%	73%	86%

Given are the *F*-statistics and the *P*-values (type 3)

*,**,*** indicate significant effect at the 5, 1 and 0.1% level

[a]Models for RML, TPM and SL28 contain (fixed) margin and margin × treatment effects

[b]Heritability is calculated under assumption of random RILs and a compound symmetry structure

[c]depends on the number of missing values (genotypes) (resp. class frequencies)

fashion with time, so that differences between genotypes at 4 weeks do not reflect relative performance later in plant development. While this possibility has not been extensively studied, it is reassuring to note that the parental differences detected here (summarized below) are consistent with differences in root distribution with depth at 56, 77 and 98 days growth in the field (Cairns et al. 2004). Importantly for the aims of this research, it is possible that this limitation of the screening system reduces the ability to detect genotype × environment interactions and hence conclusions here may understate their importance.

Effect of treatment

It is possible to predict that all the treatments imposed here would reduce total plant mass and alter the partitioning between roots and shoots. Both drought and low nitrogen would be expected to increase root partitioning while low light would be expected to reduce it. The observations reported here confirm these predictions. The low nitrogen and light experiments reduced total mass by 57 and 65%, respectively, while the drought treatment decreased total mass by 89%. The %RM was higher than the control for both the drought and low-nitrogen treatment (57 and 21% higher, respectively), while for the low-light treatment it was 16% lower. The quantity of deep roots (below 50 cm) was lower in all treatments compared to the control but was 84% lower in the low-light experiment (compared to nearer 50% lower for drought and low nitrogen). The proportion of total root mass can be calculated from the data presented. For the control, deep root mass represents 5.5% of total root mass, low nitrogen 4.8%, low light 2.4% and for drought 15.1%, indicating a fairly substantial change in the relative depth distribution between treatments (relative less deep roots in low light and more in drought). MRL was the trait least affected by treatment, although all treatments reduced it (compared to control) with low light being the most marked. Root thickness was slightly (but significantly) reduced by low nitrogen and low light but it was very markedly reduced by the drought treatment. Plant height was reduced by the low-nitrogen treatment, very substantially reduced by the drought and increased by the low-light treatment. These observations all indicate that the treatments were successful in creating substantial differences in root and shoot growth suitable for the further study of the main sources of variation, identified either using analysis of variance, as used here, or using a QTL approach which is detailed in an accompanying article.

Differences between Azucena and Bala

Azucena and Bala have been repeatedly compared using different growth media and growth conditions. In general, it is possible to conclude that Azucena is a taller plant with less tillers and roots. While MRL is sometimes greater than Bala (Price et al. 1997), it is not always (Price et al. 2002a), but root thickness is invariably higher in Azucena. Azucena also has more roots at depth. These observations are confirmed here, with Azucena being taller in every treatment, with longer and thicker roots when analysed across all treatments. Azucena also had more roots below 50 cm (although in the low light and drought experiment it was not a significant difference). Interestingly, TPM revealed significant interaction with environment (treatment) indicating that Azucena had a higher biomass than Bala in all treatments except in the control where it was the other way around. This contradicts the result obtained by Price et al. (2002a) when the same genotypes where grown in individual chambers. In that report, Azucena was a larger plant than Bala in the well-watered treatment but under drought the two were similar. The contradiction probably reflects the fact that in the present study, plants are competing with each

other for below ground resources, and that in the two studies, water deficit treatments were quite different.

Sources of variation and evidence of genotype × environment interaction

Within these experiments, there are five main sources of variation discussed in the phenotypic analysis; replication, genotype, treatment, genotype × treatment interaction and error (or seven with generation and generation × treatment, cf. Table 1). It is clear that replicate experiments did differ, as evidenced by significant difference in traits detailed in Table 1. While these differences were greater for some traits (e.g. TPM) than others (e.g. MRL), the means presented in the table illustrate that variation due to replicate was much lower than that between treatments. Thus it can be concluded that the variation caused by replication was relatively small (even if statistically significant in some instances). Variation due to treatments was generally the largest source of variation. This is most clearly shown in Table 6 for 102 genotypes where treatment effects were much higher than genotype effects. This result reflects success in producing treatments that caused a very substantial change in trait values and relatively good replication of environments across experiments.

In Tables 5 (parents) and 6 (102 genotypes), there is evidence of highly significant genotype effects for all traits indicating an important genetic component to the trait values. This conclusion is confirmed by the relatively large values for broad sense heritability obtained for all traits (Table 6) and the generally significant correlation between replicate experiments (Table 2). It is notable, however, that the drought experiment appears to display less genotypic variation as evidenced by lower correlations for traits both between replicates (Table 2) or between treatments (Table 3).

Tables 5 and 6 indicate evidence of genotype by environment interaction for all traits except MRL. It is noteworthy, however, that the genotype × treatment interaction statistic, even when highly significant, was invariably much lower than the genotype statistic, indicating that it is a relatively small component of the genotypic variation. It is also noteworthy that for the parental genotypes, there was no strong evidence of cross-over interaction except for TPM.

It is possible that these carefully managed experiments underestimate genotype × environment interactions since they use young plants (see above). Interactions might also be underestimated because the experiments do not consider combined treatments (e.g. both drought and low nitrogen), nor other stresses that interact with those chosen, such as biotic factors. It would be useful, therefore, to investigate the phenomenon of genotype or environment interaction in the field.

Conclusions

It can be concluded that the treatments employed here caused a substantial change in all traits measured in a manner consistent with prediction. Every attempt was made to produce two identical replicate experiments for each treatment. Despite the observation that replicate experiments did differ for many traits, the magnitude of variation caused by replication was very much lower than that caused by treatment. A high degree of genetic variation was observed and although genotype by treatment interaction was observed for all traits except MRL, the magnitude of its effect was lower than the genotypic effect.

Acknowledgments This document is an output from a project funded by the BBSRC (project no. P13058). The authors would like to thank the following who assisted in the harvesting and measurements of roots; Ken (Mick) Fuller, Justyn Pritchard, Alison McIntosh, Dave Hadwen, Farkhanda Khowaja, Sadeque Ahmed and Aidan Keith. We would also like to thank Allan Sim from the McCaulay Land Use Research Institute, Aberdeen, for his very considerable and considerate assistance with the growth rooms and soil management.

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