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Calcium-permeable cation channels are involved in uranium uptake in *Arabidopsis thaliana*

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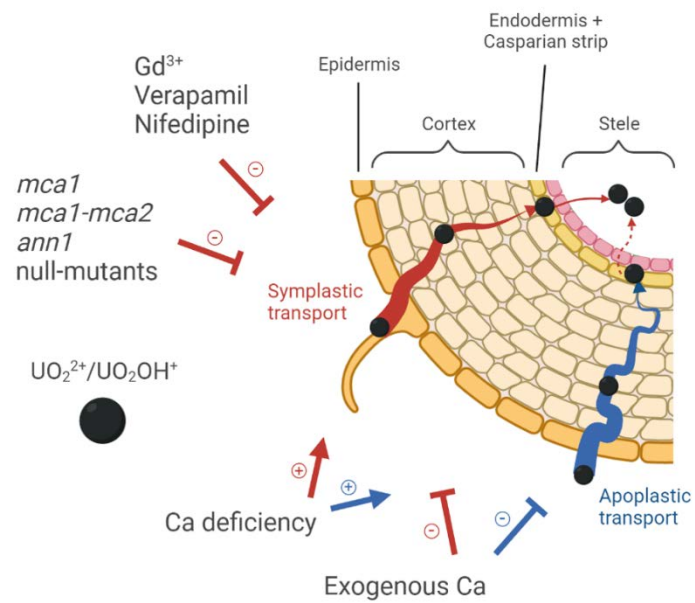
Highlights

- deprivation of *Arabidopsis* plants with calcium promotes the uranium import pathway
- external calcium competes with uranium for root uptake
- blockers of Ca²⁺-permeable channels inhibit the absorption of uranium by roots
- *Arabidopsis* mutants deficient in Ca²⁺-permeable channels accumulate less uranium

Abstract

Uranium (U) is a non-essential and toxic element that is taken up by plants from the environment. The assimilation pathway of U is still unknown in plants. In this study, we provide several evidences that U is taken up by the roots of *Arabidopsis thaliana* through Ca²⁺-permeable cation channels. First, we showed that deprivation of *Arabidopsis* plants with calcium induces a 1.5-fold increase in the capacity of roots to accumulate U, suggesting that calcium deficiency promotes the radionuclide import pathway. Second, we showed that external calcium inhibits U accumulation in roots, suggesting a common route for the uptake of both cations. Third, we found that gadolinium, nifedipine and verapamil inhibit the absorption of U, suggesting that different types of Ca²⁺-permeable channels serve as a route for U uptake. Last, we showed that U bioaccumulation in *Arabidopsis* mutants deficient for the Ca²⁺-permeable channels MCA1 and ANN1 is decreased by 40%. This suggests that MCA1 and ANN1 contribute to the absorption of U in different zones and cell layers of the root. Together, our results describe for the first time the involvement of Ca²⁺-permeable cation channels in the cellular uptake of U.

Graphical abstract



Keywords

Radionuclide, higher plants, root, accumulation, transport, competition, inhibition, mutants

Abbreviations

ANN, annexin; MCA, *mid1*-complementing activity

1 **1. Introduction**

2

3 Trace metal elements and radionuclides are important sources of environmental pollution and
4 hazards to human health. Uranium (U) is the heaviest naturally occurring radionuclide; its
5 average content in Earth's ecosystems (rocks, soils, sea and soft waters) is below 4 ppm. The
6 main sources of anthropogenic U contamination of the environment are related to the nuclear
7 industry and agricultural practices (Vandenhove, 2002). The fertilization of agricultural soils
8 with phosphate fertilizers is the main source of U dispersion (Wetterlind et al., 2012). Uranium
9 is not an essential nutrient for life but it is readily taken up by plants from the environment and
10 can contaminate the food chain, becoming a threat to public health (Anke et al., 2009).

11 The most abundant U isotope (^{238}U , 99.27%) is poorly radiotoxic but is highly chemotoxic to all
12 living organisms (Gao et al., 2019). During the last decade, significant progress has been made
13 in understanding the toxicity of U in plants (reviewed in (Chen et al., 2021)). Uranium has been
14 shown to interfere with several aspects of plant physiology, development and metabolism,
15 including photosynthesis (Misson et al., 2009; Saenen et al., 2013; Aranjuelo et al., 2014;
16 Vanhoudt et al., 2014; Serre et al., 2019). Biochemical, molecular and metabolomic studies
17 indicated that U triggers oxidative stress-related response pathways and perturbs hormones
18 synthesis and signaling, primary metabolism, iron and phosphate assimilation pathways, and
19 cell wall synthesis (Vanhoudt et al., 2011c; Vanhoudt et al., 2011a; Doustaly et al., 2014;
20 Saenen et al., 2015; Tewari et al., 2015; Lai et al., 2020; Lai et al., 2021). Also, U has a
21 significant impact on mineral nutrition, with important changes in some micro- and
22 macronutrients concentration and distribution between roots and shoots (Misson et al., 2009;
23 Vanhoudt et al., 2011b; Doustaly et al., 2014; Berthet et al., 2018; Lai et al., 2020; Sarthou et
24 al., 2020; Lai et al., 2021; Rajabi et al., 2021).

25 The uptake of non-essential elements is mediated by the assimilation pathways of essential
26 nutrients, thanks to the broad substrate specificity of some membrane carriers and to
27 similarities between the atomic structures of some elements. Uranium uptake and
28 accumulation by plants is markedly influenced by its speciation in the environment. Uranium is
29 mainly present in the U(VI) oxidation state in soil solutions and hydroponic culture media and
30 its speciation is influenced by the pH, redox potential, and presence of chelating agents (e.g.
31 carbonate, phosphate) (Chen et al., 2021). Under acidic conditions, the free uranyl species
32 (UO_2^{2+}) accumulates in roots but is poorly translocated to shoots, whereas complexation with
33 carbonate or organic acids (e.g. citrate) triggers U translocation to shoot organs (Ebbs et al.,
34 1998; Laurette et al., 2012a; Laurette et al., 2012b). Complexation with phosphate
35 considerably reduces U bioavailability (by precipitation) and limits accumulation in all plant
36 organs. In carbonate water at nearly neutral pH, calcium (Ca) has been shown to modify U
37 bioavailability and to facilitate U(VI) symplastic transport and translocation to shoots (El Hayek

38 et al., 2018; El Hayek et al., 2019). Thus, depending on U speciation in soils, several
39 assimilation pathways are anticipated for free or complexed anionic and cationic U species.
40 In spite of several studies, including transcriptomic analyses of plant responses to U stress
41 (Doustaly et al., 2014; Lai et al., 2020; Lai et al., 2021), the assimilation pathways of the
42 radionuclide are still not known. It has been postulated that the iron (Fe) uptake machinery
43 could be involved in the assimilation of U since the radionuclide interferes significantly with Fe
44 homeostasis (Doustaly et al., 2014). However, in conditions favoring free uranyl and
45 hydroxylated species, the main Fe(II) transporter at the plasma membrane, IRT1, is not
46 involved in U uptake (Berthet et al., 2018). The Ca assimilation pathway has been also
47 proposed to contribute to the uptake of U by tobacco BY-2 cells, in which U bioassociation is
48 inhibited by the cation- and Ca²⁺-channel blocker gadolinium (Rajabi et al., 2021). Moreover,
49 Ca and magnesium (Mg), but not potassium (K), inhibit uranyl uptake with a similar efficiency
50 in the green microalga *Chlamydomonas reinhardtii*, suggesting that Ca and Mg assimilation
51 routes could serve for U absorption (Fortin et al., 2007).
52 In order to identify U assimilation pathways in land plants, we used an unbiased approach
53 investigating the influence of nutrient deficiency on the capacity of *Arabidopsis thaliana* to
54 accumulate U in roots. Our working hypothesis relies on the knowledge that perturbations of
55 mineral nutrition, particularly starvations, are associated with changes in the array of plasma
56 membrane carriers involved in mineral assimilation. For example, many ion transporters and
57 channels are known to be upregulated under sulfate, nitrogen, iron, manganese, magnesium
58 or potassium deficiencies (Curie et al., 2000; Vert et al., 2002; Yoshimoto et al., 2002; Kiba et
59 al., 2012; Mao et al., 2014; Castaings et al., 2016; Lara et al., 2020). We anticipated that
60 starvation conditions promoting U uptake could identify the routes use by U to enter root cells.
61 Indeed, in conditions where free uranyl and hydroxylated species are predominant (acidic pH,
62 absence of chelators), we showed that deprivation of Arabidopsis plants with Ca is mandatory
63 for an increased bioaccumulation of U in roots, suggesting that Ca deficiency promotes the
64 radionuclide import pathway. Then, competition and inhibitor assays, together with the analysis
65 of Arabidopsis mutants impaired in Ca transport indicated, for the first time, that Ca²⁺-
66 permeable cation channels are involved in U uptake in plants.
67

68 2. Materials and methods

69

70 2.1 Plant growth conditions and uranium treatment

71 The wild-type *Arabidopsis thaliana* ecotype Columbia (Col-0) was used in this study. Seeds
72 were sterilized with chlorine gas (Lindsey et al., 2017) and stored in distilled water for 4 days
73 at 4°C in the dark. Stratified seeds were sown into homemade plates thermoprinted using the
74 amorphous polymer acrylonitrile butadiene styrene using an Ultimaker 2 3D-printer (Ultimaker
75 BV, The Netherlands). Conical holes of the plates were filled with 0.65% (w/v) agar (Agar plant
76 type A, Sigma-Aldrich) solubilized in distilled water. Plants were grown in hydropony in a
77 controlled environment with an 8 h light period at 22°C (light intensity of 110 μmol of photons
78 $\text{m}^{-2}\text{s}^{-1}$) followed by a 16 h dark period at 20°C. The nutrient solution, hereafter referred to as
79 'Gre medium', was composed of 0.88 mM K_2SO_4 , 1 mM $\text{Ca}(\text{NO}_3)_2$, 1 mM MgSO_4 , 0.25 mM
80 KH_2PO_4 , 10 μM H_3BO_3 , 0.1 μM CuSO_4 , 2 μM MnSO_4 , 0.01 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 2 μM ZnSO_4 , 10
81 μM NaCl , 0.02 μM CoCl_2 , 20 μM Fe-EDTA , and 0.25 mM MES, pH 5.6 (Table S1). Plants were
82 grown for 29 to 33 days in Gre medium with weekly solution changes until treatment. For U
83 treatment, plants were transferred to Gre media depleted with phosphate (100-fold dilution as
84 compared with Gre, i.e. 2.5 μM KH_2PO_4) and supplemented with 20 μM uranyl nitrate
85 ($\text{UO}_2(\text{NO}_3)_2$). After U treatment for 24 h, plants were harvested and rinsed once with 10 mM
86 Na_2CO_3 , then twice with distilled water to remove U that is loosely adsorbed on the root surface
87 (Laurette et al., 2012a; Laurette et al., 2012b; Doustaly et al., 2014). Roots and shoots were
88 then separated, dried on absorbent paper and fresh biomass was measured. Finally, roots and
89 shoots were dehydrated at 80°C for 24 h and dry weighed.

90 A similar procedure was used to analyze U bioaccumulation in *A. thaliana* mutant lines deficient
91 in Ca^{2+} -permeable cation channels. The *mca1*-null, *mca2*-null, and the double *mca1/mca2*-null
92 mutants were kindly provided by Prof. Iida and Prof. Miura (Nakagawa et al., 2007; Yamanaka
93 et al., 2010). Seeds of an *ann1*-null mutant (Lee et al., 2004) were obtained from the European
94 Nottingham Arabidopsis Stock Centre (N661601, SALK_015426C).

95

96 2.2 Mineral depletion assays

97 After 29 days of growth in standard Gre medium, plants were transferred for 4 days in a
98 modified medium in which one or several nutrients were depleted by a 100-fold as compared
99 with the standard concentrations (Table S1). After 4 days of starvation, plants were transferred
100 into the same modified Gre medium in which phosphate was lowered to 2.5 μM and uranyl
101 nitrate was added at 20 μM . Plants were cultivated for 24 h in these conditions and then
102 harvested. Photosystem II efficiency in dark-adapted leaves (F_v/F_m) was measured using a
103 FluorPen (FP100/D, Photon Systems Instruments, Brno, Czech Republic) and used as a proxy
104 for plant fitness in mineral-depleted media.

105

106 **2.3 Competition assays**

107 After 33 days of growth in standard Gre medium, plants were rinsed with distilled water and
108 transferred into a solution containing 0.25 mM MES (pH 5.6), 20 μ M uranyl nitrate and various
109 concentrations (20, 200 or 2000 μ M) of $\text{Ca}(\text{NO}_3)_2$. Plants were harvested after 10 h of treatment
110 in these solutions, at 22°C in the light (110 μmol of photons $\text{m}^{-2}\text{s}^{-1}$). Competition assays were
111 also performed with KNO_3 or K_2SO_4 in a similar concentration range.

112

113 **2.4 Inhibitors assays**

114 After 33 days of growth in standard Gre medium, plants were rinsed with distilled water and
115 transferred in 0.25 mM MES (pH 5.6) containing either 250 μ M gadolinium chloride (GdCl_3),
116 100 μ M verapamil hydrochloride, 50 μ M nifedipine or 1% (v/v) DMSO for 20 min at 22°C.
117 Uranyl nitrate 20 μ M was then added to different solutions and plants were harvested 2 or 6 h
118 after U addition (22°C at 110 μmol of photons $\text{m}^{-2}\text{s}^{-1}$). Verapamil and nifedipine stock solutions
119 (100-fold concentrated) were prepared in DMSO; gadolinium chloride was solubilized in
120 distilled water.

121

122 **2.5 Inductively coupled plasma mass spectrometry (ICPMS) analyses**

123 Dried samples were mineralized in 400 μL of 65% (w/v) HNO_3 (Suprapur, Merck) for 3 h using
124 a HotBlock CAL3300 (Environmental Express) digestion system. Digested samples were
125 diluted in distilled water at 0.65% (w/v) HNO_3 and analyzed using an iCAP RQ quadrupole
126 mass instrument (Thermo Fisher Scientific GmbH, Germany). Elements (^{24}Mg , ^{25}Mg , ^{31}P , ^{39}K ,
127 ^{43}Ca , ^{44}Ca , ^{55}Mn , ^{56}Fe , ^{57}Fe , ^{64}Zn , ^{66}Zn , ^{95}Mo , ^{98}Mo , ^{238}U) were analyzed using the collision
128 mode with helium as a cell gas. Elements concentrations were determined using standard
129 curves and corrected using an internal standard solution of ^{45}Sc , ^{103}Rh , ^{172}Yb .

130

131 **2.6 Predictions of uranium speciation**

132 Visual MINTEQ (version 3.1, <https://vminteq.lwr.kth.se/>) was used to predict U speciation in
133 the different media. The pH and temperature parameters were set at 5.6 and 20°C,
134 respectively. The concentrations of aqueous inorganic species (in mol/L) and the distribution
135 among dissolved and soluble species (in %) were obtained using default settings.

136

137 **2.7 Statistical analyses**

138 Statistical analyses were performed using the R Studio software (RStudio Team, 2015) and
139 the *nparcomp* package (Konietschke et al., 2015). Non-parametric statistical analysis was
140 done on our datasets that typically contain small sample sizes ($n=4$ plants in each assay) and
141 do not meet the assumptions of parametric tests (normal distribution and homogeneity of

142 variance, as determined using the Shapiro–Wilk and Fisher tests, respectively). Non-
143 parametric Tukey tests were conducted and the confidence level was set at 99% ($p < 0.01$).
144

145 **3. Results**

146

147 **3.1 Multi-element mineral deprivations improve U accumulation in roots**

148 To evaluate the impact of nutrient deprivations on the accumulation of U in *Arabidopsis*
149 *thaliana*, plants were grown for 4 weeks in a complete standard Gre medium and then
150 transferred for 4 days in a Gre medium depleted of all nutrients (100-fold dilution of Gre,
151 Gre/100 medium) (Table S1). Nutrient-deprived plants displayed shorter roots and smaller,
152 darker green leaves as compared with control plants. The photosynthetic parameter Fv/Fm
153 (photosystem II efficiency in dark-adapted leaves) was used as a proxy to evaluate plant
154 fitness. Fv/Fm of plants grown in Gre/100 was not changed as compared with plants
155 maintained in Gre (0.79 ± 0.02 in both conditions). Root and shoot ionomes were determined
156 by ICPMS following extensive washing of plant tissues with sodium carbonate and distilled
157 water to remove loosely-bound elements (Laurette et al., 2012a; Laurette et al., 2012b;
158 Doustaly et al., 2014). Nutrient deprivation had a strong impact on root and shoot ionomes,
159 with a significant decrease in the pools of Mg, P, K, Ca, Mn, Fe, Zn and Mo (Figures S1, S2;
160 Tables S2, S3). For U bioaccumulation assays, control and deprived plants were challenged
161 with a sublethal concentration of uranyl nitrate (20 μM) (Serre et al., 2019). A time-course
162 analysis of U accumulation in roots indicated that an incubation of 24 h corresponds to the
163 linear phase of the process and is suitable to analyze the effect of nutrient deprivation on the
164 uptake rate of the radionuclide by *Arabidopsis* roots (Figure S3). The terms (bio)accumulation
165 and association are used to describe the amount of U measured in roots by ICPMS;
166 (bio)accumulation and association account for U that is strongly adsorbed to the cell wall and
167 taken up by root cells (Misson et al., 2009; Laurette et al., 2012a; Laurette et al., 2012b; Rajabi
168 et al., 2021).

169 Nutrient-deficient plants displayed a 3-fold increase of U accumulated in roots as compared
170 with control plants (Figure 1). The accumulation of U in shoots was similar in the two conditions
171 (data not shown). Uranium is characterized by a low root-to-shoot translocation rate in the
172 absence of ligands (e.g. carbonate, organic acids) in the environment (Ebbs et al., 1998;
173 Laurette et al., 2012b). In our experimental conditions the translocation factor was in the range
174 $2\text{-}7 \times 10^{-4}$, meaning that less than 0.1% of U accumulated in roots was translocated to shoots.
175 Due to the very low translocation rate, the U content in shoots will not be discussed in the rest
176 of the study.

177 In order to identify which changes in nutrient supply were responsible for the increase of U
178 associated with roots in Gre/100, 4-week-old *Arabidopsis* plants were starved with either

179 micronutrients (B, Co, Cu, Fe, Mn, Mo and Zn; mi/100 medium) or macronutrients (Ca, K, Mg,
180 P, S and N; M/100 medium). After 4 days of starvation, the roots and shoots of plants grown
181 in mi/100 were significantly depleted in Fe, Mn, Mo and Zn (B, Co, and Cu were not quantified)
182 (Figures S1, S2; Tables S2, S3) but had no distinguishable phenotype as compared with plants
183 grown in Gre. Plants grown in macronutrient-deprived medium had reduced pools of Mg, P, K
184 and Ca (S and N were not quantified) (Figures S1, S2; Tables S2, S3). As compared with
185 controls, M/100 plants had shorter and more branched roots, smaller and greener shoots, but
186 comparable Fv/Fm values (0.79 ± 0.02). After 24 h of U treatment, no significant change of U
187 content was measured in roots of micronutrient-deficient plants as compared with Gre.
188 However, macronutrient-deficient plants showed a 3-fold increase of U associated with roots
189 as compared with control (Figure 1). U content in roots from M/100 plants was similar to that
190 of complete nutrient-deprived plants (Gre/100).

191 Noteworthy, the 100-fold reduction in the cations Ca^{2+} , Mg^{2+} and K^{+} in M/100 medium is
192 accompanied by important changes in the concentration of the counter-anions NO_3^- , SO_4^{2-} and
193 PO_4^{3-} (100-, 82- and 100-fold reduction, respectively) (Table S1). To analyze the effect of
194 sulfate starvation on the accumulation of U in roots, $(\text{NH}_4)_2\text{SO}_4$ was added to M/100 to recover
195 the concentration of the control Gre medium. Root accumulation of U in M/100 supplemented
196 with $(\text{NH}_4)_2\text{SO}_4$ was similar to the one in M/100 plants (Figure S4). To test the effect of
197 phosphate starvation, plants were deprived with phosphate (100-fold reduction; P/100) for 4
198 days and challenged with $20 \mu\text{M}$ uranyl nitrate for 24 h. In this condition, plants displayed no
199 phenotype and accumulated similar amount of U in roots as compared with plants in the control
200 Gre medium (Figure S4). The impact of nitrate depletion was addressed in the M/100 medium
201 supplemented with either KNO_3 or $\text{Mg}(\text{NO}_3)_2$. These conditions correspond to double cation
202 deprivations (Ca and Mg or Ca and K) and will be discussed below; they showed that nitrate
203 depletion has no impact on the amplitude of U bioaccumulation in roots (Figure S4).

204

205 **3.2 Uranium speciation is not significantly modified in nutrient-depleted media and do** 206 **not influence U bioaccumulation in roots**

207 At this stage, changes in U content in roots in Gre/100 and M/100 conditions could be due to
208 an increased capacity of roots to accumulate U because of modifications induced by the triple
209 Ca-Mg-K deprivation, and/or to changes in U speciation and bioavailability in the different
210 media. The second hypothesis was addressed by modelling U speciation using the Visual
211 MINTeq software. The chemical forms of U that are predicted in the control Gre medium (pH
212 5.6) are mainly cationic, with about 70% of hydroxylated species and 9% of free UO_2^{2+} (Table
213 S1). In M/100, these proportions are slightly changed (79% of hydroxylated forms, 7% of free
214 UO_2^{2+}) but cannot explain the 3-fold increase in U accumulation in the roots of M/100 plants
215 as compared with Gre plants. The main change was observed for the UO_2SO_4 form, which

216 represents about 8% of U species in Gre and 0.2% in M/100 (Table S1). Supplementation of
217 M/100 with $(\text{NH}_4)_2\text{SO}_4$ restored the proportion of UO_2SO_4 species at a level (6%) comparable
218 to that of the Gre medium and did not change the high accumulation of U in roots (Figure S4).
219 Together, these predictions indicated that the speciation of U has no or limited role in the
220 different capacity of roots to accumulate U in our experimental conditions.

221

222 **3.3 Calcium deprivation is essential for an increased accumulation of U in roots**

223 To assess which of the 3 elements (Ca, Mg and K) is important for the improvement of U
224 accumulation in nutrient-starved Arabidopsis roots, bioaccumulation assays were done using
225 single-nutrient deprived Gre media (Ca/100, Mg/100 or K/100). Mg/100 plants were strongly
226 deficient in Mg in both roots and shoots (40% decrease as compared with controls) (Figures
227 S1, S2; Tables S2, S3) but did not show a significant change in U accumulation in roots relative
228 to Gre (Figure 1). K/100 roots showed a moderate (<10%) decrease in K content (Figure S1;
229 Table S2) and did not accumulate more U than control plants in Gre (Figure 1). Calcium
230 deficiency was important in Ca/100 plants, with a 2-fold reduction of the root and shoot Ca
231 pools (Figures S1, S2; Tables S2, S3). A 50% increase of the accumulation of U in the roots
232 of Ca/100 plants was measured as compared with Gre plants (Figure 1).

233 The increase of U accumulation in the roots of plants deprived with Ca alone was not as
234 important as in M/100 or Gre/100 plants. Thus, we tested the effect of double nutrient
235 deprivations on the accumulation of U in roots. Plants starved with Mg and K (MgK/100
236 medium) were deficient in both cations (Figures S1, S2; Tables S2, S3) and accumulated
237 similar amount of U in roots as control plants in Gre (Figure 1). In contrast, plants starved with
238 K and Ca (KCa/100) or Ca and Mg (CaMg/100) were strongly deficient in Ca (Figures S1, S2;
239 Tables S2, S3) and showed a 50% increase of U associated with roots when compared with
240 Gre plants (Figure 1). Double-cation deprived media were also obtained using a M/100
241 medium in which KNO_3 or $\text{Mg}(\text{NO}_3)_2$ was added to restore the concentration of K^+ or Mg^{2+} to
242 standard levels. These conditions also abolished nitrate depletion due to $\text{Ca}(\text{NO}_3)_2$ limitation
243 (1/100 dilution). In these conditions, U accumulation levels in roots were similar to those
244 measured in Ca/100, KCa/100 or CaMg/100 (Figure S4).

245 Uranium speciation in the single- and double-deprived Gre media was computed using Visual
246 MINTEQ. As for Gre and M/100, the main chemical forms of U were predicted to be
247 hydroxylated species (69 to 80% of total U forms) and free UO_2^{2+} (about 8%), with little variation
248 from one medium to the other (Table S1). Significant changes were predicted for UO_2SO_4 ,
249 which represents about 5% of U species in K/100, Mg/100, KCa/100 and CaMg/100, 9% in
250 Ca/100, 0.3% in MgK/100 (Table S1). There is no correlation between the proportion of the
251 UO_2SO_4 species and the accumulation of U in roots, indicating again that U speciation does
252 not modify significantly U adsorption/absorption in our experimental conditions.

253 Together, these data showed that a decrease of Ca content in Arabidopsis plants is necessary
254 for an improved accumulation of U in roots. Perturbations of Mg and/or K homeostasis alone
255 do not have similar consequences.

256

257 **3.4 Uranium adsorption in the apoplast and absorption in the symplast are influenced** 258 **by changes in nutrient supply**

259 A bioaccumulation assay was done in the cold to discriminate between U adsorption or
260 absorption by roots, i.e. to address the distribution of the radionuclide between the apoplast
261 and the symplast of root cells. Four-week-old plants were transferred into Gre, M/100 or
262 Ca/100 for 4 days, pre-incubated for 1 h at either 4 or 22°C for acclimation, and challenged
263 with U for 24 h at 4°C or 22°C under a standard light regime. Cold-treated plants still have the
264 capacity to accumulate significant amount of U in roots in all conditions, but with a 1.4 to 1.7-
265 fold reduction as compared with plants incubated at 22°C (Figure 2). Considering that cold-
266 treatment strongly, but not completely, impairs ion transport processes, one can estimate that
267 at least 30-40% of bioaccumulated U has been taken up by root cells. The rest of U is
268 specifically adsorbed in the apoplast (roots have been thoroughly washed with sodium
269 carbonate to remove loosely-bound elements). Noteworthy, cold-treated M/100 and Ca/100
270 roots still accumulated about 3-time and 1.5-time more U than the cold-treated control roots
271 (Figure 2), respectively, meaning that the Ca-Mg-K or Ca deprivations induced changes in the
272 capacity of U adsorption and absorption through the apoplastic and symplastic routes,
273 respectively.

274

275 **3.5 Calcium competes with U for root uptake**

276 Competition assays were designed to reinforce the relationship between Ca homeostasis and
277 U accumulation by roots. Four-week-old Arabidopsis plants were challenged for 10 h with 20
278 μM uranyl nitrate in the presence of increasing concentrations of $\text{Ca}(\text{NO}_3)_2$ in 0.25 mM MES
279 (pH 5.6). A very simple incubation medium was used to limit changes in U speciation and to
280 accurately measure the effects of competition. Visual MINTEQ predictions indicated that
281 hydroxylated and free uranyl species account for 91-93% and 7-9% of total U, respectively, in
282 competition assays containing zero to 2 mM $\text{Ca}(\text{NO}_3)_2$ (Table S1). At equimolar concentration
283 (20 μM), Ca had no effect on U accumulation in roots but a significant decrease was observed
284 for 10- and 100-fold Ca excess (Figure 3). Competitions assays with similar concentrations of
285 K_2SO_4 or KNO_3 did not show any change in U accumulation (data not shown), indicating that
286 Ca^{2+} but not K^+ or NO_3^- competes with U for root accumulation.

287

288 **3.6 Uranium uptake by roots is inhibited by calcium channels blockers**

289 A pharmacological approach was used to gain insights into U absorption by roots and to test
290 the involvement of Ca channels. To this aim we used gadolinium (Gd^{3+}), a lanthanide used to
291 block non-selective cation channels (NSCCs) and mechanosensitive channels (MSCCs), and
292 verapamil and nifedipine, which are antagonists of voltage-dependent L-type Ca^{2+} -permeable
293 channels (VDCCs) (De Vriese et al., 2018). These compounds are efficient in blocking Ca
294 channels in plants (Demidchik et al., 2002; Demidchik et al., 2018). Four-week-old Arabidopsis
295 plants were preincubated with the inhibitors for 20 min and then challenged with 20 μM uranyl
296 nitrate for 2 and 6 h. Gadolinium inhibited U accumulation in roots by 40 to 60% throughout
297 the kinetic (Figure 4A). Uranium speciation was not changed in Gd-containing assays as
298 compared with controls in MES (Table S1). After 2 h of treatment, verapamil and nifedipine
299 had little impact on U accumulation in roots but the inhibition was important (30 to 45%
300 decrease) after 6 h of incubation (Figure 4B). In summary, the pharmacological approach
301 suggested that some types of Ca^{2+} -permeable channels, e.g. NSCCs, MSCCs and VDCCs,
302 are involved in U uptake by roots.

304 **3.7 Mutants in the Ca^{2+} -permeable mechanosensitive MCA1 channel and in ANNEXIN1** 305 **are impaired in U uptake by roots**

306 To gain insight into the mechanisms contributing to U uptake by roots we analyzed the
307 accumulation of the radionuclide in Arabidopsis mutants impaired in the transport of Ca. Two
308 Ca^{2+} -permeable mechanosensitive channels localized in the plasma membrane, named MCA1
309 and MCA2 for *mid1*-complementing activity, have been identified in *A. thaliana* (Nakagawa et
310 al., 2007; Yamanaka et al., 2010). Single *mca1* and *mca2* knockout mutants and the double
311 *mca1/mca2*-null line (Yamanaka et al., 2010) were grown for 4 weeks in Gre medium and
312 challenged with 20 μM uranyl nitrate in Gre depleted with phosphate. ICPMS analysis indicated
313 that root ionomes of *mca* mutants were comparable to Col-0, with only a limited increase in Fe
314 pools in *mca1* (26%) and *mca2* (14%), but not in *mca1/mca2* (Table S4). Short-time uptake
315 kinetics (6 h) showed that the *mca1* and *mca1/mca2* lines accumulated 40% less U in roots
316 than the wild-type Col-0 (Figure 5). A small (10%) but significant decrease of U
317 bioaccumulation was also measured in the *mca2* single mutant. Uranium bioaccumulation
318 assays were also done with an ANNEXIN1 *ann1*-null mutant. Members of the plant annexin
319 family function as unconventional Ca^{2+} -permeable channels with roles in development and
320 stress signaling (Davies, 2014). The *ann1* mutant showed minor changes in its root ionome as
321 compared with Col-0, with a 36% increase in the pool of Zn (Table S4). After 6 h of treatment
322 with 20 μM uranyl nitrate, the *ann1*-null mutant displayed a strong reduction (40%) of U
323 accumulation in roots as compared with wild-type plants (Figure 5). Together, these results

324 showed that mutants in the Ca²⁺-permeable mechanosensitive MCA1 channel and in
325 ANNEXIN1 are highly impaired in U uptake by roots.

326

327 **4. Discussion**

328 The link between U and the homeostasis of Ca has been previously reported in several plant
329 species (Vanhoudt et al., 2011b; Tawussi et al., 2017; El Hayek et al., 2018; El Hayek et al.,
330 2019; Rajabi et al., 2021) but no evidence was provided to support the uptake of the
331 radionuclide by the Ca assimilation machinery. In this work, we provide several lines of
332 evidence that U is taken up by the roots of Arabidopsis plants through Ca²⁺-permeable cation
333 channels. The first evidence comes from U bioaccumulation assays with Arabidopsis plants
334 starved for one or several essential nutrients. We postulated that changes in the expression of
335 ion carriers located on the plasma membrane to adapt to nutrient starvation could modify U
336 uptake and enable the identification of the route(s) used by the radionuclide for absorption by
337 roots. This assumption proved to be correct as our data showed that triple deprivation of
338 Arabidopsis plants with Ca, K and Mg was accompanied with a 3-fold increase in the capacity
339 of roots to accumulate U (Figure 1). Among the three cations, Ca was the only one for which
340 starvation was correlated with an increased U accumulation. However, single Ca deprivation
341 or double Ca-Mg or Ca-K deprivations were not as efficient as the triple Ca-Mg-K starvation to
342 trigger U bioaccumulation (1.5-fold as compared with 3-fold increase; Figure 1). At least two
343 hypotheses can explain this discrepancy, the magnitude of U accumulation in roots being
344 related to i) the degree of Ca deficiency and/or ii) to a complex interplay between the
345 homeostasis of Ca, Mg, and K. Under single and double Ca-deprivation conditions, Ca
346 deficiency was much stronger than under triple Ca-Mg-K deprivation (30-60% and 20%
347 reduction of Ca content in roots, respectively, and a comparable 40-50% decrease of Ca
348 content in shoots; Figures S1 and S2, Tables S1 and S2). This suggests that the Ca influx
349 machinery could not adapt to this drastic situation and, therefore, that U bioaccumulation could
350 not be increased more than 1.5-fold. Also, crosstalk between nutrient homeostatic controls and
351 the role of cytosolic free Ca in signaling several nutrient deficiencies, including K and Mg
352 deficiency, are well documented (Tang et al., 2015; Wilkins et al., 2016; Tang and Luan, 2017;
353 Bouain et al., 2019). Our ionic analyses confirmed the complex interaction between ions
354 when nutrient supply is unbalanced (Figures S1 and S2, Tables S1 and S2) and suggested
355 that the mechanisms implemented by Arabidopsis plants to adapt to the triple Ca-Mg-K
356 deprivation, as yet unknown, lead to an optimal situation for the accumulation of U in roots.

357 The second line of evidence for the involvement of the Ca assimilation pathway in U uptake by
358 roots comes from competition and inhibition assays. We showed that exogenous Ca inhibits U
359 accumulation in roots in a dose-dependent manner (Figure 3), without modifying U
360 bioavailability in the medium, suggesting a common route for the uptake of both cations. This

361 assumption is supported by previous reports showing that U can interact strongly with Ca-
362 binding proteins (e.g. calmodulin and osteopontin) (Qi et al., 2014; Brulfert et al., 2016; Creff
363 et al., 2019), thanks to some properties shared between UO_2^{2+} and Ca^{2+} (Garai and Delangle,
364 2020). Lanthanides (e.g. Gd^{3+}), dihydropyridines (e.g. nifedipine) and phenylalkylamines (e.g.
365 verapamil) are the most widely used blockers of Ca conductances in plants (Demidchick et al.,
366 2002; Demidchick et al., 2018). Gadolinium was found to inhibit the bioaccumulation of U in
367 Arabidopsis roots (Figure 4A) and tobacco BY2 cells (Rajabi et al., 2021), suggesting that
368 MSCCs and NSCCs Ca^{2+} -permeable channels serve as a possible route for U absorption in
369 plant cells. Uranium bioaccumulation in Arabidopsis roots was also inhibited by nifedipine and
370 verapamil (Figure 4B), two antagonists of VDCCs that bind to different receptor sites (De
371 Vriese et al., 2018). The differences in time-dependent inhibition of U uptake by Gd^{3+} on the
372 one hand and nifedipine and verapamil on the other hand (Figure 4) is likely due to the
373 different modes of actions of the blockers (physical blocking of the channel pore by Gd^{3+} ,
374 binding and inhibition for the drugs). Results obtained with Ca channels blockers should be
375 interpreted with caution because side effects have been described (De Vriese et al., 2018).
376 However, in combination with the other results described in this study, the pharmacological
377 approach is in agreement with the involvement of different types of Ca^{2+} -permeable cation
378 channels (non-specific, mechanosensitive, voltage-dependent) in the uptake of U by root cells.
379 The structural, metabolic and signaling functions of Ca in plant cells are enabled by its
380 orchestrated transport across cell membranes (Demidchik, 2018). Calcium influx across plant
381 plasma membranes is mediated by highly redundant systems, including glutamate-like
382 receptors (GLR, 20 genes in *A. thaliana*), cyclic nucleotide-gated channels (CNGC, 20 genes),
383 mechanosensitive Ca^{2+} -selective channels (MSCC, 10 genes), *mid1*-complementing activity
384 channels (MCA, 2 genes), and the unconventional Ca channels annexins (8 genes)
385 (Demidchik, 2018). In this study, we analyzed U bioaccumulation in null mutants for *MCA1*,
386 *MCA2* and *ANNEXIN1* (*ANN1*) genes (Lee et al., 2004; Nakagawa et al., 2007; Yamanaka et
387 al., 2010). We found that U bioaccumulation was reduced importantly (by 40%) in *mca1*,
388 *mca1/mca2* and *ann1*-null mutants, suggesting that the MCA1 and ANN1 channels have a role
389 in U uptake by roots (Figure 5). The MCA2 channel seems to have a limited role in U absorption
390 (10% decrease as compared to the WT, Figure 5). MCA proteins from Arabidopsis can
391 complement the Ca uptake deficiency in yeast cells lacking the high affinity Ca channel
392 composed of the Mid1 and Cch1 subunits. The proteins are located at the plasma membrane
393 and are inhibited by Gd^{3+} but not by verapamil (Nakagawa et al., 2007; Yamanaka et al., 2010).
394 Although the role of MCA1 and MCA2 in Ca nutrition is still not clear, the expression patterns
395 of the two genes suggest some distinct and overlapping functions. *MCA1* is expressed in the
396 meristem and adjacent elongation zone of the primary root, detected in the stele and
397 endodermis, but not in the cortex, epidermis, root cap, or root hairs. In the root, high levels of

398 *MCA2* expression was found in vascular tissues, stele and endodermis, but no in the cortex,
399 epidermis, root cap, meristem, elongation zone, or root hairs (Yamanaka et al., 2010). These
400 patterns, together with the ability of the *mca1*, *mca2*, and *mca1/mca2* mutants to accumulate
401 different amounts of U (Figure 5), suggest that MCA1 could contribute significantly to the
402 symplastic transport of U in the elongation zone of the primary root whereas MCA1 and MCA2
403 could contribute to the radial transfer of U through the endodermis to reach vascular tissues.
404 In our experimental conditions (acidic pH, absence of U chelators, 24 h of treatment), the
405 translocation factor of U from roots to shoots is very low ($2-7 \times 10^{-4}$), meaning that the transfer
406 of the radionuclide to the vascular tissues is poorly efficient and that a defect of MCA1 and
407 MCA2 in the endodermis and stele has little impact on U bioaccumulation in roots. ANN1 is
408 the predominantly abundant annexin in Arabidopsis and an *ann1*-null mutant displays
409 phenotypes consistent with impaired Ca uptake (lack of Ca^{2+} conductance) at the root hair
410 apex and epidermal elongation zone (Laohavisit et al., 2012). Our data suggest that ANN1
411 could be involved in the uptake of U in root hairs and the epidermal cell layer of the root
412 elongation zone. Thus, MCA1 and ANN1 could contribute to the absorption of U in different
413 zones and cell layers of the root. Additional Ca^{2+} -permeable cation channels belonging to the
414 NSCCs, MSCCs, and VDCCs are likely able to take part of U uptake in roots. To support this
415 hypothesis and the postulate of our approach (nutrient depletion inducing the upregulation of
416 ion carriers and, then, an improved U uptake), previous transcriptomic analyses have shown
417 that several genes coding Ca^{2+} -permeable channels are upregulated in Arabidopsis plants
418 starved with Ca, of which glutamate-like receptors (*GLR1.2, 1.4, 2.5, 2.8*), annexins
419 (*ANN1, 2, 4, 7*), mechanosensitive ion channel proteins (*MSL9, 10*) and cyclic nucleotide-gated
420 ion channels (*CNGC 9, 11, 19*) (Table S5) (Wang et al., 2015).

421 The partial inhibition (30 to 40%) of U accumulation in roots measured in competition and
422 inhibition assays (Figures 3 and 4) or in mutants devoid of Ca^{2+} -permeable cation channels
423 (Figure 5) suggests that other nutrient assimilation pathways are involved and/or that U does
424 not accumulate only inside root cells. Uranium bioaccumulation assays conducted at 4°C
425 allowed us to discriminate between the fraction of the radionuclide associated with the
426 symplast and the apoplast (Figure 2). The accumulation of U in the apoplast, which is a
427 temperature-insensitive adsorption and diffusion process (Barberon and Geldner, 2014),
428 accounts for 60-70% bioaccumulated U. This is in agreement with previous experiments
429 showing that plant roots or plant cells exposed to U accumulate the radionuclide principally in
430 the cell wall and intracellular spaces (Laurette et al., 2012a; Laurette et al., 2012b; El Hayek
431 et al., 2018; El Hayek et al., 2019; Lai et al., 2020; Sarthou et al., 2020; Wu et al., 2020). The
432 absorption of U by the symplastic pathway, which is mainly temperature-sensitive (reduction
433 of energy metabolism, enzymatic activities, membrane fluidity, plasmodesmatal permeability)
434 (Barberon and Geldner, 2014; Sager and Lee, 2014), accounts for 30-40% bioaccumulated U,

435 and is virtually fully inhibited by external Ca at high concentration (Figure 3) or by inhibitors of
436 NSCCs and Ca²⁺-permeable cation channels (Figure 4).

437 Our analyses of U bioaccumulation in nutrient-deprived plants provided no indication for the
438 involvement of transport systems different from NSCCs or Ca²⁺-permeable cation channels in
439 the absorption of U. In particular, Fe deficiency that is induced in the mi/100 medium (Figure
440 S1, Table S2) and known to upregulate the expression of the IRT1 and NRAMP1 Fe²⁺
441 transporters (Curie et al., 2000; Vert et al., 2002; Castaings et al., 2016), was not accompanied
442 by an improved bioaccumulation of U in roots. This finding confirms that, in an acidic
443 environment devoid of U chelators, Fe²⁺ transporters are not involved in the uptake of U cations
444 by roots (Berthet et al., 2018). Besides the Ca assimilation pathway, it is possible that
445 endocytosis contributes to some extent to U uptake by root cells. Indeed, low-dose U uptake
446 was found to be mainly mediated by endocytosis in kidney LLC-PK1 cells (Muller et al., 2008)
447 and the pinocytotic uptake of U (high dose) in the root cap of oat seedlings could be observed
448 (Wheeler and Hanchey, 1971). Also, it was found that deprivation of Ca in Arabidopsis roots
449 enhanced endocytosis (Zhang et al., 2018), suggesting that the improved absorption of U in
450 root cells of Ca-deficient plants could be due in part to this route.

451 In conditions favoring the formation of cationic species of U (acidic pH, absence of carbonate
452 or organic acids), the Ca assimilation pathway drives the main flux of the radionuclide within
453 root cells. It is likely that other transport systems are implicated in the uptake of U in
454 environments with different U speciation. For example, in carbonated water at nearly neutral
455 pH, uranyl carbonate anionic forms are predominant and possibly incorporated into cells
456 through anionic channels. Interestingly, in these conditions, Ca inhibits the radial transfer of U
457 through the apoplastic pathway but facilitates the symplastic route and results in an increased
458 translocation of U to shoot organs (El Hayek et al., 2018; El Hayek et al., 2019).

459

460 **5. Conclusion**

461 Understanding the molecular mechanisms governing the uptake of U is important for predicting
462 its absorption by plants in different environments and for phytoremediation applications
463 (Gavrilescu et al., 2009). Our study describes for the first time the involvement of Ca²⁺-
464 permeable cation channels in the cellular uptake of U. It paves the way for the identification of
465 U transport machinery in environmental conditions with changing U bioavailability as well as in
466 other organisms, in which the molecular basis of U uptake is poorly known.

467

468 **Figure legends**

469

470 Figure 1: Bioaccumulation of U in the roots of Arabidopsis plants deprived with nutrients.

471 Four-week-old Arabidopsis plants grown in a complete standard Gre medium were transferred
472 for 4 days in Gre medium depleted of one or several nutrients (100-fold reduction of the
473 standard concentration; Table S1). Plants were deprived with all elements (Gre/100),
474 macroelements (M/100), microelements (mi/100), or Ca, K, and Mg, alone or in dual
475 combination. Nutrient-deprived plants were challenged with 20 μM uranyl nitrate for 24 h,
476 thoroughly washed with sodium carbonate and distilled water to remove loosely-bound metals,
477 and U was measured by ICPMS in mineralized roots. Data from 2 to 11 replicates of the
478 experiment have been gathered, with $n=4$ plants for each condition. To normalize fluctuations
479 between replicates, U accumulated in roots was expressed as a percentage related to the
480 control condition in Gre medium. Bar plots represent mean \pm SD. Letters indicate significant
481 differences determined using a non-parametric Tukey's test with $p<0.01$. The amount of U
482 bioaccumulated in Gre medium was $727 \pm 79 \mu\text{g}\cdot\text{g}^{-1}$ fresh weight (mean \pm SD, $n=11$
483 independent experiments).

484

485 Figure 2: Effect of temperature on the accumulation of U in roots.

486 Four-week-old Arabidopsis plants were transferred in Gre, M/100 or Ca/100 media for 4 days
487 and challenged with 20 μM uranyl nitrate for 24 h at either 20 or 4°C in a standard light regime.
488 Uranium ($\text{mg}\cdot\text{g}^{-1}$ dry weight) was measured by ICPMS. Bar plots represent mean \pm SD with
489 $n=4$ biological replicates. Stars indicate significant differences determined using a non-
490 parametric Tukey's test with $p < 0.001$.

491

492 Figure 3: Competition between calcium and U for root uptake.

493 Four-week-old Arabidopsis plants were challenged for 10 h with 20 μM uranyl nitrate in
494 presence of increasing concentrations of $\text{Ca}(\text{NO}_3)_2$ in 0.25 mM MES (pH 5.6). Uranium content
495 was measured by ICPMS and standardized relative to the control condition without calcium
496 nitrate. The amount of U bioaccumulated in MES medium was $846 \pm 79 \mu\text{g}\cdot\text{g}^{-1}$ fresh weight
497 (mean \pm SD, $n=8$ measurements). Bar plots represent mean \pm SD with $n=4$ to 8 biological
498 replicates. Letters indicate significant differences determined using a non-parametric Tukey's
499 test with $p < 0.01$.

500

501 Figure 4: Effect of calcium channel inhibitors on the uptake of U by roots.

502 Four-week-old Arabidopsis plants were preincubated for 20 min at 22°C in 0.25 mM MES (pH
503 5.6) containing (A) 250 μM GdCl_3 , or (B) 100 μM verapamil, 50 μM nifedipine or 1% (v/v) DMSO
504 (used as a solvent for verapamil and nifedipine). Uranium ($\text{mg}\cdot\text{g}^{-1}$ dry weight) was measured

505 after 2 and 6 h of incubation at 22°C in the presence of 20 µM uranyl nitrate. Curves represent
506 mean ± SD with n=4 biological replicates. Letters indicate significant differences determined
507 using a non-parametric Tukey's test with p <0.01.

508

509 Figure 5: Bioaccumulation of U in the roots of Arabidopsis mutants deficient in Ca²⁺-permeable
510 cation channels.

511 The *A. thaliana* wild-type (Col-0) and the *mca1*, *mca2*, *mca1/mca2*, and *ann1* insertion mutants
512 were grown for 4 weeks in Gre medium. Plants were challenged with 20 µM uranyl nitrate for
513 6 h and U (mg.g⁻¹ dry weight) was measured by ICPMS. Bar plots represent mean ± SD with
514 n=4 biological replicates. Letters indicate significant differences determined using a non-
515 parametric Tukey's test with p <0.01.

516

517 **Supporting information**

518

519 Table S1: Composition of culture media and U speciation.

520 Table S2: Root ionomes after 4 days of nutrient deprivations.

521 Table S3: Shoot ionomes after 4 days of nutrient deprivations.

522 Table S4: Root ionomes of mutants impaired in calcium channels.

523 Table S5: Up-regulation of genes coding calcium channels and transporters in response to
524 extracellular calcium depletion.

525 Figure S1: Changes in root ionomes induced by 4 days of nutrient deprivations.

526 Figure S2: Changes in shoot ionomes induced by 4 days of nutrient deprivations.

527 Figure S3: Kinetics of U accumulation in Arabidopsis roots.

528 Figure S4: Bioaccumulation of U in the roots of Arabidopsis plants deprived with nutrients,
529 effects of supplementation with anions.

530

531 **CRedit authorship contribution statement**

532 Manon Sarthou: conceptualization, investigation, writing - original draft, review and editing;

533 Fabienne Devime: investigation; Célia Baggio: investigation; Sylvie Figuet: investigation;

534 Claude Alban: investigation, writing - review and editing; Jacques Bourguignon: funding

535 acquisition, conceptualization, investigation, writing - review and editing; Stéphane Ravel:

536 funding acquisition, conceptualization, investigation, writing - original draft, review and editing.

537

538 **Declaration of Competing Interest**

539 The authors declare that they have no known competing financial interests or personal

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541

542

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554

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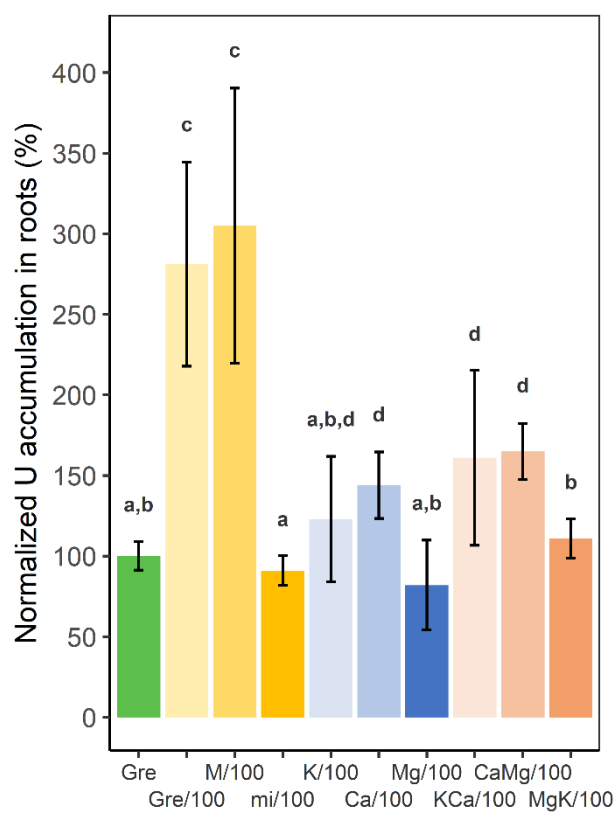


Figure 1 : Bioaccumulation of U in the roots of Arabidopsis plants deprived with nutrients.

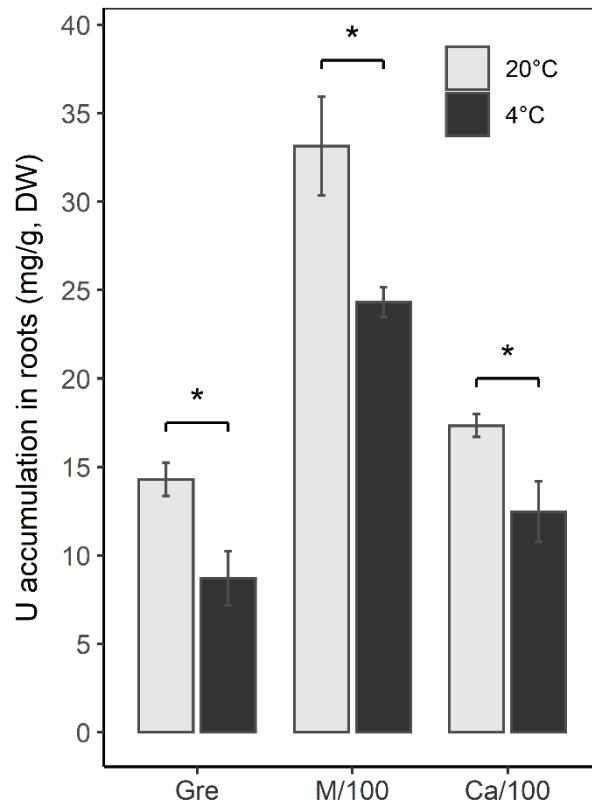


Figure 2: Effect of temperature on the accumulation of U in roots.

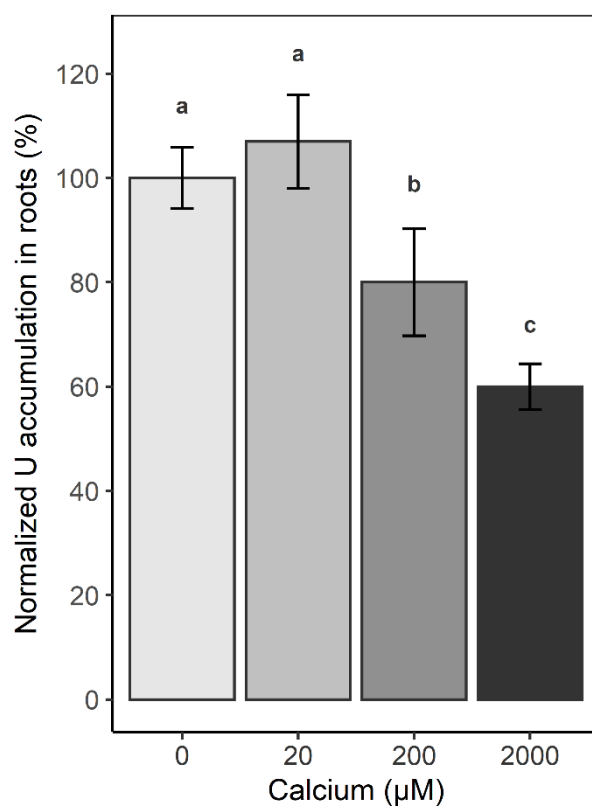


Figure 3: Competition between calcium and U for root uptake.

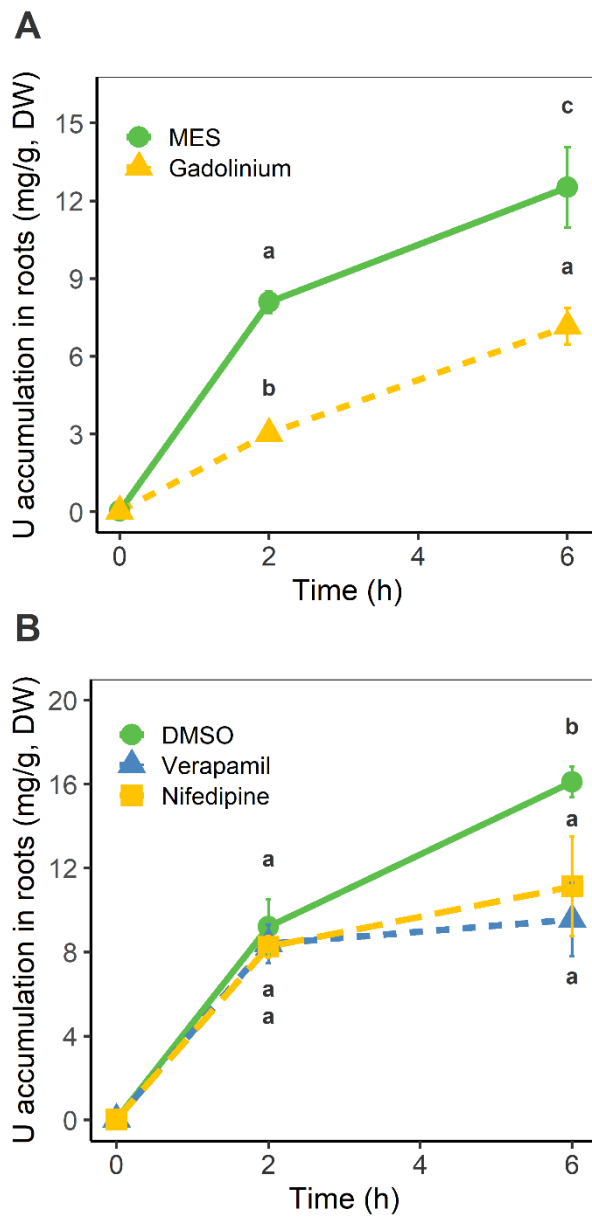


Figure 4: Effect of calcium channel inhibitors on the uptake of U by roots.

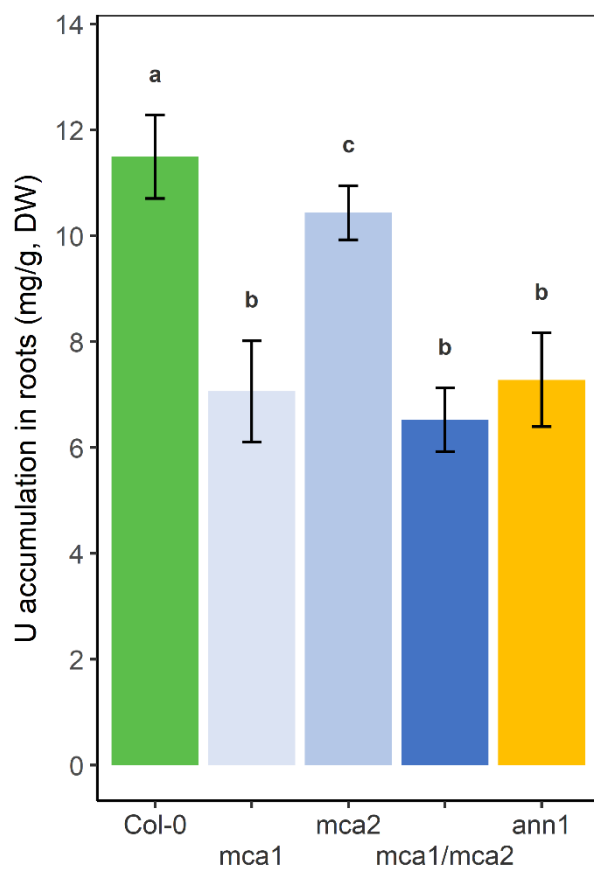


Figure 5: Bioaccumulation of U in the roots of Arabidopsis mutants deficient in Ca²⁺-permeable cation channels.

Calcium-permeable cation channels are involved in uranium uptake in *Arabidopsis thaliana*

Manon C.M. Sarthou, Fabienne Devime, Célia Baggio, Sylvie Figuet, Claude Alban, Jacques Bourguignon, and Stéphane Ravanel

Supporting information

Table S1: Composition of culture media and U speciation.
Available in a separate Excel file.

Table S2: Root ionomes after 4 days of nutrient deprivations.

Table S3: Shoot ionomes after 4 days of nutrient deprivations.

Table S4: Root ionomes of mutants impaired in calcium channels.

Table S5: Up-regulation of genes coding calcium channels and transporters in response to extracellular calcium depletion.

Figure S1: Changes in root ionomes induced by 4 days of nutrient deprivations.

Figure S2: Changes in shoot ionomes induced by 4 days of nutrient deprivations.

Figure S3: Kinetics of U accumulation in *Arabidopsis* roots.

Figure S4: Bioaccumulation of U in the roots of *Arabidopsis* plants deprived with nutrients, effects of supplementation with anions.

Table S2: Root ionomes after 4 days of nutrient deprivations.

Element ($\mu\text{g/g}$)	Culture medium									
	Gre	Gre/100	M/100	mi/100	K/100	Ca/100	Mg/100	KCa/100	CaMg/100	MgK/100
Mg	3005 \pm 183 ^a	2311 \pm 152 ^b	2249 \pm 52 ^b	3053 \pm 80 ^a	3552 \pm 66 ^c	5922 \pm 328 ^d	1878 \pm 52 ^e	4802 \pm 284 ^f	1751 \pm 102 ^e	2417 \pm 142 ^b
P	16026 \pm 1082 ^a	10848 \pm 667 ^b	11577 \pm 478 ^b	14272 \pm 226 ^c	9240 \pm 892 ^e	18312 \pm 757 ^d	13057 \pm 642 ^f	9688 \pm 486 ^e	14993 \pm 148 ^a	9383 \pm 425 ^e
K	69503 \pm 3819 ^{a,b}	52322 \pm 3105 ^{e,c}	50289 \pm 1093 ^e	72809 \pm 4295 ^a	65242 \pm 4237 ^b	55353 \pm 4320 ^c	73435 \pm 2254 ^a	63143 \pm 3222 ^b	50825 \pm 1644 ^{e,c}	46814 \pm 2230 ^d
Ca	4631 \pm 186 ^{a,b}	3583 \pm 277 ^{e,c}	3667 \pm 304 ^{e,c}	3902 \pm 208 ^e	4024 \pm 362 ^{a,e}	2205 \pm 297 ^d	4534 \pm 252 ^{a,b}	1668 \pm 215 ^f	3144 \pm 374 ^c	5187 \pm 434 ^b
Mn	79 \pm 17 ^{a,b}	31 \pm 5 ^e	155 \pm 16 ^f	29 \pm 10 ^e	58 \pm 20 ^{a,c}	94 \pm 36 ^{a,b,d}	88 \pm 8 ^b	47 \pm 9 ^c	190 \pm 16 ^g	124 \pm 7 ^d
Fe	6864 \pm 170 ^a	4205 \pm 403 ^b	5430 \pm 220 ^e	4255 \pm 185 ^b	6188 \pm 920 ^{a,e,c}	6860 \pm 325 ^a	4600 \pm 491 ^b	4074 \pm 427 ^b	5460 \pm 350 ^e	6217 \pm 432 ^c
Zn	793 \pm 63 ^{a,b}	591 \pm 55 ^e	943 \pm 80 ^c	619 \pm 125 ^{e,d,f}	782 \pm 47 ^{a,d}	450 \pm 84 ^f	651 \pm 58 ^e	667 \pm 87 ^{b,e}	633 \pm 100 ^e	1100 \pm 168 ^c
Mo	75 \pm 5 ^{a,b}	49 \pm 8 ^e	65 \pm 4 ^c	44 \pm 7 ^e	94 \pm 10 ^{d,f}	72 \pm 10 ^{a,c}	76 \pm 8 ^{a,b,c,d}	108 \pm 10 ^f	38 \pm 4 ^e	101 \pm 17 ^{b,d,f}

Four-week-old Arabidopsis plants grown in a complete standard Gre medium were transferred for 4 days in Gre medium depleted of one or several nutrients (100-fold reduction of the standard concentration; Table S1). Element concentrations (in $\mu\text{g.g}^{-1}$ dry weight) were measured in mineralized roots by ICP-MS. Deprivation experiments have been performed from 2 to 11 times. The table is a representative root ionome from two independent experiments with n=4 plants (mean \pm SD). Letters indicate significant differences determined using a non-parametric Tukey's test with $p < 0.01$.

Table S3: Shoot ionomes after 4 days of nutrient deprivations.

Element ($\mu\text{g/g}$)	Culture medium									
	Gre	Gre/100	M/100	mi/100	K/100	Ca/100	Mg/100	KCa/100	CaMg/100	MgK/100
Mg	10742 \pm 186 ^a	7627 \pm 261 ^b	6899 \pm 166 ^c	11167 \pm 259 ^d	10989 \pm 164 ^{a,d}	9496 \pm 639 ^e	6487 \pm 269 ^f	10678 \pm 465 ^{a,d,e}	5707 \pm 717 ^f	2890 \pm 6 ^g
P	6440 \pm 486 ^a	4967 \pm 256 ^b	4168 \pm 227 ^c	6467 \pm 382 ^a	3958 \pm 191 ^c	6294 \pm 261 ^a	6042 \pm 320 ^a	4715 \pm 233 ^b	5694 \pm 515 ^a	2049 \pm 50 ^d
K	31001 \pm 1216 ^a	29009 \pm 554 ^b	25067 \pm 1042 ^c	29015 \pm 1775 ^{a,b}	25586 \pm 2053 ^c	24833 \pm 1641 ^{c,d}	31131 \pm 1000 ^a	22950 \pm 720 ^d	28008 \pm 2524 ^{a,b,c}	13937 \pm 537 ^e
Ca	27615 \pm 1735 ^a	17312 \pm 1492 ^b	13915 \pm 771 ^c	26081 \pm 1187 ^a	26153 \pm 1071 ^a	14794 \pm 445 ^{c,d}	31889 \pm 1743 ^e	16900 \pm 1977 ^{b,d}	14724 \pm 1093 ^{c,d}	17238 \pm 281 ^b
Mn	101 \pm 6 ^a	72 \pm 6 ^{b,c,d}	127 \pm 4 ^e	64 \pm 3 ^b	97 \pm 4 ^a	83 \pm 7 ^c	119 \pm 5 ^f	97 \pm 5 ^a	116 \pm 6 ^f	69 \pm 2 ^d
Fe	74 \pm 3 ^a	80 \pm 17 ^a	61 \pm 4 ^b	65 \pm 9 ^{a,b}	73 \pm 2 ^a	51 \pm 1 ^c	72 \pm 7 ^a	71 \pm 5 ^a	72 \pm 32 ^{a,b}	37 \pm 1 ^d
Zn	114 \pm 10 ^{a,b}	122 \pm 10 ^{a,b,c}	132 \pm 17 ^{a,c}	87 \pm 9 ^d	121 \pm 8 ^a	103 \pm 11 ^b	112 \pm 3 ^b	146 \pm 16 ^c	121 \pm 13 ^{a,b}	73 \pm 11 ^d
Mo	14,5 \pm 0,9 ^a	11,0 \pm 1,9 ^b	11,3 \pm 1,0 ^b	10,7 \pm 0,7 ^b	14,2 \pm 1,4 ^{a,c}	10,7 \pm 2,3 ^{b,d}	15,1 \pm 0,9 ^a	12,0 \pm 1,1 ^{b,c}	10,9 \pm 1,1 ^b	7,6 \pm 0,7 ^d

Four-week-old Arabidopsis plants grown in a complete standard Gre medium were transferred for 4 days in Gre medium depleted of one or several nutrients (100-fold reduction of the standard concentration; Table S1). Element concentrations (in $\mu\text{g.g}^{-1}$ dry weight) were measured in mineralized shoots by ICP-MS. Deprivation experiments have been performed from 2 to 11 times. The table is a representative shoot ionome from two independent experiments with $n=4$ plants (mean \pm SD). Letters indicate significant differences determined using a non-parametric Tukey's test with $p<0.01$.

Table S4: Root ionomes of mutants impaired in calcium channels.

Element ($\mu\text{g/g}$)	Line				
	Col-0	<i>mca1</i>	<i>mca2</i>	<i>mca1/mca2</i>	<i>ann1</i>
Mg	4917 \pm 325 ^{a,b}	4553 \pm 312 ^{a,b}	5086 \pm 346 ^a	4833 \pm 300 ^a	5515 \pm 711 ^b
P	16879 \pm 1008 ^a	17627 \pm 1149 ^a	17163 \pm 1190 ^a	16419 \pm 1489 ^a	18179 \pm 3443 ^a
K	109916 \pm 10350 ^a	110303 \pm 8228 ^a	99742 \pm 4641 ^a	97690 \pm 11194 ^a	102947 \pm 16144 ^a
Ca	4979 \pm 553 ^a	4711 \pm 1328 ^a	5309 \pm 490 ^a	4779 \pm 250 ^a	5164 \pm 1131 ^a
Mn	100 \pm 47 ^{a,b}	98 \pm 24 ^a	116 \pm 24 ^a	76 \pm 19 ^{a,b}	57 \pm 16 ^b
Fe	6851 \pm 84 ^a	8671 \pm 774 ^b	7824 \pm 1133 ^b	7471 \pm 636 ^{a,b}	8432 \pm 2628 ^{a,b}
Zn	1239 \pm 113 ^a	1391 \pm 172 ^{a,b}	1428 \pm 145 ^{a,b}	1282 \pm 123 ^{a,b}	1694 \pm 327 ^b

Arabidopsis plants were grown in a complete standard Gre medium for 4 weeks. Element concentrations (in $\mu\text{g}\cdot\text{g}^{-1}$ dry weight) were measured in mineralized roots by ICP-MS. Values are mean \pm SD with n=4 biological replicates. Letters indicate significant differences determined using a non-parametric Tukey's test with $p < 0.01$.

Table S5: Up-regulation of genes coding calcium channels and transporters in response to extracellular calcium depletion.

- Data from Wang *et al* (2015) Arabidopsis transcriptional response to extracellular Ca²⁺ depletion involves a transient rise in cytosolic Ca²⁺. *J Int Plant Biol* 57, 138-150.

Upregulated genes involved in Ca mobilization (24 h Ca depletion in 11-day-old *Arabidopsis thaliana* seedlings). Data from Table 2.

Gene ID	Average fold-change	Symbol	Annotation
At2g29110	6.2	GLR2.8	Glutamate receptor cation channel 2.8
At5g48410	5.1	GLR1.2	Glutamate receptor cation channel 1.2
At5g11210	3.2	GLR2.5	Glutamate receptor cation channel 2.5

- Transcriptomic data GSM1655511 (unpublished).

Arabidopsis thaliana plants were grown on perlite with a complete nutrient solution in which Ca concentration was 3 mM. After 17 days Ca was suppressed for 2 days.

Upregulated genes in Ca-deficient plants (>1.5-fold change)

Gene ID	Average fold-change	Symbol	Annotation
At3g07520	4.17	GLR1.4	Glutamate receptor cation channel 1.4
At3g22910	4.12	ACA13	Putative calcium-transporting ATPase 13
At3g17690	3.95	CNGC19	Putative cyclic nucleotide-gated ion channel 19
At5g65020	3.23	ANN2	Annexin 2
At1g35720	2.30	ANN1	Annexin 1
At5g19520	2.24	MSL9	Mechanosensitive ion channel protein 9
At4g30560	1.92	CNGC9	Putative cyclic nucleotide-gated ion channel 9
At3g51860	1.78	CAX3	Vacuolar cation/proton exchanger 3
At5g10230	1.68	ANN7	Annexin 7
At2g29100	1.59	GLR2.9	Glutamate receptor cation channel 2.9
At2g38750	1.55	ANN4	Annexin 4
At2g46440	1.55	CNGC11	Cyclic nucleotide-gated ion channel 11
At5g12080	1.50	MSL10	Mechanosensitive ion channel protein 10

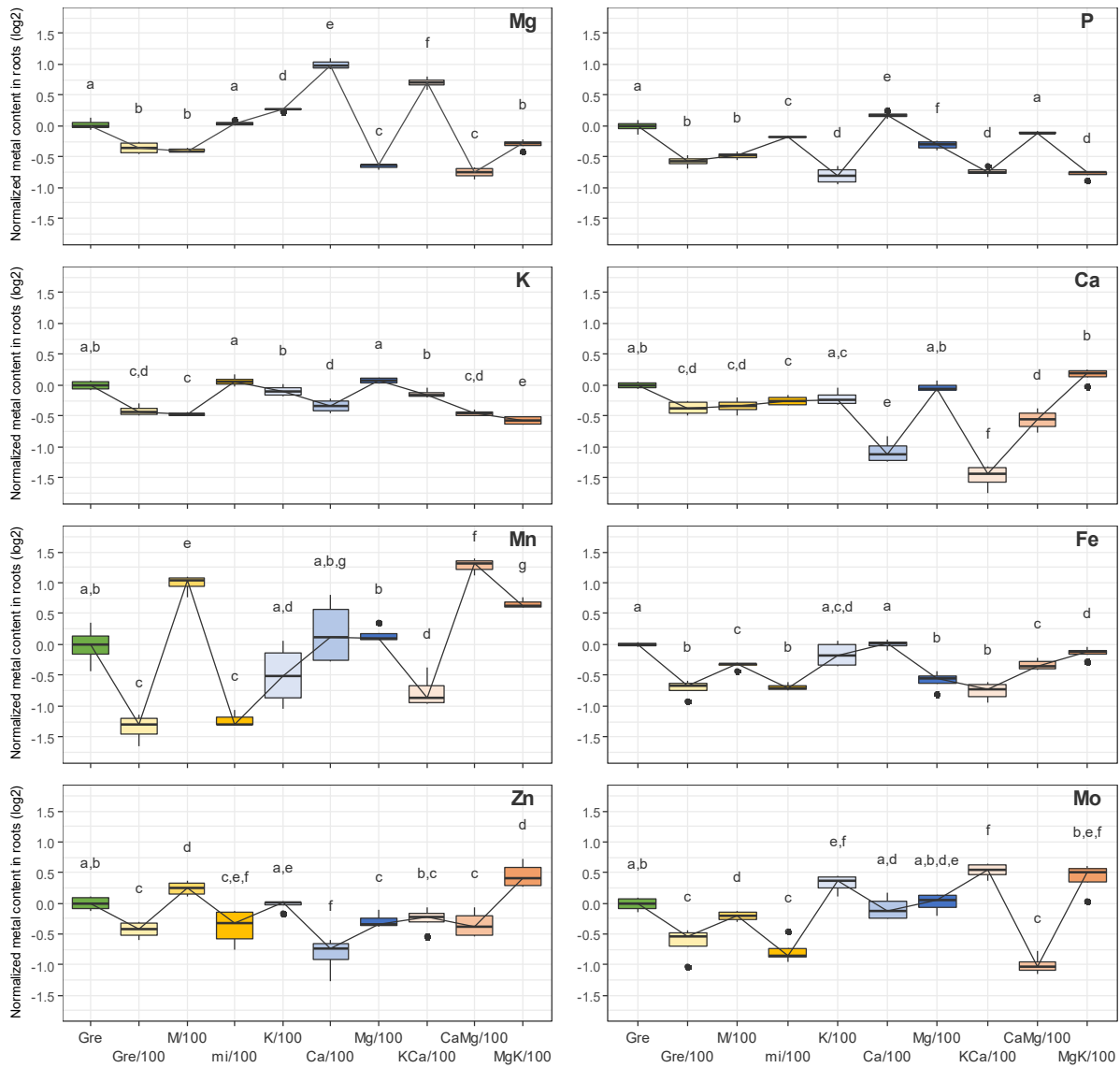


Figure S1: Changes in root ionomes induced by 4 days of nutrient deprivations.

Modifications of the root ionomes from two independent representative deprivation experiments (Table S2) are shown. Changes in mineral content are expressed as a log₂ of the ratio relative to the control condition (Gre medium). Box plots represent minimum, first quartile, median, third quartile and maximum. Letters indicate significant differences determined using a non-parametric Tukey's test with p < 0.01.

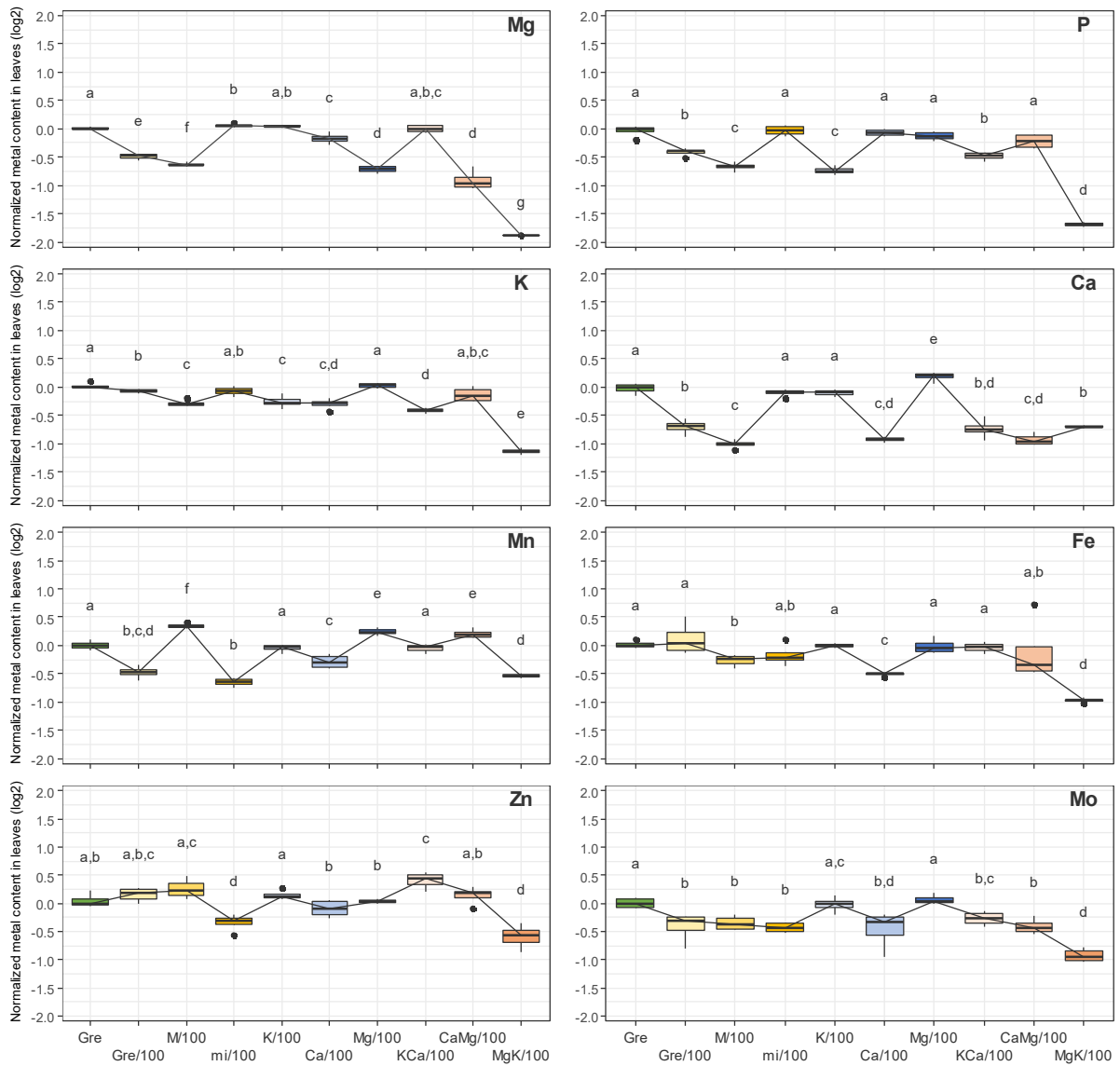


Figure S2: Changes in shoot ionomes induced by 4 days of nutrient deprivations.

Modifications of the shoot ionomes from two independent representative deprivation experiments (Table S3) are shown. Changes in mineral content are expressed as a log₂ of the ratio relative to the control condition (Gre medium). Box plots represent minimum, first quartile, median, third quartile and maximum. Letters indicate significant differences determined using a non-parametric Tukey's test with $p < 0.01$.

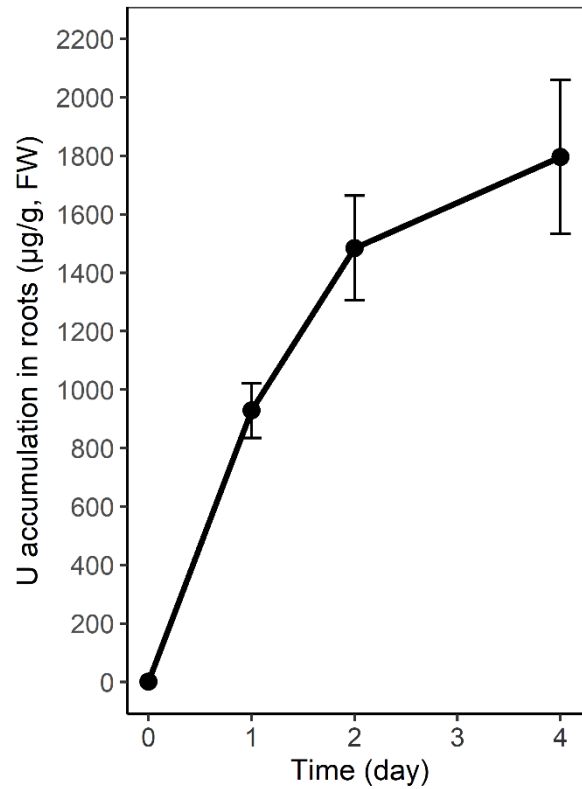


Figure S3: Kinetics of U accumulation in Arabidopsis roots.

Four-week-old Arabidopsis plants grown in a complete standard Gre medium were challenged with 20 µM uranyl nitrate for 24 to 72 h at 20°C under a standard light regime. Uranium content was measured by ICPMS. The curve shows mean ± SD with at least n=8 biological replicates.

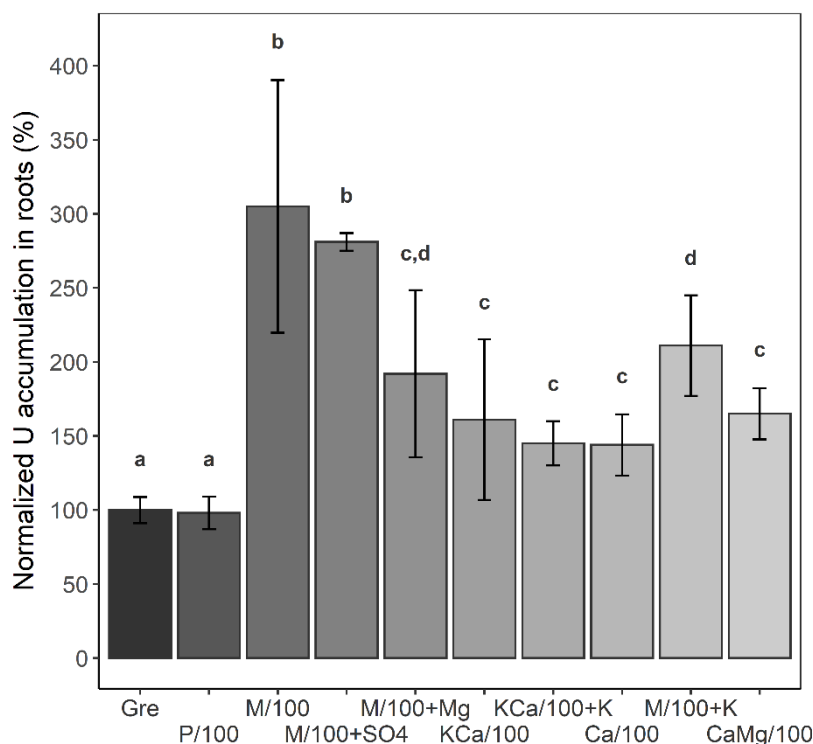


Figure S4: Bioaccumulation of U in the roots of Arabidopsis plants deprived with nutrients, effects of supplementation with anions.

Four-week-old Arabidopsis plants grown in a complete standard Gre medium were transferred for 4 days in Gre medium depleted of one or several nutrients (100-fold reduction of the standard concentration; Table S1). Various media have been tested to modify the nutrient supply of plants. “P/100” is a Gre medium in which phosphate has been reduced by 100-fold. “M/100+SO₄” is a M/100 medium supplemented with 1 mM (NH₄)₂SO₄ to restore sulfate supply to control conditions. “KCa/100+K” is a KCa/100 medium supplemented with 2 mM KNO₃, leading to a medium comparable to Ca/100 but with a standard nitrate concentration. “M/100+K” is the M/100 medium with 2 mM KNO₃, leading to a medium comparable to CaMg/100 but with a standard nitrate concentration. Nutrient-deprived plants were challenged with 20 μM uranyl nitrate for 24 h, thoroughly washed with sodium carbonate and distilled water to remove loosely-bound metals, and U was measured by ICPMS in mineralized roots. Uranium accumulation in roots was expressed as a percentage related to the control condition in Gre medium. Bar plots represent mean ± SD. Letters indicate significant differences determined using a non-parametric Tukey’s test with p<0.01. The amount of U bioaccumulated in Gre medium was 727 ± 79 μg.g⁻¹ fresh weight (mean ± SD, n=11 independent experiments).