Calcium in Carbonate Water Facilitates the Transport of U(VI) in *Brassica juncea* Roots and Enables Root-to-Shoot Translocation

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Additional Materials and Methods:

Analysis of U Content in Plant Tissue. After exposure for 30 days, the seedlings were washed with distilled water followed by three rinses with 18 MΩ water. Shoot and root tissues were separated and the fresh weight was measured before being dried until a constant dry mass at 70 °C for 48 h. The concentration of U in the shoots and roots was measured in triplicates after acid digestion in 10 ml reagent-grade HNO_3 under progressive heating until total dissolution according to Alexander at al. method.[1,2] Prior to ICP-MS/OES analyses, samples were diluted and filtered using a 0.45 μm filter. Laboratory blanks and QA/QC measures were periodically taken during samples analysis and checked to ensure quality data.

Statistical Analyses. Statistical analyses were conducted to assess the significance of the effect of Ca concentration on U accumulation in plant roots and shoots using the software XLSTAT. Nonparametric tests were performed because of the non-normality of our data sets as determined using the Shapiro-Wilk test. Kruskal-Wallis test was complemented with multiple pairwise comparison using Conover-Iman test in order to assess the significant difference between Ca treatments. The statistically significant level was set at $\alpha = 0.05$ ($p < 0.05$) for all the statistical tests. All treatments had three replicates, with results presented as mean ± SD (standard deviation). The calculated p-value by Kruskal-Wallis test are presented in Table S1.

Chemical Equilibrium Modeling. Visual MINTEQ was used to determine the aqueous U species and to ensure that the total added U concentration (80 μM) in the exposure solutions is under dissolved condition. The aqueous species of U were determined under atmospheric equilibrium with using the inputs based on the chemical composition of the exposure solutions (MgSO4 0.5 mM, NH_4NO_3 2mM, KCl 1mM, and $NaHCO_3$ 1mM) at 80 μM U and three different Ca concentrations (0, 0.3 and 6 mM). The pH of solutions was set at 7.5 under 25°C.

Microscopy and Spectroscopy Analyses. *Brassica juncea* roots were washed thoroughly with 18 MΩ water. Root samples were then prefixed for 48h at 4 °C in 2.5% glutaraldehyde prepared in 0.1M sodium cacodylate buffer (pH=7.4), post-fixed for 1h in 1% osmium tetroxide, dehydrated in a graded series of acetone, and finally embedded in resin (Hard Plus Resin-812). Ultrathin sections of 80 to 120 nm were cut by a Reichert Ultracut E ultramicrotome and collected on copper TEM mesh grids covered with a holey carbon support film. A grid from each sample was stained with uranyl acetate and Reynold's lead citrate to allow for observation of cell morphology in a Hitachi HT7700 TEM operated at 80kV; images were acquired with an AMT XR16M digital camera. The grids for energy dispersive X-ray spectroscopy (EDS) analyses were not stained.

In order to conduct EDS analyses on regions of interest, unstained microtome sections were observed on a JEOL 2010F FASTEM field emission gun scanning TEM (FEGSTEM/TEM) instrument equipped with an Oxford AZtec EDS analysis system using an Oxford X-Max N 80 mm^2 ultrathin window SDD EDS detector. The unstained microtome sections were also characterized using bright-field TEM (BF-TEM), high-angle annular dark-field (HAADF) STEM, electron diffraction and single spot EDS analyses on regions of interest. All TEM observations using the JEOL 2010F were carried out at an accelerating voltage of 200 kV. In addition, some

single spot EDS analyses were also conducted using a JEOL JXA-8200 SuperProbe electron probe microanalyzer (EPMA) with wavelength dispersive X-ray spectroscopy (WDS) at an accelerating voltage of 10 kV with a 10 μm probe diameter and 30 nA probe current.

Tables.

Table S1. The content of U in plant roots and shoots at the three different Ca treatments were compared according to Kruskal-Wallis test which was used as a nonparametric test alternative to ANOVA. $p < 0.05$ indicates that the compared treatments (n=3) are significantly different.

Comparison of U content in the roots of plant at different Ca treatments (0, 0.3 and 6 mM)	**p-value < 0.0001**
Comparison of U content in the shoots of plant at different Ca treatments (0, 0.3 and 6 mM)	**p-value < 0.0001**

Table S2. The measured mass of accumulated U (mg) with considering the dry biomass of plant roots and shoots, and the percentage of U mass in roots or shoots at the three different Ca treatments.

Ca concentration (mM) at each treatment	**U mass in shoots (mg)**	**U mass in roots (mg)**	**Total U mass in plants (U mass in shoots + U mass in roots)**	**Percentage of U mass in shoots (the ratio of U mass in shoots over the total U mass accumulated in plants)**	**Percentage of U mass in roots (the ratio of U mass in roots over the total U mass accumulated in plants)**
0	0.0042± 0.002	3.35±0. 8	3.3542	0.12%	99.88%
0.3	0.022±0. 005	4.2±1.3	4.222	0.5%	99.5%
6	0.133±0. 7	0.37±0. 1	0.503	26.4%	73.6%

Table S3. Percent (%) distribution of U species calculated using Visual MINTEQ for solutions at pH 7.5, 25 °C and under atmospheric equilibrium, and containing 80 µM U**,** 1 mM HCO_3^- with different Ca concentrations (0, 0.3 and 6 mM). The U-CO_3 complex represents the sum of $(UO_2)_2CO_3(OH)_3^-$, $UO_2(CO_3)_2^{-2}$, UO_2CO_3 and $UO_2(CO_3)_3^{-4}$. The Ca-U-CO_3 complexes are presented separately in the table as the negatively charged $CaUO_2(CO_3)_3^{-2}$ and the neutrally charged $Ca_2UO_2(CO_3)_3$. The UO_2-OH complex represents the sum of UO_2OH^+, $UO_2(OH)^{3-}$, $UO_2(OH)_{2\,(aq)}$ and $(UO_2)_3(OH)^{5+}$.

		% Distribution of U Aqueous Species			
Ca mM	**U µM**	**U-CO_3 (aq)**	**$Ca_2UO_2(CO_3)_3$ (aq)**	**$CaUO_2(CO_3)_3^{-2}$**	**UO_2-OH (aq)**
0	**80**	99.5	0	0	0.5
0.3	**80**	97.1	0.7	1.8	0.4
6	**80**	15	71.9	13	0.1

References.

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