Theor Appl Genet (2002) 104:743–750
DOI 10.1007/s00122-001-0815-y

B. L. Rauh · C. Basten · E. S. Buckler IV

Quantitative trait loci analysis of growth response to varying nitrogen sources in *Arabidopsis thaliana*

Received: 4 January 2001 / Accepted: 30 April 2001 / Published online: 13 March 2002

Abstract Nitrogen absorption and assimilation is variable among plants as a result of two factors: the source of nitrogen available and the genetic variation among species within the resulting nitrogen pathways. Several genes involved in nitrogen cycling have been identified, yet little is known about the genes that control quantitative responses to different nitrogen sources. With quantitative trait loci (QTL) mapping in *Arabidopsis thaliana* recombinant inbred lines (Columbia × Landsberg *erecta*) we have identified chromosomal regions controlling aerial mass, root mass, and root length when plants are grown in nitrate, ammonium, ammonium nitrate, or low nitrogen treatments. A total of 16 QTL ($P < 0.01$) were identified among the nitrogen treatments. Most of the QTL were specific to a single treatment. The percentage additive genetic effects of significant QTL were as high as 17%. Five significant QTL corresponded to the locations of candidate genes associated with nitrogen assimilation, while a few QTL corresponded with candidate genes in the developmental pathways. Most QTL were not shared across treatments, suggesting that there is no optimal genotype for all nitrogen sources.

Keywords Quantitative trait mapping · QTL × environment interaction · *Arabidopsis* · Nitrogen utilization · Roots

Communicated by H.C. Becker

B.L. Rauh · E.S. Buckler IV (✉)
USDA-ARS, Department of Genetics,
North Carolina State University, Raleigh,
NC 27695-7614, USA
e-mail: buckler@statgen.ncsu.edu
Fax: +1-919-5153355

C. Basten
Program in Statistical Genetics, Department of Statistics,
North Carolina State University, Raleigh,
NC 27695-8203, USA

E. S. Buckler IV
Plant Science Research Unit, USDA-ARS, Raleigh,
NC 27695-7614, USA

Introduction

Nitrogen is the most limiting nutrient required for plant growth due to high demand by plants (Mattson et al. 1991) and often needs to be supplemented for proper plant growth and development. Overall, an estimated sixty-five billion kilograms of nitrogen are applied annually to the world's crops, which provides large agricultural benefits (Peoples and Crasswell 1992); however, up to two-thirds of applied nitrogen accumulates as runoff, resulting in detrimental consequences to the environment (Frink et al. 1999). Nitrogen supplementation has led to the development of several types of inorganic nitrogen fertilizers. The first fertilizer to be put into widespread production was ammonium sulfate ($(NH_4)_2\ SO_4$; Russel 1984). Ammonium nitrate ($NH_4\ NO_3$) and other more complex nitrogen mixes are the most common fertilizers used today. Despite its importance, the molecular quantitative genetic response to these basic sources of nitrogen remains largely unknown.

The research reported herein examined the quantitative genetics of nitrogen use and plant growth. Critical towards this goal is establishing how genetic backgrounds interact with nitrogen sources (genotype by environment interaction, G×E) and determining which individual genes interact with various nitrogen sources. The determination of individual genes will permit results to be applied to a wide range of taxa. *Arabidopsis* provides a model system, where the prevalence of genotype and nitrogen source interactions and the underlying genetic loci can be rapidly identified.

Quantitative trait loci (QTL) mapping is an efficient method to determine the genomic regions that contribute to growth in different nitrogen environments. Composite interval mapping (CIM; Zeng 1993) has become the most powerful QTL mapping approach in widespread use, as it controls for the genetic effects of multiple genomic regions simultaneously, resulting in a higher resolution of QTL mapping. QTL effects across environments can also be compared to evaluate the effects of loci in different treatments (Jiang and Zeng 1995; Zeng

1994). A general problem with QTL studies across multiple treatments is determining whether the absence of a QTL in a treatment is the result of low statistical power or biological reality. In this research, we explicitly tested the hypothesis that quantitative effects are different between treatments (QTL by environment interaction, Q×E) through a new implementation of composite interval mapping (Basten et al. 1998; Jiang and Zeng 1995). Mapping in four treatments may enable determination of whether QTL were more likely to be involved in developmental versus nitrogen use. For example, QTL that show up consistently across nitrogen treatments are most likely to be genes responsible for the development of the plant, while QTL with large environmental interactions are most likely to be conferred by genes in the nitrogen use pathways.

In this research, we establish the heritability of aerial and root growth for multiple nitrogen sources in both *Arabidopsis* recombinant inbred lines and ecotypes. The prevalence of genotype by nitrogen treatment interactions was also established for a large group of lines. By QTL mapping, we identified genomic regions that contribute to aerial and root growth, but most were specific to single treatments. These QTL suggested a group of positional candidate genes that may allow the future identification of the genes controlling the observed interactions with nitrogen source.

Experimental procedures

Plant material and growth conditions

Seeds of 56 ecotypes collected from Europe and 99 recombinant inbred lines (RIL) of the cross Columbia (Col) × Landsberg *erecta* (L*er*) (Lister and Dean 1993) were obtained from the Arabidopsis Biological Resource Center. Plants were grown in Metromix 200 soil-less planting media containing a starter fertilizer (approximately 0.32, 3.61, 3.83 kg/m^3, respectively, for nitrogen, phosphorus, and potassium, as determined by the North Carolina Department of Agriculture) in Rootrainer (Spencer-Lemaire Industries, Edmonton, Alta, Canada) pots (3.8×5.1×20.3 cm). The greenhouse was maintained at 23°±3 °C and was supplemented with fluorescent light 24 h per day. Seeds were cold-treated at 4 °C for 48 h before planting. One plant of each of the 99 RI lines and their two parents were arranged in a completely randomized block design across each of four treatments and replicated five times. Pots were initially misted daily with tap water containing an average of 0.35 mg/l nitrate and no readable nitrites to maintain moisture. Most seeds germinated between days 5 and 7. Any plants that had not germinated by day 7 were excluded from the analysis. Fertilization treatments were initiated 10 days after planting, and 25-ml aliquots of solutions of either 8 m*M* $(NH_4)_2\ SO_4$, 16 m*M* KNO_3, or 8 m*M* $NH_4\ NO_3$, or of the control solution – tap water as used in misting – were applied on alternating days for a total of nine applications. The concentration of fertilizer applied was determined by the amount necessary to supply equal molar amounts of N. Twenty-eight days after planting, each replication was collected by washing soil from the roots and recording the root length to the nearest millimeter. The plant was then divided into aerial and root portions, and the roots were rinsed of any remaining soil and blotted dry. The fresh weights of the aerial and root portions were then measured to the nearest milligram.

Some of the plants flowered within 23 days; however, a regression analysis indicated that flowering time was not correlated with the main traits of interest and, therefore, was not included in further analysis. It was also observed that the length measurements might have been slightly compromised, as some control plants and some plants grown in KNO_3 were reaching the bottom of the pots.

Statistical analyses

Type-III sum of squares was used to partition out replication and treatment effect with GLM analysis using SAS/STAT software, version 7 of the SAS System for Windows (SAS Institute, Raleigh, N.C.). Correlation coefficients were calculated using data averaged across replications with the correlation procedure of the SAS system. Broad-sense heritabilities were calculated using one-way ANOVA as outlined by Lynch and Walsh (1998) on the same data. Molecular mapping data used in this study were obtained from the *Arabidopsis thaliana* database at http://genome-www.stanford.edu/Arabidopsis/. A subset of 189 total markers for the five chromosomes was used, with a maximum intermarker distance of 10.5 cM and an average distance of 3.01 cM. The list of markers used in this study can be accessed at http://statgen.ncsu.edu/buckler/. Molecular markers were analyzed for skewness to either parent by conducting a Chi-square test on the marker data, assuming a 50% contribution by each parent.

QTL mapping was used to identify the genomic regions controlling growth characteristics. Composite interval mapping was chosen to identify the responsible QTL because it is statistically powerful, has better resolution of the QTL peaks, and is able to control for a number of background markers (Zeng 1993; Zeng and Weir 1996). QTL analyses were calculated with the software package QTL CARTOGRAPHER v1.13a (Basten et al. 1998) using forward-backward regression, a maximum of five background parameters, and the default window size of 10 cM. The experiment-wise LOD (log of the odd ratio) threshold significance level was determined by computing 1,000 permutations of each morphological character (Churchill and Doerge 1994), as implemented by QTL CARTOGRAPHER. These permutations can account for non-normality in marker distribution and trait values. The levels of significance for QTL in this study were determined to be $P \leq 0.05$: LOD 2.79, $P \leq 0.01$: LOD 3.56, and $P \leq 0.001$: LOD 4.53.

QTL by environment interactions were estimated using the multitrait mapping method of Jiang and Zeng (1995) that is implemented in the QTL CARTOGRAPHER module JZmapqtl. When identical genotypes are grown in different treatments, it is equivalent to what Jiang and Zeng refer to as design I. For each trait in turn, phenotypes from four treatments were analyzed simultaneously. Experiment-wise significance levels were determined by the permutation test (Churchill and Doerge 1994) with 1,000 permutations.

Results

To examine *Arabidopsis* aerial and root growth response to different fertilizers, we applied four treatments of $(NH_4)_2\ SO_4$, KNO_3, $NH_4\ NO_3$, or a control (low nitrogen) to 99 RIL and 56 ecotypes. The aerial and root portions of plants grown in $(NH_4)_2\ SO_4$ were smaller in appearance when compared to other treatments for most all traits; the only exception occurred with the ecotypes in the control treatment (Table 1). The plants grown in KNO_3 were vigorous, with long, thick, fibrous roots, as exhibited by these plants ranking highest in root length. In comparison, plants treated with $NH_4\ NO_3$ had shorter root lengths than plants from the KNO_3 treatment, although, aerial mass in the former was highest of all treatments. Control plants treated with low nitrogen tap water grew roots of a length similar to those treated with $NH_4\ NO_3$, yet the root mass and aerial mass were lower than plants fertilized with either $NH_4\ NO_3$ or KNO_3. In general, the growth response of the RIL and ecotypes were similar for each of the fertilizers; however, the ecotypes were substantially larger on average. Root length, aerial mass, and root mass were all positively correlated to one another among the RIL in all of these treatments; for the various comparisons Pearson correlations (r) ranged from 0.45 to 0.85.

Table 1 Means for three morphological traits measured across four nitrogen treatments in two studies using either recombinant inbred lines of the cross Columbia × Landsberg *erecta* or 56 diverse ecotypes

Trait	Treatment[a]			
	$(NH_4)_2\ SO_4$	KNO_3	$NH_4\ NO_3$	Control (low N)
Recombinant inbred lines				
Root length (mm)	124.6[c]	189.5[a]	175.5[b]	176.2[b]
Aerial mass (mg)	109[d]	225[b]	258[a]	137[c]
Root mass (mg)	93[d]	193[a]	166[b]	136[c]
Ecotypes				
Root length (mm)	173.1[c]	213.3[a]	209.4[a]	202.3[b]
Aerial mass (mg)	370[c]	695[b]	881[a]	213[d]
Root mass (mg)	197[d]	308[b]	353[a]	247[c]

[a] Values in horizontal rows followed by different letters are significantly different from each other at $P \leq 0.05$ as determined by the t-test

To evaluate the contribution of genetic background on each of these traits, broad-sense heritability was estimated. Heritabilities were moderate for most traits in all treatments and ranged from 0.18 to 0.58. Root mass was generally the least heritable, which may reflect measurement error. The highest levels of heritability were generally found in the two treatments with nitrate. Overall, the ecotypes exhibited substantially higher heritabilities relative to the RIL.

The relative importance of genetic background, fertilizer treatments, and experimental replication was examined by an analysis of variance (Table 2). As expected, treatment effects were extremely important in both the RIL and ecotype experiments, with the largest effect on aerial mass. Replication was also an important factor contributing to the observed phenotypic variation, suggesting a significant variation in the greenhouse environment. Genetic background (RIL or ecotype) was also very important in explaining the overall variation and sometimes had a larger effect than did treatment. The relative importance of genetic background, replication, and treatment was similar for both the RIL and ecotypes, except for the root mass trait. This analysis also indicated that for most traits there was significant genotype by treatment effect (Table 2). This G×E effect was less significant in the ecotypes.

To evaluate how prevalent the genotype by treatment effect was among the lines, the contribution of each line to the overall genotype by treatment effect was estimated. A general linear model was used to remove the main

Table 2 Type-III sum of squares from analysis of variance for three growth traits measured in two studies using either 99 recombinant inbred lines (RIL) of the cross Columbia × Landsberg *erecta* or 56 diverse ecotypes

Source of variation	*df*	Root length	Aerial mass	Root mass
(Line) RIL	98–100	$3.81\times10^{5***}$	2.793^{***}	1.526^{***}
Replication	4	$1.47\times10^{5***}$	1.526^{***}	4.571^{***}
Treatment	3	$9.78\times10^{5***}$	5.313^{***}	1.946^{***}
RIL × replication	389–391	$9.17\times10^{5**}$	3.246^{***}	2.860^{**}
RIL × treatment	300	$6.51\times10^{5*}$	2.224^{***}	2.439^{***}
Replication × treatment	12	$9.17\times10^{5***}$	0.904^{***}	0.402^{***}
(Line) Ecotype	55	$3.95\times10^{5***}$	20.44^{***}	6.761^{***}
Replication	4	$0.43\times10^{5***}$	5.59^{***}	1.272^{***}
Treatment	3	$2.51\times10\ ^{5***}$	80.08^{***}	4.080^{***}
Ecotype × replication	165	$2.14\times10^{5**}$	15.81^{***}	5.084^{**}
Ecotype × treatment	220	2.07×10^{5}	12.74^{*}	4.093
Replication × treatment	12	$1.50\times10^{5***}$	6.98^{***}	3.051^{***}

*, **, *** Values are significant at the 0.05, 0.01, and 0.001 levels of probability, respectively

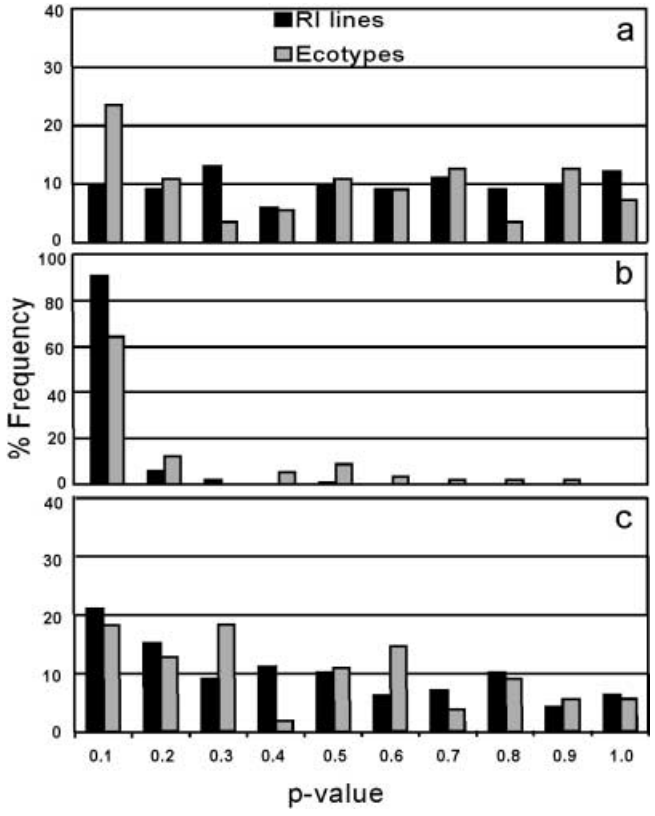

Fig. 1 Genotype × treatment effect estimated using analysis of variance on the residuals of the three phenotypes among 99 RIL (*black*) and 56 diverse ecotypes (*gray*): **a** root length, **b** aerial mass, **c** root mass Note: plot **b** (aerial mass) is not on the same scale as plots **a** and **c**

treatment and replication factors, and then the residuals of this analysis were used to evaluate significant G×E interactions for each genotype by a second series of ANOVAs. Only a few ecotypes contribute to the G×E effect seen in the root traits (Fig. 1). However, the G×E effect was extremely prevalent among genotypes for aerial mass, where 66% of all the lines had a significant G×E effect ($P<0.05$). The same basic G×E pattern was seen for both the RIL and ecotypes.

QTL mapping

To identify some of the genomic regions that control aerial and root growth and the interaction of growth traits with nitrogen treatment, we mapped the QTL in the recombinant inbred lines. Segregation of the framework markers was not the expected 1:1 ratio across much of the genome (data not presented). Many of the molecular markers chosen for the QTL analysis of chromosome 1 were skewed toward Columbia, from 50 cM to the end. All markers chosen on chromosome 2 were skewed toward Landsberg *erecta*, as well as those from 0 to 71 cM on chromosome 5. The marker bias may decrease the power of QTL analysis within these chromosomal regions.

A total of 19 QTL were identified for various traits in the four treatments ($P<0.05$; Table 3), of these there were seven that were significant at the $P<0.01$ level. In all, there were 15 loci with more than a 10% additive effect on phenotype. There were four QTL for root length, ten for aerial mass, and five for root mass. Five of the QTL were present in the $(NH_4)_2$ SO_4 treatment, eight were present in the NH_4 NO_3 treatment, and three loci were present in both the KNO_3 treatment and the control. It is important to note that the QTL were quite different treatment to treatment (Fig. 2).

There were three regions of strong effect in the $(NH_4)_2$ SO_4 treatment among its five significant QTL. On chromosome 2, approximately 77 cM from the proximal end of the chromosome (IIcM77-79), the Columbia allele reduced root length, aerial mass, and root mass, while the Columbia alleles on the upper arm of chromosome 3 (IIIcM0-17) and on the upper arm of chromosome 5 (VcM0-6 and VcM9-12) had a 16% increase in aerial mass and positive effects on root length and root mass. There were only two significant QTL detected under KNO_3 treatment. The first QTL was located at IIcM18-28 where the Columbia alleles increased aerial mass with little effect on root traits. The Columbia alleles were also responsible for the last QTL, which was also located on chromosome 2 (IIcM62-66). This QTL was responsible for a 10% decrease in root length with little effect on the mass traits.

Eight significant QTL were identified in the NH_4 NO_3 treatment. Alleles from the Columbia parent were responsible for an increase in mass traits with little effect on root length in regions IcM80-86, IIcM18-28, IIcM31-35, and IIcM37-39. The remaining locus was located at IIIcM23-36, with the allele coming from the Landsberg parent; it substantially decreased aerial mass (12%) and also had a deleterious effect on root length (5%) and root mass (7%).

The control contained just three QTL, one of which was highly significant. One of the QTL that increased root mass was located at IIcM31-35 and was also responsible for a slight increase in aerial mass. The last two QTL were located at VcM26-29 and were responsible for significant increases in aerial mass and root mass, with no effect on root length.

Composite interval mapping is sensitive to the number of background markers included in the analysis. The relatively low number of five background markers was used in the analysis described above, as heritability for these traits was moderate and the inclusion of too many background markers can over parameterize the model. However, for comparison, QTL identified using ten background markers were also examined. All but one of the QTL identified using five background markers were also significant when ten background markers were used, however, an additional 15 QTL were identified. It is likely that some of these QTL are real. These results can be accessed at www.statgen.ncsu.edu/buckler. The number of background markers may also have an effect on QTL position. For example, using ten background markers, we

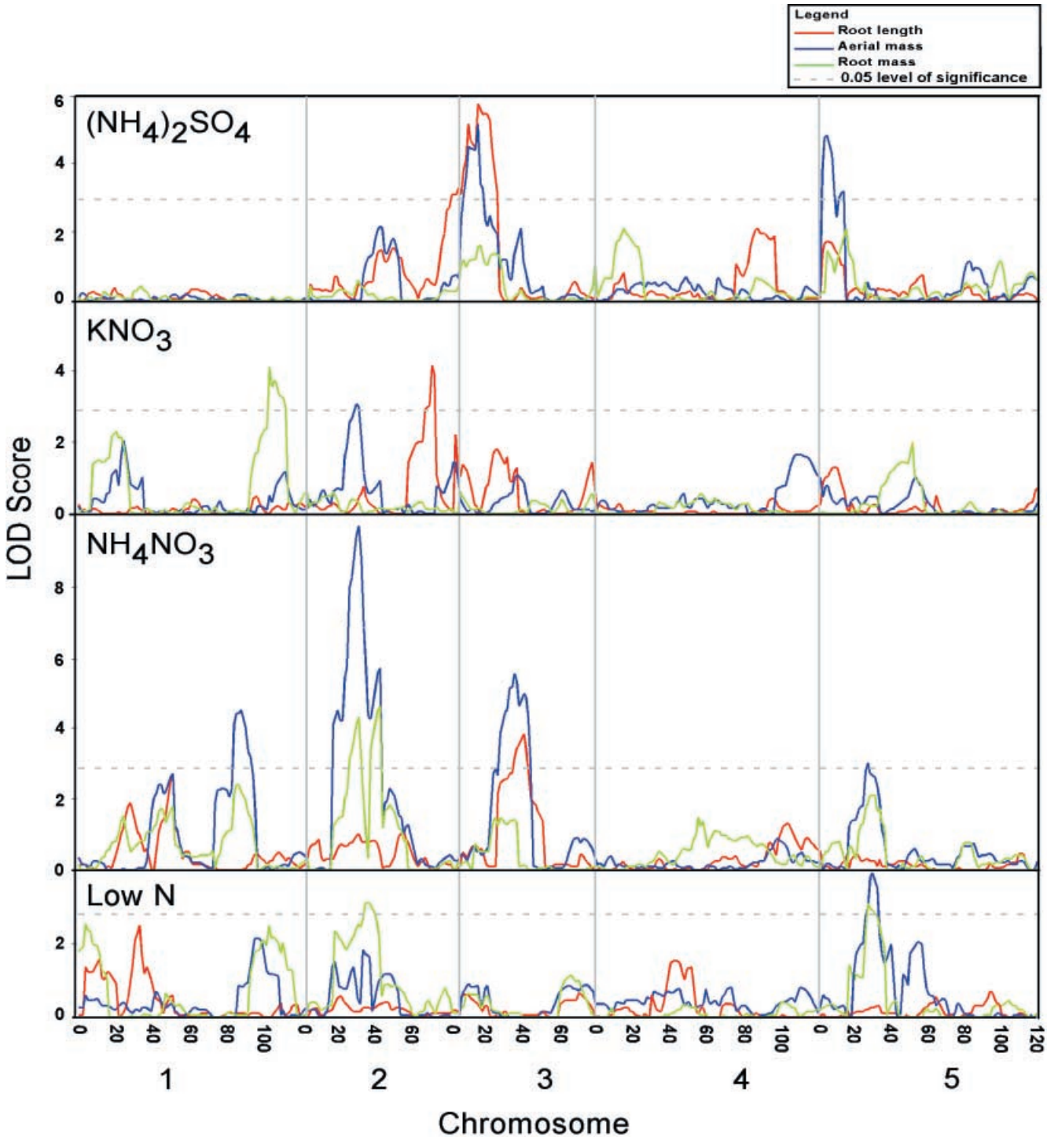

Fig. 2 QTL likelihood plots (LOD) of three traits in four different nitrogen treatments where chromosomal position is measured in centiMorgans.

identified three QTL in the middle of chromosome 2. However, using five background markers, we identified only two QTL. One of the shifted/fused QTL is probably the *erecta* locus. This effect of background marker choice was probably strongest in regions with many QTL and large-scale marker bias (e.g., middle of chromosome 2).

To examine QTL by environment interactions (Q×E), we analysed the phenotypes from pairs of treatments (Table 3). Three QTL shared Q×E interactions for root mass (IcM98-106, IIcM31-35, IIcM51), four for aerial mass (IIcM31-35, IIcM37-39, IIcM51, IIIcM38), and one QTL for root length (IIIcM33). Most QTL appear to indicate that the relative performance of Columbia or Landsberg alleles was unique in most of the nitrogen treatments. For example, the pairwise comparisons for QTL at positions IIcM31-35, IIcM39, IIcM51, and

Table 3 Major QTLs, percentage of mean additive genetic effect[a], and positional candidate genes that are all, associated with growth characteristics across four environments.

QTL position[b]	Nearest associated marker	Root length				Aerial mass				Root mass				Q × E[c]	Positional candidate loci
		$(NH_4)_2SO_4$	KNO_3	NH_4NO_3	Control	$(NH_4)_2SO_4$	KNO_3	NH_4NO_3	Control	$(NH_4)_2SO_4$	KNO_3	NH_4NO_3	Control		
IcM80-86	mi209	1	−1	−1	−1	3	−2	11**	6	2	2	10	2		SAB(88)
IcM98-106	g4552	0	−1	−2	−2	−1	5	2	7	13**	−2	3	10	r: 12, 23, 24	TIR2(102)
IIcM18-28	g4532	3	2	3	1	−1	8*	17***	7	4	6	16**	10		
IIcM31-35	m216	3	2	3	2	7	4	14***	8	−1	4	9	12*	a: 13, 23, 34 r: 13, 23, 34	STP1(34),
IIcM37-39	O802F	6	1	3	2	10	5	14***	7	−3	1	16***	10	a: 13, 23	SUR1(35), CHL2(36), CP2(38), T30L20.1-3(40)
IIcM51	er	4	0	0	0	0	−3	−3	5	6	1	2	4	r: 13, 23, 34 a: 13, 23, 34	ER (48)
IIcM62-66	ve015	5	−10**	−2	−1	−2	−1	2	−2	−2	−1	1	4		
IIcM77-79	m336	−8*	5	1	−1	−6	5	2	−1	−1	−5	4	2		T19C21.22(74)
IIIcM0-17	mi172	13***	4	−2	2	17**	1	5	6	4	10	−6	5		ABI8(11), SPY(12), GS-kB6(22),
IIIcM23-36	m105	−3	−4	−5*	0	−11	−5	−12***	0	−2	−5	−7	2		AXR2(33)
IIIcM38	mi225	−1	−4	0	0	0	4	−4	−9	0	−1	1	0	a: 13, 34 rl: 13, 23, 34	
VcM0-6	g3715	6	3	1	0	16***	4	2	4	1	14	−6	−4		
VcM9-12	nga225	5	3	1	1	13*	3	1	4	1	12	−7	−4		GLU1(10), CBB3(15)
VcM26-29	ve033	−2	1	1	1	6	3	9	13**	−3	−5	9	11*		ASN3(25), ASP3(25), TIP1(27), GA3(33), GDH1(33)

*, **, *** Significance at the 0.05, 0.01 and 0.001 probability levels, respectively, as determined by 1,000 permutations

[a] Positive effect represents favorable alleles from the parent Columbia; negative effects represent deleterious alleles from the Columbia parent

[b] QTL position is indicated by first the chromosome number then the centiMorgan (cM) region cumulative from the distal end of the chromosome according to the published map (Lister and Dean 1993)

[c] Q×E effects that were significant at $P > 0.05$ were listed where r, a, and rl refer to root mass, aerial mass, and root length, respectively. Treatment with $(NH_4)_2SO_4$ corresponds to treatment 1, treatment with KNO_3 to treatment 2, treatment with NH_4NO_3 to treatment 3, and the control is represented as treatment 4

IIIcM38 were significant ($P<0.05$) between treatments of $(NH_4)_2\ SO_4$ and $NH_4\ NO_3$ (treatments 1 and 3, respectively), KNO_3 and $NH_4\ NO_3$ (treatments 2 and 3, respectively), and the low N control and $NH_4\ NO_3$ (treatments 4 and 3, respectively). This indicated that the Columbia and Landsberg alleles in this region responded differently to the $NH_4\ NO_3$ treatment relative to all other treatments. Another QTL (IcM98-106) showed specific Q×E interactions involving effects between the $(NH_4)_2\ SO_4$ and KNO_3 treatments in root mass. Additional QTL may exhibit G×E, but statistical power was limited to find significant G×E.

Discussion

The overall growth of these plants for various nitrogen sources was similar to what has been observed in other studies (Marschner 1995) and, as often happen, plants grown with NH_4^+ as the only source of nitrogen may have been pH stressed (Schubert and Yan 1997). Beyond these basic growth patterns with various nitrogen sources, this study clearly established the strength and prevalence of genotype by nitrogen regime interactions.

Genetic variation played an important role in how these lines responded to the nitrogen fertilizers tested, but the genetic interactions with nitrogen treatments often appeared to explain even more variation (Table 2). This indicated that many of the genotypes interacted differently with the nitrogen treatments. The ubiquity of these interactions was demonstrated by examining interactions line by line (Fig. 1). This was especially true for aerial mass, which exhibited major G×E interactions in almost all lines. The reason that only aerial mass commonly exhibits G×E interactions is unclear, but it has important implications. It suggests that few genotypes grow well across all nitrogen sources.

Positional candidate gene identity

Half of the QTL for the phenotypes measured in the Columbia × Landsberg *erecta* population were specific to individual nitrogen treatment. The lack of consistent QTL across treatments could have been the product of little statistical power (Lynch and Walsh 1998), although the five significant QTL by environment interactions suggested that there are some alleles that perform well in specific nitrogen treatments and that these interactions may be ubiquitous. These QTL by environment interactions suggest that the metabolic rate-limiting step for nitrogen utilization varies tremendously with the nitrogen source. In contrast, genes involved in basic development would be expected to show up in multiple treatments. The positions of the significant QTL regions were compared to the positions of mutants in *Arabidopsis* and genomic sequence data. This comparison identified many possible candidates genes for these QTL (Table 3), including many nitrogen-related genes.

The positional candidate genes fell into two classes – genes involved with growth, and genes involved in nitrogen pathways: First, nitrogen utilization is dependent upon the development of the plant relative to soil nutrients. In order to take full advantage of inorganic nitrogen present, plants employ two physical features of root architecture, root length and number of lateral roots. Some QTL were positioned close to root mutants that appear to modify root length such as *superroot* (Boerjan et al. 1995) and *spindly* (*SPY*; Jacobsen and Olszewski 1993). Longer roots may better position the plant for nitrogen uptake. Lateral roots are responsible for the "foraging" ability of the plant; consequently, an increased number of lateral roots per plant may be beneficial. Nitrate has also been shown to be an essential signal for initiation of lateral roots (Zhang et al. 1999), although one of the most interesting candidate genes, *ANR1* (Zhang and Forde 1998), had no nearby QTL.

Second, nitrogen utilization in crop plants depends upon the efficient utilization of inorganic nitrogen available, in particular NO_3^- and NH_4^+. The plasticity of genes within these two pathways is likely to be responsible for the genetic basis of many Q×E interactions. One QTL fell near the *Arabidopsis* GmSAT1-like transporter (Kaiser et al. 1998), which could be involved in initial ammonia uptake. Nitrate is broken down via nitrate reductase in the presence of the molybdenum-pterin cofactor (MoCo) encoded by the *CHL2* locus, which is near one of the QTL (LaBrie et al. 1992). Ammonium is assimilated into amino acids through a cyclic pathway involving three enzymes. QTL have been identified near representative genes of each of these three enzymes: glutamine synthetase (GS-kb6; Peterman and Goodman 1991), glutamate synthase (*GLU1*; Lam et al. 1995), and glutamate dehydrogenase (*GDH1*; Lam et al. 1995).

It is unfortunate that the regulatory genes that control these enzymes are not well understood as these genes would be likely candidates for these QTL. A more refined list of positional candidates will be produced by comparative sequencing between Columbia and Landsberg and through expression profiling. Combining *Arabidopsis* genomic sequencing with QTL mapping and candidate gene association approaches will allow these quantitative traits to be dissected at the single nucleotide level.

Conclusions

We found significant differences in QTL depending on the nitrogen treatment used. If these patterns observed in *Arabidopsis* are similar to those in crops, it suggests that breeding crops for increased nitrogen efficiency will involve adapting each variety to the nitrogen source that will be used in production, rather than to a range of nitrogen sources. Evaluating the Q×E effects presents a promising method of distinguishing developmental genes from those involved in a specific nitrogen pathway. The combining of this QTL mapping with *Arab-*

idopsis genomics made it possible to rapidly suggest a list of positional candidate genes.

Acknowledgements We would like to thank Dianne Beattie and Melissa Fabiano for their help in data collection. This research benefited also from many discussions with Michael Purugganan, Ben Bowen, David Remington, Jeff Thornsberry, and Sherry Whitt. This research was generously supported by Pioneer Hi-Bred International and USDA-ARS. We hereby declare that the experiments herein comply with the current laws of the country in which the experiments were performed.

References

Basten CJ, Weir BS, Zeng ZB (1998) QTL CARTOGRAPHER: a reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, N.C.

Boerjan W, Cervera MT, Delarue M, Beeckman T, Dewitte W, Bellini C, Caboche M, Vanonckelen H, Vanmontagu M, Inze D (1995) Superroot, a recessive mutation in *Arabidopsis*, confers auxin overproduction. Plant Cell 7:1405–1419

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Frink CR, Waggoner PE, Ausubel JH (1999) Nitrogen fertilizer: retrospect and prospect. Proc Natl Acad Sci USA 96:1175–1180

Jacobsen SE, Olszewski NE (1993) Mutations at the *SPINDLY* locus of *Arabidopsis* alter gibberellin signal transduction. Plant Cell 5:887–896

Jiang CJ, Zeng ZB (1995) Multiple-trait analysis of genetic-mapping for quantitative trait loci. Genetics 140:1111–1127

Kaiser BN, Finnegan PM, Tyerman SD, Whitehead LF, Bergersen FJ, Day DA, Udvardi MK (1998) Characterization of an ammonium transport protein from the peribacteroid membrane of soybean nodules. Science 281:1202–1206

LaBrie ST, Wilkinson JQ, Tsay YF, Feldmann KA, Crawford NM (1992) Identification of two tungstate-sensitive molybdenum cofactor mutants, *chl2* and *chl7*, of *Arabidopsis thaliana*. Mol Gen Genet 233:169–176

Lam HM, Coschigano K, Schultz C, Melooliveira R, Tjaden G, Oliveira I, Ngai N, Hsieh MH, Coruzzi G (1995) Use of *Arabidopsis* mutants and genes to study amide amino-acid biosynthesis. Plant Cell 7:887–898

Lister C, Dean C (1993) Recombinant inbred lines for mapping RFLP and phenotypic markers in *Arabidopsis thaliana*. Plant J 4:745–750

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Assoc, Sunderland, Mass.

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, San Diego

Mattson WJ, Haack RA, Lawrence RK, Slocum SS (1991) Considering the nutritional ecology of the spruce budworm in its management. Forest Ecol Mgmt 39:183–210

Peoples MB, Crasswell ET (1992) Biological nitrogen fixation: Investments, expectations, and actual contributions to agriculture. Plant Soil 141:13–39

Peterman TK, Goodman HM (1991) The glutamine-synthetase gene family of *Arabidopsis thaliana* – light regulation and differential expression in leaves, roots and seeds. Mol Gen Genetics 230:145–154

Russel D (1984) Conventional nitrogen fertilizers. In: Hauck R (ed) Nitrogen in crop production. ASA-CSSA-SSSA, Sheffield, Ala. pp 183–194

Schubert S, Yan F (1997) Nitrate and ammonium nutrition of plants: effects on acid/base balance and adaptation of root cell plasmalemma H+ ATPase. Z Pflanz Bod 160:275–281

Zeng Z-B (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976

Zeng Z-B (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468

Zeng Z-B, Weir BS (1996) Statistical methods for mapping quantitative trait loci. Acta Agron Sin 22:535–550

Zhang H, Forde BG (1998) An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. Science 279:407–409

Zhang HM, Jennings A, Barlow PW, Forde BG (1999) Dual pathways for regulation of root branching by nitrate. Proc Natl Acad Sci USA 96:6529–6534