Supplemental data.

Mutant	Orientation	5' → 3'			
H211A	Sense	CTCCGCCGTTGTC <u>GCT</u> ATGGTCGGCGGC			
	Antisense	GCCGCCGACCAT <u>AGC</u> GACAACGCCGGAG			
H211E	Sense	CTCCGGCGTTGTC <u>GAG</u> ATGGTCGGCGGCG			
	Antisense	CGCCGCCGACCAT <u>CTC</u> GACAACGCCGGAG			
H377A	Sense	GCGGCGCAGCTG <u>GCT</u> GGAGGGTGCGG			
	Antisense	CCGCACCCTCC <u>AGC</u> CAGCTGCGCCGC			
H377E	Sense	GCGGCGCAGCTG <u>GAG</u> GGAGGGTGCGGC			
	Antisense	GCCGCACCCTCC <u>CTC</u> CAGCTGCGCCGC			
H125A	Sense	CGGCCTCACAGACATC <u>GC</u> CGCCCAGAATTTAGAC			
	Antisense	GTCTAAATTCTGGGCG <u>GC</u> GATGTCTGTGAGGCCG			
H125R	Sense	GGCCTCACAGACATCC <u>G</u> CGCCCAGAATTTAGAC			
	Antisense	GTCTAAATTCTGGGCG <u>C</u> GGATGTCTGTGAGGCC			

 Table S1. Primers employed for the generation of the PvAMT1;1 mutants.

The underline bases are those that were changed to obtain the required mutation.

1) Calculation of the voltage dependence of PvAMT1; 1 affinity (K_m) for ammonium (δ) .

As shown in figure 2G of the main text, the affinity of PvAMT1;1 for ammonium (K_m), was affected by voltage and pH, observing that at acidic pH (5.5) the affinity for ammonium showed a strong voltage dependence, decreasing at more positive membrane potentials; dependence that became smaller as the pH increased to pH 7.0, and becoming almost voltage independent at pH 8.0 (Fig. 2G, main text). Assuming a single binding site for ammonium, as indicated by the Michaelis-Menten kinetics, we made use of the method of Woodhull {Document not in library: (1)} (equation 1) to evaluate the voltage dependence of K_m,

$$K_m(\delta) = K_m^{(0 mV)} * exp \left(\delta * e * \frac{V}{k*T}\right)$$
(1)

where δ is the fractional electrical distance, *e* is the elementary charge, *V* is the membrane potential, *k* is Boltzmann's constant, and *T* is the absolute temperature {Document not in library: (1)}. Employing the program Origin 8.5 (OriginLab Co., MA, USA) we adjusted the results from figure 2G to an exponential ($r^2 = \geq 0.95$) to derive the value of δ from the slope that allowed us to determine that the location of the putative ammonium binding site within the membrane electrical field seems to move towards the extracellular side as the external pH is alkalinized, changing from (δ) 34% at pH 5.5 to 30% at pH 7.0 and to 21% at pH 8.0, a response that may be due to a screening effect caused by the higher concentration of protons associated with acidic pH.

II) Determination of the Stoichiometry of *Pv*AMT1;1

As demonstrated in figures 2C and 2D of the main text, the reversal potential showed sub-Nernstian responses to changes in both, extracellular ammonium and proton concentrations (pH) with slope values of 33 ± 3.7 and 26 ± 3.5 mV, respectively. According to our interpretation, the thermodynamic functioning of *PvAMT1;1* can be explained by equation (2) that defines the activity of a symporter (2), rather than by the Nernst equation.

$$E_r = \frac{1}{1+r} \left[E_H + r E_{NH_4^+} \right]$$
(2)

where the stoichiometric ratio r = m/n and the Nernst potential for each ion, E_{H+} and E_{NH4+} , are given by equation 3 and 4, respectively:

$$n\mathrm{NH}_{4}^{+}{}_{\mathrm{o}} + m\mathrm{H}_{\mathrm{o}}^{+} \qquad \blacktriangleleft n\mathrm{NH}_{4}^{+}{}_{\mathrm{i}} + m\mathrm{H}_{\mathrm{i}}^{+} \qquad (3)$$

$$E_{\chi} = \frac{RT}{z_{\chi}F} \ln\left(\frac{[X]_o}{[X]_i}\right) \tag{4}$$

where z_x is the ionic valance, R is the gas constant, T is temperature, F is Faraday's constant and [X] the ion activities out (o) or inside (i) the cell.

Applying equation (2) to data from figures 2C and 2E we calculated an *r* value of 0.8 ± 0.2 (n=6), that indicates *Pv*AMT1;1 functions as an H⁺/NH₄⁺ symporter with a 1:1 stoichiometry. This ratio value correlates with the intracellular acidification caused by the presence of ammonium, and the stimulation of the inward ammonium currents induced by low pH, and clearly supports the H⁺/NH₄⁺ symport mechanism for *Pv*AMT1;1.

1. Woodhull, a M. (1973) The Journal of general physiology 61, 687-708

2. Accardi, A. and Miller, C. (2004) Nature 427, 803-7





Supplentary Figure 1

				I
Phaseolus vulgaris	FYY <mark>l</mark> fgfaf-	AFGSPS	NGFIGKH-	- YFGLTDIHA 126
Populus trichocarpa Populus trichocarpa	FYYLFGFAF-	AFGTPS	NGF IGKH-	- NEGLKNEPS 123
Populus trichocarpa Populus trichocarpa	FYYLFGFAF-	AFGTPS	NGF IGKH-	- NFGLKAFPS 126
Populus trichocarpa	FYYLFGFAF-	AFGTPS	NGFIGKH-	- NFGLKAFPS 126
Ricinus communis	FYYLFGFAF-	AFGGPS	<mark>NGFIGK</mark> H-	- HFGLESIPS 126
Ridinus communis	FYYLFGFAF-	AFGGPS	NGFIGKH-	- HFGLESIPS 126
Lotus japonicus	FYYLFGFAF-	AFGAPS	NGF IGRH-	- FFGLKDVPT 126
Lotus japonicus	FYYLFGFAF-	AFGAPS	NGF I GRH-	- FFGLKDVPT 127
Citrus sinensis x Poncirus trifoliata	FYYLFGFAF-	AFGTPS	NGF IGRH-	- NFALKSEPT 129
Vitis vinitera Vitis vinitera	FYYLFGFAF-	AFGSPS	NGE IGKH-	FEALKSIPT 123
Vitis vinifera	FYYLFGFAF-	AFGSPS	NGFIGKH-	- FFALKSIPT 123
Vitis vinifera	FYY <mark>l</mark> fgfaf-	AFG <mark>SPS</mark>	<mark>N</mark> GFIGKH-	- FFALKSIPT 123
Brassica napus	FYYLFGYAF-	AFGGSS	EGFIGRH-	- NFAL RDEPT 124
Arabidonsis thaliana	EYYLEGYAE-	AFGGSS	FGFIGRH-	- NEAL RDEPT 124
Arabidopsis thaliana	FYYLFGYAF-	AFGGSS	EGFIGRH-	- NFALRDFPT 124
Arabidopsis thaliana	FYY <mark>l</mark> fgyaf-	AFGGSS	EGFIGRH-	- NFALRDFPT 124
Arabidopsis thaliana Arabidopsis thaliana	FYYLFGYAF-	AFGESS	DGFIGRH-	- NEGLONEPT 124
Arabidopsis thaliana	FYYLFGYAF-	AFGSPS	NGFIGKH-	- YFGLKDIPT 120
Solanum lycopersicum	FYYLFGFAF-	ALGGPS	<mark>N</mark> GFI <mark>GR</mark> H-	- FFGLKEIPS 121
Populus tremula x Populus tremuloides	SYYLFGYAF-	AFGSPS	NGF IGRH-	- FFGLGDFPS 122
Populus trichocarpa Bicinus communia	STTEFGTAF-	AFGSPG	NGELGRH-	EFGLODEDS 195
Camellia sinensis	SYYLFGFAF-	AFGSGSRS	NAFIGHY-	- SFALTGVPS 128
Solanum lycopersicum	SYYLFGFAF-	AFGAPS	<mark>NGF I G</mark> KH-	- FFGLKEFPS 126
Vitis vinifera	SYYLFGFAF-	AFGSPS	NGF IGRH-	- FFGLKDFPS 126
Lotus japonicus Arabidonais thaliana	SYYLEGEAE.	AFGAPS	···NGFIGHH	SEEALSSYDE 132
Arabidopsis thaliana	SYYLFGFAF-	AFGTPS	NGF IGRHH	SFFALSSYPE 132
Camellia sinensis	FYYLFGFAF-	AF <mark>G</mark> SPS	NGFIGSH-	- FFALKSIPT 125
Camellia sinensis	FYYLFGFAF-	AFGSPS	NGFIGSH-	- FFALKSIPT 125
Ridinus communis	FYYIFGFAF-	AFGSPS	NGEV GGH-	- SFGLSKEPS 126
Lotus japonicus	FFYIFGFAL-	AFGTPS	NGFIGKH-	- FFGLNEFPS 126
Populus trichocarpa	FYYLFGFAF-	AFGSPS	NGFIGKQ-	- FFGLESFPS 125
Oryza sativa Onza sativa Japonica Group	FYYLFGFAF-	AFGTPS	NGE IGKO-	- FFGLKHMP- 112
Oryza sativa Japonica Group	FYYLFGFASL	RDCLRTPS	NGF IGKO-	- FFGLKHMPA 116
Oryza sativa Japonica Group	FYYLFGFAF-	AFGTPS	NGFIGKQ-	- FFGLKHMP - 112
Oryza sativa Indica Group	FYYLFGFAF-	AFGTPS	NGFIGKO-	- FFGLKHMP- 112
∠ea mays Zea mays	FYYLFGFAF-	AFGTPS	NGF I GKO-	FEGLORI D. 112
Triticum aestivum	FYYLFGFAF-	AFGTPS	NGF IGKH-	-FFGLKDMP- 113
Oryza sativa Japonica Group	FYY <mark>L</mark> FGFAF-	AFGAPS	<mark>N</mark> GFI <mark>GK</mark> H-	- FFGLKOVP- 112
Oryza sativa Japonica Group	FYYLFGFAF-	AFGAPS	NGF IGKH-	- FFGLKOVP- 112
Uryza sativa Triticum sestivum	EXVLEGEAE.	AFGTDS	NGELGKH.	FEGLEDVP- 112
Zea mays	FYYLFGFAF-	AYGTPS	NGFIGKH-	- FFGLKRLP- 111
Oryza sativa Japonica Group	FYYLFGFAF-	AFGTPS	KGFIGKO-	- FFGLKHMP- 114
Oryza sativa	FYYLFGFA	SRRTPS		- FFGLKHMP- 113
Vitis vinifera	FTTLFGFAF-	AFGBPB		
Triticum aestivum	FYYLFGFAF-	AFGTPS	<mark>N</mark> G <mark>F I</mark> G <mark>K</mark> H-	- FFGLKDMPO 114
Nepenthes alata				
Vitis vinifera Vitis vinifera	SYYFEGEAF-			DYFALKDIPN 94
Ricinus communis	SYYLFGFAF-	AFGVASSS	NPFIGT	CYFALKDIPN 94
Populus trichocarpa	SYYLFGFAF-	AFGDGTNS	NPFIGT	TFFALKDIPN 94
Solanum lycopersicum	SYYLFGFAF-	AFGDS	NPFIGA	SYFALKDIPS 91
Cydonia obionga Cucumis selivus	EYYLEGEAE.	AFGTPS	NPELGRH-	- FEGLKSIPS 55
Chlamydomonas reinhardtii	MWYLVGFGF-	AYGIGDNP	NGF IG D	ALFGLSRYSS 91
Populus trichocarpa			· · · · · · · · · · · · · · · ·	
Consensus	FYYLFGFAF-	AFGTPS	NGFIGKH-	- FFGLKDFPS
1.00	s VI AT F	IFĂ e⊼	ER 10 o	
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Supplementary Figure 2





Supplementary Figure 3

Legends to Supplementary Figures

Supplementary Figure 1. Inward currents were not activated in water-injected oocytes by ammonium or in *PvAMT1;1*-injected oocytes in the absence of the cation at different pH. A Current-voltage (I-V) relationships showing that increasing ammonium concentrations (\blacksquare 10, \bullet 20, \blacktriangle 80, \triangledown 250 and \diamond 1000 μ M) did not activate inward currents in water-injected oocytes. B Current-voltage relationships showing that changes in extracellular pH (\bullet 5.5, \circ 7.0 and \bigstar 8.0) had no effect on the currents recorded in the absence of ammonium in oocytes expressing *Pv*AMT1;1. Data represent the mean \pm SD from more than 7 oocytes derived from more than three frogs.

Supplementary Figure 2. H125 is present exclusively in *Pv***AMT1;1 among the plant ammonium transporters.** Sequence analysis of plant ammonium transporters showing the extracellular loop between transmembrane domains II and III. The arrow indicates the unique H125 in *Pv*AMT1;1; this residue is conserved among all the plant transporters and corresponds to Proline.

Supplementary Figure 3. Amino acids involved in ammonium transport in *Ec*AmtB are conserved in *Pv*AMT1;1., Pore region of *Ec*AmtB (A), *Pv*AMT1;1 (B) and *Pv*AMT1;1H211E (C) showing that the amino acids proposed to be involved in ammonium transport in *Ec*AmtB are conserved and maintain similar positions in the common bean homologue *Pv*AMT1;1 and the mutant PvAMT1;1H211E. The green spheres in (A) represent NH_4^+/NH_3 molecules. Ribbon representation of the *Ec*AmtB (D) and *Pv*AMT1;1 (E) monomers, observe the longer extracellular loops in the latter. In all the figures the periplasmic/extracellular side is above.