Mapping quantitative trait loci associated with selenate tolerance in *Arabidopsis thaliana*

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**Summary**

- Selenium is essential for many organisms, but is toxic at higher levels. To investigate the genetic basis of selenate tolerance in *Arabidopsis thaliana*, quantitative trait loci (QTL) associated with selenate tolerance in accessions Landsberg erecta and Columbia were mapped using recombinant inbred lines (RILs).
- The selenate tolerance index (TID₁₀ = root growth + 30 µM selenate/root growth control × 100%) was fourfold higher for parental line Col-4 (59%) than for parent Ler-0 (15%). Among the 96 F₈ RILs, TID₁₀ ranged from 11 to 75% (mean 37%).
- Using composite interval mapping, three QTL were found on chromosomes 1, 3 and 5, which together explained 24% of variation in TID₁₀ and 32% of the phenotypic variation for the difference in root length +/- Se (RL₁₀). Highly significant epistatic interactions between the QTL and markers on chromosome 2 explained additional variation for both traits. Potential candidate genes for Se tolerance in each of the QTL regions are discussed.
- These results offer insight into the genetic basis of selenate tolerance, and may be useful for identification of selenate-tolerance genes.

**Key words:** *Arabidopsis thaliana*, composite interval mapping (CIM), epistatic interactions, quantitative trait loci (QTL), recombinant inbred lines (RIL), selenium (Se), tolerance.


**Introduction**

Selenium is an essential micronutrient for animals and bacteria (Birringer *et al*., 2002), but also has long been known for its toxicity at higher concentrations (Draize & Beath, 1935; Schwarz & Foltz, 1957). Selenium is essential because certain proteins require selenocysteine in their active site (Stadtman, 1990, 1996). Essential selenoproteins have been found in bacteria, archaea and eukaryotes. In higher plants there is no evidence for the essential nature of Se, although Se is essential for certain algae (Doblin *et al*., 1999; Fu *et al*., 2002; Obata & Shiraiwa, 2005). Selenium toxicity is thought to be caused by its chemical similarity to sulfur, leading to the replacement of S by Se in proteins and other S compounds. Both Se deficiency and toxicity occur worldwide, depending on Se availability in the environment. Selenium deficiency in humans and animals occurs in several low-Se areas of the world, including a region in China stretching from the north-east to the south-west (Tan *et al*., 2002), as well as the eastern USA (Combs, 2000). The toxic effects of excess Se have been found in several regions, including Hubei Province, China (Tan *et al*., 2002) and the western USA (Terry & Zayed, 1998).

Both the environmental and health concerns related to Se could potentially be alleviated by modifying plant Se metabolism. Plants with enhanced Se tolerance and accumulation could be used for environmental clean-up, and may also have added nutritional value, as a diet rich in Se has been shown to reduce the risk of cancer and various infectious diseases (Combs *et al*., 1997; Orser *et al*., 1999). In order to maximize the plant's capacity to accumulate Se in a form that is most...
desirable, we need to know more about the factors that control plant Se metabolism. Selenium enters plants in the form of selenate (SeO₄²⁻) or selenite (SeO₃³⁻) and is metabolized via the S-assimilation pathway (Terry et al., 2000; Ellis & Salt, 2003). Certain plant species, such as Astragalus bisulcatus (Byers, 1936) and Stanleya pinnata (Feist & Parker, 2001), have been found to tolerate and accumulate extraordinary levels of Se (0.1–0.5% DW) even from low external concentrations. Other species, such as many Brassicaceae, can tolerate and accumulate Se to high levels, but do not hyperaccumulate from low external concentrations. These plants serve as good models to investigate the physiological and genetic basis of Se metabolism in plants.

Genetic studies are a useful approach for the isolation of novel Se-tolerance genes from plants. For traits that are controlled by multiple genes, quantitative trait locus (QTL) mapping is a powerful approach to identify the number, position and effect of genetic factors that contribute to phenotypic variation. QTL mapping is performed by testing for statistical associations between the genotype of molecular markers distributed across the genome and the phenotypic values of the trait of interest. This approach has become easier to implement because of the advancement of PCR-based molecular markers (Kearsey & Farquhar, 1998); new statistical procedures (Lander & Botstein, 1989; Zeng, 1993); and the availability of software programs (Basten et al., 1994; Manly & Olson, 1999; Sen & Churchill, 2001). QTL mapping is a first step towards an understanding of the molecular genetic mechanisms behind phenotypic complexity. It has become a particularly fruitful approach in the sequenced model plant Arabidopsis thaliana (Lukowitz et al., 2000; Maloof, 2003). Recently, QTL studies have been conducted in A. thaliana for photomorphogenesis (Borevitz et al., 2002); salt tolerance (Quesada et al., 2002); aluminium and caesium tolerance and accumulation (Kobayashi & Koyama, 2002; Hoekenga et al., 2003; Payne et al., 2004); and root growth and architecture (Mouchel et al., 2004; Olivier et al., 2005).

In Se hyperaccumulator plants, no genetic mapping analyses have been done because of the lack of molecular markers. As A. thaliana shows intraspecific variation in Se tolerance (as shown in our previous studies), and many molecular tools are available for this species, QTL mapping in this model plant may identify new genes involved in Se tolerance. Recombinant inbred lines, which are generated by repeatedly selfing the offspring from a cross (F2 until F6 or greater) until > 95% of the loci are homozygous, have been generated for various Arabidopsis accessions. These lines provide a convenient population for QTL mapping. We used RILs from a cross between Ler-0 and Col-4 (Lister & Dean, 1993) to map the QTL responsible for variation in selenate tolerance. Several QTL were identified in this population. Further analysis of these chromosomal regions should increase our understanding of the mechanisms that control plant Se tolerance.

**Materials and Methods**

**Plant material**

Recombinant inbred lines (RILs) from a cross between Ler-0 and Col-4 [Lister & Dean, 1993, Arabidopsis Biological Resource Center (ABRC), Columbus, OH, USA, stock number Cs1899] were used to map the QTL responsible for variation in selenate tolerance. Parent lines Ler-0 and Col-4 were also obtained from the ABRC (stock numbers Cs20 and Cs933, respectively).

**Selenate tolerance of parental lines and RILs**

Selenate tolerance was estimated by measuring root length in the presence of selenate, compared with plants grown simultaneously under identical conditions lacking selenate. To assess selenate tolerance for both parental lines and to determine the selenate concentration giving the maximal tolerance difference, the parental lines Ler-0 and Col-4 were grown from seed on agar medium with different selenate concentrations. For each treatment, 30 seeds from each of the accessions were sterilized, vernalized at 4°C for 3 d, and sown as 2 × 15-seed replicates on half-strength Murashige and Skoog (MS) medium (Murashige & Skoog, 1962) containing 10 g l⁻¹ sucrose and 4 g l⁻¹ agar gel (Sigma, St Louis, MO, USA), supplied with selenate (30, 40 or 50 µM) or without selenate. Plates were placed vertically in a growth chamber and incubated at a light intensity of 40 µmol m⁻² s⁻¹ in a 12 h photoperiod at 24°C. After 10 d the seedlings were harvested and seedling root length was measured as a parameter for Se tolerance (Murphy & Taiz, 1995). The 30-µM selenate concentration was chosen to test 100 RILs (F₅ generation) from a cross between Ler-0 and Col-4, as well as the parental lines. The RIL evaluations were conducted twice, using the experimental conditions described above. For each evaluation, 15 seeds per RIL were sown on half-strength MS medium with or without selenate (30 µM); four RILs failed to germinate and 96 gave useful data.

Data were recorded or calculated for the following traits:

- RL₉₁₀ (root length after 10 d growing on control medium);
- RL₉₅₁₀ (root length after 10 d growing on 50 µM selenate);
- RL₉₃₁₀ (root length after 10 d growing on 30 µM selenate);
- RL₂₉₁₀ (root length after 10 d growing on 20 µM selenate); and
- TI₉₅₁₀ (tolerance index, TI₉₅₁₀ = RL₉₅₁₀ − RL₉₅₁₀/RL₂₉₁₀ × 100%).

**Statistical methods**

**General**

Means, standard errors, mean comparisons, correlations and distributions were determined with the software package JMP-IN version 3.2.6 of SAS Institute (Cary, NC, USA). ANOVA was followed by statistical comparison of each pair of means (t-test, α = 0.05). Distributions were examined for normality using the Shapiro–Wilk W-test.

**QTL analysis**

A total of 119 molecular markers were chosen from among the available ‘framework’ markers to provide...
complete coverage of all five chromosomes at intervals of less than 5 centiMorgan (cM). The genotypic data for the
96 lines and the 119 marker positions were obtained from
the Nottingham Arabidopsis Stock Center website (http://
nasc.nott.ac.uk/new_ri_map.html). The genotypic and
phenotypic data sets were imported into the computer
program QTL CARTOGRAPHER version 2.5 (Wang et al.,
2005). Composite interval mapping (CIM) was used, which
takes background genetic variation into account, for more accurate
QTL detection and characterization. To conduct CIM, Model 6 was used of the Zmapqtl procedure of QTL CARTOGRAPHER,
specifying an RI1 population (RILs derived by selfing) and
scanning the genome every 2 cM. The default settings for
Model 6 were used (five background cofactors determined by
forward stepwise regression and a 10 cM window). This
procedure estimated the log-likelihood (LOD) score, additive
effect, and percentage phenotypic variance explained every
2 cM along each chromosome. QTL locations were determined
as the positions with peak LOD score exceeding the threshold
significance level (2.5–2.8). Empirical threshold levels for
declaring the presence of a QTL at the genome-wise significance
levels of 0.05 and 0.01 were obtained by analyzing 1000
 permutations of the data, according to the method of Churchill
et al. (2006). To determine the percentage phenotypic
variance accounted for by all the QTL for a given trait, the
marker closest to each QTL was identified, and these markers
were combined in multiple-locus models with the GLM
procedure of SAS/STAT version 8.2 (SAS Institute). Pairwise
epistatic interactions between the identified QTL and all other
markers were evaluated with the SAS/STAT GLM procedure,
using the EPISTACY program (Holland, 1998) and a significance
threshold of \( P < 0.001 \). Finally, the significant epistatic
interaction terms were combined with the markers most
closely linked to the QTL in multiple locus models (SAS/STAT
GLM procedure).

Verification of QTL using SSLP markers
To determine the genotypes around the molecular markers
ABI3 and g4560, two simple sequence-length polymorphism
(SSLP) markers, ciw11 and ciw8, were selected and used for
cosegregation analysis. These markers were not part of the
119 markers used for the QTL study, nor were they used for
genotyping by Lister & Dean, 1993 (http://nasc.nott.ac.uk/
new_ri_map.html). Marker ciw11 is located 776 kb from
ABI3 on chromosome 3, and ciw8 is 157 kb from g4560 on
chromosome 5. Ten each of the extreme selenate-tolerant and
selenate-sensitive RILs (judged from TID10) were screened.
The selected selenate-sensitive RILs were from the same RIL
population: Cs1914, Cs1919, Cs1926, Cs1932, Cs1943,
Cs1948, Cs1949, Cs1952, Cs1954 and Cs1986. The selected
selenate-tolerant RILs were: Cs1906, Cs1927, Cs1946,
Cs1947, Cs1957, Cs1974, Cs1989, Cs1995, Cs1996 and
Cs1997. The selenate tolerance indices were between 51 and
75%, and 11–25% for the 10 Se-tolerant RILs and the 10
Se-sensitive RILs, respectively. Leaf samples from the 20
selected RILs were used for DNA isolation, following the
’shorty’ protocol described by the University of Wisconsin
Arabidopsis knockout facility (http://www.biotech.wisc.edu/
NewServicesAndResearch/Arabidopsis FindingYourPlant.asp).
DNA from the RILs was then used as a template for
amplification by PCR using the SSLP markers described
above. Parental lines Cs20 (Ler-0) and Cs933 (Col-4) were
included for reference. Detailed information about the primer
sequences, the PCR conditions, as well as the sizes of the PCR
fragments of Cs20 and Cs933 accessions can be accessed online at http://carnegiedpb.stanford.edu/publications/
methods/psuppl.html. The PCR products were separated
on high-resolution gels in order to resolve the small size
differences; 4% gels were used prepared with low-melting
point agarose purchased from ISC BioExpress (Kaysville, UT,
USA).

Selenium and sulfur accumulation studies
Six plant types were used for Se and S accumulation analysis:
the two parental lines, two selenate-sensitive RILs (Cs 1948,
Cs 1949), and two selenate-tolerant RILs (Cs 1927, Cs 1989).
100 plants of each type were grown from surface-sterilized
seeds on MS agar medium containing selenate (30 µM).
The plates were incubated horizontally in a growth chamber
(24°C, 12 h photoperiod) for 3 wk. Root and shoot materials
were harvested separately, rinsed with distilled water, and
dried at 70°C overnight. Three to five replicates consisting of
several seedlings each (20 mg per replicate for shoots and
10 mg for roots) were acid-digested and analyzed for Se and
S by inductively coupled plasma atomic emission spectrometry
(ICP-AES) as described by Pilon-Smits et al. (1999).

Results
Scoring selenate tolerance of parental lines and RILs
Parent line Col-4 (Cs933) grew much better in the presence
of selenate than line Ler-0 (Cs20); it also grew somewhat
better on control medium (Fig. 1a). To correct for differences
under control conditions, the Se-tolerance index (TID10 = root
growth + 30 µM selenate/root growth control × 100%) was
compared between the parental lines. The TID10 was significantly
higher for Col-4, and this difference was greatest when grown
on medium containing 30 µM selenate (fourfold, Fig. 1b).
Therefore these selenate concentration was chosen for subsequent
experiments, scoring the selenate tolerance of offspring obtained
from crosses of Col-4 and Ler-0.

The root length was scored for the parental lines as well as
(\( F_2 \)) RILs after 10 d growth on control medium and on
medium supplied with 30 µM selenate. On the selenate medium,
Col-4 had five times longer roots than Ler-0 (Table 1); the
root length of Col-4 on control medium was again somewhat higher than that of Ler-0. The root lengths of the RILs varied approximately threefold on control medium and approximately sevenfold on selenate medium (Table 1). On selenate medium, the RILs showed a normal distribution ($P > 0.05$; Fig. 2a) with an average intermediate between the parent lines (Table 1).

The Se-tolerance index $T_{SI10}$ was calculated for each RIL and the parental lines. The $T_{SI10}$ of Ler-0 was fourfold lower than that of Col-4, and the $T_{SI10}$ for the RILs varied approximately sevenfold, with a normal distribution and an average that was intermediate between both parental lines (Table 1; Fig. 2b). The coefficients of variation (CV) for both parameters root length on the +Se medium and TI were more than twofold higher than the CV on the control medium (Table 1). Thus the genetic variation may be larger for the Se treatment than the control treatment. However, as CV is variance expressed relative to the mean, then for a given level of precision of measurement, if the mean is only half as great, then CV will be twice as high. This may also explain in part the higher CV observed on selenate medium. Almost all the RILs segregated between Ler-0 and Col-4, suggesting that the major Se-tolerance alleles are from the Col-4 parent and the Se-sensitive alleles are from Ler-0.

**QTL mapping**

Two RIL evaluations were conducted to analyze QTL responsible for selenate tolerance. The tolerance indices from experiments 1 and 2 were significantly correlated ($P < 0.0001$, $r^2 = 0.62$), and the same QTL regions were detected using these two data sets. Judged from combined ANOVA, using the data from both experiments, the heritability of the TI was 0.76, indicating

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Table 1 Root growth and selenium-tolerance index ($T_{SI10} = \text{root growth} + 30 \mu\text{M selenate/root growth control} \times 100\%$) of *Arabidopsis thaliana* recombinant inbred lines (RILs) and the two parental lines after 10 d on MS medium in the presence or absence of 30 µM selenate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>+Selenate</th>
<th>Se-tolerance index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ler-0</td>
<td>45.5 ± 4.2</td>
<td>7.0 ± 0.6</td>
<td>15.4 ± 1.4</td>
</tr>
<tr>
<td>Col-4</td>
<td>60.3 ± 4.0</td>
<td>35.6 ± 1.6</td>
<td>59.1 ± 2.7</td>
</tr>
<tr>
<td>Range of RILs</td>
<td>26–88.5</td>
<td>7.4–54.6</td>
<td>11.2–75.3</td>
</tr>
<tr>
<td>Mean of RILs</td>
<td>67.4 ± 1.2</td>
<td>24.7 ± 1.0</td>
<td>37.4 ± 1.6</td>
</tr>
<tr>
<td>CV</td>
<td>17.9</td>
<td>40.8</td>
<td>42.0</td>
</tr>
</tbody>
</table>

*Se-tolerance index = root growth + Se/control × 100%. Values represent mean ± SE. CV, coefficient of variation.
that 76% of the observed variation was from genetic causes, and 24% was caused by environmental effects or measurement errors. It was decided to present the QTL results of the second evaluation here as, in this experiment, more RILs provided useful data (the number of seeds sown per RIL were adjusted to compensate for low germination rates).

To identify loci controlling the observed differences in Se tolerance between Ler-0 and Col-4 and among the RILs, three phenotypic variables related to selenate tolerance were used for QTL analysis, and root length on control medium was also analyzed. The Se-tolerance variables used were: (i) root length after 10 d growth on medium containing 30 µM selenate (RLSe10); (ii) difference in root length between plants grown on control medium and plants grown on medium with selenate (RLD10 = RLC10−RLSe10); (iii) relative root length when grown +Se/−Se (Se tolerance index or TID10 = RLSe10/RLC10 × 100%). Because parental lines Ler-0 and Col-4 grew differently on the control medium, the difference in root length and the Se tolerance index should be the better traits to use for QTL analysis. The three Se tolerance-related traits, as well as growth under control conditions, were analyzed separately with CIM using QTL CARTOGRAPHER model 6 (Zeng, 1993, 1994). QTL were considered significant when LOD scores exceeded the calculated threshold value (2.5–2.8; Fig. 3).

One significant QTL was detected for root length under control conditions (Fig. 3a). It was located on chromosome 1, 2 cM from the closest marker (m253), and explained 12.5% of the phenotypic variation for the trait (Table 2). No significant QTL was found using simple root length of plants grown on 30 µM selenate. However, three major-effect QTL

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Fig. 3 Genome scans for selenium tolerance quantitative trait loci (QTL) as identified by composite interval mapping. Log-likelihood (LOD) score for two Se-tolerance traits, as well as root growth under control conditions, are plotted against the five chromosomes of Arabidopsis. Significance of QTL is indicated by a LOD score above the threshold values determined by permutation analysis at the genome-wide significance level of 0.05. (a) Root length on control medium; (b) Se-tolerance index (TID10 = root growth + 30 µM selenate/root growth control × 100%); (c) difference in root length (RL) between recombinant inbred lines (RIL) grown on media −Se and +Se (RLD10 = RLC10 − RLSe10). For each QTL the most closely linked marker and its two flanking markers are shown. The markers indicated on chromosome 2 showed epistatic interactions with one or more QTL (Table 3). Left to right: G6842; GPA1; O5841.
were mapped for RLD10, and two of these same QTL were found for trait TID10 (Fig. 3b,c). Individually, the Se-tolerance QTL accounted for 11.3–18.6% of trait variation (Table 2).

The QTL around 40 cM on chromosome 3, found for both TID10 and RLD10, was most closely linked (2 cM) to marker ABI3, with flanking markers mi386 and GAPA. The other QTL found for both TID10 and RLD10, near 37 cM on chromosome 5, was detected directly at marker g4560, flanked by markers KG31 and m291. As mentioned, these two QTL on chromosomes 3 and 5 were not found for root length on control medium. The QTL for RLD10 near 50 cM on chromosome 1 was most closely linked to the same marker as the QTL for root length on control medium (Table 2; Fig. 3a,b). The genome scan for TID10 also showed some indication of a QTL on chromosome 1 (Fig. 3c), but the LOD peak of 1.67 did not reach the significance threshold. Together, the three QTL explained 32% of the phenotypic variation in RLD10 and the two QTL explained 18% of the variation in TID10 (24% if the locus on chromosome 1 was included).

For both ABI3 and g4560, the additive effect values for the Col-4 allele (the average effect of replacing a Ler-0 allele with a Col-4 allele) were positive for trait TID10 and negative for RLD10. As high selenate tolerance results in a high TID10 and a low RLD10, this indicates that the tolerance allele at both QTL is, indeed, contributed by tolerant parent Col-4. Interestingly, the chromosome 1 QTL for RLC10 showed a positive additive effect, indicating that the Col-4 allele conferred better growth under control conditions, while the chromosome 1 QTL for RLD10 showed a positive additive effect, indicating that the Ler-0 allele conferred selenate tolerance.

Analysis of epistasis revealed three interactions for RLD10 and two for TID10 (all significant at \( P < 0.0006 \)) involving the detected quantitative trait loci (QTL) regions and markers elsewhere in the genome (Table 3). For both traits, a region on the long arm of chromosome 2 interacted with the QTL on chromosomes 1 and 3. The interactions involving chromosome 2 are quite similar: only when the A (Col-4) allele is present at the chromosome 2 marker do the QTL regions have a significant effect on the phenotype. For example, the O5841 × mi386 interaction was highly significant (\( P = 0.00055 \)) for RLD10. When the A allele is present at O5841, the mi386 allele has a large effect on the phenotype (34.0 vs 51.7 mm for the AA/AA and Table 2

**Summary of significant quantitative trait loci (QTL) results from composite interval mapping**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Chromosome</th>
<th>cM</th>
<th>Nearest marker</th>
<th>LOD score</th>
<th>Additive effect</th>
<th>( R^2 \times 100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLC10 (mm)</td>
<td>1</td>
<td>53.9</td>
<td>M253</td>
<td>2.77*</td>
<td>+4.3</td>
<td>12.5</td>
</tr>
<tr>
<td>RLD10 (mm)</td>
<td>1</td>
<td>53.9</td>
<td>M253</td>
<td>3.61*</td>
<td>+5.1</td>
<td>13.9</td>
</tr>
<tr>
<td>RLD10 (mm)</td>
<td>3</td>
<td>40.1</td>
<td>ABI3</td>
<td>3.96**</td>
<td>−5.1</td>
<td>13.8</td>
</tr>
<tr>
<td>RLD10 (mm)</td>
<td>5</td>
<td>36.9</td>
<td>g4560</td>
<td>4.00**</td>
<td>−5.6</td>
<td>12.5</td>
</tr>
<tr>
<td>TID10 (%)</td>
<td>3</td>
<td>40.1</td>
<td>ABI3</td>
<td>4.56**</td>
<td>+6.8</td>
<td>18.6</td>
</tr>
<tr>
<td>TID10 (%)</td>
<td>5</td>
<td>36.9</td>
<td>g4560</td>
<td>3.18*</td>
<td>+5.6</td>
<td>11.3</td>
</tr>
</tbody>
</table>

Traits analyzed were root length under control conditions (RLC10); difference in root length of RILs grown +/− selenium (RLD10); and Se-tolerance index (TID10 = root growth + 30 µM Se/control × 100%).

LOD score = \( \log_{10} \left[ \text{maximum likelihood (QTL present)}/\text{maximum likelihood (QTL absent)} \right] \right) \times 100$: percentage of phenotypic variation explained by QTL. *, **, Genome-wise significance at 5 and 1% level, respectively.

†Average additive effect of a Col-4 allele at each locus.
The Cs933 (selenate-tolerant) and Cs20 (selenate-sensitive) parental lines are included for comparison. Tolerant RILs and the 10 Se sensitive RILs, respectively. Genotypes were determined at the SSLP markers ciw11 on chromosome 3 and ciw8 on chromosome 5. The loci identified using QTL analysis for selenate tolerance were verified using different SSLP markers in the identified QTL regions that have not been tested before in this population. As shown in Fig. 4, seven out of 10 selenate-tolerant RILs contained the Col-4 (Cs933) allele at each of the QTL on chromosomes 3 and 5, and all the tolerant RILs had at least one of the resistant alleles. Ten out of 10 sensitive RILs contained the Ler-0 (Cs20) allele on chromosome 3; and nine out of 10 contained the Ler-0 allele on chromosome 5. These results, while obtained from the same RIL population, further demonstrate that the QTL on chromosomes 3 and 5 are, indeed, two major genes controlling selenate tolerance in this population.

Potential genes responsible for selenate tolerance

As selenate is chemically similar to sulfate, and is known to be transported and assimilated via the same mechanisms, it is feasible that differences in selenate tolerance are caused by qualitative or quantitative differences in sulfate transporters, resulting in differences in S and Se accumulation. To investigate whether selenate tolerance was correlated with Se accumulation in the parental lines and RILs, tissue Se and S concentrations were determined in Ler-0, Col-4, and four extremely selenate-tolerant or -sensitive RILs (Fig. 5). The selenate-sensitive Ler-0 parent had significantly higher shoot Se levels than Col-4; shoot S levels were also approx. 30% higher, but not significantly. There was no difference in root Se or S levels between the two parental lines. A significant correlation between selenate tolerance and shoot Se accumulation was not found in the four selected RILs (Fig. 5).

The major tolerance mechanism in this population may also involve differences in metabolic Se detoxification. To identify possible candidate genes in the QTL regions involved in S/Se metabolism, we examined gene annotation information. Potential Se-related genes on the major-effect QTL of chromosome 3 are a selenocysteine methyl transferase (SMT) homolog (At3g22740); an ATP sulfurylase (APS1, At3g22890); an Se-binding protein (At3g23800); and a serine acetyl transferase (SAT-1, At3g31110). A cystathionine gamma-synthase (CGS, At1g33320) is located in the identified QTL region on chromosome 1. No obvious candidate genes for selenate tolerance were located in the QTL region on chromosome 5. On chromosome 2 there are two potentially interesting SAT genes (At2g17640 and At2g34970) that may be responsible for the observed epistatic effects. No sulfate-transporter genes are annotated in any of the regions identified.

Discussion

Judged from the segregation of selenate tolerance in offspring obtained from Ler-0 and Col-4 crosses, it appears that selenate tolerance in these populations is controlled by multiple genes. QTL analysis of selenate tolerance in the RILs indicated that this trait was controlled by three major-effect loci under our experimental conditions. These QTL accounted for 32% of the phenotypic variation in RL_D10 across the population, and 18–24% of the phenotypic variation in TID10. In addition to these major QTL, there were epistatic interactions of the QTL on chromosomes 1 and 3 with two markers on chromosome 2. The combined effects of all QTL and the epistasis effects explained 43–48% of the variation in selenate tolerance. As the total phenotypic variance includes environmental variance and measurement errors, the actual genetic variance attributable to major QTL is even larger. Two replicate experiments were conducted to analyze QTL responsible for selenate tolerance, both under the same experimental conditions. The tolerance indices calculated from both experiments were significantly

![Fig. 4 Cosegregation of simple sequence-length polymorphism (SSLP) markers in the quantitative trait loci (QTL) regions on chromosomes 3 and 5 with selenate tolerance. Ten selenate-tolerant and 10 selenate-sensitive recombinant inbred lines (RILs) were screened. Selenate tolerance indices (TID10 = root growth + 30 µM selenate/root growth control × 100%) were between 51 and 75% and 11–25% for the 10 selenium-tolerant RILs and the 10 Se sensitive RILs, respectively. Genotypes were determined at the SSLP markers ciw11 on chromosome 3 and ciw8 on chromosome 5. The Cs933 (selenium-tolerant) and Cs20 (selenium-sensitive) parental lines are included for comparison.](image-url)
correlated, and the same QTL regions were detected from separate analysis of each experiment. Moreover, these same QTL were detected using two different parameters for selenate tolerance. Thus the results were consistent and replicable. These results are significant, as this is the first report on a genetic analysis of plant Se tolerance, and one of the first QTL studies on metal or metalloid tolerance.

The two major-effect QTL on chromosomes 3 and 5 contributing to root growth on selenate medium did not control root length on the control medium, and thus appear specifically to control selenate tolerance in this RIL population. For both these QTL, the tolerance allele was contributed by the Col-4 parent, based on additive effect values.

The QTL on chromosome 1, linked with marker m253, was found both for RL_{D10} and for root length on control medium. If the chromosome 1 QTL found for RL_{C10} and RL_{D10} are the same locus, this gene may be involved with general root growth, rather than specifically with selenate tolerance. However, the additive effects indicated that the Col-4 allele conferred better growth under control conditions, while the Ler-0 allele conferred better growth on selenate medium. Therefore it could also be hypothesized that this locus is involved in S acquisition, positively affecting growth on control medium, but negatively affecting growth on selenate medium because of enhanced selenate uptake. Total shoot S and Se levels, however, were lower in Col-4 than in Ler-0, and there were no differences in root S and Se levels. Also, no correlation was found between tolerance and accumulation in the four selected RILs, and no sulfate-transporter genes were found in the tolerance-related QTL regions. In other studies using 19 Arabidopsis accessions, there was also a lack of correlation between selenate tolerance and accumulation (L. Z. and E.A.H.P.-S., unpublished results).

The selenate-tolerance mechanism in these accessions may also involve Se detoxification through metabolism, possibly through S-assimilation enzymes. In this context, some potentially interesting genes in the identified QTL region on chromosome 3 are: (i) APS1, encoding the chloroplast (main) isoform of ATP sulfurylase; (ii) SAT-A, encoding a chloroplast isoform of serine-O-acetyl-transferase (SAT); (iii) HMT1, a homologue of the SeCys methyltransferase gene in Se hyperaccumulator Astragalus species (Neuhierl et al., 1999); and (iv) a predicted Se-binding protein. It is feasible that these loci would confer Se tolerance, as overexpression of these enzymes was shown earlier to confer plant Se tolerance (Pilon-Smits et al., 1999; LeDuc et al., 2004; Agalou et al., 2005; John Freeman, Colorado State University, pers. comm.). It is also possible that more than one selenate-tolerance gene contribute to the QTL in this region.

In addition to the three QTL on chromosomes 1, 3 and 5, there appear to be one or two loci on chromosome 2 that contribute to selenate tolerance via epistatic interactions. The tolerance alleles for chromosome 2 loci were contributed by the Col-4 parent. The chromosome 2 loci appear to be epistatic over both chromosome 3 and 1 QTL, and the chromosome 3 QTL is epistatic over the chromosome 1 QTL. Interestingly, some of these same regions on chromosomes 1, 2 and 5 were identified as QTL in earlier studies investigating
aluminium tolerance in this same RIL population (Kobayashi & Koyama, 2002; Hockenga et al., 2003). The aluminium QTL on chromosomes 1 (54.6 cM) and 2 (48.9 cM) were associated with the traits root length on control medium and root length on aluminium medium, but not with tolerance index; Col-4 contributed both tolerance alleles (Kobayashi & Koyama, 2002). The QTL on chromosome 5 (37.7 cM) was associated with the difference in root length between control and aluminium treatments, and thus with aluminium tolerance; Col-4 contributed the tolerance allele (Hockenga et al., 2003). If these Al- and Se-related QTL are indeed the same, they may control a general root growth or stress-tolerance mechanism in this population. To our knowledge, the Se-related QTL on chromosome 3 was not found in any other study, and may be the most Se-specific of the QTL identified.

In future studies, multiple QTL analysis using other RIL populations of Arabidopsis or crop species may be performed to determine whether these QTL also control selenate tolerance in other genetic backgrounds and experimental conditions. Also, to provide more mechanistic insight into selenate tolerance, results from QTL mapping of selenate tolerance may be compared with results from biochemical studies, mutant studies, and studies on the effects of overexpression of key enzymes from the S/Se assimilation pathway. Furthermore, genes of interest in the identified QTL regions may be revealed through comparative expression analyses of RILs and parent lines that differ in selenate tolerance, for example using transcriptomics.

In addition to enhancing our understanding of plant Se metabolism, the results from these studies may ultimately be applicable for breeding plants with higher levels of Se tolerance and plants that accumulate higher levels of selenocompounds. Such plants would be useful for environmental clean-up (phytoremediation) and may potentially have anticarcinogenic properties.

References


