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Supplemental Information

The *Dionaea muscipula* Ammonium Channel *DmAMT1* Provides NH_4^+ Uptake Associated with Venus Flytrap's Prey Digestion

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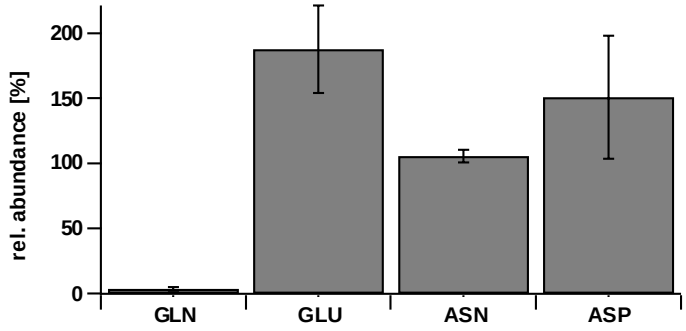
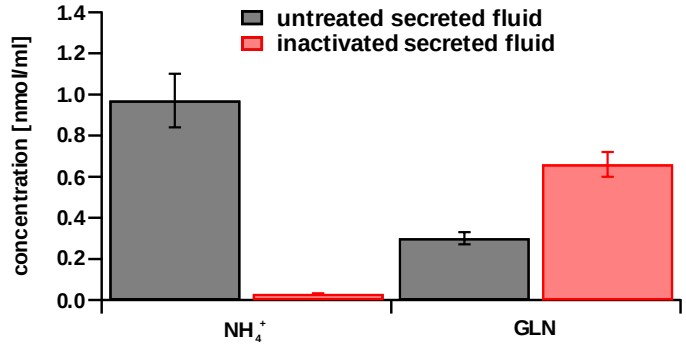
A

Concentration of NH_4^+ [mM], derived after digestion		
input	insect powder (*Creatonotos transiens*)	protein standard (casein)
5 mg	0.04 ± 0.01	0.61 ± 0.08
10 mg	0.09 ± 0.01	1.17 ± 0.09
20 mg	0.21 ± 0.07	2.65 ± 0.54

B



C



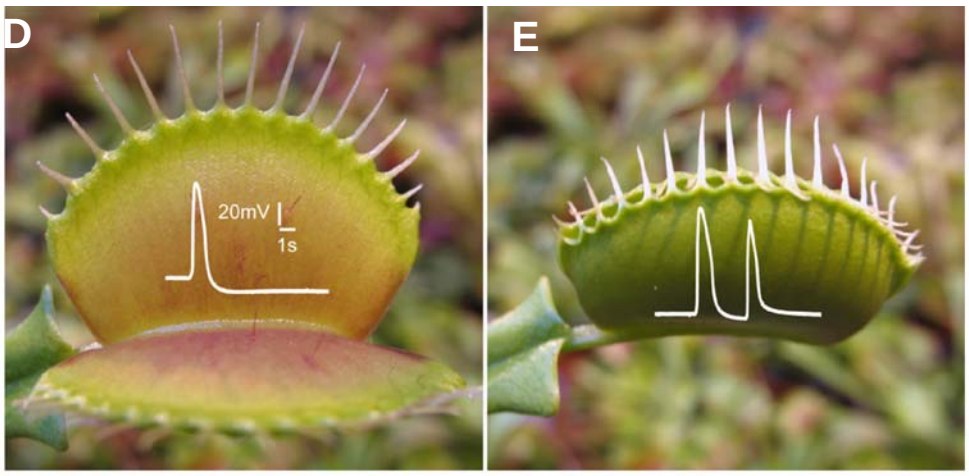
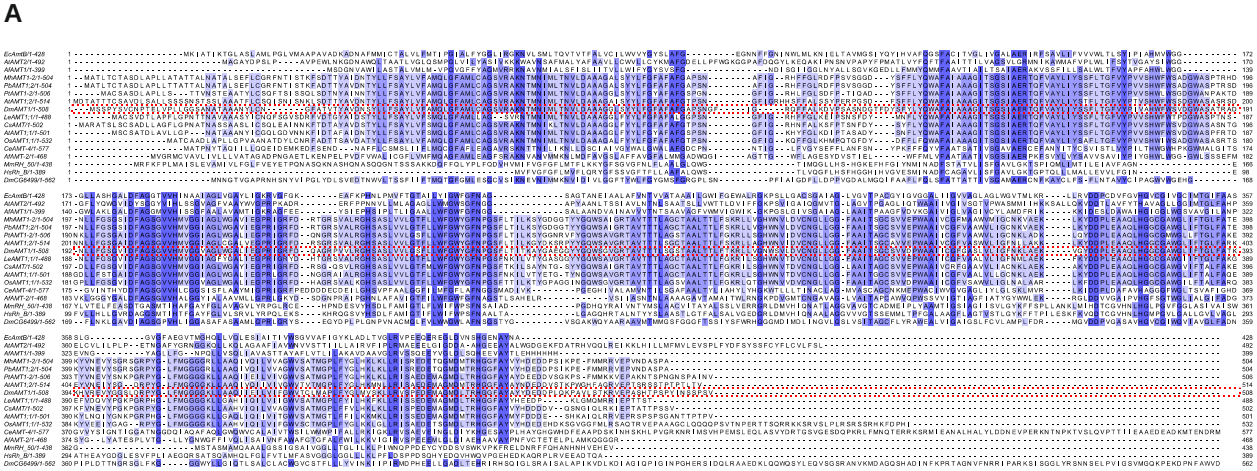
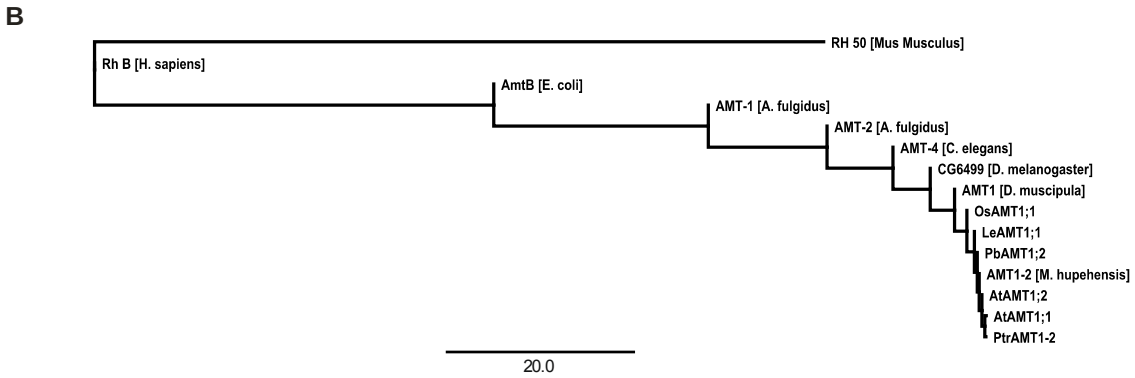


Figure S1. Related to Figure 1

(A): Production of NH_4^+ as a result of enzymatic reaction of insect powder or casein in the secreted fluid of *Dionaea muscipula* (n=4, mean ± SD). **(B):** Detected AS level in the casein digest normalized to its abundance in the protein. While asparagine (ASN) and aspartate (ASP) could be detected with values close to the abundance in casein (105.47 ± 8.44% and 150.72 ± 81.81%), glutamine (GLN) was almost absent (3.41 ± 2.25%) and the level of glutamate (GLU) was increased (187.76 ± 58.09%) (n=12, mean ± SD). **(C):** Ammonium and glutamine concentration in secreted fluid of *Dionaea muscipula*, after digestion of GLN (0.8 nmol/ml) in untreated or inactivated secretion fluid. Inactivation was performed by freezing (10 min/-20 °C) followed by addition of SDS (sodium dodecyl sulfate) (1 mM) and boiling (5 min) (n=3, mean ± SD). **(D):** Mechano-stimulation of trigger hairs by a prey evokes an action potential (AP), which spreads over the entire leaf lobe that is densely packed with gland cell complexes. **(E)**: Two action potentials close the Venus flytrap.





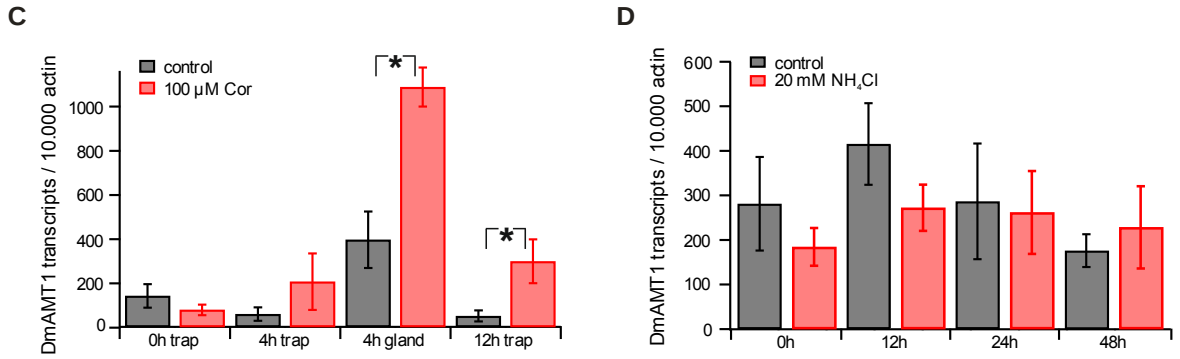


Figure S2. Related to Figure 2

(A) Sequences of AMT/MEP/Rh homologs. Alignment of 17 AMT/MEP/Rh proteins of various origins by clustralw2. AMT1 from *Dionaea muscipula* is boxed in red. Identical amino acids were highlighted in blue (jalview 2.7). EcAmtB: *E. coli* AmtB; AtAMT2: *A. thaliana* AMT2; AfAMT1: *A. fulgidus* AMT-1; MhAMT1-2: *M. hupehensis* AMT1-2; PbAMT1;2: *P. betulifolia* AMT1;2; PtAMT1-2: *P. trichocarpa* AMT1-2; AtAMT1;2: *A. thaliana* AMT1;2; DmAMT1: *Dionaea muscipula* AMT1;

LeAMT1;1: *L. esculentum* AMT1;1; CsAMT: *C. sinensis* ammonium transporter; AtAMT1;1: *A. thaliana* AMT1;1; OsAMT1;1: *O. sativa* AMT1;1; CeAMT-4: *C. elegans* AMT-4; AfAMT-2: *A. fulgidus* AMT-2; MmRH_50: *M. musculus* RH 50; HsRh_B: *H. sapiens* Rh B; DmCG6499: *D. melanogaster* ammonium transporter. **(B)** Phylogeny of ammonium transporters. This phylogenetic tree bases on an alignment with selected protein sequences of mammalian, plant, bacterial and fungal ammonium permeases of the AMT/MEP/Rh family. The tree was constructed with geneious using Neighbor-Joining [S1]. Scale bare indicates the genetic distance. Among the ammonium permeases DmAMT1 closes the gap between the plant field and other members of this family in different kingdoms. Although it shares a high identity-score (71) to OsAMT1;1 whereas it´s homolog in Drosophila has only a score of 28 (calculated by clustralw2). **(C)** DmAMT1 expression was upregulated in isolated glands already 4h after application of 100 µM coronatine. Transcripts of DmAMT1 were normalized to 10.000 molecules of DmACT. Asterisks indicate statistically significant upregulation by coronatine ($P < 0.01$ by one-way ANOVA)($n \geq 3$, mean ± SD). **(D)** Quantification of DmAMT1 transcript levels in response to ammonium. DmAMT1 transcripts in traps of *Dionaea muscipula* were normalized to 10.000 molecules of DmACT and expression was measured after application of H_2O (control) or 200 µl NH_4Cl at indicated time points. No significant differences could be observed between water and ammonium treatment ($P > 0.9$ by one-way ANOVA)($n \geq 5$, mean ± SE).

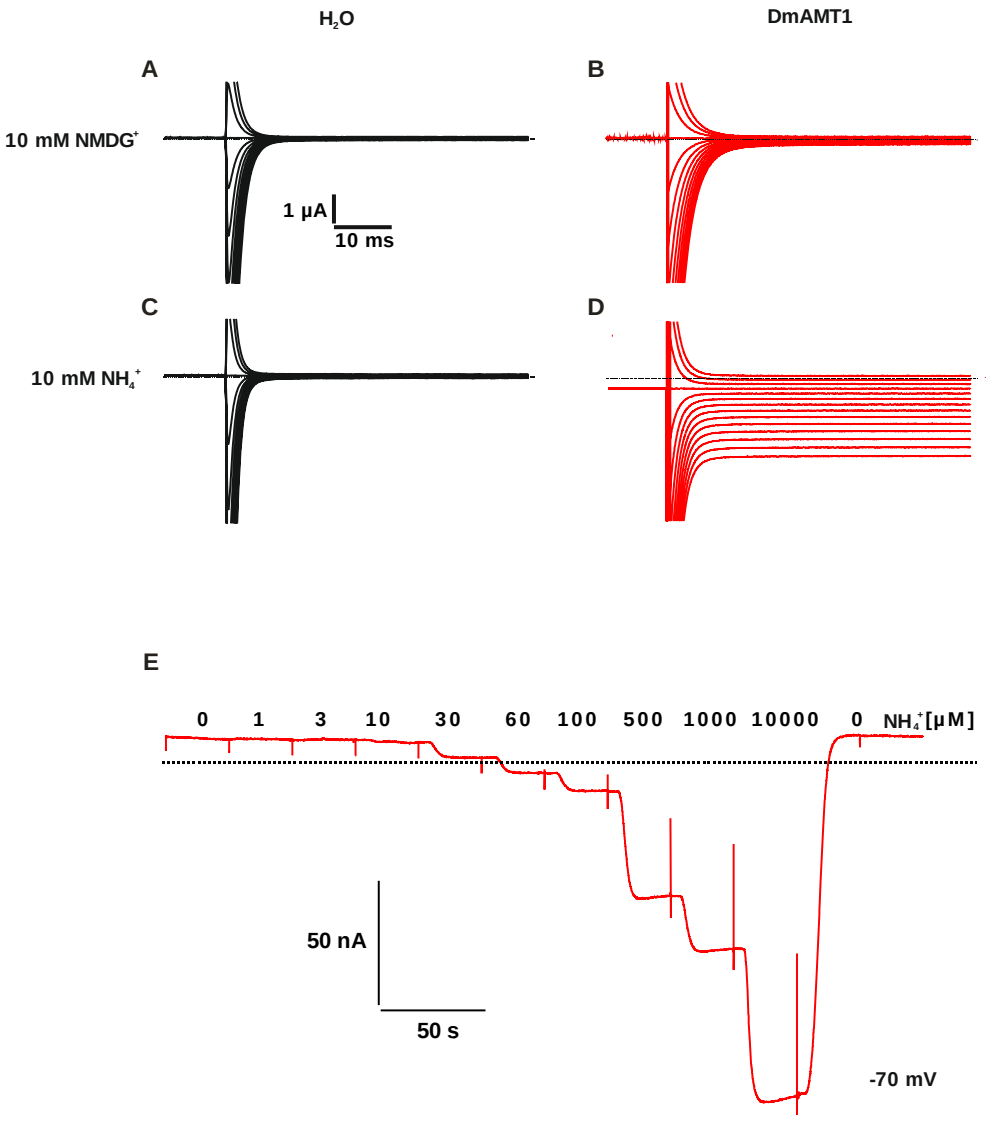


Figure S3. Related to Figure 3

Raw currents recorded at a typical voltage protocol changing the membrane potential from +60 to -200 mV in 20 mV decrements. Holding potential was 0 mV and dotted lines indicate 0 µA. No macroscopic currents are detectable with water-injected oocytes **(A)** as well as in DmAMT1 expressing oocytes without ammonium available **(B)**. Addition of 10 mM NH_4^+ leads neither to macroscopic currents in water-injected oocytes **(C)**, but an ammonium influx of up to 3 µA was detectable in DmAMT1 expressing oocytes **(D)**. **(E)** DmAMT1 mediated currents in *Xenopus* oocytes at the clamped membrane potential of -70 mV. NH_4^+ concentration in the bath medium was exchanged as indicated and the monovalent cation concentration was complemented with K^+ to 10mM. Dotted line represents 0 mV. Currents were recorded in standard bath solution.

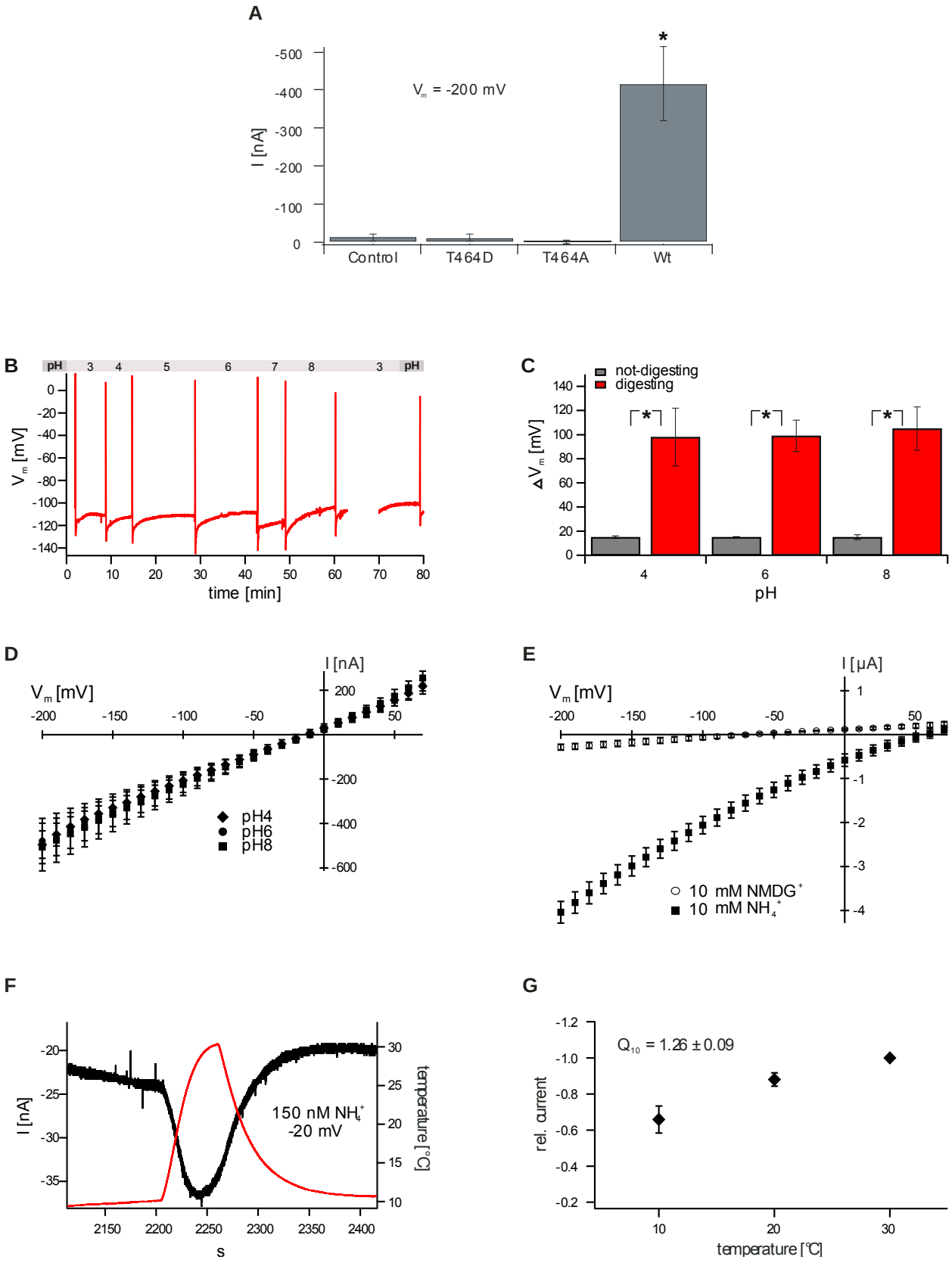

A
I [nA]
-500
-400
-300
-200
-100
0
V_m = -200 mV
*
Control
T464D
T464A
Wt
B
pH
3
4
5
6
7
8
3
pH
V_m [mV]
0
-20
-40
-60
-80
-100
-120
-140
0
10
20
30
40
50
60
70
80
time [min]
C
not-digesting
digesting
ΔV_m [mV]
140
120
100
80
60
40
20
0
*
*
*
4
6
8
pH
D
I [nA]
V_m [mV]
-200
-150
-100
-50
50
200
-200
-400
-600
pH4
pH6
pH8
E
I [µA]
V_m [mV]
-200
-150
-100
-50
50
1
-1
-2
-3
-4
10 mM NMDG+
10 mM NH4+
F
I [nA]
-20
-25
-30
-35
150 nM NH4+
-20 mV
30
25
20
15
10
temperature [°C]
2150
2200
2250
2300
2350
2400
s
G
rel. current
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
Q10 = 1.26 ± 0.09
10
20
30
temperature [°C]

Figure S4. Related to Figure 4

(A) Point mutation at T464 leads to inactive DmAMT1. Oocytes were injected with water, DmAMT1 T464D, T464A, and wild type RNA. Macroscopic currents were only recorded with wild type DmAMT1 expressing oocytes in standard bath solution containing 10 mM NH_4Cl. Statistically significant differences were only observed for wt- against mutant- and control-currents ($P < 0.01$ by one-way ANOVA)(n=6, mean ± SD). **(B)**: Membrane voltage responses of unstimulated *Dionaea muscipula* gland cells to different proton concentrations (as indicated). Just before each pH change an AP was mechanically evoked to confirm intracellular localization of measuring electrode. Changing the proton concentration of up to 100.000-fold did only weakly affect the resting potential. **(C**) pH dependency of ammonium-induced depolarization in glands. Significant differences were only observed between digesting and not-digesting plants, whereas depolarization within this two groups is not pH dependent ($P < 0.01$ by one-way ANOVA)(n=3, mean ± SD). **(D)** Current/voltage dependency under different pH values. Varying H^+ concentration by a factor of 10.000 didn't affect the voltage independent currents of DmAMT1. The reversal potentials (V_{rev}pH4 = 13.9 ± 0.88, V_{rev}pH6 = 11.8 ± 0.6, and V_{rev}pH8 = 14.2 ± 0.8 mV) as well as the positive outward currents at membrane potentials positive from V_{rev} suggest that the oocytes harbored contents of ammonium. Experiments were performed in standard bath solution containing 10 mM NH_4Cl (n=5, mean ± SD). **(E)** Ammonium influx in DmAMT1 expressing oocytes. Macroscopic currents of up to 4 µA were only detectable with 10 mM NH_4^+ in the bath solution. If $NMDG^+$ is presented instead only small ohmic leakage currents were investigated (n=5, mean ± SD). **(F)** Temperature dependency of DmAMT1 mediated ammonium currents. To minimize changes in the oocyte internal NH_4^+ concentration during measurement caused by large ammonium currents, low external concentration (150 µM) and a small electrical gradient (V_m = -20 mV) was used. Increasing the bath temperature from 10 to 30 °C (red line) resulted in rapid increasing inward-currents from 24 to 37 nA. This behavior was reversible by lowering the temperature again. **(G)** DmAMT1 functions as channel. Relative currents were recorded at V_m = -200 mV at 3 different temperatures. Q_{10} value was calculated at membrane potentials which resulted in distinct currents (V_m = -100 - -200 mV) and as mean factor between currents recorded at 10°C - 20°C, 20°C – 30°C and √ 10°C -30°C. Experiments were performed in standard bath solution containing 150 µM NH_4Cl (n=5, mean ± SD).

Supplemental References

S1. Ko JH, Yang SH, Han KH (2006) Upregulation of an *Arabidopsis* RING-H2 gene, *XERICO*, confers drought tolerance through increased abscisic acid biosynthesis. Plant J 47: 343-355.