Supplementary data

Table S1. Primers used for gene expression analysis by quantitative real time polymerase chain reaction (qRT-PCR). List of genes, gene accessions, primers and references.

Gene	Gene accessions of pea or legume homologs	Primer sequences (5’-3’)	References
Amino acid permease 1 (AAP1)	AY956395	For: GCTGGAACCATTACTGGAGTAAATGA Rev: GACTCTGACGGTGGTGGTGCTTTTAC	Tegeder *et al*., 2000
Amino acid permease 2 (AAP2)	AY956396	For: AGCAAGCCACGAGGATAAGTATAGGC Rev: CAGTGAGTAAGTTTCCTGGCGATGTG	Tegeder *et al.,* 2000
Amino acid permease 3 (AAP3)	KX620905	For: CGTCACAGATTATTGAACATCAAGCA Rev: GGGTCAAAATCACGGGTTGAAATAGA	Tan *et al.,* 2010
Amino acid permease 6 (AAP6)	KX620908	For: GTTAACAGGAGTGCAAGTTGGGGTGG Rev: ATGATTTGGTTCTCTGGAGGGCTTGA	Dhandapani *et al.,* 2016
Cationic amino acid transporter 6 (CAT6)	Partial clone (cf. Tan 2010)	For: GGTTCGGAGTGTTTTCGGCTGTTACG Rev: TGAAACTTTGGTCCACTCTTTCTTTTGA	Tan, 2010
Asparagine synthetase 1 (AS1)	X52179	For: CTGTCACTGCTAGATACCTTGCTGGT Rev: GAGACATCAGAAACATAGGTGTTGCA	Tsai and Coruzzi, 1990
Asparagine synthetase 2 (AS2)	X52180	For: CCATCACTTCTCGCTACCTAGCAACC Rev: TCGACATGAGAAACATAGGCGTGCTT	Tsai and Coruzzi, 1990
L-Asparaginase (ASNase)	*Glycine max*; AY096000 *Arachis hypogaea*; DQ889570 *Phaseolus vulgaris*; DQ300284	For: GTYATGGAHAARTCYCCDCATTCCTA Rev: AMCARGATTGYDTTTGCYTCCTTTGC	Tan *et al.,* 2010
Glutamate synthase-NADH dependent (GOGAT)	*Phaseolus vulgaris;* AF314925 *Medicago sativa;* L37606	For: CACAGATTGCATWGGAACATCCATTC Rev: CCATTTTCATCTCCCAMAAACCTCTTAG	Tan *et al.,* 2010
Glutamine synthetase 2, chloroplastic (GS2)	M20664	For: GAAAATGGCACCATCAATAGGGTAGAG Rev: AGGGATGCGAAACAGGCTTTGATATG	Tingey *et al.,* 1988
Ammonium facilitator 3 (AMF)	*Glycine max*; Glyma08g06880 *Medicago truncatula;* Medtr2g010350 *Pisum sativum*; CD860511.1	For: CTCGCCTCTTGTCGGAGTTGTCC Rev: CGGTGGTTGTACTTGTCTCCTCTAC	-
Nodulin26-like intrinsic protein 1 (NIP1)	AJ243308	For: CACCGATAACAGAGCGATTGG Rev: TCCATATTCCTCGATATTCATTGTG	Shuurmans *et al.,* 2003
Elongation factor 1α (EF1α)	X96555	For: CAGTGGGACGTGTTGAAACTGGTGTT Rev: ATCGAACATTGTCTCCTGGAAGAGCC	Cong *et al.,* 2000
Ubiquitin (Ubi)	L81139, L81140, L81141, L81142	For: GGCAAAAATACAGGACAAGGAGGGAA Rev: CGCAAMACHAGGTGRAGAGTRGACTC	Xia and Mahon, 1998

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Table S2. Concentrations and spectrum of free amino acids in the xylem and roots of nodulated *PsAAP6*-miR plants. Data are presented as means with standard deviation (± SD). Significant differences are indicated by asterisks (Student's *t*-test; *, p<0.05; **, p<0.001). Values for **%**Δ describe the percentage change between *PsAAP6*-miR and *GFP*-miR control plants.

Amino acids	**Xylem** (µM)			**Root** (nmol/mg DW)		
	***GFP*-miR**	***AAP6*-miR**	**%Δ**	***GFP*-miR**	***AAP6*-miR**	**%Δ**
Ala	38.05 ± 5.14	46.75 ± 10.10		0.60 ± 0.11	0.49 ± 0.08	
Arg	188.24 ± 12.10	167.47 ± 16.37*	-11	1.42 ± 0.25	1.08 ± 0.14*	-24
Asn	**11543.22 ± 987.22**	**8352.38 ± 2648.52***	**-28**	**12.13 ± 2.50**	**7.62 ± 0.91***	**-37**
Asp	3153.10 ± 380.06	2773.79 ± 519.33		1.93 ± 0.84	1.32 ± 0.23	
Cys	7.58 ± 1.03	8.37 ± 1.24		ND	ND	
Glu	199.81 ± 32.39	160.12 ± 12.93*	-20	4.14 ± 0.91	2.69 ± 0.68*	-35
Gln	3993.25 ± 508.25	3110.05 ± 787.87*	-22	4.48 ± 1.08	3.47 ± 0.80*	-23
Gly	16.19 ± 4.44	16.30 ± 2.45		0.69 ± 0.08	0.75 ± 0.01	
His	79.35 ± 17.21	60.81 ± 23.37		0.19 ± 0.03	0.19 ± 0.02	
Hse	991.85 ± 81.14	860.34 ± 244.88		10.87 ± 2.79	5.12 ± 0.28*	-53
Ile	37.58 ± 4.57	42.88 ± 8.92		0.20 ± 0.04	0.18 ± 0.03	
Leu	42.19 ± 8.95	48.83 ± 13.64		0.23 ± 0.04	0.22 ± 0.04	
Lys	64.10 ± 15.71	73.80 ± 22.47		0.37 ± 0.08	0.35 ± 0.06	
Met	0.46 ± 0.26	0.55 ± 0.17		0.07 ± 0.01	0.05 ± 0.01*	-17
Phe	26.30 ± 6.47	23.38 ± 4.12		0.29 ± 0.01	0.27 ± 0.01*	-7
Pro	9.69 ± 2.55	10.73 ± 2.85		0.11 ± 0.02	0.11 ± 0.01	
Ser	33.10 ± 10.93	31.95 ± 3.69		0.71 ± 0.14	0.75 ± 0.14	
Thr	71.64 ± 9.04	70.66 ± 21.18		0.88 ± 0.13	0.74 ± 0.10*	-16
Tyr	12.02 ± 2.76	9.12 ± 2.43		0.61 ± 0.40	0.65 ± 0.39	
Val	85.12 ± 16.91	86.98 ± 17.09		0.55 ± 0.07	0.53 ± 0.06	
Total	**21323.571 ± 1201.81**	**17327.23 ± 2549.27***	**-19**	**41.42 ± 7.47**	**28.49 ± 2.93***	**-31**

Table S3. Concentrations and spectrum of free amino acids in the stem and leaves of nodulated *PsAAP6*-miR plants. Data are presented as means with standard deviation (± SD). Significant differences are indicated by asterisks (Student's *t*-test; *, $p<0.05$; **, $p<0.001$). Values for **%**∆ describe the percentage change between *PsAAP6*-miR and *GFP*-miR control plants.

Amino acids	**Stem** (nmol/mg DW)			**Leaf** (nmol/mg DW)		
	***GFP*-miR**	***AAP6*-miR**	**%∆**	***GFP*-miR**	***AAP6*-miR**	**%∆**
Ala	3.49 ± 0.87	2.82 ± 0.54		3.35 ± 0.51	1.16 ± 0.4**	-65
Asn	13.15 ± 2.39	12.85 ± 1.85		0.35 ± 0.16	0.19 ± 0.06*	-45
Asp	2.43 ± 0.36	2.21± 0.51		2.66 ± 0.22	1.09 ± 0.36**	-59
Cys	0.02 ± 0.01	0.02 ± 0.01		0.25 ± 0.08	0.93 ± 0.65*	270
Glu	12.1 ± 2.42	9.83 ± 1.64		13.18 ± 2.18	4.99 ± 2.08**	-62
Gln	3.76 ± 1.55	3.18 ± 0.98		0.90 ± 0.42	0.30 ± 0.10**	-66
Gly	0.82 ± 0.37	0.71 ± 0.36		2.84 ± 0.51	1.01 ± 0.47**	-64
His	0.30 ± 0.11	0.05 ± 0.02*	-41	0.15 ± 0.04	0.05 ± 0.02**	-69
Hse	7.73 ± 2.94	3.75 ± 1.09*	-51	1.98 ± 0.66	0.72 ± 0.04*	-63
Ile	0.51 ± 0.15	0.33 ± 0.06*	-35	0.20 ± 0.06	0.05 ± 0.02**	-73
Leu	0.304 ± 0.11	0.20 ± 0.06*	-34	0.19 ± 0.06	0.07 ± 0.04*	-60
Lys	0.47 ± 0.12	0.44 ± 0.08		0.27 ± 0.05	0.15 ± 0.05*	-45
Phe	0.34 ± 0.04	0.26 ± 0.02*	-24	0.73 ± 0.11	0.23 ± 0.10**	-68
Pro	4.36 ± 3.36	0.59 ± 0.43*	-86	1.23 ± 0.47	0.38 ± 0.18*	-70
Ser	2.08 ± 0.79	1.74 ± 0.43		1.22 ± 0.42	0.52 ± 0.15*	-58
Thr	1.69 ± 0.47	1.09 ± 0.27*	-36	1.08 ± 0.25	0.33 ± 0.15**	-69
Tyr	0.03 ± 0.01	0.07 ± 0.05		1.49 ± 0.14	0.68 ± 0.48*	-54
Val	0.90 ± 0.18	0.67 ± 0.20		0.43 ± 0.06	0.15 ± 0.05**	-65
Total	**56.15 ± 15.39**	**43.55 ± 6.86***	**-22**	**32.96 ± 8.52**	**16.15 ± 7.30***	**-51**

Fig. S1. Comparison of pea and Arabidopsis amino acid transporter cDNA sequences. For gene accessions see Table S1 and http://aramemnon.uni-koeln.de/.

	PsAAP6	***PsAAP1***	***PsAAP2***	***PsAAP3***	***PsCAT6***	***AtAAP1***	***AtAAP2***	***AtAAP3***	***AtAAP4***	***AtAAP5***	***AtAAP6***	***AtAAP7***	***AtAAP8***
PsAAP6	**100**	60.9	56.4	56.4	43	65.6	59.7	62.2	60.1	60.3	71.8	57.9	64.4
PsAAP1	60.9	**100**	60.4	63.6	43.3	59.2	68.4	68.5	65	66.9	61.8	58.6	58.7
PsAAP2	56.4	60.4	**100**	65.7	39.1	53.8	60.4	61.4	62.6	59.1	56.4	52.5	53.2
PsAAP3	56.4	63.6	65.7	**100**	45	55.8	64.3	61.5	60.7	59.1	57	51.5	54.2
PsCAT6	43	43.3	39.1	45	**100**	44.6	42.4	43.4	38.9	41.8	40.4	41.8	42.1
AtAAP1	65.6	59.2	53.8	55.8	44.6	**100**	59.9	60.7	59.3	59.7	69.7	55.6	74.5
AtAAP2	59.7	68.4	60.4	64.3	42.4	59.9	**100**	68.8	77.5	64.9	59.5	55.7	58.3
AtAAP3	62.2	68.5	61.4	61.5	43.4	60.7	68.8	**100**	68.7	68.9	63	59.8	62.2
AtAAP4	60.1	65	62.6	60.7	38.9	59.3	77.5	68.7	**100**	63.5	60.2	54.6	59.4
AtAAP5	60.3	64.9	59.1	59.1	41.8	59.7	64.9	68.9	63.5	**100**	61.5	55.9	60
AtAAP6	71.8	61.8	56.4	57	40.4	69.7	59.5	63	60.2	61.5	**100**	56.7	66.1
AtAAP7	57.9	58.6	52.5	51.5	41.8	55.6	55.7	59.8	54.6	55.9	56.7	**100**	58.4
AtAAP8	64.4	58.7	53.2	54.2	42.1	74.5	58.3	62.2	59.4	60	66.1	58.4	**100**

Fig. S2. Organ-expression analysis of *PsAAP6.* RT-PCR was performed using total RNA from nodulated pea plants grown in potting soil (Sunshine potting mix LC-1 consisting of 70% peat, 20% perlite and 10% limestone) in the greenhouse. Cot, cotyledon; Lf, leaf; Nd, nodule; PW, pod wall; Rt, root; SC, seed coat; St, stem; *18S*, *18 S* ribosomal RNA.

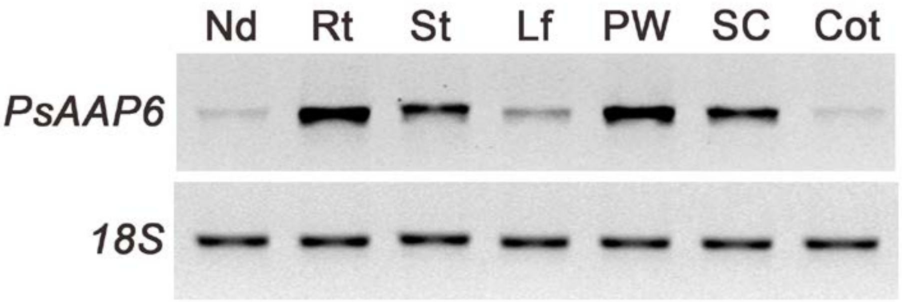

Fig. S3. Alignment of *PsAAP6* amiRNA sequence with pea *AAP* and *CAT6* sequences. Mismatches are marked yellow.

GGCGCGTGCTGTAAGGACAT **- PsAAP6 miRNA ‘targeting’ coding sequence**
GGAGCGTGCTGTATGGACAT **- PsAAP6 sequence targeted**
20 nucleotides (nt) – 2nt mismatches

GGCG_CGTGCTGTAAGGACAT **- PsAAP6 miRNA ‘targeting’ coding sequence**
GGATACGCAGCGTTTGGAAAT - **PsAAP1 sequence**
10nt mismatches (50% difference)

GGCGC_GTGCTGTAAGGACAT **- PsAAP6 miRNA ‘targeting’ coding sequence**
GGAGCAGTTGGGTTTTGGCCT **- PsAAP2 sequence**:
10nt mismatches (50% difference)

GGCGCGTGCTGTAAGGACAT **- PsAAP6 miRNA ‘targeting’ coding sequence**
GGGGAGTTTTCCAAGGTCTT **- PsAAP3 sequence**
10nt mismatches (50% difference)

GGCGCGTGCTGTA_AGGACAT **- PsAAP6 miRNA ‘targeting’ coding sequence**:
GGAGAGTTCAGTGGTGAATAT **- PsCAT6 sequence**:
9nt mismatches (45% difference)