The Use of Hydroponic Growth Systems to Study the Root and Shoot Ionome of *Arabidopsis thaliana*

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1. Introduction

Plants provide an important source of minerals for human and animal consumption. There is a general worldwide deficiency of human intake of several essential minerals, including iron, zinc, copper, calcium, magnesium, selenium, and iodine (White & Broadley, 2009). This deficiency exists not only in developing countries, but also in the developed world. On the other hand, excessive amounts of heavy metals in crop plants can endanger the health of humans and livestock. It is, therefore, important to develop crop plants with balanced levels of minerals. Understanding of the genes and processes that control the mineral content of plants can provide the basis for engineering crops with the optimal concentrations of minerals in various organs and tissues. In recent years, there has been increasing interest in determining the elemental composition (ionome) of various plant species and identifying the genes that dominate this composition [reviewed by Baxter (2009), Salt et al. (2008), and Williams & Salt (2009)]. The agriculturally related plants whose ionomes were investigated include the crops rice (Norton et al., 2010) and *Brassica napus* (Liu et al., 2009), as well as the plant *Lotus japonicus*, which serves as a model species for legume crops (Chen et al., 2009).

Although *Arabidopsis thaliana* is not a crop plant, the availability of a wide range of genetic and genomic tools in this species makes it a useful model organism for identifying the genes that determine the plant ionome. This can be done by correlating ionomic data with genetic information and data about gene expression in genetically divergent *A. thaliana* lines. The commonly utilized genetically divergent *A. thaliana* populations are natural accessions, recombinant inbred lines (RIL), and mutant lines. Such populations were used in several high-throughput studies, which resulted in the identification of many quantitative trait loci (QTLs) controlling the *A. thaliana* ionome (Buescher et al., 2010; Ghandilyan et al., 2009; Lahner et al., 2003; Prinzenberg et al., 2010). Due to the high-throughput nature of these studies, the *A. thaliana* plants were grown in soil in many cases, and the elemental composition was determined in shoots but not in roots. Since minerals are absorbed into plants through roots, it is important to gain knowledge on the root ionome. Hydroponic growth systems make it possible to obtain clean roots suitable for mineral analysis.

Moreover, hydroponic systems enable the application of accurate and relatively stable concentrations of minerals. RIL populations of *A. thaliana* grown in hydroponic systems were utilized to identify QTLs that correlate the mineral composition of roots, leaves, and seeds with phytate content and growth-related traits (Ghandilyan et al., 2009; Prinzenberg et al., 2010). The parental *A. thaliana* accessions utilized in these studies were Ler-3, Kas-2, Kon, An-1, and Eri-1.

In this chapter, we describe in detail the system we use for the growth of *A. thaliana* plants in hydroponics. We then provide data on the mineral composition of roots, rosette leaves, and inflorescence stems (the latter two are referred to hereafter as leaves and stems, respectively) of the Col-0 accession of *A. thaliana*. Col-0 is widely used as a model accession in a variety of genetic, genomic, and gene-expression studies of *A. thaliana*. We discuss the inter-and intra-experimental variations we observed in the hydroponic system. We then compare the results obtained in this system with those reported in a high-throughput analysis of plants grown in soil. In previous studies, the *A. thaliana* stem ionome was not investigated *per se*. We discuss the differences in the composition of the leaf and stem ionome, and the significance of this point for the design of ionomic studies. We also provide data on the differences between the root and leaf ionome of the Col-0 and C24 accessions of *A. thaliana,* as determined using a hydroponic growth system.

2. A hydroponic system for the growth of *A. thaliana* plants

Hydroponic systems for the growth of *A. thaliana* plants were described in several publications (for example, David-Assael et al., 2005; Gibeaut et al., 1997; Hermans & Verbruggen, 2005; Laganowsky et al., 2009). Although these systems are basically similar, there are some variations between them. Hydroponic growth media are usually based on different dilutions of the media described by Hoagland & Arnon (1938), Johnson et al. (1957), or others [reviewed by Laganowsky et al. (2009)]. We describe here the system and media we use for the growth of *A. thaliana* plants in hydroponics, but other systems and growth media can be utilized as well.

The *A. thaliana* plants are germinated in Jiffy pellets or in MS plates. After about 10 days, when the roots can still be easily removed from the Jiffy pellets or agar, the seedlings are transferred into a 1:1 mixture of sand (salt-free sand designed for agricultural use) and perlite (course particles), and watered in the x1 basic hydroponic solution (see below). After about 10-12 days (Fig. 1A), the seedlings are carefully removed from the sand and perlite mixture, and their roots are manually washed in the hydroponic solution to eliminate all sand or perlite particles. The plants are transferred into a hydroponic growth system consisting of 5- or 1.3-liter containers containing the same basic mineral solution. The plants are inserted into holes in polystyrene surfaces freely floating in the containers. Each 5- or 1.3-liter container can contain 25-50 or 10-20 plants, respectively. To prevent algal growth, the containers and polystyrene surfaces should be black or brown (Fig. 1B), and the gaps between the polystyrene surfaces and the containers should be covered in aluminum foil (Fig. 1D, E). Aquarium pumps connected to rubber hoses and Pasteur pipettes are used to maintain aeration (Fig. 1D). The solutions are refilled each day according to their consumption, and are changed completely at least once a week. Fig. 1C shows a mature, hydroponically grown plant, and Fig. 1E shows a 5-liter container containing 50 plants photographed at 5-6–day intervals.
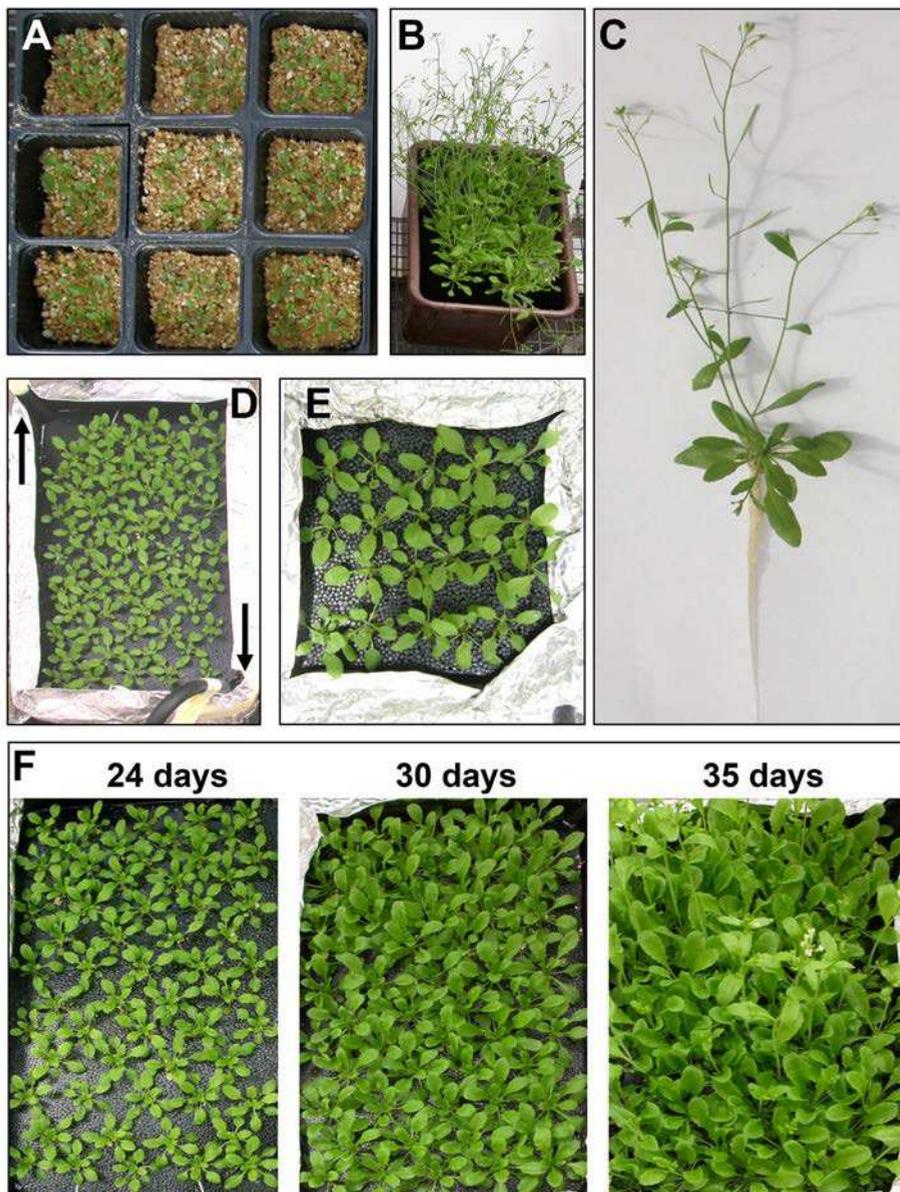
A. Twenty-four-day–old *A. thaliana* plants grown in a 1:1 mixture of sand and perlite. B. A typical 5-liter
container. C. A mature plant that was grown hydroponically. D. A polystyrene surface including about
50 plants floating in a 5-liter container, with aluminum foil covering the gaps between the surface and
the container. The arrows indicate two Pasteur pipette connected rubber hoses. E. A polystyrene surface
including about 20 plants floating in a 1.3 liter container. F. A container containing 50 *A. thaliana* plants
photographed at different days after germination. A to E are Col-0 plants, and F are C24 plants.

Fig. 1. The hydroponic system

2.1 Preparation of the hydroponic medium

2.1.1 Preparation of x4000 micronutrient stock

Mineral	Gram for separate stocks of 500 ml	Concentration of each separate stock (mM)	Amount from each separate stock for 1 liter of x4000 micronutrient stock
$CoCl_2 \cdot 6H_2O$	0.475	4	1 ml
$CuSO_4 \cdot 5H_2O$	62.500	500	1 ml
H_3BO_3	19.325	625	40 ml
$MnSO_4 \cdot H_2O$	70.500	844	6 ml
$Na_2MoO_4 \cdot 2H_2O$	40.500	335	1 ml
$ZnSO_4 \cdot 7H_2O$	143.750	1000	2 ml
KCl			3.728 gram

2.1.2 Preparation of 10 liters of x20 solution I

Mineral	Stock concentration	ml from stock
K_2HPO_4	1 M	50
K_2SO_4	0.5 M	100
KNO_3	1 M	600
$MgSO_4 \cdot 7H_2O$	1 M	100
Fe-EDDHA (see notes 1, 2)	20 mM	50
x4000 micronutrient stock	x4000	50

Add dH_2O to reach a final volume of 10 liters.

2.1.3 Preparation of 10 liters of x20 solution II

Dissolve 17.2 g $CaSO_4 \cdot 2H_2O$ in dH_2O to reach a final volume of 10 liters.

2.1.4 Preparation of x1 basic hydroponic solution

Add 9 liters of dH_2O into a 10-liter bottle, then add 500 ml of x20 Solution I and mix, then add 500 ml of x20 Solution II. Mix and adjust pH to 5.5 with ~40% H_2SO_4.

2.1.5 Final concentrations in the x1 basic hydroponic solution

Mineral	Final concentration
$CaSO_4$	0.5 mM
K_2HPO_4	0.25 mM
K_2SO_4	0.25 mM
KNO_3	3 mM

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Mineral	Final concentration
$MgSO_4$	0.5 mM
Fe-EDDHA	5 μM
$CoCl_2$	0.001 μM
$CuSO_4$	0.125 μM
H_3BO_4	6.25 μM
KCl	12.5 μM
$MnSO_4$	1.27 μM
Na_2MoO_4	0.084 μM
$ZnSO_4$	0.5 μM

Notes:

1. All stock solutions prepared in steps 1 and 2 should be autoclaved, except Fe-EDDHA.
2. Fe-EDDHA is Sequestrene 138 from Novartis. We prepare the 20 mM stock solution by taking 18.7 grams per liter from the material we have, which includes 6% iron. The solution should be well mixed before each use.
3. The use of two separate x20 stocks, and preparation of the final (x1) solution as described, are essential for prevention of $CaSO_4$ precipitation.

3. Mineral analysis

Plant material is harvested, divided into the different tissues, and washed two times in distilled water and two times in double-distilled water. Roots are washed once in tap water prior to these washes. The material is immediately transferred into a 70°C incubator and dried for three days. Dry plant material is crushed into a fine powder. Samples of 250 mg are ashed by incubation for 10 h at 500°C, and dissolved in 10 ml of 0.1 M nitric acid ("Suprapur" grade, Merck). After 30 min at 80°C, samples are brought to 25 ml with H_2O. When the available amount of dry plant material is less than 250 mg, the samples are brought to a final volume of less than 25 ml, but not less than 10 ml. Each sample is then filtered through 1 mm Whatman paper. Mineral content was determined by inductively coupled plasma atomic emission spectrometry (using an ICP-AES model `Spectroflame' from Spectro, Kleve, Germany).

4. Inter-and intra-experimental variations in the mineral composition of roots, leaves, and stems of Col-0 plants

The mineral content of roots, leaves, and stems of the Col-0 accession of *A. thaliana* was examined in the hydroponic growth system. We and others found that there is variation in the mineral content observed in different experiments, even when the experimental conditions are kept as constant as possible. This is due to inevitable variations in the environmental conditions that alter the physiological state of the plants. It is, therefore, interesting to find if there are minerals that exhibit relatively low inter- and/or intra-experimental variations. We present here the results of five independent experiments, carried out in different seasons and years, in which the mineral composition of Col-0 plants

was determined. Table 1 shows the levels in roots, leaves, and stems of the major minerals present in the hydroponic solution: the macronutrients potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), and sulfur (S); the micronutrients iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo), and cobalt (Co); and the mineral sodium (Na). It should be noted that Na is not classified as an essential macro- or micronutrient, but as a beneficial mineral element (Marschner 1995). The extent of inter-experimental variation (the variation between different experiments) is indicated by the coefficient of variation (CV) (see the legends of Table 1 for details how the values presented were calculated). To visualize the differences in CV values, values larger that 50% (an arbitrary rank) were indicated in bold letters in Table 1. The CV values of the macronutrients were generally lower than those of the micronutrients. Particularly high inter-experimental variations were observed in the root levels of all micronutrients analyzed. This suggests that the root levels of these micronutrients are more prone to variations due to unintended differences in the precise physiological state of the plants in different experiments compared to the leaf or stem levels of the same minerals, or compared to both the root and shoot levels of the macronutrients. It is not surprising that the intra-experimental variations (i.e., the variations between different samples of the same experiment) in the root and leaf levels of most minerals analyzed were lower compared to the inter-experimental variations (Tables 1 and 2, and data not shown). Particularly low CV values are highlighted in gray in Tables 1 and 2. The levels of the corresponding minerals can be used as internal standards in experimental designs that allow this manipulation (that is, when the levels of the utilized minerals are not supposed to vary).

Table 3 shows a comparison between the mineral concentrations observed by us in leaves of hydroponically grown Col-0 plants and the concentrations observed by Buescher et al. (2010) in shoots of soil-grown Col-0 plants. This comparison is valid since the shoot material harvested by Buescher et al. (2010) included only leaves. The data of Buescher et al. (2010) were based on 3-6 independent experiments, and our data were based on 5 independent experiments. Table 3 shows that there is a high similarity in the concentrations of the macronutrients analyzed in the two sets of experiments [K, Ca, Mg, and P; the S content was not reported by Buescher et al. (2010)]. This similarity is notable due to the large differences between the experimental conditions in the two sets of experiments. It is, therefore, possible that the concentrations (or at least the average concentrations observed in several experiments) of some of these macronutrients are relatively stable in leaves of Col-0 plants, at least under non-extreme physiological conditions.

Most ionomic studies include examination of leaves (besides other tissues). The concentrations of K showed relatively low inter- as well as intra-experimental variations (14% and 4%, respectively) (Tables 1 and 2). The leaf levels of K also showed high similarity between plants grown in hydroponics and those grown in soil under the experimental conditions used by us and by Buescher et al. (2010), respectively (Table 3). This suggests that leaf K levels are particularly suitable to serve as internal standards in experimental designs that allow such manipulation (i.e., when the levels of K are not supposed to vary).

5. Mineral distribution in roots, leaves, and stems of Col-0 plants

In most ionomic studies of *A. thaliana*, the mineral composition of stems was not determined. Figure 2 presents the ratio between the mineral content of roots, leaves, and stems

		Roots			Leaves			Stems		
	Mineral	Average	SD	CV	Average	SD	CV	Average	SD	CV
Macro-nutrients	K	62.938	17.684	28	49.2695	7.0722	14	68.4901	18.381	27
	Ca	6.701	0.7227	11	43.2389	14.698	34	14.5582	1.2711	9
	Mg	2.4165	0.3782	16	11.8031	2.7514	23	5.1593	0.545	11
	P	10.494	1.7523	17	8.6381	1.6471	19	7.6944	2.4785	32
	S	19.2347	4.6952	24	10.4239	1.662	16	9.1496	4.3863	48
	Na	6.0529	4.2422	**70**	6.842	3.1237	46	4.0972	4.1012	**100**
Micro-nutrients	Fe	1.0366	1.1379	**110**	0.0614	0.0219	36	0.0327	0.019	**58**
	Zn	1.1301	0.7509	**66**	0.1882	0.0811	43	0.0955	0.0195	20
	Mn	0.2753	0.379	**138**	0.053	0.0287	**54**	0.0192	0.0049	25
	Cu	0.017	0.0104	**61**	0.0054	0.0011	21	0.0063	0.0004	7
	Mo	0.1016	0.088	**87**	0.0101	0.0043	42	0.0084	0.005	**60**
	Co	0.0097	0.0046	**48**	0.003	0.0024	**78**	0.0002	0.0001	35

This table presents the results of five independent experiments. In each experiment, *A. thaliana* Col-0 plants were germinated in Jiffy pellets, transferred to sand and perlite, and then to hydroponics, and harvested when they were about six-weeks old. Plants were maintained in a greenhouse at 24°C with a photoperiod of 16 h light and 8 h darkness. In each experiment, plants were grown in four separate 5-liter hydroponic containers, each containing about 25 plants. The organs (roots, leaves or stems) of all plants grown in the same container were harvested together and treated as one sample. For each experiment, we calculated the average concentration (g·kg^{-1} dry weight) of each mineral in the four samples of each organ (derived from the four containers). The five values obtained for the content of each mineral in each organ (derived from the five independent experiments) were used to calculate the average and standard deviation (SD) values presented in the table. The coefficient of variation (CV) is the percentage of the SD from the average. CV values larger than 50% (an arbitrary rank) are indicated in bold letters. The lowest CV value among those obtained for the macronutrients analyzed in each organ is highlighted in gray.

Table 1. The elemental composition of roots, leaves, and stems of Col-0 plants as determined in five experiments

	Mineral	Roots			Leaves		
		Average	SD	CV	Average	SD	CV
Macro-nutrients	K	59.2833	7.1657	12	55.0859	2.1145	4
	Ca	6.2584	0.7591	12	31.6133	1.1190	4
	Mg	2.2233	0.1298	6	8.7049	0.3059	4
	P	10.9846	0.9259	8	9.3453	1.1018	12
	S	19.4929	1.4356	7	11.0383	1.0447	9
	Na	13.1094	2.3329	18	6.1646	1.3165	21
Micro-nutrients	Fe	3.0601	0.3414	11	0.0985	0.0138	14
	Zn	2.1601	0.1500	7	0.2411	0.0224	9
	Mn	0.9521	0.3136	33	0.0609	0.0039	6
	Cu	0.0322	0.0124	39	0.0066	0.0031	48
	Mo	0.2165	0.0449	21	0.0107	0.0032	30
	Co	0.0163	0.0103	**63**	0.0058	0.0011	18

The table presents the results of one experiment in which *A. thaliana* Col-0 plants were germinated in Jiffy pellets, transferred to sand and perlite, and then grown in four separate 5-liter containers, each containing about 25 plants. Plants were maintained in a greenhouse at 24°C with a photoperiod of 16 h light and 8 h darkness. The organs (roots or leaves) of all plants grown in the same container were harvested together and treated as one sample. The table shows the average concentration (g . kg^{-1} dry weight) and the SD of each mineral in the four samples (derived from the four containers) of each organ. The CV is the percentage of the SD from the average. CV values larger than 50% (an arbitrary rank) are indicated in bold letters. The lowest CV values among those obtained for the different macronutrients analyzed in each organ are highlighted in gray.

Table 2. The elemental composition of roots and leaves of Col-0 plants as determined in one experiment

	Mineral	Leaf content in this work	Leaf content in the work of Buescher et al. (2010)	Leaf content in this work relative to the data of Buescher et al. (2010)
Macro-nutrients	K	49.2695	46.1000	**1.0688**
	Ca	43.2389	45.0000	**0.9609**
	Mg	11.8031	12.9000	**0.9150**
	P	8.6381	9.7000	**0.8905**
	Na	6.8420	0.8600	7.9559
Micro-nutrients	Fe	0.0614	0.1000	0.6140
	Zn	0.1882	0.0610	3.0847
	Mn	0.0530	0.0636	0.8336
	Cu	0.0054	0.0018	2.9524
	Mo	0.0101	0.0055	1.8384
	Co	0.0030	0.0019	1.6353

This table shows the levels (g · kg^{-1} dry weight) of minerals in Col-0 leaves obtained in the present study (average values of the five experiments reported in Table 1) and in the ionomic study of Buescher et al. (2010). The right column presents the ratio between the mineral content of leaves in the present study and in the study of Buescher et al. (2010). Ratios close to 1 are indicated in bold letters.

Table 3. Comparison between the data obtained in the present study and in soil-grown plants

of hydroponically grown Col-0 plants. It should be noted that these ratios reflect the total amounts of each mineral in the indicated tissues. These values can be considerably different from the free levels of the same minerals in different cellular compartments. For example, a major proportion of the minerals in roots is deposited in complexes in the root apoplast. Figure 2 shows that the amounts of Ca and Mg are, by far, higher in leaves than in roots or stems. The distribution between roots and leaves seen here for Ca is close to the distribution of this element in most dicot plants [reviewed by Conn & Gilliham (2010)]. The fact that the highest concentration of Ca is found in the leaves is related to the fact that this element
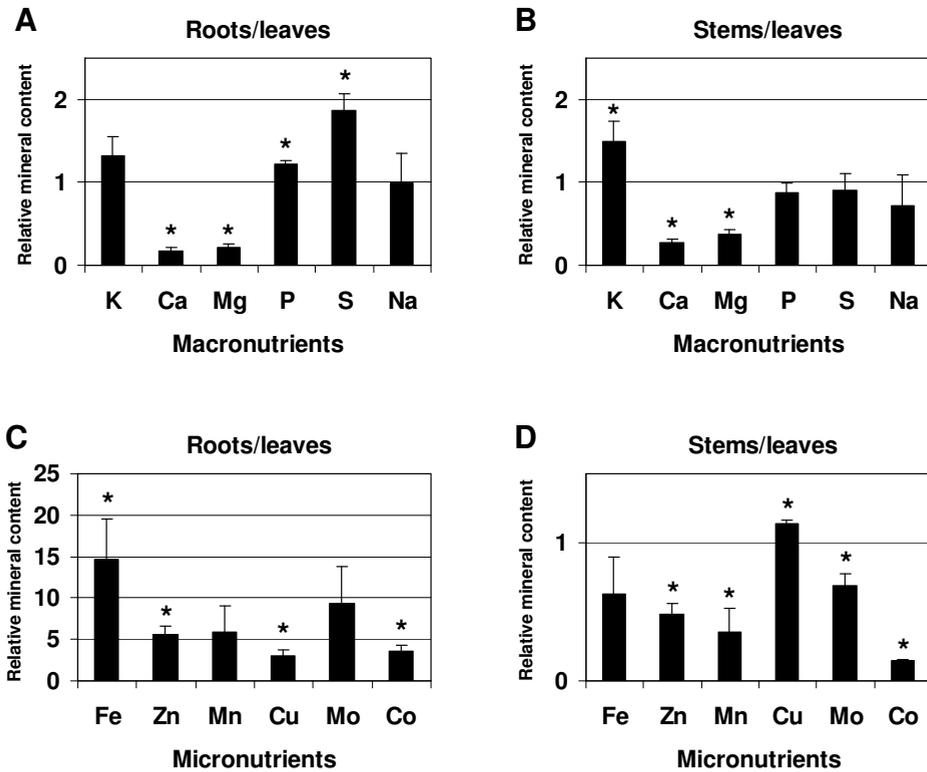
The ratios between the mineral content (that was determined in terms of $g \cdot kg^{-1}$ dry weight) of roots and leaves, or stems and leaves, were separately calculated for each of the five experiments described in Table 1. Each column shows the average and the standard error (SE) of the ratios obtained in each of the independent experiments. The graphs present the root-to-leaf (A, C) and stem-to-leaf (B, D) levels of the macronutrients (A, B) and micronutrients (C, D). The mineral Na is shown together with the macronutrients. The asterisks indicate statistically significant differences between the content of each mineral in roots and leaves (A, C) or stems and leaves (B, D).

Fig. 2. The ratio between the mineral content of roots, leaves and stems of Col-0 plants grown hydroponically

is phloem-immobile and is, therefore, not readily redistributed after deposition in the shoot [reviewed by Conn & Gilliham (2010)]. The K levels are about 50% higher in stems than in leaves (Fig. 2B). The mineral P shows slight, but statistically significant, higher levels in roots than in leaves. The mineral S is unique among the macronutrients with respect to its concentrations that are two times higher in roots than in leaves (Fig. 2).

The concentrations of most micronutrients analyzed (Fe, Zn, Cu, and Co) are higher in roots than in leaves (Fig. 2C). Among the four indicated minerals, the Zn and Co levels are also significantly lower in stems than in leaves (Fig. 2D). This suggests that mobilization of Zn

and Co in the vascular system is particularly low, at least in Col-0 plants grown in the physiological conditions described here. The data presented in Figure 2C and D suggest that this is also the case for Fe, Mn, and Mo, although apparently with higher inter-experimental variations since the differences in the content of these minerals in the different organs were not always statistically significant. One outcome of this situation is a restriction in the levels of heavy metals that reach the sensitive reproductive organs. The restricted mobility of the micronutrients in the vascular system is related to processes occurring at both the xylem and phloem. These processes include (among others) accumulation in the xylem parenchyma of roots and shoots, and control of mineral loading into the xylem and phloem (Marschner 1995). The mobility of most micronutrients in the phloem is lower than that of most macronutrients (Marschner 1995).

In some ionomic studies of *A. thaliana*, the stems, when present, were harvested as part of the shoots. The data presented here show that the leaf and stem contents of many minerals, including K, Ca, Mg, Zn, Mn, Cu, Mo, and Co, are significantly different (Fig. 2B, D). It is, therefore, important that shoot material harvested for ionomic analysis will be divided into separate leaf and stem samples or will include, as much as possible, constant proportions of leaves and stems. However, the study of the stem ionome *per se* is an essential step towards achieving a better understanding of long-distance transport processes in plants.

6. The differences between the root and leaf ionome of the Col-0 and C24 accessions of *A. thaliana*

Ionomic analysis of different *A. thaliana* accessions can provide valuable information. Comparing the shoot ionome of different *A. thaliana* accessions was utilized for identification of over 100 QTLs for mineral accumulation (Buescher et al., 2010). Identification of differences in the root ionome between different accessions can contribute to the discovery of novel genes that control mineral uptake and transport in plants. Towards this goal, we present here the differences between the root and leaf ionome of the Col-0 and C24 accessions of *A. thaliana* [C24 was not included among the 12 accessions whose shoot ionome was analyzed by Buescher et al. (2010)]. Figure 3 shows both the absolute and relative levels of each mineral in roots and leaves of plants grown hydroponically. The levels of Ca, P, and Zn were almost identical in both roots and leaves of the two accessions. Both roots and leaves of the C24 accession exhibited significantly lower levels of Mo and Fe compared to Col-0. A statistically significant reduction was observed in the levels of K, Mg, Mn, and Cu in leaves of C24 compared to Col-0. However, the differences in the leaf content of K and Mg were minor. The only mineral that showed a significant reduction in its root but not leaf content in C24 plants was S.

Among the minerals that differed between the two accessions, a particularly big difference was seen in the root and leaf content of Mo. A strong reduction in the content of this mineral in shoots of various *A. thaliana* accessions, as compared to Col-0, was previously observed by Buescher et al. (2010). In a subsequent study it was shown, using hydroponic growth systems, that the Ler accession had a reduced content of Mo not only in shoots but also in roots, when compared to Col-0 (Baxter et al., 2008). This suggested that the low Mo content in shoots was not due to enhanced accumulation of Mo in the roots, but rather to reduced Mo uptake by the roots (Baxter et al., 2008). Grafting experiments demonstrated that the differences in Mo content in shoots were driven solely by the roots. It was found that the mitochondrial Mo transporter (MOT1) is responsible for the variation in Mo content in both
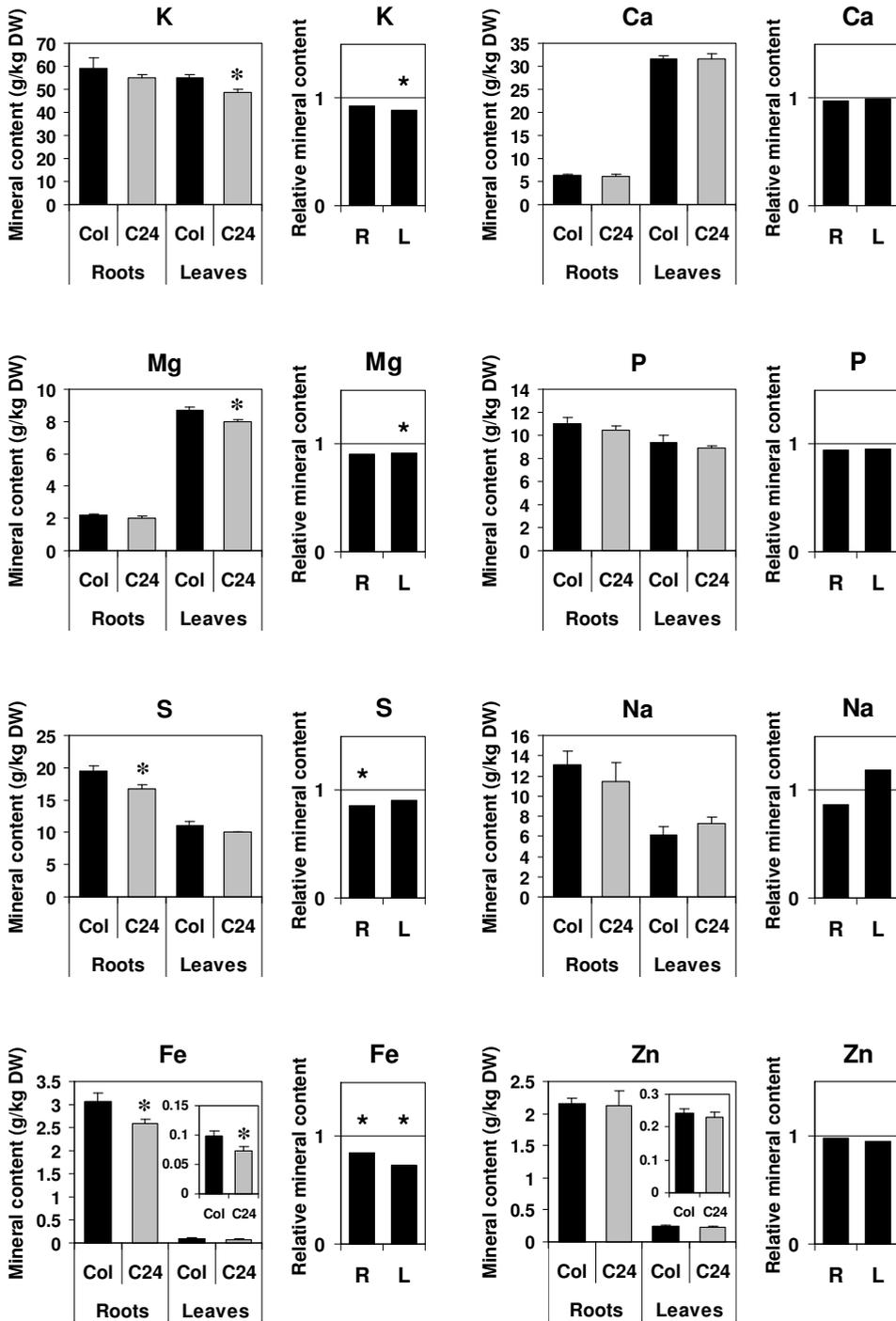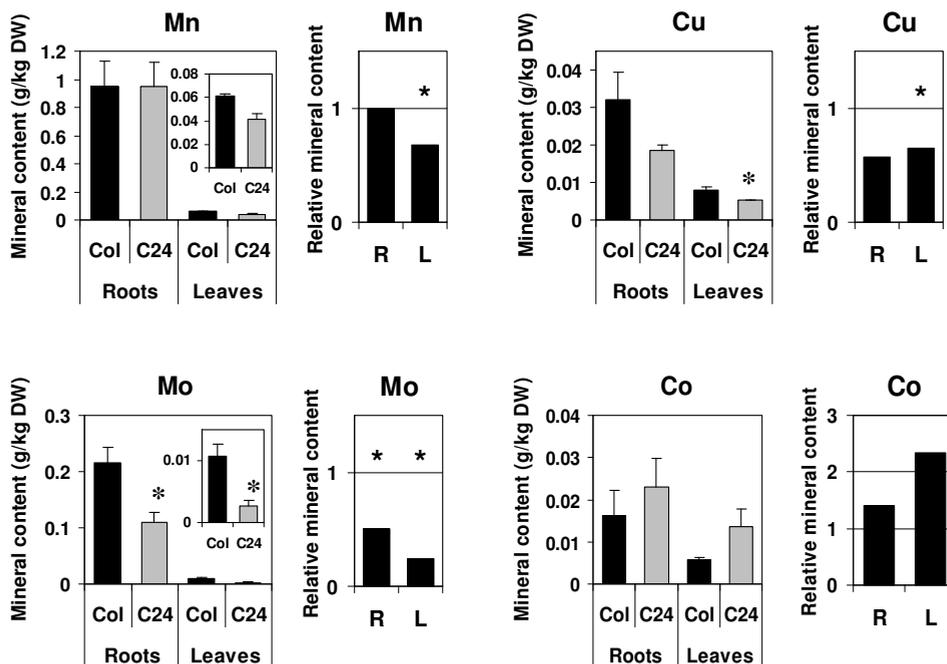
A. thaliana plants (accessions Col-0 and C24) were germinated in Jiffy pellets, transferred to sand and perlite, and then to hydroponics. For each accession, plants were grown in four separate 5-liter containers containing about 25 plants each. Plants were maintained in a greenhouse at 24ºC with a photoperiod of 16 h light and 8 h darkness. The organs (roots or leaves) of all plants grown in the same container were harvested together and treated as one sample. Two graphs are presented for each mineral. One graph presents the averages and standard errors of the mineral concentrations (g · kg^{-1} dry weight) obtained in the four containers of each accession. The second graph presents the relative levels of each mineral (C24 relative to Col-0) in each organ. The asterisks, which are presented in both graphs, indicate a statistically significant difference ($p<0.05$ in Student's *t*-test) between the levels of minerals in the two accessions. R, roots; L, leaves.

Fig. 3. The differences between the root and leaf ionome of the Col-0 and C24 accessions

shoots and roots of the various accessions (Baxter et al., 2008). This study demonstrated the significance of investigating the mineral content of roots in order to enhance understanding of the mechanisms involved.

Interestingly, Mn content is significantly lower in leaves, but not roots, of C24 as compared to Col-0 (Fig. 3). This suggests that the trait(s) that governs the difference in Mn content between Col-0 and C24 is not related to uptake in roots but to transport and/or accumulation in leaves. The mechanisms for uptake, transport, and internal sequestration of Mn in plants are now starting to be understood (Cailliatte et al., 2010; reviewed by Pittman, 2005). It will be necessary to determine if the indicated difference between Col-0 and C24 is related to a novel trait(s) or to some of the currently identified proteins involved in internal sequestration of Mn. The latter proteins include CAX2 (Hirschi et al., 2000), the ER Ca/Mn pump ECA1 (Wu et al., 2002), and putative homologs of the *Stylosanthes hamata* Mn transporter MTP1 (Delhaize et al., 2003).

Co levels were apparently higher in leaves of C24 compared to Col-0 (Fig. 3). The difference was not statistically significant (p=0.09 in Student's t-test), possibly due to the difficulty to accurately determine Co amounts, which were close to the detection limit of the ICP machine. The Fe and Co transporter FPN2, which sequesters Co into the root vacuoles and lowers its mobilization to the shoot, was identified as the protein responsible for the increased levels of Co in the Ts-1 and Se-0 accessions of *A. thaliana* as compared to Col-0 (Morrissey et al., 2009). The Ts-1 and Se-0 accessions have a truncated version of the *FPN2* gene, and it is possible that the C24 accession shares this property.

An interesting finding reported here is the lower content of Cu in leaves, and perhaps also roots, of the C24 compared to the Col-0 accession (the p values in Student's t-test were 0.009 and 0.076 for leaves and roots, respectively) (Fig. 3). Among the *A. thaliana* accessions studied by Buescher et al. (2010), no accession showed a reduction in its Cu content relative to Col-0. The only accession whose Cu content differed from that of Col-0 was Ler-2, which showed a 69% increase in the Cu content of shoots. This makes Ler-2 and C24 interesting candidates for the search for putative novel genes that control the Cu content of plants. It is, of course, possible that the difference in the Cu content of the indicated accessions is related to one of the currently identified genes that control Cu homeostasis in plants [reviewed by Palmer & Guerinot (2009) and by Pilon (2011)]. Our data suggest, although not with complete certainty, that the reduction in the Cu content of C24 is related to processes occurring also, or possibly solely, in roots. The currently identified transport proteins that affect the Cu content of roots include COPT1, which is involved in Cu uptake into roots (Sancenon et al., 2004), HMA5, whose knock-out increased the Cu content of roots but not shoots (Andres-Colas et al., 2006), and COPT5, which functions in the inter-organ allocation of Cu (Klaumann et al., 2011).

A statistically significant reduction in Fe content was observed in both leaves and roots of C24 as compared to Col-0 (Fig. 3). Among the *A. thaliana* accessions investigated by Buescher et al. (2010), no accession showed a reduction in its Fe content compared to Col-0, whereas two accessions showed increased Fe content. It will be interesting to determine whether the gene(s) responsible for the differences in the Fe content of the indicated accessions is among the many Fe-homeostasis genes identified thus far [reviewed by Conte & Walker (2011), Jeong & Guerinot (2009), and by Palmer et al. (2009)]. Our data suggest that the reduction in the Fe content of C24 compared to Col-0 is related to processes occurring also, or possibly solely, in roots.

7. Conclusions

This chapter emphasizes the practical and potential contribution of hydroponic growth systems to the identification of genes and processes governing the plant ionome and, in particular, to the study of processes occurring in roots. Hydroponic systems are a useful tool for obtaining sufficient amounts of clean roots for mineral analyses. These systems also enable the application of accurate concentrations of minerals. Nevertheless, the absolute levels of minerals in plants can vary between different experiments due to unavoidable differences in the precise physiological conditions of the plants. The data presented here indicate that the root levels of the micronutrients are more prone to variations due to unintended differences in the precise physiological state of the plants in different experiments compared to the leaf or stem levels of the same minerals, or compared to both the root and shoot levels of the macronutrients.

Interesting conclusions were reached by comparing the leaf ionome reported by Buescher et al. (2010) with that observed by us in Col-0 plants grown in soil or hydroponics, respectively. Despite the large differences in the experimental conditions, the concentrations of all macronutrients analyzed (K, Ca, Mg, and P) were very similar in the two sets of experiments. In contrast, the concentrations of all micronutrients analyzed in the two systems (Fe, Zn, Cu, Mo, Co, and – to a lesser extent – Mn) were relatively different (Table 3). This suggests that the macronutrient content in *A. thaliana* leaves varies to a lower extent due to altered environmental conditions compared to the micronutrient content. In particular, the leaf content of K showed low inter- and intra-experimental variation in the hydroponic system, as well as similarity with the values obtained in plants grown in soil in the experimental system described by Buescher et al. (2010). This suggests that the leaf content of K can be utilized as an internal standard in experimental designs that allow this manipulation (that is, when the levels of K are not supposed to vary). The data presented here also showed that for many minerals, the ion composition of the *A. thaliana* stem is considerably different from that of the leaf. This point should be taken into consideration in the design of ionomic studies. The data reported here about the differences between the root and leaf ionomes of the Col-0 and C24 accessions may contribute to the efforts to utilize the natural variations between different *A. thaliana* accessions for identification of novel genes that control mineral uptake and transport in plants. Since minerals are absorbed into plants through roots, it is also important to gain knowledge of the root ionome, and hydroponic systems can act as a useful tool in such a study.

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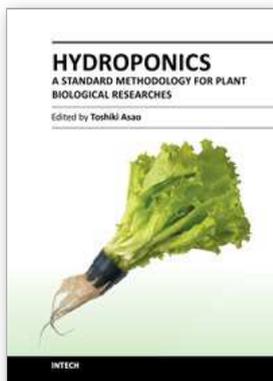
Hydroponics - A Standard Methodology for Plant Biological Researches
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Hydroponics-A standard methodology for plant biological researches provides useful information on the requirements and techniques needs to be considered in order to grow crops successfully in hydroponics. The main focuses of this book are preparation of hydroponic nutrient solution, use of this technique for studying biological aspects and environmental controls, and production of vegetables and ornamentals hydroponically. The first chapter of this book takes a general description of nutrient solution used for hydroponics followed by an outline of in vitro hydroponic culture system for vegetables. Detailed descriptions on use of hydroponics in the context of scientific research into plants responses and tolerance to abiotic stresses and on the problems associated with the reuse of culture solution and means to overcome it are included. Some chapters provides information on the role of hydroponic technique in studying plant-microbe-environment interaction and in various aspects of plant biological research, and also understanding of root uptake of nutrients and thereof role of hydroponics in environmental clean-up of toxic and polluting agents. The last two chapters outlined the hydroponic production of cactus and fruit tree seedlings. Leading research works from around the world are brought together in this book to produce a valuable source of reference for teachers, researcher, and advanced students of biological science and crop production.

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