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RESEARCH PAPER

# A strong effect of growth medium and organ type on the identification of QTLs for phytate and mineral concentrations in three *Arabidopsis thaliana* RIL populations

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## Abstract

**The regulation of mineral accumulation in plants is genetically complex, with several genetic loci involved in the control of one mineral and loci affecting the accumulation of different minerals. To investigate the role of growth medium and organ type on the genetics of mineral accumulation, two existing (L*er*×Kond, L*er*×An-1) and one new (L*er*×Eri-1) *Arabidopsis thaliana* Recombinant Inbred Line populations were raised on soil and hydroponics as substrates. Seeds, roots, and/or rosettes were sampled for the determination of their Ca, Fe, K, Mg, Mn, P or Zn concentrations. For seeds only, the concentration of phytate (IP6), a strong chelator of seed minerals, was determined. Correlations between minerals/IP6, populations, growth conditions, and organs were determined and mineral/IP6 concentration data were used to identify quantitative trait loci (QTLs) for these traits. A striking difference was found between QTLs identified for soil-grown versus hydroponics-grown populations and between QTLs identified for different plant organs. Three common QTLs were identified for several populations, growth conditions, and organs, one of which corresponded to the *ERECTA* locus, variation of which has a strong effect on plant morphology.**

## Introduction

Plants generally obtain the minerals for their growth from the media they live on. The (bio)-availability of essential minerals depends on their solubility in the growth media and on their binding strength to soil particles. Many minerals are cationic metals, which are generally taken up as hydrated ions and/or as metal–chelate complexes (Clemens *et al.*, 2002). Factors like soil structure and pH affect the bio-availability of minerals to plants. Mineral requirements and the capacity to accumulate them are species-dependent. Mineral uptake, translocation, and storage processes in various tissues and cellular compartments are vital for the plant and need to be maintained within appropriate physiological limits (Clemens, 2001). Therefore, firm regulatory mechanisms are in place to control mineral uptake at the organ and cellular level. At the moment, little is known about the genes controlling the variation within species for cationic mineral uptake, distribution, phytate biosynthesis and storage in plants (Maser *et al.*, 2001; Raboy, 2003; Ghandilyan *et al.*, 2006). Identification of these genes will increase our understanding of the mineral uptake and distribution process and may facilitate the improvement of plant nutrient content and use efficiency with potentially beneficial effects on crop yield and quality.

Improving our knowledge about the genetic control of plant mineral concentration and the concentration of anti-nutrients can also contribute to improved human health.

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Cereals, vegetables, and fruits, and the products made from them, make up a large part of human nutrition, especially in vegetarian or vegan diets. Nutritional deficiencies account for almost two-thirds of childhood deaths worldwide (Welch and Graham, 2004). The major cause of mineral malnutrition found among humans is the predominant consumption of plant-based foods that contain inadequate levels of bioavailable minerals. The bioavailability of many cationic minerals from food for human consumption is severely reduced by the presence of anti-nutrients in food, which can form strong complexes with cationic minerals. One of these anti-nutrients is phytate also known as inositol hexakisphosphate (IP6). For plants, phytate is the major source of phosphorus for germinating seeds and hence is important for seedling vigour, but for cereal-derived food products it is a major anti-nutrient.

*Arabidopsis thaliana* (*Arabidopsis*) is a molecularly and genetically well-characterized plant species, which is very suitable for large-scale genetic analysis of mutants and natural variants. Given the availability of well-genotyped mapping populations, such as Recombinant Inbred Line (RIL) populations, Quantitative Trait locus (QTL) analysis is a powerful technique to study complex traits of the genetic differences that are present within the species *Arabidopsis* (Koornneef *et al.*, 2004).

Particularly since RIL populations represent an 'immortal' genetic resource (homozygous lines that can easily be propagated after self-fertilization), many replicates of identical lines can easily be studied in many different environments, and thus investigate thoroughly the genetic component of the environmental response.

The aim of the research presented here is to study the genetic variation for the accumulation of minerals in seeds, rosettes, and roots of *Arabidopsis* grown on different media. It is expected that there is a genetic control of mineral accumulation in *Arabidopsis* organs, and it is conceivable that this control will be specific for the type of organ under investigation. In addition, it is also expected that this genetic control depends on the substrate used for plant cultivation, since mineral bioavailability can vary. In this study, a situation of high minerals bioavailability (plants raised on hydroponic medium) was compared with a situation more related to an agronomic scenario with reduced mineral bioavailability (soil-grown plants).

Given that accessions differ in their genetic composition, the analysis of similar traits in different populations derived from contrasting accessions enables us to sample the genetic variation and basis of a specific trait within a species. Three different RIL populations grown on different media were studied. The soil medium was common for the three populations, whereas the hydroponic system was used for two populations that were also grown on soil. For each growing scenario, accumulation was quantified for seven minerals elements (Ca, Fe, K, Mg, Mn, P, and Zn) and for phytate in different organs (seed, rosette, and root) and QTLs for this set of traits were detected. Mapping QTLs using the same populations under different conditions would enable us to distinguish common QTLs which are involved in

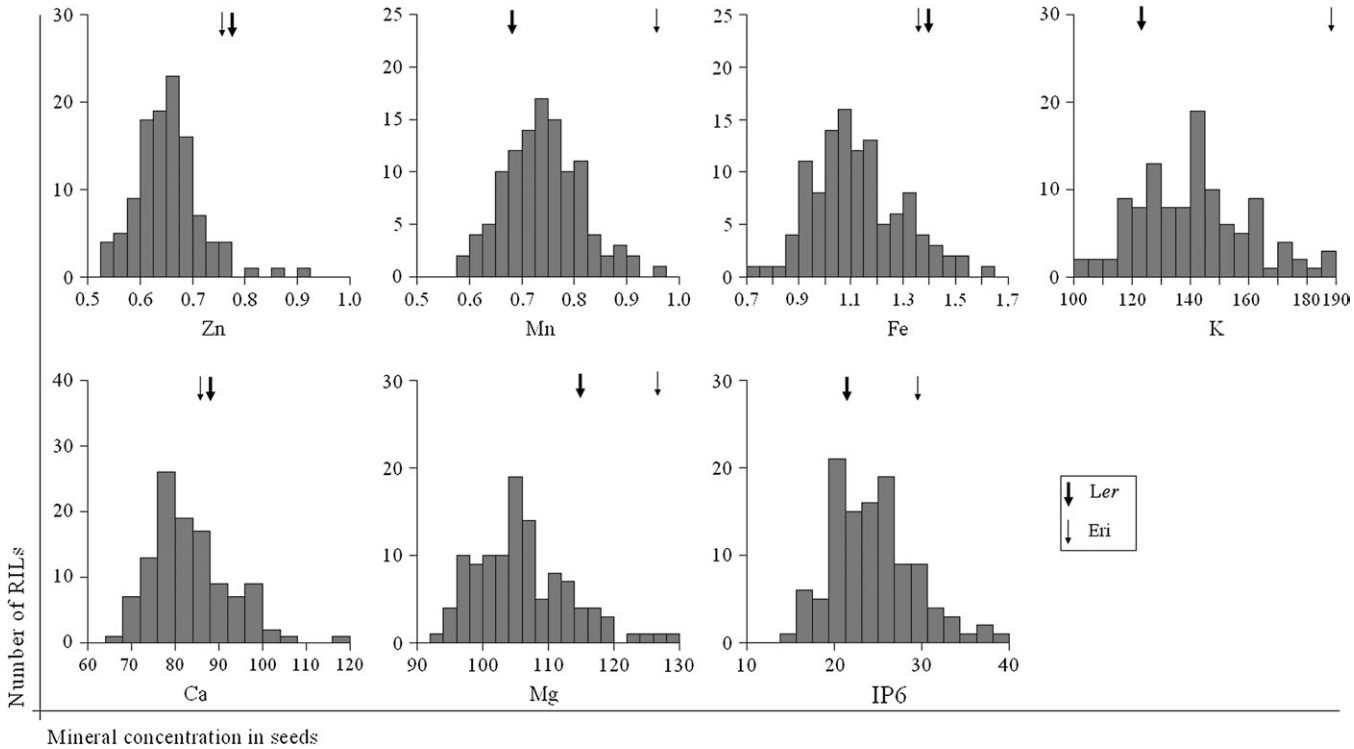


**Fig. 1.** Frequency distributions of the mineral (Zn, Mn, Fe, K, Ca, and Mg; μmol $g^{-1}$ DW) and phytate (IP6; mg $g^{-1}$ DW) concentrations in seeds of the L*er*/Eri-1 RIL population grown on soil. Arrows indicate the levels in the parental lines, with the thick arrows indicating L*er* and the slim arrows indicating Eri-1.

mineral homeostasis, whatever the growing scenario, and/or QTLs which are organ- or environment-specific. Based on previous data for mineral levels in seeds analysed in 21 accessions (Vreugdenhil *et al.*, 2004), Landsberg *erecta* (L*er*), Kondara (Kond), and Antwerp-1 (An-1) accessions were selected and the available RIL populations derived from the inter-accession crosses L*er*/Kon and L*er*/An-1 (El-Lithy *et al.*, 2006) were analysed. In addition, a new mapping population, derived from the cross between L*er* and accession Eringsboda-1 (Eri-1) was generated.

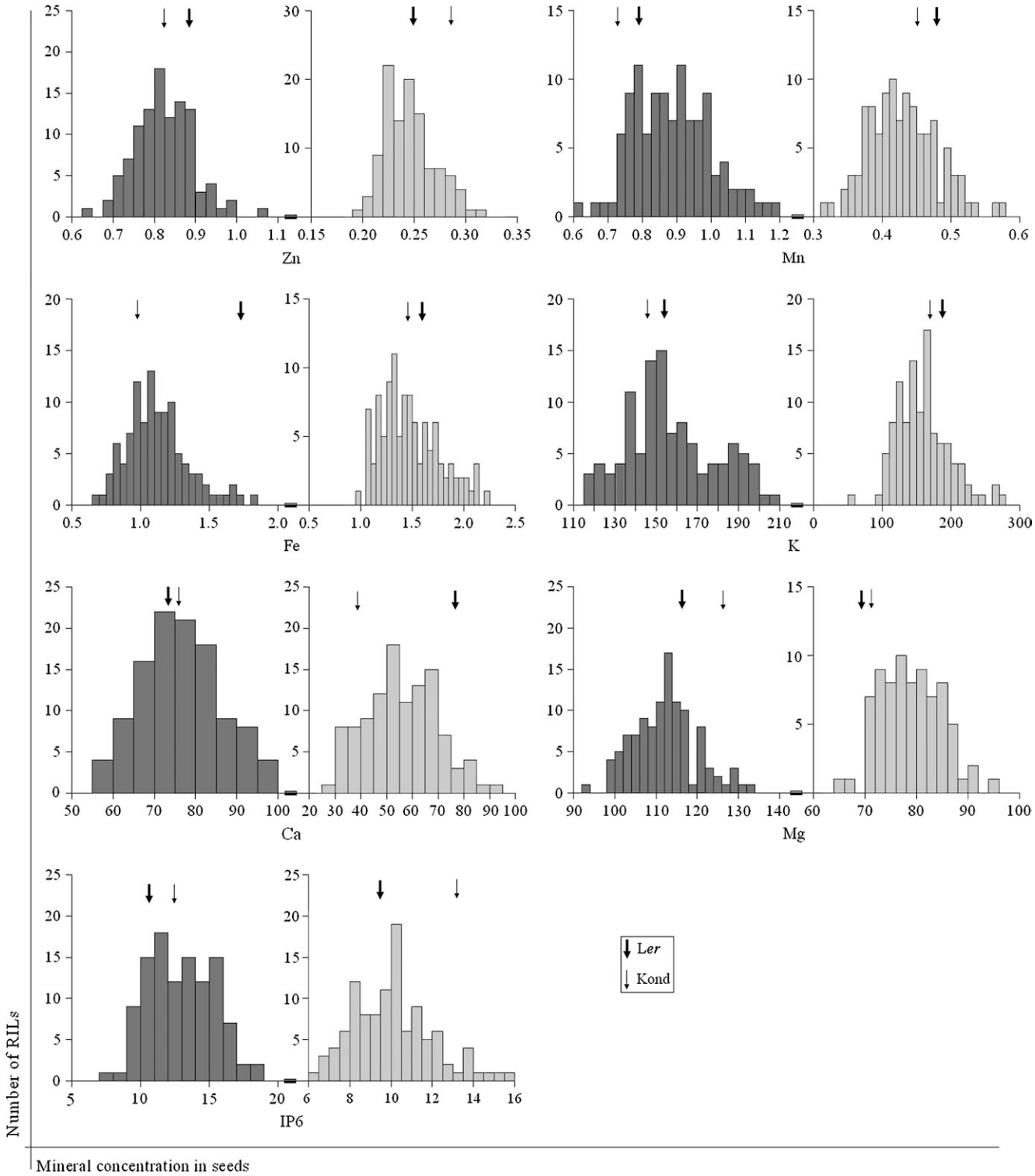


**Fig. 2.** Frequency distributions of the mineral (Zn, Mn, Fe, K, Ca, and Mg; μmol $g^{-1}$ DW) and phytate (IP6; mg $g^{-1}$ DW) concentrations in seeds of the L*er*/Kond RIL population grown on soil (dark) and hydroponics (light). Arrows indicate the levels in the parental lines, with the thick arrows indicating L*er* and the slim arrows indicating Kond.

## Materials and methods

### *Plant material and growing conditions*

*Arabidopsis thaliana* accessions Landsberg *erecta* (L*er*, N20), Eringsboda-1 (Eri-1, CS22548; collected in South Sweden), Kondara (Kond, CS6175; collected in Tadjikistan), and Antwerp (An-1, N944; collected in Belgium) and the RIL populations L*er*/Eri-1, L*er*/Kond, and L*er*/An-1, were grown in the experiments described by El-Lithy *et al.* (2006). The parents and populations were grown once on soil (in a greenhouse) and once on hydroponics (in a climate chamber). The L*er*/Eri population was only grown on soil. All populations were grown in the same greenhouse and the same climate chamber under the same settings

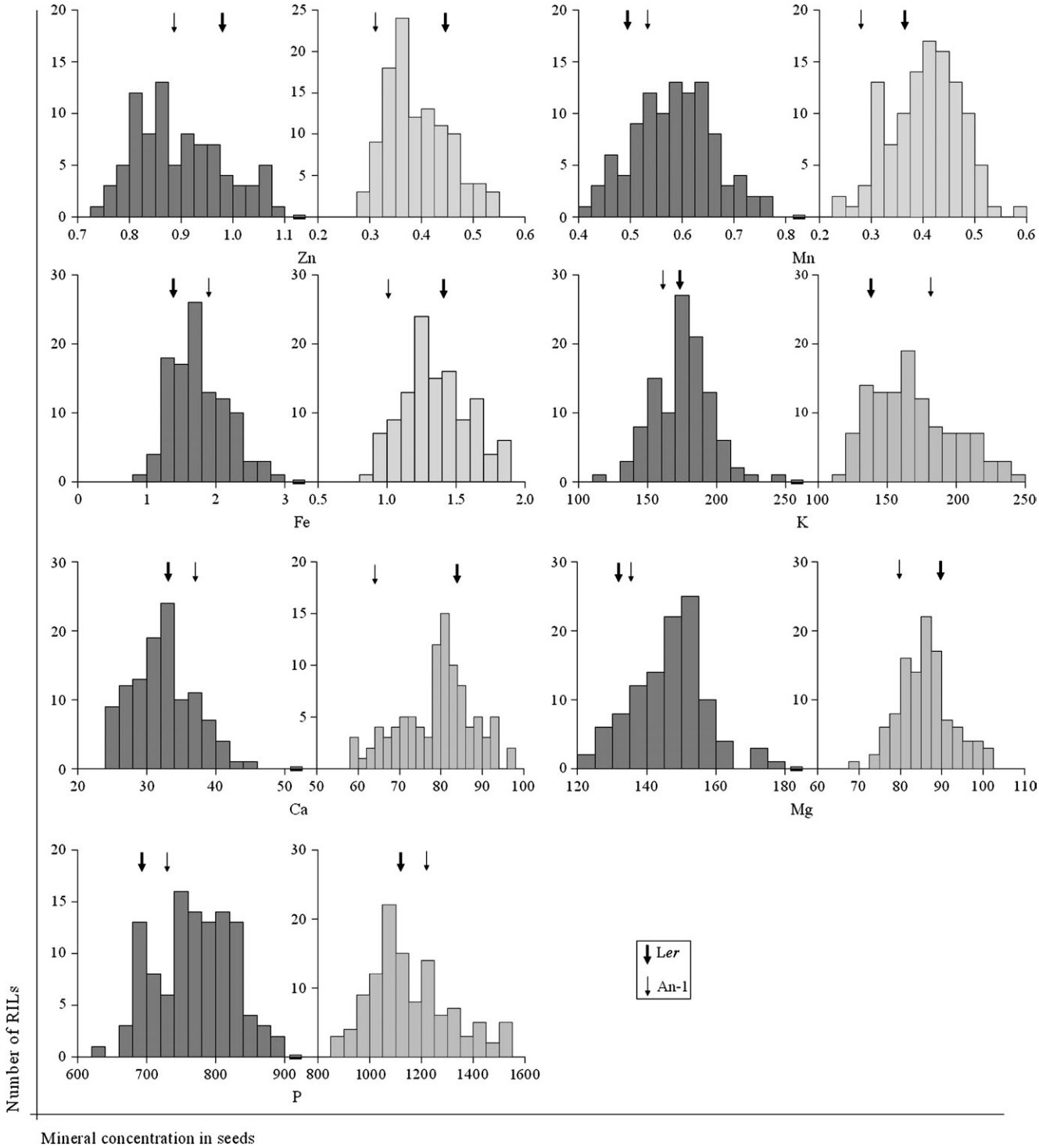


**Fig. 3.** Frequency distributions of the mineral (Zn, Mn, Fe, K, Ca, Mg, and P; μmol $g^{-1}$ DW) concentrations in seeds of the L*er*/An-1 RIL population grown on soil (dark) and hydroponics (light). Arrows indicate the levels in the parental lines, with the thick arrows indicating L*er* and slim arrows indicating An-1.

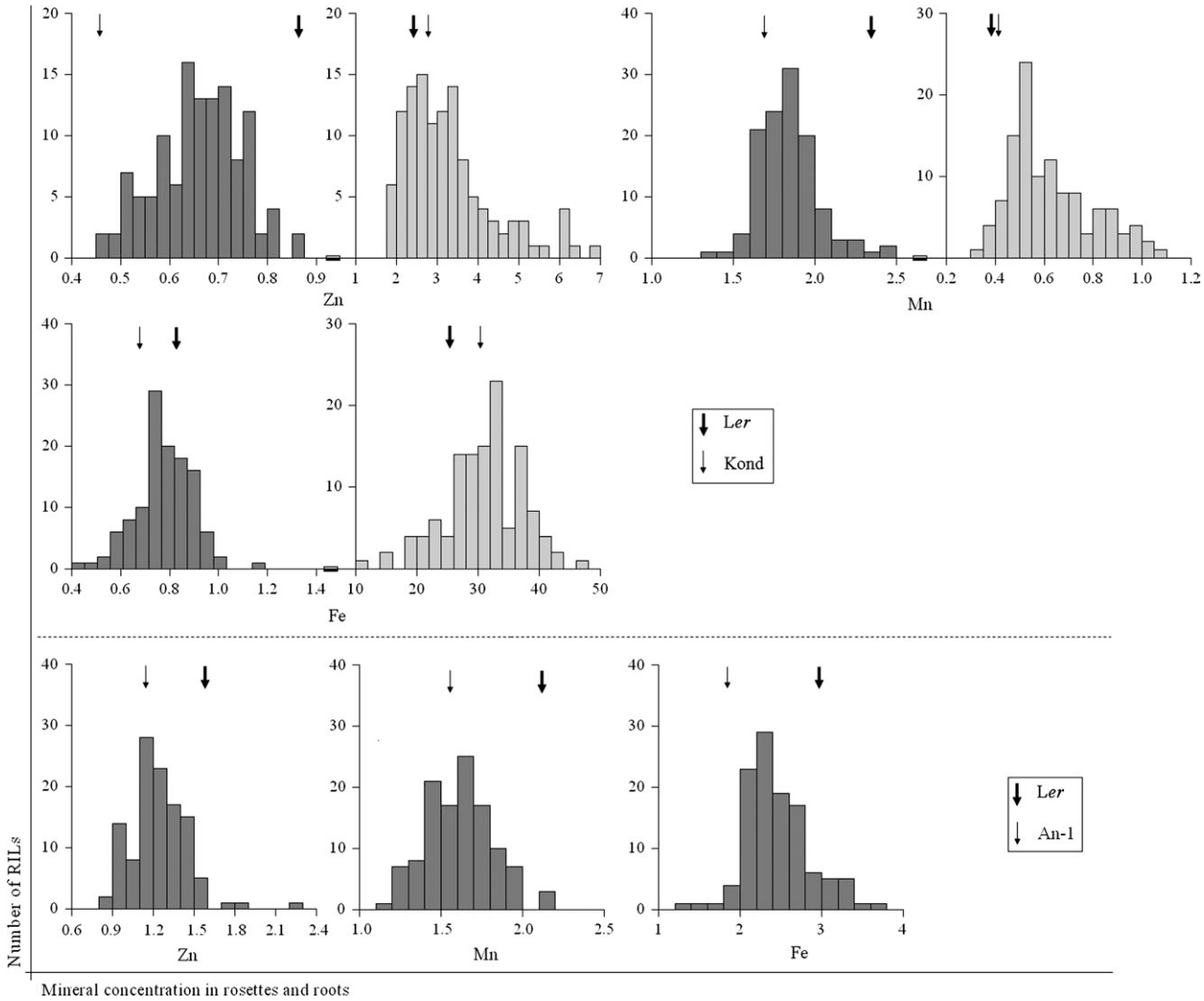


**Fig. 4.** Frequency distributions of Zn, Mn, and Fe (μmol $g^{-1}$ DW) concentrations in rosettes (dark) and roots (light) of the L*er*/Kond RIL population (above the dashed line) and in rosettes of the L*er*/An-1 RIL population grown on hydroponics (below the dashed line). Arrows indicate the levels in the parental lines, with the thick arrows indicating L*er* and the slim arrows indicating Kond or An-1.

(daylength, temperature, RH; see below), but not at the same time.

For plants growing on soil, seeds were placed on demi-water-soaked filter paper in 6 cm Petri dishes and kept for 4 d in the cold (4 °C) to break any residual seed dormancy and to ensure uniform germination. Afterwards, the Petri dishes were transferred to 24 °C in light for 1 d to initiate germination. Germinated seedlings were placed on soil [a peat:perlite (4:1 v/v) or a peat:sand (3:1 v/v) mixture], one plant per 6 cm clay pot, six plants per genotype in two replications. Replications were randomized in the plot using a randomized two-block design to reduce environmental effects. Plants grew in an air-conditioned greenhouse, with 70% relative humidity, supplemented with additional light (model SON-T plus 400 W, Philips, Eindhoven, The Netherlands) providing long-day conditions (16 h light), and maintained at a temperature of 22–25 °C during the day and 18 °C at night. For plants growing on hydroponics, seeds were grown on a standard hydroponics solution suggested for *Arabidopsis* (Tocquin *et al.*, 2003), in a phytotron at 20 °C with a relative humidity of 70% and a light intensity of 40 W $m^{-2}$ for 12 h $d^{-1}$. The hydroponics set-up consisted of a 9.0 l tray (liquid medium container) covered with a firm, non-transparent black plastic lid containing nine rows of nine holes. Each hole received a 0.5 ml microfuge tube, the tip of which was cut off. The tubes were filled with 0.55% agar (weight/volume) prepared with deionized water. Seeds were placed on the agar surface of the microfuge tubes, one seed per tube to yield nine plants per genotype in two replications. Replications were randomized in the trays using a randomized two-block design to reduce environmental effects.

For soil-grown plants of the L*er*/Kond and L*er*/An-1 populations, samples of ripe dry seeds were harvested from each of the two blocks for further analysis, each consisting of the seeds coming from six plants. For the hydroponically-grown L*er*/An-1 population, samples of ripe dry seeds were harvested from each of the two blocks for further analysis, each consisting of the seeds coming from eight plants. The ninth plant of each line in each block was grown

for 3 weeks only and the full rosette of each plant was harvested for further analysis. For the hydroponically-grown L*er*/Kond population, samples of ripe dry seeds were harvested from each of the two blocks for further analysis, each consisting of the seeds from the nine plants per line. To obtain rosette material, the experiment was repeated, but plants were only grown for 3 weeks, after which nine rosettes and nine root systems per line were harvested for further analysis.

### *Phenotypic analysis*

Tissue concentrations of Zn, Mn, Fe, K, Ca, and Mg were measured using Atomic Absorption Spectrometry (AAS). Two replicate samples per line were analysed for seed and rosette minerals, one sample was analysed for root minerals. Each sample consisted of approximately 100 mg oven-dried and ground rosette material from nine plants, up to 60 mg oven-dried and ground root material from 18 plants, or 100 mg of seeds from the bulk harvest of six plants. Tissues were put in a Teflon cylinder together with 2 ml acid-mix ($HNO_3$:HCl, 4:1 v/v), closed tightly and mineralized for 7 h at 140 °C. After cooling, each digest was diluted with 3 ml deionized water and transferred to a sterile 15 ml tube. Different dilution samples were made, depending on the expected concentration of each mineral before measuring the minerals with an Atomic Absorption Spectrophotometer (Perkin Elmer AAS 1100; Perkin Elmer, Rodgau-Judesheim, Germany). For seeds of the L*er*/An-1 population, the total P concentrations were measured using a spectrophotometric method described by Chen *et al.*, (1956). For seeds of the L*er*/Eri and L*er*/Kond populations, the phytate (myo-inositol-1,2,3,4,5,6-hexakisphosphate, IP6) concentrations were measured, rather than total P, as previously described by Bentsink *et al.* (2003).

All Zn, Mn, Fe, K, Ca, Mg, and P mineral concentrations are presented in μmol $g^{-1}$ DW units, which is most common in mineral analysis. These convert to μg $g^{-1}$ DW units, as follows: 1 μg $g^{-1}$ is 65.4 μg $g^{-1}$ for Zn, 54.9 μg $g^{-1}$ for Mn, 55.8 μg $g^{-1}$ for Fe, 39.1 μg $g^{-1}$ for K, 40.1 μg $g^{-1}$ for Ca, 24.3 μg $g^{-1}$ for Mg, and 31 μg $g^{-1}$ for P. The phytate concentrations are presented in mg $g^{-1}$ DW. 1 mg $g^{-1}$ phytate ($C_6H_{12}O_{24}P_6$) corresponds to 654 mmol $g^{-1}$.

### *Construction of the Ler/Eri mapping population and genotyping*

An $F_2$ population derived from a cross between L*er* (maternal parent) and Eri-1 (paternal parent) (CS22548) was propagated by single seed descent for nine successive generations. 110 Recombinant Inbred Lines were obtained. For genotyping, the flower buds of three $F_9$ plants per line were collected. DNA extraction used the Wizard® Magnetic 96 DNA Plant System (Promega; www.promega.com) according to the manufacturer's instructions. Genomic DNA was used for genotyping using AFLP and SSLP markers. 90 AFLP markers were obtained using one primer combination (E, *Eco*RI primer GACTGCGTACCAATTC and M, *Mse*I primer GATGAGTCCTGAGTAA). In addition, a set of 39 SSLP markers distributed over the five *Arabidopsis* chromosomes were used to genotype all the lines (see Supplementary Table 1 at *JXB* online).

A genetic map has been created using JoinMap® 4 (www.kyazma.nl). All the genetic information from AFLP and SSLP markers has been used. To avoid similarities of loci due to a low frequency of recombination between markers within the population, 40 co-segregating AFLP markers have been removed from the analysis. A total of 89 markers have then been used to build the genetic map. The grouping was based upon the JoinMap®4 test for independence with the LOD score as the statistic. The Kosambi function was used in a regression mapping algorithm (Stam, 1993) to build the genetic map. The known physical positions of the SSLP markers based on the reference sequence of *Arabidopsis thaliana* (TAIR: www.*Arabidopsis*.org) was used to assign each linkage group to a specific chromosome.

### *Statistical tests and QTL mapping*

For all statistical analyses the SPSS package version 15.0 was used. Differences in mean trait values of the genotypes were analysed by Univariate Analysis of Variance using the Dunnett's pairwise multiple comparison *t* tests in the

**Table 1.** Concentration ratios for minerals (Zn, Mn, Fe, K, Ca, Mg, and P) and phytate (IP6) for three RIL populations (L*er*/Kond, L*er*/An-1, and L*er*/Eri-1) corresponding to different organs (seed, rosette or root)

Seed concentrations are considered for plants grown on soil or hydroponics (hydrop). For ratios with one organ/growth condition combination, the value represents the highest average mineral or phytate concentration of any RIL, divided by the lowest average mineral or phyate concentration of any RIL. The ratios shown for comparisons of different organs or growth conditions represent the max/min ratio for the first organ/growth condition divided by the max/min ratio for the second organ/growth condition. Rosette and root values were only obtained for plants grown on hydroponics.

| Ratio | L*er*/Kond | | | | | | | L*er*/An-1 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zn | Mn | Fe | K | Ca | Mg | IP6 | Zn | Mn | Fe | K | Ca | Mg | P |
| Seed soil | 1.6 | 1.9 | 2.8 | 1.8 | 1.8 | 1.4 | 2.6 | 1.5 | 1.8 | 3.0 | 2.1 | 1.8 | 1.4 | 1.4 |
| Seed hydrop. | 1.6 | 1.8 | 2.3 | 5.0 | 3.3 | 1.5 | 2.5 | 1.9 | 2.6 | 2.1 | 2.1 | 1.6 | 1.5 | 1.7 |
| Rosette | 1.9 | 1.8 | 2.7 | | | | | 2.7 | 1.9 | 2.7 | | | | |
| Root | 3.6 | 3.3 | 3.9 | | | | | | | | | | | |
| Seed soil/ hydrop. | 1.0 | 1.1 | 1.2 | 0.4 | 0.5 | 0.9 | 1.0 | 0.8 | 0.7 | 1.4 | 1.0 | 1.1 | 0.9 | 0.8 |
| Rosette/seed | 1.2 | 1.0 | 1.2 | | | | | 1.4 | 0.7 | 1.3 | | | | |
| Root/seed | 2.3 | 1.8 | 1.7 | | | | | | | | | | | |
| Root/rosette | 1.9 | 1.8 | 1.4 | | | | | | | | | | | |

| | L*er*/Eri-1 | | | | | | |
|---|---|---|---|---|---|---|---|
| | Zn | Mn | Fe | K | Ca | Mg | IP6 |
| Seed soil | 1.7 | 1.6 | 2.2 | 1.8 | 1.9 | 1.4 | 2.6 |

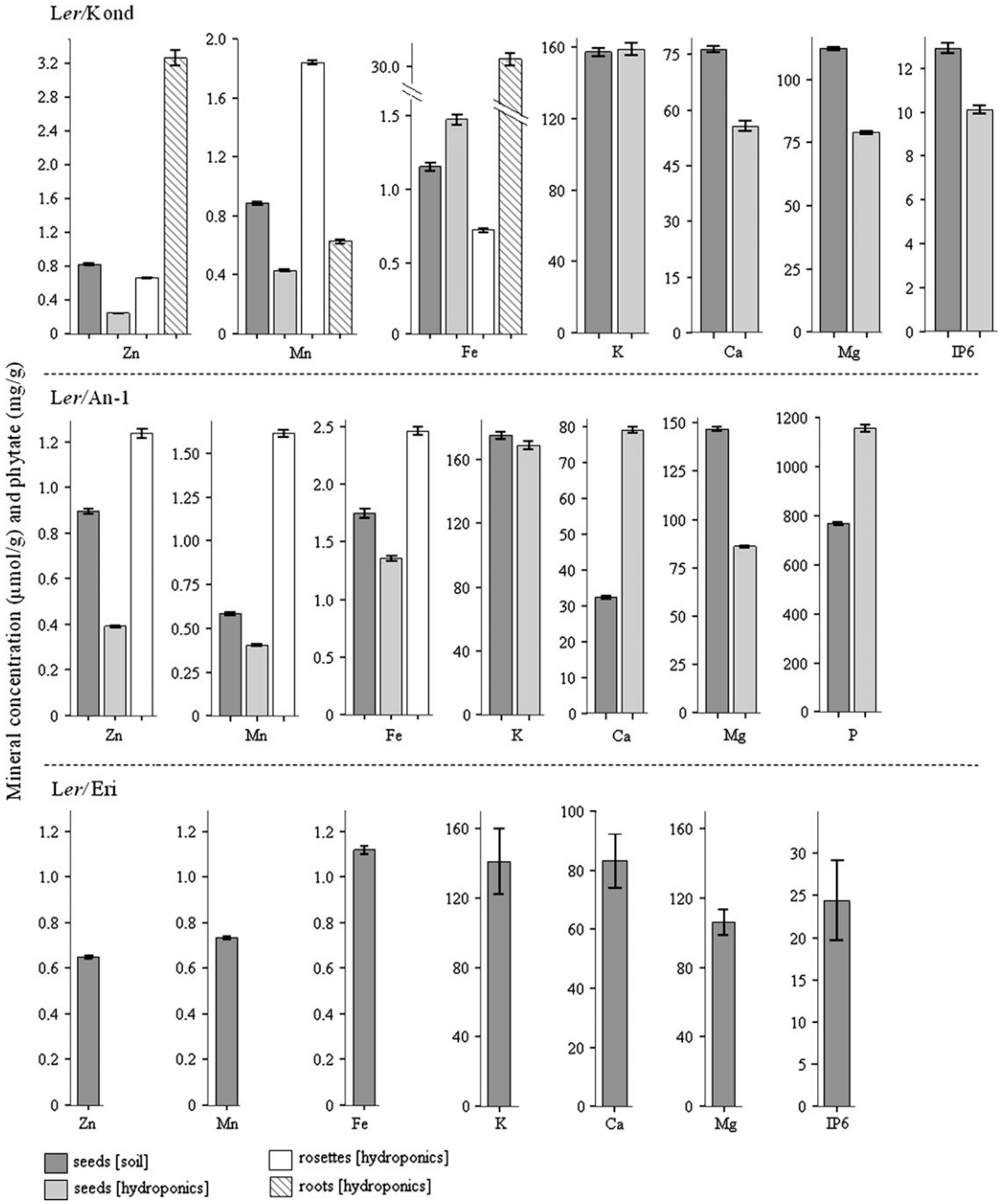


**Fig. 5.** Average seed, rosette, and root mineral (Zn, Mn, Fe, K, Ca, Mg, and P) and phytate (IP6) concentrations (±SE) in the L*er*/Kond, L*er*/An-1, and L*er*/Eri-1 RIL populations grown on soil and on hydroponics. Except for the K concentrations in seeds, all differences between mineral concentrations for different organs or conditions were significant ($P$ <0.05).

General Linear Model module of the package. For each analysis, trait values were used as dependent variables and genotypes were used as fixed factors. Tests were performed 2-sided with a significance threshold level of 0.05. The independent samples $t$ test of the package was used to determine mean differences between two individual lines. Correlation analyses were performed by calculating the Pearson or Spearman correlation coefficients.

QTL mapping was performed using the MapQTL® software version 5.0 (www.kyazma.nl) and a complete pair-wise search for conditional and co-adaptive epistatic interactions for each trait was done ($P$ <0.001, determined by Monte Carlo simulations) using the EPISTAT Statistical Package (Chase *et al.*, 1997). In addition, interactions among QTLs were analysed using co-factors (taken as the markers closest to a QTL) as fixed factors and the traits as dependent variables in a Univariate Analysis of Variance. Models included marker main effects and interactions among them.

## Results

To investigate the genetics of seed, rosette, and root mineral and phytate homeostasis, immortal RIL populations were

**Table 2.** Correlation coefficients (*r*) of mineral (Zn, Mn, Fe, K, Ca, and Mg) and phytate (IP6) concentrations in seeds of the L*er*/Eri-1 RIL population grown on soil

Significant negative correlation coefficients are highlighted in bold. Significance threshold levels: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

| | Mn | Fe | K | Ca | Mg | IP6 |
|---|---|---|---|---|---|---|
| Zn | 0.48*** | 0.58*** | 0.06 | 0.01 | 0.24** | 0.05 |
| Mn | | 0.20* | 0.10 | 0.33*** | 0.23* | 0.33*** |
| Fe | | | –0.04 | –0.11 | 0.12 | **–0.20*** |
| K | | | | **–0.20*** | 0.41*** | 0.38*** |
| Ca | | | | | 0.20* | 0.24* |
| Mg | | | | | | 0.27** |

studied that were derived from the inter-accession crosses L*er*×Kond, L*er*×An-1 (El-Lithy *et al.*, 2006), and L*er*×Eri-1 (a new population), which were grown on soil and, for two populations, that were also grown in a hydroponics system.

### *Variation in plant mineral and phytate concentrations*

The traits studied, which are the Ca, Fe, K, Mg, Mn, P, Zn, and phytate concentrations of seeds, rosettes, and roots, demonstrated large segregations in all three RIL populations grown in both soil and hydroponic conditions (Figs 1, 2, 3, 4). For several traits, such as seed Zn and K concentrations in L*er*/Kond RILs, the segregations were transgressive, meaning that values for the RILs were substantially larger and/or smaller than both of the parents, whereas for some others, such as rosette Zn concentrations in L*er*/Kond RILs, the RIL values were intermediate to the parental values. Mineral concentrations varied 1.4–3.0-fold for seeds of the three soil-grown populations and 1.5–5.0-fold for seeds of the two hydroponics-grown populations. For the rosettes of hydroponically grown plants, mineral concentrations varied 1.8–2.7-fold in both populations. The root mineral concentrations of the L*er*/Kond RIL population grown on hydroponics varied 3.3–3.9-fold (Figs 1, 2, 3, 4; Table 1). In general, the concentration ranges were comparable for most minerals when comparing populations and conditions. Only for seed K and Ca concentrations for hydroponically grown plants, was the variation in the L*er*/Kond population about twice as large as in the L*er*/An-1 population and also as in the soil-grown L*er*/Kond population. The levels of seed mineral concentrations also depended on the growth conditions (Fig. 5). For both populations grown on soil and on hydroponics, the maximum difference between concentrations was 3.4-fold. In particular, seed K concentrations were similar for both conditions. For the L*er*/Kond population the seed mineral concentrations were higher when the RILs were grown on soil than on hydroponics, except for Fe and K. For Fe, the seed concentrations were higher in the hydroponically grown population. For the L*er*/An-1 population the seed Zn, Mn, Fe, and Mg concentrations were higher when the RILs were grown on soil, and the seed Ca and P concentrations were higher for the hydroponically grown plants. In general, the Zn and Fe concentrations in the roots were higher than in rosettes and seeds (Fig. 5). The seed Fe concentrations were higher than rosette Fe concentrations in the L*er*/Kond population, whereas it was the reverse for the L*er*/An-1 population.

All these results indicate that, in general, mineral and phytate concentrations and their variation levels depended on the sampled organ, population, and/or growing conditions.

### *Relationship between the traits*

Both negative and positive correlations were observed between traits (Tables 2, 3). The correlation of the seed Zn concentration with the seed Mn, Fe, K, Mg and P concentrations in the L*er*/An-1 RILs, was observed in the population grown on both soil and hydroponics. Root Zn and Fe concentrations (L*er*/Kond) were always negatively correlated and no correlations were observed between the seed and rosette Zn and Fe concentrations in the L*er*/Kond RILs grown on hydroponics. Many of the correlations observed within a population, like the seed Zn and Fe concentrations in the L*er*/Kond RILs, were not stable over the two different environments, but only observed in one condition (in this case when the population was grown on soil). Seed P and Mn concentrations were positively correlated with all the other seed mineral concentrations in L*er*/An-1 RILs grown on soil, whereas some of the correlations for soil-grown plants were not significant when the population was grown on hydroponics. Also the reverse can be observed, such as for the seed IP6 and Zn concentrations, which were correlated in the L*er*/Kond RIL population only when grown on hydroponics. Where often positive correlations were found between mineral concentrations in seeds of both soil-grown populations, they were often negative between soil and hydroponics-grown plants of the L*er*/Kond population. In general, correlations of Zn concentrations with other mineral concentrations were different from those between concentrations of Fe and the other minerals.

Overall, it was observed that (i) the concentrations of a mineral in the same organ generally did not correlate between the two growth conditions; (ii) the concentrations of different minerals, measured in the same organs, grown under the same conditions generally did not correlate; and (iii) the concentrations of a mineral when measured in different organs (whether or not grown under the same conditions) generally did not correlate. Also, even if correlations were found to be statistically significant, the correlation was often not very high, seed mineral concentrations of soil-grown plants being an exception. This general absence of robust correlations between mineral concentrations over all conditions, organs, and populations suggests that there are many condition-, organ-, and population-specific (genetic) factors to control mineral concentrations.

### *Genetic map of the Ler/Eri-1 population*

This report describes a new RIL population, made from a cross between L*er* and Eri-1. The genetic map obtained

**Table 3.** Correlation coefficients (*r*) of mineral (Zn, Mn, Fe, K, Ca, Mg, and P) and phytate (IP6) concentrations in seeds, rosettes, and roots of the L*er*/Kond (upper right diagonal half) and in seeds and rosettes of the Ler/An-1 RILs (bottom left diagonal half) grown on soil and hydroponics

Negative significant correlation *r* values are highlighted (grey). Significance threshold levels: * $P \leq 0.05$;** $P \leq 0.01$;*** $P \leq 0.001$.

| | | Seed (soil) | | | | | | | Seed (hydrop) | | | | | | | Rosette | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Zn** | **Mn** | **Fe** | **K** | **Ca** | **Mg** | **IP6 or P[a]** | **Zn** | **Mn** | **Fe** | **K** | **Ca** | **Mg** | **IP6 or P[a]** | **Zn** | **Mn** | **Fe** | **Zn** | **Mn** | **Fe** |
| Seed | Zn | | 0.30** | 0.49*** | –0.06 | 0.09 | 0.12 | –0.04 | 0.09 | –0.04 | –0.01 | 0.06 | –0.16 | –0.02 | 0.13 | 0.01 | 0.09 | 0.04 | –0.03 | –0.04 | 0.04 |
| (soil) | Mn | 0.34** | | 0.02 | 0.12 | 0.57*** | 0.23* | 0.32*** | –0.27** | 0.28** | –0.13 | 0.02 | 0.06 | 0.23 | 0.26* | –0.05 | 0.08 | 0.18 | –0.15 | 0.02 | 0.13 |
| | Fe | 0.53*** | 0.34*** | | –0.20* | –0.03 | 0.14 | –0.15 | 0.18 | –0.23* | 0.11 | –0.01 | –0.29** | 0.10 | 0.05 | 0.04 | –0.07 | 0.11 | 0.01 | –0.12 | 0.04 |
| | K | 0.26* | 0.31** | 0.09 | | –0.16 | 0.49*** | 0.33*** | –0.14 | 0.11 | –0.35*** | 0.24* | –0.16 | –0.09 | 0.11 | –0.02 | 0.12 | 0.24* | –0.14 | 0.17 | 0.07 |
| | Ca | 0.17 | 0.41*** | 0.23* | –0.30** | | –0.09 | 0.16 | –0.14 | 0.34*** | 0.03 | 0.12 | 0.33*** | 0.06 | –0.12 | 0.25** | 0.12 | 0.09 | –0.07 | –0.05 | 0.02 |
| | Mg | 0.40*** | 0.40*** | 0.33*** | 0.55*** | –0.08 | | 0.37*** | –0.16 | 0.06 | –0.30** | 0.28** | –0.51*** | 0.23 | 0.37*** | –0.43*** | 0.07 | 0.24* | –0.15 | –0.01 | 0.25** |
| | IP6 or P[a] | 0.34** | 0.56*** | 0.24* | 0.36*** | 0.43*** | 0.60*** | | –0.38*** | 0.09 | –0.32** | 0.01 | –0.09 | 0.15 | 0.24* | –0.07 | 0.21* | 0.09 | –0.30** | –0.09 | 0.18 |
| Seed | Zn | 0.08 | 0.16 | 0.25* | 0.30** | 0.03 | 0.23* | 0.09 | | –0.01 | 0.16 | 0.07 | –0.11 | 0.04 | –0.21* | 0.10 | 0.08 | –0.19* | 0.15 | 0.09 | –0.10 |
| (hydrop) | Mn | 0.08 | 0.21* | 0.22* | 0.35*** | –0.04 | 0.29** | 0.07 | 0.30** | | 0.19* | –0.03 | 0.30*** | 0.13 | 0.02 | 0.01 | 0.20* | 0.00 | 0.13 | 0.09 | –0.01 |
| | Fe | 0.01 | –0.03 | 0.40*** | 0.04 | –0.07 | 0.04 | –0.24* | 0.37*** | 0.51*** | | –0.46*** | 0.24* | 0.23* | –0.18 | 0.03 | 0.02 | –0.03 | 0.11 | 0.02 | –0.08 |
| | K | 0.19 | 0.24* | 0.11 | 0.30** | 0.10 | 0.24* | 0.40*** | 0.42*** | –0.20* | –0.12 | | –0.43*** | –0.16 | 0.15 | –0.02 | 0.11 | –0.07 | –0.03 | –0.03 | –0.13 |
| | Ca | –0.15 | –0.07 | –0.07 | –0.12 | 0.07 | –0.05 | –0.16 | –0.11 | 0.32** | 0.30** | –0.63*** | | –0.07 | –0.34*** | 0.38*** | 0.04 | –0.20* | 0.07 | 0.11 | –0.12 |
| | Mg | 0.12 | 0.22* | 0.22* | 0.16 | –0.01 | 0.32*** | 0.34*** | 0.47*** | 0.05 | 0.10 | 0.60*** | –0.27** | | 0.06 | –0.13 | –0.05 | 0.02 | 0.11 | –0.13 | –0.12 |
| | IP6 or P[a] | 0.12 | 0.24* | 0.07 | 0.23* | 0.17 | 0.27** | 0.42*** | 0.56*** | –0.10 | –0.13 | 0.72*** | –0.39*** | 0.78*** | | –0.34*** | 0.10 | 0.25** | –0.05 | –0.24* | 0.06 |
| Rosette | Zn | –0.11 | 0.02 | –0.11 | 0.16 | –0.18 | –0.09 | –0.17 | 0.17 | 0.07 | 0.07 | 0.00 | –0.08 | –0.11 | –0.06 | | 0.41*** | 0.08 | 0.00 | 0.10 | –0.15 |
| | Mn | 0.24* | 0.11 | 0.26** | 0.24* | –0.05 | 0.25** | 0.06 | 0.31*** | 0.22* | 0.24** | 0.24** | –0.18 | 0.33*** | 0.23* | 0.21* | | 0.28** | –0.24** | 0.20* | –0.05 |
| | Fe | 0.00 | –0.03 | –0.04 | 0.11 | –0.15 | –0.06 | –0.27** | 0.22* | 0.18 | 0.08 | –0.03 | –0.10 | –0.02 | –0.07 | 0.23* | 0.27** | | –0.18* | 0.03 | 0.23* |
| Root | | | | | | | | | | | | | | | | | | | Zn | –0.02 | –0.20* |
| | | | | | | | | | | | | | | | | | | | Mn | | 0.01 |

[a] Total P concentrations for L*er*/An-1 and IP6 concentrations for L*er*/Kond RILs.

for this L*er*/Eri-1 RIL population has a length of 365 cM (Fig. 6), which is in the same range as *Arabidopsis* genetic maps obtained from other, different crosses (Alonso-Blanco *et al.*, 1998; Loudet *et al.*, 2002; El Lithy *et al.*, 2006). The markers are distributed along the five chromosomes with a genetic distance between two successive markers of 4 cM, on average, with a maximum of 12 cM (between markers T2N18 and F17A22 on chromosome 2; between markers DF.76L and BH.120L-Col on chromosome 3; between markers M4-36 and G3883 on chromosome 4; Fig. 6). No significant allelic distortion has been observed for this new mapping population.

### *Genetic analyses of mineral concentrations*

To estimate the proportion of phenotypic variation in a population that can be attributed to genetic variation, the broad-sense heritability values for all traits (Fig. 7) were calculated. The values varied between 10.6%, for seed Fe concentrations in the L*er*/Eri-1 population and 89.2% for seed P concentrations in the L*er*/An-1 population. Heritabilities for the mineral concentrations were higher in the L*er*/An-1 population, which is most likely a population effect, as it is seen for both soil and hydroponically grown plants, although differences in the growing conditions cannot be ruled out, since populations were tested at different times. To identify the genetic factors responsible for the mineral concentrations in roots, rosettes, and seeds, a QTL analysis was performed. QTLs were identified for each trait in at least one of the three populations tested (Figs 6, 8; Tables 4, 5, 6). The total phenotypic variances explained by the identified QTLs were over 50% of the heritability values for most of the traits (Fig. 7). No major QTL was identified for seed Zn and Fe concentrations in the L*er*/Eri-1 population, which was in line with the low heritability values of these traits in this population. The trait variation was not a good indicator for the number of QTLs that could be identified or the total explained variances for a trait. For instance, the fold difference between RILs for seed Mg concentrations (1.5-fold) in the L*er*/Kond population grown on soil was one of the lowest in comparison to other traits, but the total trait variance explained by QTLs was the highest (53.5). Also for seed K concentration, the ranges observed among the L*er*/Kond RILs were twice as high when compared to the L*er*/An-1 RILs. However, the heritability values, the number of identified QTLs, and their total explained phenotypic variance, were similar.

Many of the QTLs identified for the same trait in the three populations co-located. However, the total number of QTLs detected in the L*er*/An-1 populations was much larger than for the other two populations. Four hotspots

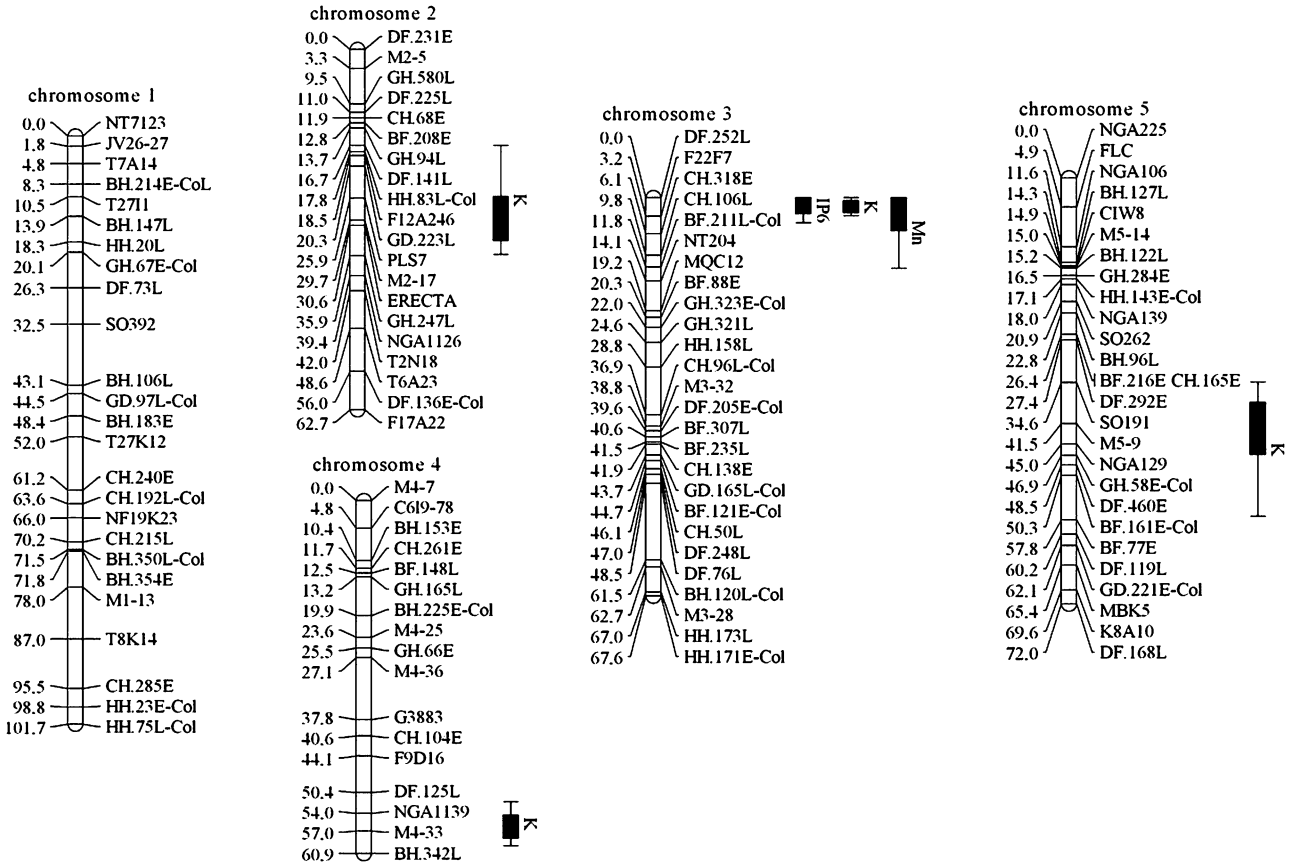


**Fig. 6.** Genetic map of the L*er*/Eri-1 RIL population with QTLs identified for seed mineral (Zn, Mn, Fe, K, Ca, and Mg) concentrations of plants grown on soil. QTLs are indicated on the right of the chromosomes with thin lines indicating 1-LOD intervals and the thick bars indicating 2-LOD interval for each QTL.

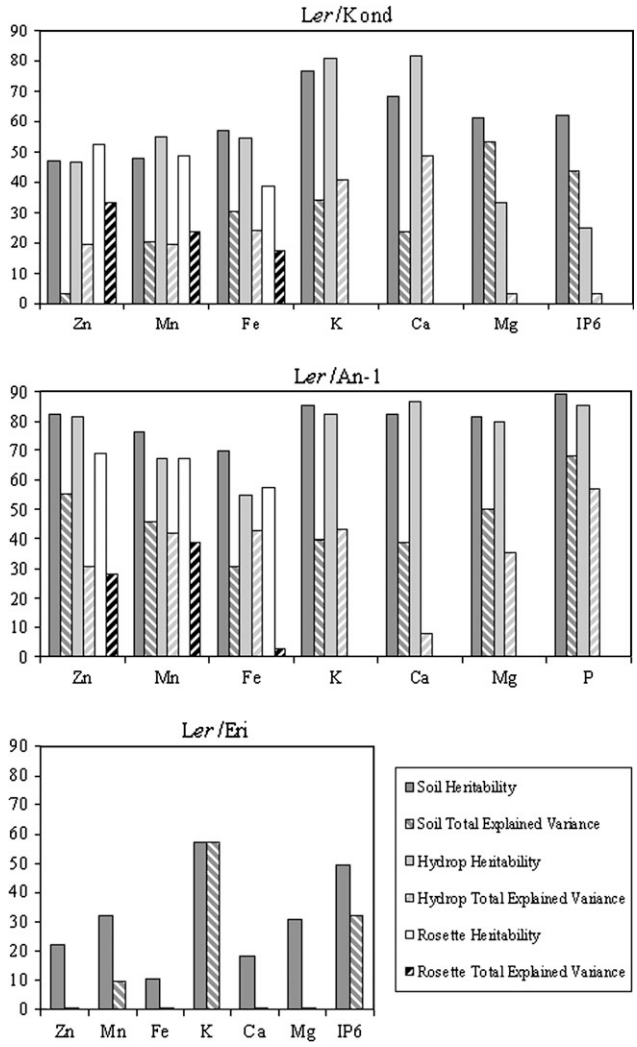


**Fig. 7.** Heritabilities and explained phenotypic variances (Total Explained Variance) for the mineral (Zn, Mn, Fe, K, Ca, and Mg) and phytate (IP6) concentrations in the L*er*/Kond, L*er*/An-1, and L*er*/Eri-1 RIL populations. Data are provided for seed mineral concentrations of soil and hydroponically (hydrop) grown plants and for rosette mineral concentrations of hydroponically grown plants.

were found for co-locating QTLs: the region of chromosome 2 around the *ERECTA* gene; the top of chromosome 3, around marker NGA172; and two regions on chromosome 5, respectively, around markers SNP236 and MBK5. The co-locations identified were not specific to macro-elements (e.g. K and Ca) or micro-elements (e.g. Zn and Fe), but often involved both groups of elements.

Epistatic interactions among many loci were detected in many cases, although most interactions did not explain a major part of the phenotypic variance compared to the main effects (Table 7). Several QTL positions for the populations grown on soil were previously identified for the same traits in the L*er*/Cvi population (Bentsink *et al.*, 2003;Vreugdenhil *et al.*, 2004). These include K and Ca QTLs on chromosome 1 and the Zn and Mn QTLs around *ERECTA*. In addition, the QTLs located at the top of chromosome 3, including the strong IP6 and P QTLs that were found in all populations and which co-located with K, Zn, and Mn QTLs at least in some of the studied populations. The cluster of QTLs at the MBK5 marker (at the bottom of chromosome 5), was only detected in the L*er*/Kond and L*er*/Eri-1 populations.

Despite many QTL co-localizations when comparing different populations grown under the same condition, only a few QTLs co-localized when comparing the same population grown under two conditions (hydroponics or soil). This again illustrates that there is considerable difference between the genetic control of mineral accumulation when plants are grown on hydroponic medium or on soil. It also illustrates the large effect of mineral bioavailability, pH or other factors like root architecture on mineral concentration occurring between the growing conditions. Besides the main effect of the environment (growth condition) on the traits, many interactions were also detected between loci and environment (growth condition) (Table 8). Significant interactions with growth conditions were detected for all the seed mineral concentrations (including P), in particular, at the Erecta marker (Fig. 9). The mineral concentrations varied depending on the allele at the Erecta marker (L*er*, Kond, or An-1); depending on the growth condition (hydroponics or soil); and depending on the interaction between the locus and the growth condition (Genotype× Environment interaction). For example, on hydroponics, seed Fe concentrations were highest in plants carrying the Kond allele at the Erecta marker, compared to plants carrying the L*er* or An-1 alleles. On soil, however, the seed Fe concentrations were lowest in plants carrying the Kond allele at the Erecta marker, while the lines carrying the L*er* and An-1 alleles showed comparable differences between seed Fe concentrations as on hydroponics. Thus, for Fe concentration there is a clear G×E interaction. G×E interactions at the Erecta marker were not found for seed IP6 concentrations.

## Discussion

Micronutrients, such as iron, zinc, vitamin A, and iodine, are required by humans in small amounts only, but are essential for good health. Women and children in Sub-Saharan Africa, South and South-East Asia, Latin America, and the Caribbean are especially at risk of disease, premature death, and impaired cognitive abilities because of diets lacking essential micronutrients (http://www.harvestplus.org/). In order to allow breeding for varieties with a higher mineral content (bio-fortification) or a more efficient uptake of minerals from the soil, knowledge on the genetic variation of cationic mineral homeostasis and the genes underlying allelic variation needs to be expanded. In the past, little attention has been paid to breeding for enhanced mineral content. With the Harvest Plus initiative that has changed. Recent efforts to breed for micronutrient-enhanced rice grains showed that, although there appears to be sufficient genetic variation for micronutrient content in rice, it is a difficult trait to breed for (Gregorio *et al.*, 2008). The main difficulties are the

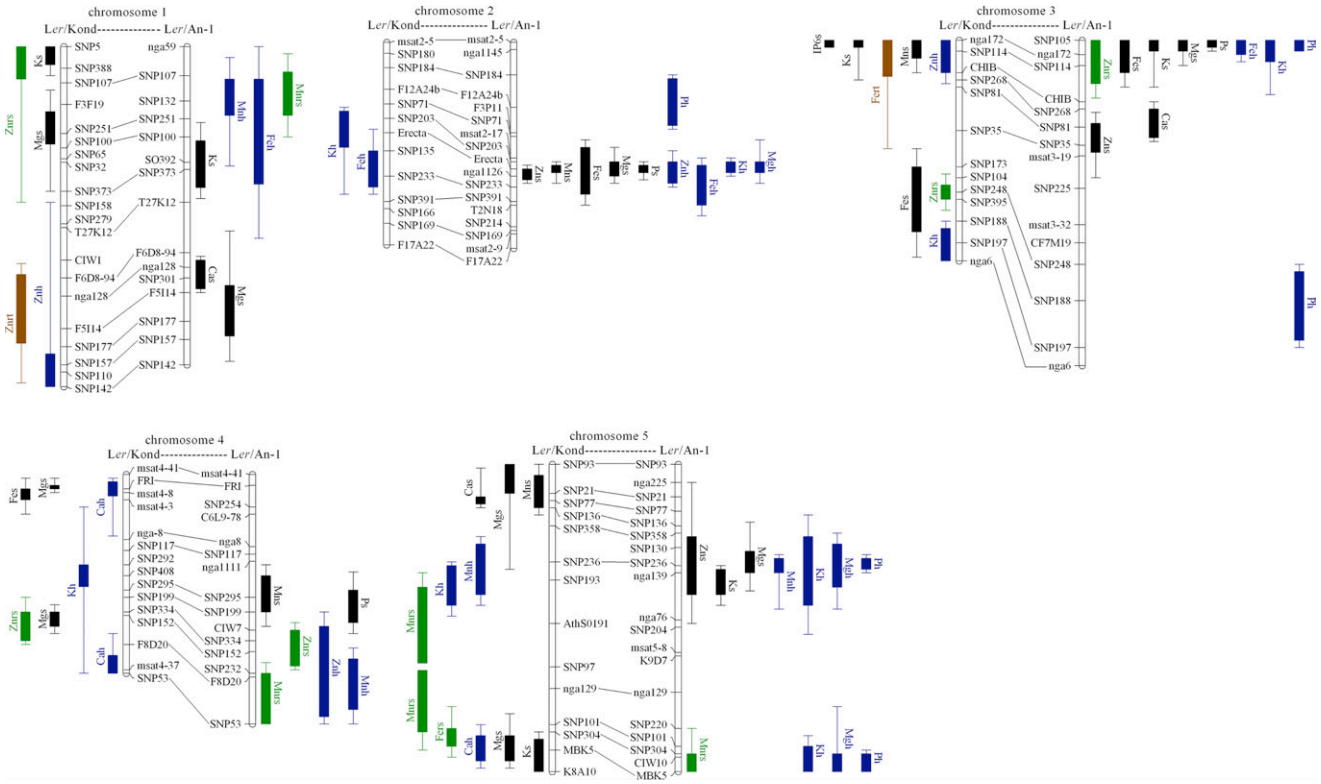


**Fig. 8.** Genetic map of the L*er*/Kond and L*er*/An-1 RIL populations with QTLs identified for seed (s) mineral (Zn, Mn, Fe, K, Ca, and Mg) and phytate (IP6) concentrations of plants grown on soil (in black) or hydroponics (in blue) and for rosettes (rs; in green netted) and roots (rt; in brown striped) of hydroponically grown plants. QTLs are indicated on the left of the chromosomes for L*er*/Kond and on the right of the chromosomes for L*er*/An-1 with the thick bars indicating 1-LOD intervals and thin lines indicating 2-LOD interval for each QTL.

complexity of the traits, low heritabilities, and the high environmental influence. Therefore we set about to investigate this in the model plant species *Arabidopsis thaliana*.

Studying mineral homeostasis in plants grown on different media will help to understand the differences in regulation due to growth conditions, whereas studying mineral homeostasis in different plant organs will help to understand the differences in the regulation due to organ specificity. To study the genetics behind mineral and phytate homeostasis in plants, segregating *Arabidopsis* populations were used based on crosses of accessions Landsberg *erecta* (L*er*) with Kondara (Kond) and Antwerp (An-1) (El-Lithy *et al.*, 2006), for which it was known that the parents differed significantly in seed mineral concentrations (Vreugdenhil *et al.*, 2004). In addition, a new mapping population was included in this study, which is derived from a cross between accessions L*er* and Eringsboda. In addition to the previously used accession Cape Verde Islands (Cvi; Vreugdenhil *et al.*, 2004), these four accessions cover a wide range of ecological niches and should provide a good representation of available genetic variation for mineral concentrations in *Arabidopsis*.

Variations in mineral concentrations of plants will depend on variations in many parameters, like mineral mobilization, uptake, trafficking, and sequestration, which are all relevant processes in the mineral transport pathway from roots to shoots (Clemens, 2001). Roots excrete compounds to acidify the environment and, in roots and shoots, ligands and chelates are present to bind minerals. These processes depend on the substrate on which the plant is grown. The two growth media used here for cultivating the RIL populations, soil and hydroponics, are expected to differ in terms of mineral (bio)-availability, buffering, and ion exchange capacities, with more variation for soil conditions than for hydroponics and, especially for hydroponics, a generally higher bio-availability. Thus, in the latter medium, the intention was to remove one of the 'bottlenecks' limiting plant mineral acquisition and accumulation. Although none of the growth conditions led to obvious mineral deficiency or excess symptoms, it is still possible that there were fewer or more (bio)-available minerals present in one substrate ompared with the other, which caused differences in plant mineral concentrations, although both growth conditions were considered as optimal. It is realized that the different bio-availabilities of the various minerals in the growth substrates might also cause differences in competition between the minerals for mobilization, uptake, translocation, and sequestration levels, considering the presence of the large proportion of mineral high- and low-affinity transporters and chelates shared among minerals (Maser *et al.*, 2001;Clemens *et al.*, 2002). Our hypothesis was that QTLs identified for the soil-grown populations, but not in the hydroponics populations,

**Table 4.** QTLs affecting mineral (Ca, Fe, K, Mg, Mn, P, and Zn) and phytate (IP6) concentrations identified in the L*er*/Kond RIL population

Seed QTLs are determined for the population when grown on soil or hydroponics as indicated in brackets. Rosette and root QTLs are only determined for the hydroponically grown population. For each QTL the chromosome number is indicated (Chrom), the genetic position (in cM), the closest genetic marker (Locus), the additive logarithm of odds value (LOD), the percentage of explained phenotypic variance (% Expl. var.) and the parental allele that increases the trait value (Effect).

| | Trait | Chrom | Position | Locus | LOD | % Expl. var. | Effect |
|---|---|---|---|---|---|---|---|
| Seed (soil) | Mn | 3 | 0 | nga172 | 3.60 | 12.9 | Kond |
| | Mn | 5 | 10.4 | SGCSNP77 | 2.57 | 9.1 | *Ler* |
| | Fe | 3 | 49.8 | SGCSNP188 | 3.20 | 10.7 | *Ler* |
| | Fe | 4 | 4.7 | msat4-8 | 5.84 | 20.6 | Kond |
| | K | 1 | 0 | SGCSNP5 | 4.02 | 12.8 | *Ler* |
| | K | 3 | 0 | nga172 | 2.80 | 8.6 | Kond |
| | K | 5 | 79.1 | MBK5 | 3.95 | 12.5 | *Ler* |
| | Ca | 5 | 10.4 | SGCSNP77 | 6.25 | 23.5 | *Ler* |
| | Mg | 1 | 24.2 | SGCSNP251 | 3.97 | 8.7 | Kond |
| | Mg | 4 | 4.1 | FRI | 4.40 | 9.6 | Kond |
| | Mg | 4 | 39.1 | SGCSNP152 | 5.09 | 11.5 | Kond |
| | Mg | 5 | 0 | SGCSNP93 | 2.72 | 5.7 | Kond |
| | Mg | 5 | 79.1 | MBK5 | 4.61 | 10.0 | *Ler* |
| | IP6 | 3 | 0 | nga172 | 13.46 | 43.6 | Kond |
| Seed (hydroponics) | Zn | 1 | 94.1 | SGCSNP142 | 2.48 | 9.8 | Kond |
| | Zn | 3 | 0 | nga172 | 3.06 | 12.2 | *Ler* |
| | Mn | 5 | 27.4 | SGCSNP236 | 5.07 | 19.7 | *Ler* |
| | Fe | 2 | 36.5 | SGCSNP233 | 6.27 | 24.1 | Kond |
| | K | 2 | 21.5 | SGCSNP203 | 5.39 | 14.7 | *Ler* |
| | K | 3 | 56.3 | SGCSNP197 | 4.75 | 13.1 | *Ler* |
| | K | 4 | 27.8 | SGCSNP408 | 4.19 | 11.5 | Kond |
| | K | 5 | 31.6 | SGCSNP193 | 5.40 | 15.1 | *Ler* |
| | Ca | 4 | 4.1 | FRI | 8.57 | 28.2 | *Ler* |
| | Ca | 4 | 55.5 | SGCSNP53 | 4.40 | 15.8 | *Ler* |
| | Ca | 5 | 79.1 | MBK5 | 4.91 | 12.4 | Kond |
| Rosettes | Zn | 1 | 0 | SGCSNP5 | 2.60 | 7.5 | *Ler* |
| | Zn | 3 | 42.4 | SGCSNP248 | 3.74 | 10.7 | *Ler* |
| | Zn | 4 | 39.1 | SGCSNP152 | 5.61 | 16.7 | *Ler* |
| | Mn | 5 | 43.8 | AthS0191 | 3.95 | 15.0 | *Ler* |
| | Mn | 5 | 72.0 | SGCSNP101 | 3.83 | 14.4 | *Ler* |
| | Fe | 5 | 73.8 | SGCSNP304 | 4.67 | 17.8 | *Ler* |
| Roots | Zn | 1 | 68.9 | nga128 | 2.80 | 10.9 | *Ler* |
| | Fe | 3 | 2.8 | SGCSNP114 | 3.94 | 14.5 | Kond |

would correspond to loci involved in enhancing mineral bio-availability.

Variation in mineral concentrations was observed between different organs when comparing RILs of one population. This suggests that mineral concentrations are maintained in plants in an organ specific manner. The rosette concentrations were determined for plants which had not yet bolted. Potentially, minerals accumulated in rosette leaves could be available for later loading into the inflorescence and, eventually, seeds, although Waters and Grusak (2008*a*) recently showed that seed loading in particular does not depend on remobilization but rather on direct uptake by roots from soil. It is not clear if this also holds for mobilization to developing inflorescences. Considerable transgression of phenotypic values in both directions for seed mineral concentrations were detected in specific RILs, as was also previously observed in L*er*/Cvi RILs (Vreugdenhil *et al.*, 2004), suggesting that both accessions carry genes with alleles contributing to an increased or a decreased content of all minerals tested.

Both negative and positive correlations were observed among traits. Most robust are the correlations between Zn, Fe, and Mn concentrations, which are largely independent of the organ, population, or environment. These three minerals are known to share components of their respective mechanism for uptake from soil, transport into and out of cells and cell organelles, and for chelation during vascular transport (Maser *et al.*, 2001), which could account for such correlations. However, whereas most correlations for these minerals in seeds of soil-grown plants are positive (suggesting co-transport and co-chelation), when comparing other organs, negative correlations are also found, suggesting a limited availability of transport proteins or chelator molecules causing competition between minerals. Also IP6

**Table 5.** QTLs affecting mineral (Ca, Fe, K, Mg, Mn, P, and Zn) and phytate (IP6) concentrations identified in the L*er*/An-1 RIL population

Seed QTLs are determined for the population when grown on soil or hydroponics as indicated in brackets. Rosette QTLs are only determined for the hydroponically grown population. For each QTL the chromosome number is indicated (Chrom), the genetic position (in cM), the closest genetic marker (Locus), the additive logarithm of odds value (LOD), the percentage of explained phenotypic variance (% Expl. var.) and the parental allele that increases the trait value (Effect).

| | Trait | Chrom | Position | Locus | LOD | % Expl. var. | Effect |
|---|---|---|---|---|---|---|---|
| Seed (soil) | Zn | 2 | 36.6 | nga1126 | 11.06 | 36.8 | L*er* |
| | Zn | 3 | 29 | SNP35 | 5.33 | 15.1 | An-1 |
| | Zn | 5 | 28.4 | SNP236 | 2.86 | 7.6 | L*er* |
| | Mn | 2 | 34.8 | Erecta | 12.29 | 37.2 | L*er* |
| | Mn | 4 | 34.4 | SNP295 | 2.98 | 7.4 | An-1 |
| | Fe | 2 | 32.8 | SNP203 | 3.22 | 10.9 | L*er* |
| | Fe | 3 | 0 | SNP105 | 5.39 | 19.6 | An-1 |
| | K | 1 | 34.3 | SNP373 | 3.24 | 9.3 | L*er* |
| | K | 3 | 0 | SNP105 | 3.45 | 9.8 | An-1 |
| | K | 5 | 30 | nga139 | 7.98 | 24.8 | L*er* |
| | Ca | 1 | 61.3 | nga128 | 8.22 | 25.5 | L*er* |
| | Ca | 3 | 24.1 | SNP81 | 6.30 | 19.0 | An-1 |
| | Mg | 1 | 76.4 | SNP177 | 3.02 | 7.2 | An-1 |
| | Mg | 2 | 34.8 | Erecta | 4.14 | 9.9 | L*er* |
| | Mg | 3 | 0 | SNP105 | 7.99 | 21.0 | An-1 |
| | Mg | 5 | 18.6 | SNP358 | 6.34 | 16.0 | L*er* |
| | P | 2 | 34.8 | Erecta | 5.12 | 8.4 | L*er* |
| | P | 3 | 0 | SNP105 | 23.28 | 56.6 | An-1 |
| | P | 4 | 37.6 | SNP199 | 2.96 | 4.9 | An-1 |
| Seed | Zn | 2 | 36.6 | nga1126 | 5.86 | 19.0 | L*er* |
| (hydroponics) | Zn | 4 | 46 | SNP334 | 3.52 | 10.9 | L*er* |
| | Mn | 1 | 15 | SNP132 | 2.71 | 7.2 | L*er* |
| | Mn | 4 | 55.2 | SNP232 | 2.93 | 7.8 | L*er* |
| | Mn | 5 | 28.4 | SNP236 | 9.93 | 30.5 | L*er* |
| | Fe | 1 | 32 | SO392 | 2.47 | 5.9 | L*er* |
| | Fe | 2 | 36.6 | nga1126 | 4.31 | 10.8 | L*er* |
| | Fe | 3 | 0 | SNP105 | 9.04 | 25.1 | L*er* |
| | K | 2 | 34.8 | Erecta | 5.25 | 13.5 | L*er* |
| | K | 3 | 0 | SNP105 | 2.38 | 5.8 | An-1 |
| | K | 5 | 23.3 | SNP130 | 3.38 | 9.6 | An-1 |
| | K | 5 | 79.7 | SNP304 | 2.61 | 7.3 | L*er* |
| | Mg | 2 | 34.8 | Erecta | 4.19 | 12.4 | L*er* |
| | Mg | 5 | 28.4 | SNP236 | 2.82 | 8.1 | An-1 |
| | Mg | 5 | 84.6 | MBK5 | 3.35 | 9.7 | L*er* |
| | P | 2 | 17.8 | F12A24b | 3.42 | 6.3 | L*er* |
| | P | 3 | 0 | SNP105 | 7.31 | 14.8 | An-1 |
| | P | 3 | 72.2 | SNP188 | 2.77 | 5.1 | L*er* |
| | P | 5 | 28.4 | SNP236 | 8.62 | 17.9 | An-1 |
| | P | 5 | 81.3 | CIW10 | 4.59 | 8.7 | L*er* |
| Rosettes | Zn | 3 | 7.1 | SNP114 | 2.74 | 8.5 | L*er* |
| | Zn | 4 | 48.8 | SNP152 | 6.77 | 22.5 | L*er* |
| | Mn | 1 | 15 | SNP132 | 4.03 | 10.8 | L*er* |
| | Mn | 4 | 55.7 | F8D20 | 4.03 | 10.6 | L*er* |
| | Mn | 5 | 84.6 | MBK5 | 2.99 | 7.7 | L*er* |

concentrations in seeds are frequently found to be correlated with other minerals, particularly Zn, K, and Mg, in line with the physical co-locations of IP6 and these minerals

**Table 6.** QTLs affecting seed mineral (Ca, Fe, K, Mg, Mn, P, and Zn) and phytate (IP6) concentrations identified in the L*er*/Eri-1 RIL population

QTLs are determined for the population when grown on soil. For each QTL the chromosome number is indicated (Chrom.), the genetic position (in cM), the closest genetic marker (Locus), the additive logarithm of odds value (LOD), the percentage of explained phenotypic variance (% Expl. var.) and the parental allele that increases the trait value (Effect).

| Trait | Chrom. | Position | Locus | LOD | % Expl. var. | Effect |
|---|---|---|---|---|---|---|
| Mn | 3 | 3.1 | F22F7 | 2.48 | 9.8 | Eri |
| K | 2 | 29.7 | M2-17 | 2.96 | 5.6 | Eri |
| K | 3 | 0.0 | DF.252L | 13.43 | 32.0 | Eri |
| K | 4 | 56.7 | M4-33 | 5.14 | 10.1 | Eri |
| K | 5 | 45.5 | DF.460E | 3.01 | 5.7 | Eri |
| IP6 | 3 | 0.0 | DF.252L | 9.25 | 31.9 | Eri |

in different parts of *Arabidopsis* developing seeds (Otegui *et al.*, 2002).

A genetic basis for mineral concentrations in the populations was founded by identifying many QTLs in each population, often co-locating between the RIL populations. For example, in all populations studied, the presence of a L*er* allele on the top of chromosome 3 resulted in lower seed P/IP6 concentrations and, consequently, in QTLs. These results suggest that the L*er* allele at this locus differs from the alleles in the other parental accessions. The same was previously found by Waters and Grusak (2008*b*).

Regarding our initial hypothesis that QTLs identified for both soil and hydroponics would represent loci not involved in mineral bio-availability, this only holds for three loci at which several soil and hydroponics seed mineral QTLs co-localize. These three mineral QTL hotspots are associated with the markers Erecta (chromosome 2), NGA172 (chromosome 3), and MBK5 (chromosome 5). These three loci were also hotspots for QTLs of life history traits found in the L*er*/Cvi population (Alonso-Blanco *et al.*, 1999;Ungerer *et al.*, 2002). Co-localization of the QTLs for different traits suggests a single pleiotropic locus to be involved in the homeostasis of multiple traits. A straightforward explanation for this co-localization would be if such a locus represents a gene for which variation has a striking effect on plant morphology or development, as that will very likely also affect mineral homeostasis. For the *Erecta* locus such an effect can easily be envisioned. The L*er* parent, which was used for all three populations, as well as the L*er*/Cvi population, carries a mutant allele of the *ERECTA* gene. The *ERECTA* gene encodes a receptor protein kinase protein, mutation of which has a drastic effect on plant morphology (Torii *et al.*, 1996). Previously the *Erecta* locus has been identified as a major QTL for various traits (including mineral concentrations; Waters and Grusak, 2008*b*) in populations with L*er* as one of the parents (Llorente *et al.*, 2005; Masle *et al.*, 2005; Tisné *et al.*, 2008). The *ERECTA* gene, which was previously also shown to affect seed yield-associated factors like plant total seed number in *Arabidopsis* (Alonso-Blanco *et al.*, 1999), was

**Table 7.** Epistatic interactions ($P$ <0.005) between two loci in the L*er*Kond, L*er*/An-1, and L*er*/Eri-1 populations

Traits are mineral (Ca, Fe, K, Mg, Mn, P, and Zn) or phytate (IP6) concentrations, with (h) or (s) indicating, respectively, seed traits of hydroponics and soil-grown plants and (rs) indicating rosette mineral traits. For each interaction the additive $P$-value of the interaction is indicated. Only statistically significant ($P$ <0.05) interactions are shown. The percentage of phenotypic variance that is explained by the interaction is indicated (% Expl. var.).

| | Trait | Locus 1 | Locus 2 | Additive *P*-value | % Expl. var. |
|---|---|---|---|---|---|
| L*er*/ | Fe(h) | SNP101 | SNP295 | 0.0005 | 3.6% |
| Kond | Fe(rs) | nga128 | SNP104 | 0.0014 | 7.0% |
| | Fe(s) | nga6 | SNP203 | 0.0008 | 6.0% |
| | IP6(s) | MBK5 | SNP388 | 0.0005 | 5.6% |
| | K(h) | SNP114 | SNP395 | 0.0001 | 8.1% |
| | K(h) | SNP135 | SNP388 | 0.0009 | 8.2% |
| | Mg(h) | F12A24b | SNP93 | 0.001 | 4.8% |
| | Mg(h) | SNP166 | SNP93 | 0.0023 | 6.2% |
| | Mn(h) | msat4-3 | SNP251 | 0.0024 | 3.9% |
| L*er*/An-1 | Ca(h) | nga139 | SNP220 | 0.0003 | 7.7% |
| | Ca(s) | SNP177 | SNP35 | 0.0013 | 24.9% |
| | Ca(s) | SNP157 | SNP204 | 0.0029 | 21.5% |
| | Ca(s) | SNP136 | SNP304 | 0.0003 | 3.4% |
| | Fe(s) | SNP301 | M4-41 | 0.0007 | 6.3% |
| | Mg(s) | SNP81 | F6D8-94 | 0.0021 | 13.5% |
| | Mn(rs) | T27K12 | SNP169 | 0.0003 | 8.2% |
| | Mn(s) | SO392 | SNP184 | 0.0002 | 5.2% |
| | Mn(s) | F12A24b | SNP373 | 0.0005 | 12.4% |
| | Mn(s) | M2-17 | M2-5 | 0.0083 | 31.7% |
| | P(h) | SNP268 | SNP295 | 0.0004 | 7.9% |
| | P(h) | M4-41 | SNP77 | 0.0012 | 3.1% |
| | Zn(h) | F12A24b | SNP295 | 0.0009 | 5.1% |
| | Zn(rs) | nga6 | SNP236 | 0.0016 | 3.4% |
| | Zn(rs) | SNP114 | CIW7 | 0.0002 | 6.0% |
| | Zn(s) | SNP391 | CIW7 | 0.0006 | 15.0% |
| L*er*/Eri-1 | Zn(s) | SO191 | T6A23 | 0.0002 | 1.6% |
| | Zn(s) | NGA106 | NGA129 | 0.0001 | 1.2% |
| | Mn(s) | BH.354E | DF.119L | 0.0008 | 5.3% |
| | Mn(s) | CH.318E | NGA106 | 0.0005 | 5.1% |
| | Mg(s) | SO262 | T6A23 | 0.0001 | 5.1% |
| | Fe(s) | NGA129 | T6A23 | 0.0001 | 2.7% |
| | Fe(s) | NGA106 | T6A23 | 0.0003 | 9.6% |
| | Fe(s) | CIW8 | GH.58E-Col | 0.0010 | 2.4% |

**Table 8.** Overview of the QTL×environment interactions identified in the L*er*/Kond and L*er*/An-1 populations

Traits are seed mineral (Zn, Mn, K, Fe, Ca, Mg, and P) concentrations. For the QTLs, the chromosome number (Chrom.), their physical position (based on the Columbia genome; in Mb) and their closest genetic marker (Locus) is provided, as is the probability ($P$) value of the interaction. For co-locating QTLs, of which only one QTL showed interaction, the $P$-value of the other QTL is indicated as non-detected (nd).6

| Trait | Chrom | Position | Locus | *P*-value |
|---|---|---|---|---|
| Mn | 1 | 3.1 | SNP107 | $P$ <0.001 |
| K | 1 | 3.1 | SNP107 | Nd |
| Fe | 1 | 11.4 | SNP373 | $P$ <0.001 |
| Ca | 1 | 20.6 | nga128 | $P$ <0.001 |
| Mg | 1 | 26 | SNP177 | $P$ <0.001 |
| Zn | 1 | 29.8 | SNP142 | $P$ <0.001 |
| P | 2 | 7.3 | F12A24b | $P$ <0.012 |
| Zn | 2 | 11.2 | Erecta | $P$ <0.004 |
| Mn | | | | $P$ <0.001 |
| Fe | | | | $P$ <0.001 |
| K | | | | $P$ <0.001 |
| Mg | | | | $P$ <0.001 |
| P | | | | $P$ <0.039 |
| Zn | 3 | 0.8 | nga172 | $P$ <0.012 |
| Mn | | | | $P$ <0.001 |
| Fe | | | | $P$ <0.001 |
| K | | | | $P$ <0.011 |
| Mg | | | | $P$ <0.001 |
| P | | | | nd |
| IP6 | | | | $P$ <0.001 |
| Ca | 3 | 5.8 | SNP81 | $P$ <0.001 |
| Mg | 3 | 5.8 | SNP81 | $P$ <0.001 |
| Zn | 3 | 8.2 | SNP35 | nd |
| P | 3 | 19.6 | SNP188 | nd |
| Fe | 3 | 19.6 | SNP188 | $P$ <0.001 |
| K | 3 | 21.9 | SNP197 | nd |
| Fe | 4 | 0.3 | FRI | $P$ <0.001 |
| Ca | 4 | 0.3 | FRI | $P$ <0.001 |
| Mg | 4 | 5.3 | SNP117 | $P$ <0.001 |
| Mn | 4 | 7.8 | SNP295 | $P$ <0.001 |
| K | 4 | 7.8 | SNP295 | nd |
| P | 4 | 8.9 | SNP199 | nd |
| Zn | 4 | 12.4 | SNP334 | nd |
| Mn | 4 | 16.9 | F8D20 | $P$ <0.001 |
| Ca | 4 | 17.5 | SNP53 | $P$ <0.001 |
| Mn | 5 | 3.5 | SNP77 | $P$ <0.001 |
| Ca | 5 | 3.5 | SNP77 | $P$ <0.001 |
| Zn | 5 | 7.7 | SNP236 | $P$ <0.001 |
| Mn | | | | $P$ <0.001 |
| K | | | | $P$ <0.023 |
| Mg | | | | $P$ <0.001 |
| P | | | | $P$ <0.001 |
| K | 5 | 25.5 | MBK5 | $P$ <0.042 |
| Ca | | | | $P$ <0.001 |
| Mg | | | | $P$ <0.001 |
| P | | | | $P$ <0.003 |

similarly located in the QTL interval for Zn and Mn concentrations in plants identified in the L*er*/Cvi population (Vreugdenhil *et al.*, 2004).

Co-localization of QTLs did not always reflect the correlations observed between the traits. For instance, although the correlation coefficient between seed IP6 and Mg concentrations in L*er*/Kond RILs grown on soil was higher than between IP6 and Mn and K concentrations, the identified QTL for seed IP6, Mn, and K concentrations did co-locate, whereas the QTL for seed Mg concentrations did not. This is most likely due to the presence of other QTLs for these minerals. Often the same QTLs were not found in these hotspot clusters. This can be explained by the fact that a QTL close to the border of significance can appear in one but might not appear in another population. However, the identification of generally different QTLs for mineral accumulation in the same populations, when grown on soil

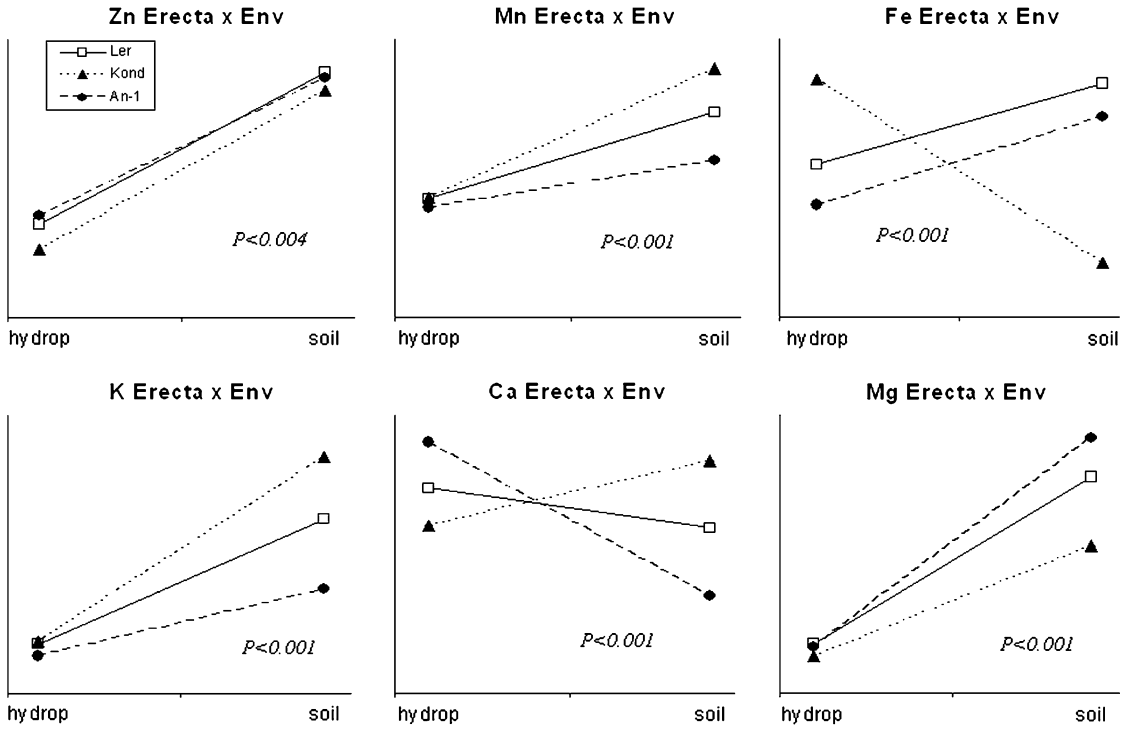


**Fig. 9.** Genotype×environment interactions of QTLs at the Erecta marker. Each graph shows the relative differences of seed mineral (Zn, Mn, Fe, K, Ca, and Mg) concentrations grown on hydroponics (hydrop) and soil (soil). To compare all minerals, the scales of the *y*-axis of each graph are made proportional to each other and represent mineral concentrations in μmol $g^{-1}$ DW. For all minerals shown there was significant ($P$ <0.05) interaction between the growth condition (Env) and the QTL (Erecta).

or on hydroponics, indicates the relevance of mapping QTL for traits in different growth conditions and/or populations to understand the environmental effect on mineral homeostasis in plants better.

The latter is especially important when QTL mapping is performed in crop plants. If commercial crops are grown under certain conditions, then most relevant results will be obtained when also mapping mineral QTLs in those crops using populations grown under the same conditions. QTLs identified in populations which were grown under dissimilar growth conditions might not be used to improve the crop value under commercial growth conditions. Despite the ease of growing plants reproducibly on hydroponics medium in a climate chamber, we do not advocate the use of such growing conditions for mineral QTL analysis when trying to identify relevant genetic loci controlling mineral accumulation for plants grown on soil.

## Supplementary data

Supplementary data are available at *JXB* online.

**Supplementary Table 1.** List of SSLP markers and PCR primers used for genotyping of the L*er*×Eri-1 RIL population.

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