Supplemental Data

The assembly of the plant urease activation complex and the essential role of the urease accessory protein G (UreG) in delivery of nickel to urease

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Figure S1. Optimization of urease expression using an optimal start codon context and viral enhancer sequences.

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Table S1. Primer list.

References in Supplemental Data

1. Rangan, L., Vogel, C., and Srivastava, A. (2008) Analysis of context sequence surrounding translation initiation site from complete genome of model plants. *Mol. Biotechnol.* **39 (3),** 207–213
2. Witte, C.P., Rosso, M. G., and Romeis, T. (2005) Identification of three urease accessory proteins that are required for urease activation in Arabidopsis. *Plant Physiol.* **139 (3),** 1155–1162

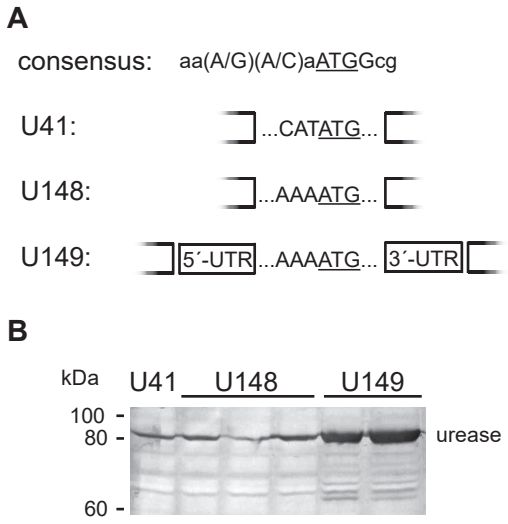

FIGURE S1. **Optimization of urease expression using an optimal start codon context and viral enhancer sequences.**

A, the sequence upstream of the start codon of the original construct for AtUrease expression (U41) was optimized according to the consensus sequence surrounding start codons in dicot plants (U148). Additionally, 5' and 3' UTR enhancer sequences of cowpea mosaic virus (CPMV) RNA-2 flanking the urease coding sequence were introduced (U149). B, protein abundance during transient expression in leaves of *N. benthamiana* employing the different constructs described in A was monitored by Western blot using an anti-urease antibody. The blot was loaded on equal leaf fresh weight basis. Each lane contains the proteins from an independent transient expression.

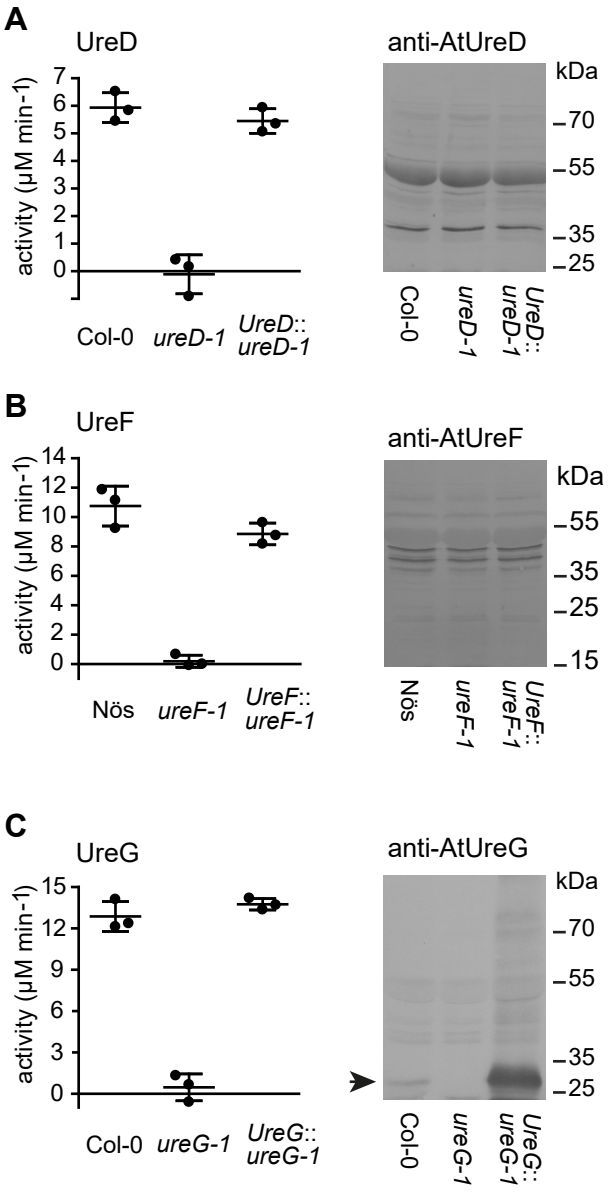

FIGURE S2. **Urease activity and UAP detection in wild types and respective UAP knockout and complementation lines.**

A, urease activity (left panel) and Western blot developed with anti-UreD antiserum (right panel) in Col-0, the *ureD-1* mutant (SALK_105718; 15) and a complementation line expressing a *ureD* cDNA under 35S promoter in mutant background. Note that the UreD protein is not detected with the UreD antiserum in wild type and UreD overexpressing Arabidopsis plants. B, as A but for UreF. The *ureF-1* line (RIKEN 15-1020-1; 15) was used as mutant line which was complemented. Also the UreF protein is not detected on the Western blot. C, as A and B but for UreG. The *ureG-1* line (Gabi-Kat 294B06; 15) was used as mutant line which was complemented. For each experiment three biological replicates per genotype each containing four plants were analyzed. All replicates were pooled for the Western blots. 15 µl supernatant were loaded per lane. Plants were grown in peat soil in pots with 8 cm diameter and fertilized with 0.4 mM $NiCl_2$ (2 mL) either 5 days (A and B) or 10 days (C) before the experiment. Note that full activity was not reached in A and B because of the shorter exposure time to nickel.

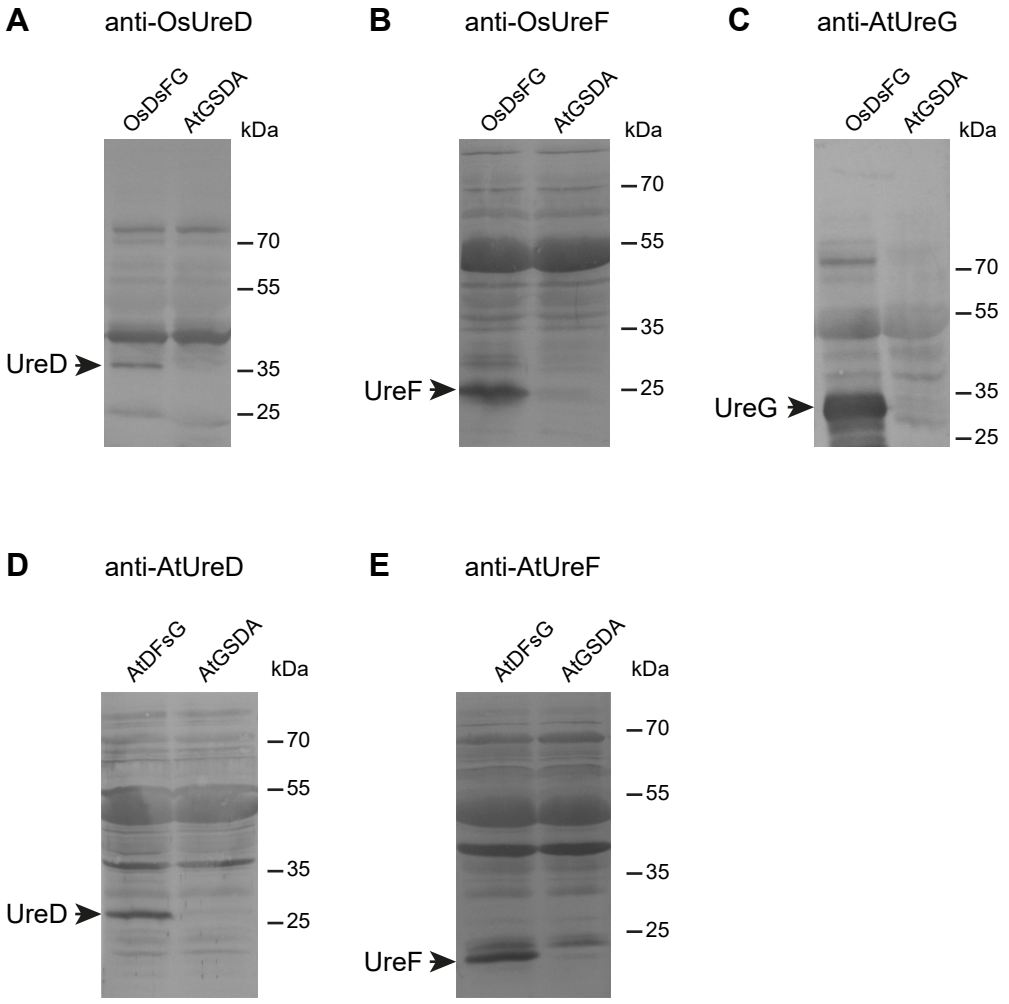

FIGURE S3. **Specificity of the antisera developed against Arabidopsis and rice UAPs tested in *N. benthamiana*.**

Antisera developed against A, OsUreD; B, OsUreF; C, OsUreG; D, AtUreD; and E, AtUreF were evaluated on Western blots prepared from leaf extracts of *N. benthamiana* either overexpressing Strep-tagged OsUreD, OsUreF and OsUreG (A, B, C) or AtUreD, Strep-tagged AtUreF and AtUreG (E, F). Guanosine deaminase from Arabidopsis (AtGSDA; 43) was expressed as negative control. Note that the specificity of the AtUreG antiserum for AtUreG was evaluated by comparing Western blot signals from Col-0, *UreG* knockout and overexpressing plants (Figure S2).

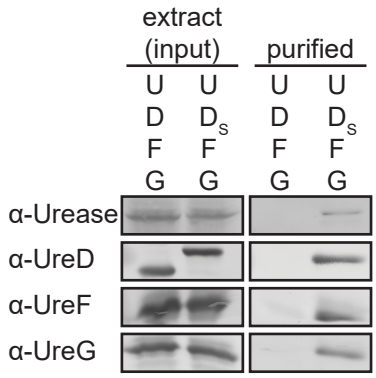

FIGURE S4. **Interaction analysis of rice urease and its accessory proteins.**
Urease (U) and the UAPs from *O. sativa* were coexpressed in *N. benthamiana*. The complex was purified using StrepII-tagged UreD (D_S) or untagged UreD (D) as negative control. Clarified crude extracts (left panel) and purified samples (right panel) were analyzed by SDS gel electrophoresis and Western blot using specific antibodies. The experiment was repeated three times and a representative repeat is shown.

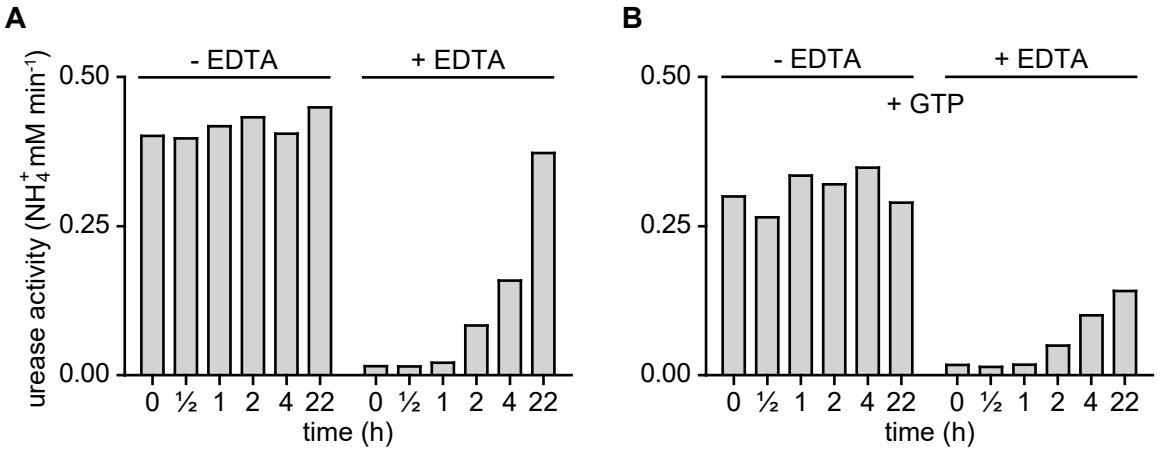

FIGURE S5. **EDTA resistance of *in vitro* activated urease.**

A, Arabidopsis urease and the UAPs were transiently co-expressed in *N. benthamiana* and the complex affinity purified using N-terminally StrepII-tagged UreF. Purified samples were incubated with $NiCl_2$ and $NaHCO_3$ (final concentration: 100 µM and 25 mM, respectively) at 50°C. At several time points (0-22 h) a sample was taken, mixed with urea (final concentration: 5 mM) and the urease activity determined as described by Witte et al. (2005). To examine the EDTA resistance of the activated urease, the activity assay was performed in the presence or absence of 1 mM EDTA (+ EDTA or – EDTA). B, the experiment was repeated in the presence of GTP (final concentration: 200 µM) in addition to $NiCl_2$ and $NaHCO_3$.

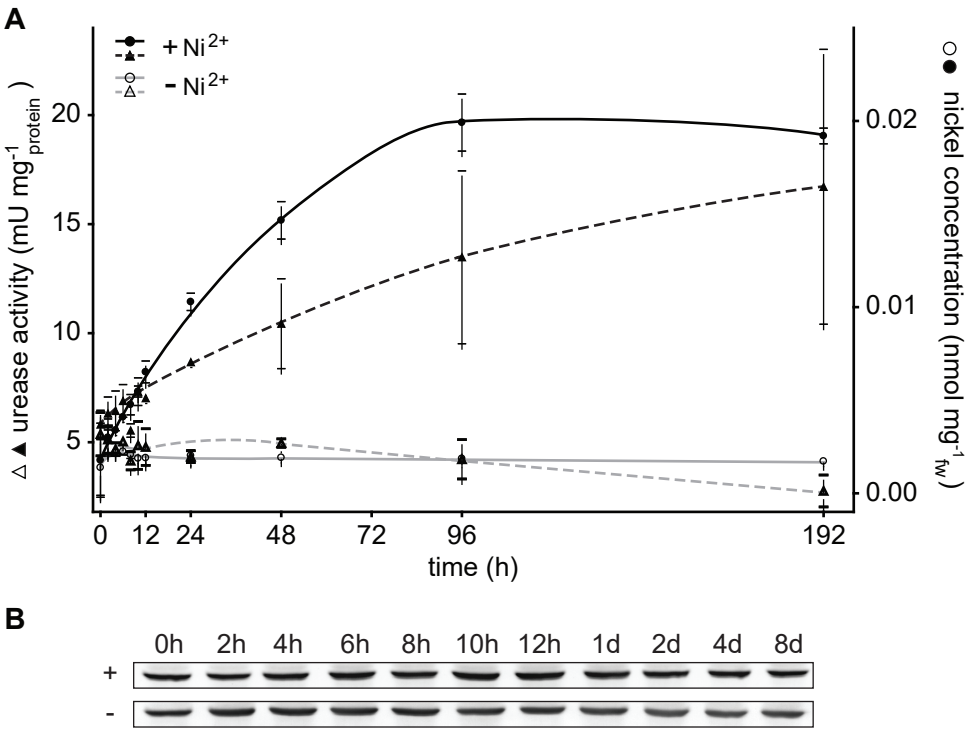

FIGURE S6. **Nickel-dependent activation of urease *in vivo*.**

A, Arabidopsis wild type plants were hydroponically grown without nickel. After 5½ weeks, the hydroponic media was supplemented with $NiCl_2$ (final concentration: 10 µM). The urease activity (dashed lines) and nickel concentration (solid lines) were monitored in Ni-fertilized plants (black lines) as well as in control plants (grey lines). B, for each time point, the protein amount of urease in the rosette of Ni-fertilized (+) and control (-) plants was analyzed by Western blot using a urease-specific antibody. Error bars are SD of three biological replicates containing three plants each.

TABLE S1. **Primer list.**

Project	No.	Sequence	Comments
AtUrease	N1	TGAATTCAAAATGAAGTTGTTGCCGCGAG	forward primer to amplify *AtUrease* with optimised consensus sequence before start codon; introducing EcoRI site
	569	TCCCGGGTTAAAAGAGGAAATAGTTCC	reverse primer to amplify *AtUrease*; introducing XmaII site
CPMV 5´-UTR	N2	TCTCGAGTATTAAAATCTTAATAGGTTTTG	forward primer to amplify CPMV 5´-UTR; introducing XhoI site
	N3	TGTCGACCGCGAATTTGGGCAGAAT	reverse primer to amplify CPMV 5´-UTR; introducing SalI site
CPMV 3´-UTR	N4	TACTAGTTTAACTCTGGTTTCATTAAATTTTC	forward primer to amplify CPMV 3´-UTR; introducing SpeI site
	N5	TTCTAGAAATAAAATTAAAATCTTTTTGT	reverse primer to amplify CPMV 3´-UTR; introducing XbaI site
N-terminal AtUreG deletions	1760	TACCATGGCGTCGTGGGTGGGGAAG	forward primer to amplify *AtUreG* with deletion of the first 26 aa, introducing NcoI site and Met in the aa-sequence
	1761	TACCATGGGACTCGCACCTCACTCAC	forward primer to amplify *AtUreG* with deletion of the first 41 aa, introducing NcoI site and Met in aa-sequence
	1762	TACCATGGGACCCATCTACTCTCCTGGC	forward primer to amplify *AtUreG* with deletion of the first 49 aa, introducing NcoI site and Met-Gly in aa-sequence
	1763	TACCATGGGTAACTTCTCTGAGAGAGCTTTTACC	forward primer to amplify *AtUreG* with deletion of the first 67 aa, introducing NcoI site and Met-Gly in aa-sequence
	1759	TACTGCAGTCACTGGCGCTTCTTCC	reverse primer to amplify AtUreG; introducing PstI site
pET30nco-CTH	1779	CTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATG G	1st annealing primer to generate pET30-CTH derivative (V48); introducing NcoI site instead of NdeI site
	1780	AATTCCATGGTATATCTCCTTCTTAAAGTTAAACAAAATTATTT	2nd annealing primer to generate pET30-CTH derivative (V48); introducing NcoI site instead of NdeI site