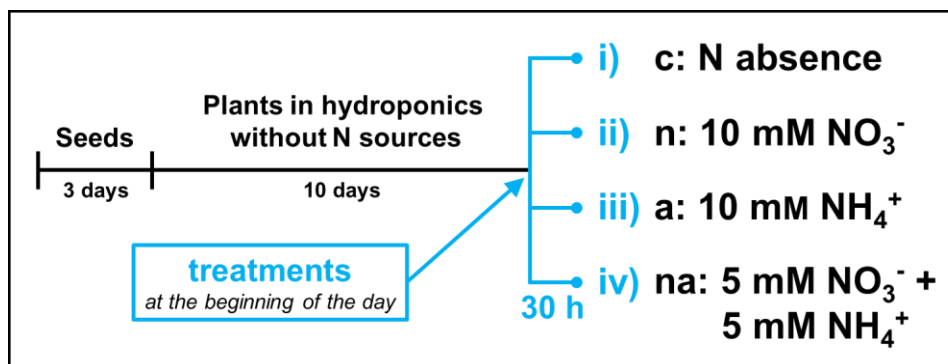


ADDITIONAL FILE 1

Additional material of the manuscript “Mineral nitrogen sources differently affect root glutamine synthetase isoforms and amino acid balance among organs in maize”

Bhakti Prinsi and Luca Espen



Additional Figure A1. Experimental design. T-250 maize seeds germinated in the dark. After 3 days, the seedlings were grown in a hydroponic system for 10 days in absence of N. Afterwards the plants were incubated for further 30 h in four different conditions: i) N absence (c); ii) 10 mM NO₃⁻ (n); iii) 10 mM NH₄⁺ (a); iv) 5 mM NO₃⁻+5 mM NH₄⁺ (na). For details, see the materials and methods section and Additional Table 1.

Additional Table A1. Composition of the hydroponic solutions used for the 30 h treatments. Each solution was composed of 400 µM CaSO₄, 175 µM KH₂PO₄, 100 µM MgSO₄, 20 µM Fe-EDTA, 5 µM KCl, 2.5 µM H₃BO₃, 0.2 µM CuSO₄, 0.2 µM ZnSO₄, 0.2 µM MnSO₄, 0.05 µM Na₂MoO₄ added according to the following treatments and adjusted to a pH=6.1.

Abbreviation	Condition	Specific composition	
c	Control in nitrogen absence	K ₂ SO ₄	5 mM
n	10 mM NO ₃ ⁻	KNO ₃	10 mM
a	10 mM NH ₄ ⁺	(NH ₄) ₂ SO ₄	5 mM
		K ₂ SO ₄	5 mM
na	5 mM NO ₃ ⁻ +5 mM NH ₄ ⁺	KNO ₃	5 mM
		(NH ₄) ₂ SO ₄	2.5 mM
		K ₂ SO ₄	2.5 mM

Additional Figure A2. 1D-electrophoretic profiles of leaf protein samples and mass spectrometry characterization of the most prominent band in the profiles. Leaf samples from plants grown for 10 days without N sources and then exposed for the last 30 h to absence of N (c), to 10 mM NO₃⁻ (n), to 10 mM NH₄⁺ (a) or to 5 mM NO₃⁻+5 mM NH₄⁺ (na) were analysed by 1D-SDS-PAGE. The bands in the dotted boxes were individually picked from the gel and analysed by LC-nESI-MS/MS. In red are reported the sequenced peptides. The data are the sums of all the analyses reporting the unique peptides with the best score assignment. **n**: spectrum number; **%f**: frequency of identification of the peptides among all the analyses; **z**: charge state; **Local FDR (%)**: Local False Discovery Rate (%); **SPI (%)**: Score Peak Intensity (%); **Sequence map**: forward-slashes for locations of y-ions, backslashes for locations of b-ions, vertical lines for locations of both b- and y-ions. The abundance (%) was evaluated as the percentage of the sum of spectra related to pyruvate orthophosphate dikinase respect the total spectra intensity of the band. No peptide related with nitrate reductase was ever detected.

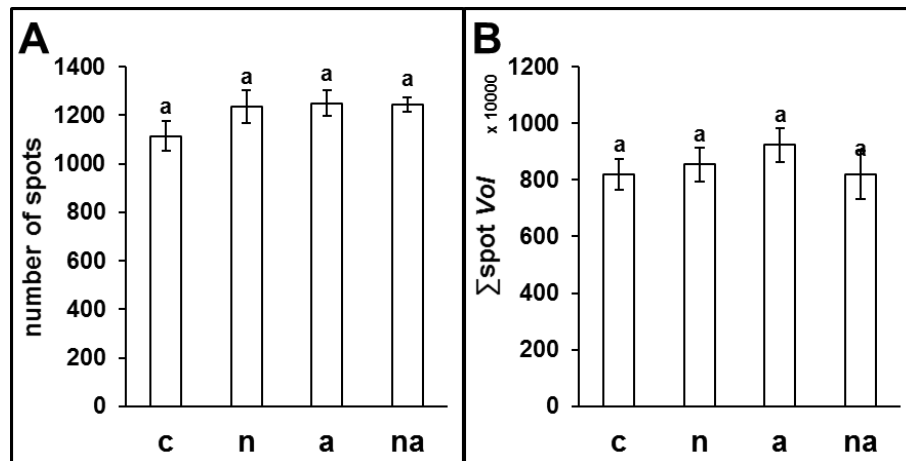


462/947 AA: 48.8%. Total peptide (unique): 149 (29). Reproducibility: 100%. Abundance (%) in 1D-SDS-PAGE band: 65.6% ± 2.8.

n	%f	z	Score	Local FDR (%)	DB Fwd-Rev Score	SPI (%)	Sequence map	MH ⁺ Matched	MH ⁺ Mass Shift (Da)	MH ⁺ Error (ppm)
1	1/4	3	15.35	<0.1%	15.35	85.7	(R)AALVADEIAEQAEFFSFGTNDLTQMTFGYSR(D)	3429.594	-0.0049	-1.4
2	3/4	2	23.26	<0.1%	23.26	90.9	(R)A EIT/S/P E/D/V/G/G M H/A/A/V/G I/LT E/R(G)	2140.034	0.0074	3.5
3	4/4	2	20.26	<0.1%	20.26	94	(K)A G/L/D/Y/V/S/C/S/P F/R(V)	1371.636	0.0036	2.6
4	2/4	2	17.72	<0.1%	12.22	84.5	(R)A/m/D/I/L/P V/T/I/R(L)	1072.582	15.9988	3.6
5	1/4	2	17.11	<0.1%	17.11	73.5	(K)C/C/V/S/G/C/S/G I/R(V)	1155.47	0.0106	9.1
6	3/4	2	24.37	<0.1%	24.37	98.2	(R)E G E/W/L/S/L/N/G/S T/G E/V/I/L/G K(Q)	1888.965	-0.0028	-1.5
7	4/4	2	21.63	<0.1%	21.63	98	(K)E M/Q/D/I/E/F/T/V/Q/E/N(R)	1638.743	0.0037	2.3
8	4/4	2	21.49	<0.1%	21.49	93	(K)E m/Q/D/I/E/F/T/V/Q/E/N(R)	1638.743	15.9977	1.7
9	1/4	2	16.87	<0.1%	16.87	71	(K)F I/P V Y/L/A Q/G I/L/Q/H/D/P F E/V/L/D/Q R(G)	2598.372	0.002	0.8
10	4/4	2	22.03	<0.1%	22.03	97.2	(R)F L/D/M/F/G/N V/V/M/D/I/P R(S)	1653.813	0.0086	5.2
11	4/4	2	23.14	<0.1%	23.14	94.8	(R)F L/D/M/F/G/N V/V/M/D/I/P R(S)	1653.813	15.9984	2.1
12	4/4	2	25.13	<0.1%	25.13	97.9	(R)F L/D/M/F/G/N V/V/M/D/I/P R(S)	1653.813	16.0014	3.9
13	4/4	2	23.01	<0.1%	23.01	87.8	(R)F L/D/M/F/G/N V/V/M/D/I/P R(S)	1653.813	31.9908	0.6
14	4/4	2	22.2	<0.1%	22.2	95	(K)G/L/K/N/D/I/T D/LT/A/S/D/L/K(E)	1490.77	0.0038	2.6
15	4/4	2	26.74	<0.1%	26.74	97.3	(R)GTAVNVQCmVFGNMGNTSGTGVLFRTR(N)	2718.28	0.0051	1.9
16	1/4	2	18.6	<0.1%	18.6	87.1	(R)GTAVNVQCmVFGNMGNTSGTGVLFRTR(N)	2718.28	15.9931	-0.7
17	2/4	2	23.7	<0.1%	23.7	86.2	(R)GTAVNVQCmVFGNMGNTSGTGVLFRTR(N)	2718.28	15.9931	-0.7
18	4/4	2	23.38	<0.1%	19.31	98.6	(K)I A/V/D/M/V/N/E/G/L/V/EIP R(S)	1541.799	0.0033	2.2
19	4/4	2	24.99	<0.1%	21.72	96.1	(K)I A/V/D/M/V/N/E/G/L/V/EIP R(S)	1541.799	16.0001	3.3
20	1/4	3	15.98	<0.1%	15.98	93.6	(K)K Q L E L A V/LA/V/F/N/S/W E/S/P R(A)	2087.128	0.0012	0.6
21	4/4	2	23.66	<0.1%	19.89	96.7	(R)I G/I/S/Y/P E/L/T/E/M/Q A R(A)	1607.81	0.0028	1.7
22	4/4	2	23.98	<0.1%	18.75	92.5	(R)I G/I/S/Y/P E/L/T/E/M/Q A R(A)	1607.81	15.9994	2.7
23	1/4	3	21.36	<0.1%	21.36	91.7	(R)LLDPPLHEFLPEGNIEDIVSELCAETGIANQEDALAR(I)	3975.928	-0.0032	-0.8
24	4/4	2	23.25	<0.1%	15.54	100	(K)L/S/E/V/N/P M/L/G F/R(G)	1262.656	0.0032	2.5
25	1/4	2	20.77	<0.1%	20.77	95.2	(K)L/S/E/V/N/P M/L/G F/R(G)	1262.656	15.9994	3.5
26	1/4	2	20.8	<0.1%	14.88	96.6	(K)L/V/T/I/G/G H V/L/R(E)	1064.658	0.0028	2.7
27	4/4	2	27.03	<0.1%	27.03	96.6	(K)I Y/G E/F/L/V/N/A/Q/G E/D/V/V/A/G I/R(T)	2050.06	0.0051	2.5
28	1/4	2	13.21	<0.1%	13.21	76.2	(K)M V/E/P G H L D/Q/L L/H/P Q/F E/N/P S A Y/K(D)	2550.245	0.001	0.4
29	4/4	2	17.94	<0.1%	17.94	85.9	(K)N/D/I/T/D/L/T/A/S/D/L/K(E)	1192.569	0.0029	2.5
30	3/4	2	19.03	<0.1%	19.03	92.2	(R)N/N/G/A/Q/G/I/G/L/C/R(T)	1159.564	0.0029	2.5
31	4/4	2	24	<0.1%	24	100	(K)Q L E/L/A/V/LA/V/F/N/S/W/E/S/P R(A)	1959.033	0.0029	1.5
32	3/4	3	20.64	<0.1%	20.64	96.1	(K)Q P L S P P A L/SIG D/L/G/T/F/M/A/W/V/D D/V/R(K)	2472.223	0.004	1.6
33	2/4	2	24.69	<0.1%	20.89	89.1	(K)Q P/L/S/P P A L/S/G D/L/G T F/m A W/V/D/D/V/R(K)	2472.223	15.9942	-0.3
34	4/4	2	24.75	<0.1%	24.75	90.3	(R)SGAAVSMGMMMDTVLNLGLNDEVAAGLAAK(S)	2903.431	-0.0002	-0.1
35	3/4	2	24.32	<0.1%	24.32	82.8	(R)SGAAVSMGMMMDTVLNLGLNDEVAAGLAAK(S)	2903.431	15.9946	-0.1
36	1/4	2	24.92	<0.1%	24.92	90.8	(R)SGAAVSMGMMMDTVLNLGLNDEVAAGLAAK(S)	2903.431	15.9952	0.1
37	4/4	2	21.38	<0.1%	21.38	96.5	(R)T/E/H/I/M/F/I/A/S D/E/R(I)	1369.584	0.0034	2.5
38	4/4	2	21.4	<0.1%	14.63	100	(R)T/P/E/D/L/D/A/M/K(D)	1019.471	0.0025	2.5
39	3/4	2	18.96	<0.1%	18.96	78.8	(R)T/P/E/D/L/D/A/M/K(D)	1019.471	15.9981	3.1
40	1/4	2	14.3	<0.1%	14.3	74.5	(K)V/G I C G E/H/G/G/E/P S S V/A/F/I/A/K(T)	1948.922	0.0036	1.8
41	2/4	2	17.69	<0.1%	14.38	90.1	(K)V/G/T/I/M/I/E/I/P/R(A)	1015.561	0.0034	3.4
42	1/4	2	17.31	<0.1%	17.31	82.2	(K)V/G/T/I/M/I/E/I/P/R(A)	1015.561	15.9974	2.5
43	4/4	2	23.22	<0.1%	23.22	100	(K)V/LA/N/A/D/TIP D D/A LT A R(N)	1542.776	0.0027	1.7

Evaluation of the 2D-electrophoretic procedure

Additional Figure A3. Evaluation of 2D-electrophoretic reproducibility among protein samples from root system of maize plants exposed to the four nutritional treatments. The graph shows the number of spots **(A)** and the sum of volume of all the spots **(B)** in the 2D-profile obtained from maize plants grown for 10 days without N sources and then exposed for the last 30 h to absence of N (c), to 10 mM NO_3^- (n), to 10 mM NH_4^+ (a) or to 5 mM NO_3^- +5 mM NH_4^+ (na). Data are expressed as average values $\pm$ SE (n=6). The upper letters indicate no differences among the four treatments according to Student's t-test ($p < 0.05$).



Spot characterization by LC-nESI-MS/MS

Supplementary data about spot identification by LC-nESI-MS/MS analysis. The results derive from four independent biological samples analysed in triplicate (n = 12). The data are the sums of all the replicates. In red are reported the sequenced peptides, among which the underlined ones are those that allowed discriminating among the different isoforms. The putative transit peptide is reported in green. In the table are reported the unique peptides with the best score assignment. **n**: spectrum number; **s**: specificity, those with the * are the discriminating peptides; **%f**: frequency of identification of the specific peptides among all the replicates (n=12); **z**: charge state; **Local FDR (%)**: Local False Discovery Rate (%); **SPI (%)**: Score Peak Intensity (%); **Sequence map**: forward-slashes for locations of y-ions, backslashes for locations of b-ions, vertical lines for locations of both b- and y-ions. Spot numbers and acronyms refer to Fig. 3, Fig. 4 and Table 1 of the manuscript.

Additional Table A2

SPOT 1 – GS2a

>gil121341|sp|P25462.1|GLNAC_MAIZE RecName: Full=Glutamine synthetase, chloroplastic; AltName: Full=GS2; AltName: Full=Glutamate--ammonia ligase; Flags: Precursor

MAQAVVPAMQCRVGVKAAAGRVWSAGRTTRTGRGGASPGFKVMAVSTGSTGVVPRLEQLLNMDTTPYTDK VIAEYIWWGGSGIDIR
SKSR TISKPVEDPSELPK WNYDGSSTGQAPGEDSEVILYPQAFKDPFRGGNNVLVICDTYTPQGEPLPTNKRHR AAQIFSDPKVGEQ
VPWFGEIEQYETLLQKDVNWPLGWPVGGFPGPQGPYYCAVGADKSFGR DISDAHAKLYAGINISGTNGEVMPGQWEYQVGPSVG
IEAGDHIWISRYILERITEQAGVVLTLDPKPIQGDWNGAGCHTNYSTKTMR EDGGFEEIKRAILNLSLRHDLHISAYGEGNERRLTGK H
ETASIGTFWSGVANR GCSIRVGRDTEAKGKGYLEDRRPASNMDPYIVTGLLAETTLWQPSLEAEALAAKKLALKV

94/423 AA: 22.2%

Total peptides (unique): 58 (9)

Isoform specific peptides: 9

Specificity reproducibility (%): 100%

n	s	%f	z	Score	Local FDR (%)	Database Fwd-Rev Score	SPI (%)	Sequence Map	MH ⁺ Matched (Da)	MH ⁺ Mass Shift (Da)	MH ⁺ Error (ppm)
1	*	83	2	19.48	<0.1%	19.48	95.6	(R)A/AQ F S/D P K(V)	976.51	0.0043	4.4
2	*	58	2	17.24	<0.1%	4.21	94.3	(R)A/I L N L S/L/R(H)	899.567	0.0044	4.9
3	*	33	2	19.17	<0.1%	10.13	97.8	(R)D/I S D/A H Y K(A)	948.442	0.0042	4.4
4	*	8	2	15.86	<0.1%	15.86	77.4	(R)E/D G/G/F/E/E I/K(R)	1023.463	0.0084	8.2
5	*	25	2	23.41	<0.1%	17.39	91.8	(R)E/D G/G/F/E/E I/K/R(A)	1179.564	0.0047	4
6	*	25	3	17.88	<0.1%	17.88	93.4	(R)H D L\H I S A Y G/E G N E R(R)	1597.735	0.0088	5.5
7	*	17	3	19.16	<0.1%	19.16	97.9	(K)H E T A S I G T F S W G V A N R(G)	1732.84	0.0078	4.5
8	*	50	2	24	<0.1%	24	100	(R)L E Q L L N M D T T P Y T D K(V)	1781.863	0.0077	4.3
9	*	25	3	20.77	<0.1%	15.97	97.3	(R)T/I S K/P V/E D P S E/L P K(W)	1539.826	0.0087	5.6

Additional Table A3

SPOT 2 – GS2b

>gil121341|sp|P25462.1|GLNAC_MAIZE RecName: Full=Glutamine synthetase, chloroplastic; AltName: Full=GS2; AltName: Full=Glutamate--ammonia ligase; Flags: Precursor

MAQAVVPAMQCRVGVKAAAGRVWSAGRTTRTGRGGASPGFKVMAVSTGSTGVVPRLEQLLNMDTTPYTDK VIAEYIWWGGSGIDIR
SKSR TISKPVEDPSELPK WNYDGSSTGQAPGEDSEVILYPQAFKDPFRGGNNVLVICDTYTPQGEPLPTNKRHR AAQIFSDPKVGEQ
VPWFGEIEQYETLLQKDVNWPLGWPVGGFPGPQGPYYCAVGADKSFGR DISDAHAKLYAGINISGTNGEVMPGQWEYQVGPSVG
IEAGDHIWISRYILERITEQAGVVLTLDPKPIQGDWNGAGCHTNYSTKTMR EDGGFEEIKRAILNLSLRHDLHISAYGEGNERRLTGK H
ETASIGTFWSGVANR GCSIRVGRDTEAKGKGYLEDRRPASNMDPYIVTGLLAETTLWQPSLEAEALAAKKLALKV

*: single mismatch (G → A) from >unnamed protein product (CAY33789.1). This mutation does not modify the pI of the protein.

115/423 AA: 27.2% coverage

Total peptides (unique): 95 (10)

Isoform specific peptides: 10

Specificity reproducibility (%): 100%

n	s	%f	z	Score	Local FDR (%)	Database Fwd-Rev Score	SPI (%)	Sequence Map	MH ⁺ Matched (Da)	MH ⁺ Mass Shift (Da)	MH ⁺ Error (ppm)
1	*	100	2	20.06	<0.1%	20.06	97	(R)A A Q I F S/D P/K(V)	976.51	0.0023	2.4
2	*	92	2	18.37	<0.1%	3.23	97.5	(R)A/I L N L S/L/R(H)	899.567	0.0018	2.1
3	*	42	2	19.09	<0.1%	11.44	95.2	(R)D/I S D/A H Y K(A)	948.442	0.0014	1.5
4	*	92	2	18.24	<0.1%	18.24	87.8	(R)E/D G/G/F/E/E I/K(R)	1023.463	0.0044	4.3
5	*	67	2	20.35	<0.1%	20.35	83.7	(R)E/D G/G/F/E/E I/K/R(A)	1179.564	0.0057	4.8
6	*	33	3	15.03	<0.1%	15.03	85.2	(K)H E T A S I G T F S W G V A N R(G)	1732.84	0.0018	1
7	*	100	2	27.25	<0.1%	27.25	100	(R)L E Q L L N M D T T P Y T D K(V)	1781.863	16.0087	7.7
8	*	92	2	18.58	<0.1%	18.58	98.4	(R)T I/S K P V E D P S E L P/K(W)	1539.826	0.0081	5.2
9	*	33	3	19.94	<0.1%	19.94	85	(K)V A E Q V P W F G W E Q E Y T L L Q K(D)	2278.175	0.0029	1.3
10	*	25	2	15.66	<0.1%	15.66	83.3	(K)V I A E Y I W V G G S G I D/I R(S)	1747.938	0.0056	3.2

Additional Table A4

SPOT 3 – GS2c

>gi|121341|sp|P25462.1|GLNAC_MAIZE RecName: Full=Glutamine synthetase, chloroplastic; AltName: Full=GS2; AltName: Full=Glutamate--ammonia ligase; Flags: Precursor

MAQAVVPAMQCRVGVKAAAGRVSAGRTTRGRGGASPGFKVMAVSTGSGTVVPRLEQLLNMDTTPYTDK VIAEYIWWGGSGIDIR
SKSRITISKPVEDPSELPK WNYDGSSTGQAPGEDSEVILYPQAIKDPFRGGNNVLVICDTYTPQGEPLPTNKRHRAAQIFSDPKVGEQ
VPWFGIEQEYTLQKDVNWPLGWPGVGGFPGPGPYCAVGADKSFGRDISDAHYKACLYAGINISGTNGEVMPGQWEYQVGPSVG
IEAGDHIWISRYILERITEQAGVVLTDPKPIQGDWNGAGCHTNYSTKTMREDGGFEEIKRAILNLSLRHDLHISAYGEGNERRLTGKH
ETASIGTFSWGVANRGCSIRVGRDTEAKGKGYLEDRRPASNMDPYIVTGLLAETTLWQPSLEAEALAAKKLALKV

46/423 AA: 10.9% coverage

Total peptides (unique): 46 (4)

Isoform specific peptides: 4

Specificity reproducibility (%): 100%

n	s	%f	z	Score	Local FDR (%)	Database Fwd-Rev Score	SPI (%)	Sequence Map	MH ⁺ Matched (Da)	MH ⁺ Mass Shift (Da)	MH ⁺ Error (ppm)
1	*	100	2	20.89	<0.1%	20.89	98.5	(R)A/A Q I F S D P K(V)	976.51	0.0003	0.3
2	*	42	2	18.94	<0.1%	10.03	91.3	(R)D/I S D/A/H Y/K(A)	948.442	0.0024	2.5
3	*	100	2	24.14	<0.1%	18.84	98.4	(R)L E Q L N J M D/T T P Y/T/D/K(V)	1781.863	0.0043	2.4
4	*	25	2	16.66	<0.1%	16.66	87.3	(R)T I/S K P V/E D P S E L P K(W)	1539.826	-0.0013	-0.9

Additional Table A5

SPOT 4 – GS1-3/4

Co-migration of GS1-3 + GS1-4

>gi|585204|sp|P38562.1|GLNAA_MAIZE RecName: Full=Glutamine synthetase root isozyme 4; AltName: Full=GS107; AltName: Full=Glutamate--ammonia ligase

MACLTDLVNLNLSDDTEKIAEYIWIWGGSGMDLRSKAR*TLPGPVTDPSPKLPKWNVDGSSTGQAPGEDSEVILYPQAIKDPFRRGNLIL
VMDCCYTPAGEIPTNKRYSAAKIFSSPEVAAEEPPWYIGIEQEYTLQKDTNWPLGWPIGGFPGPGPYCGIGAEEKSFGRDIVDAHY
KACLYAGINISGINGEVMPGQWEFQVGPSVGISGSDQVWVARYILERITEIAGVVVTFDPKPIPGDWNGAGAHTNYSTESMRKEGGY*
*FVIAKAAIEKLKLRHKEHIAAYGEGNERRLTGRHETADINTFSWGVANRGASVAVGQTEQNGKGYFEDRRPASNMDPYVVTSMIAETTI
VWKP

* in GS1-3: TLSGPVTDPSPK, in GS1-4: TLPGPVTDPSPK. Both sequences were sequenced.

** in GS1-3: RPASNMDPYVVTSMIAETTIWKP, in GS1-4: RPASNMDPYVVTSMIAETTIWKP. Both sequences were sequenced.

GS1-3: 166/356 AA: 46.6%

GS1-4: 180/355 AA: 50.7%

GS1-3 Total peptides (unique): 145 (10)

GS1-4 Total peptides (unique): 178 (12)

Isoform specific peptides: 6 common + 3 for GS1-3 + 3 for GS1-4

Specificity reproducibility (%): 100%

n	s	%f	z	Score	Local FDR (%)	Database Fwd-Rev Score	SPI (%)	Sequence Map	MH ⁺ Matched (Da)	MH ⁺ Error (ppm)
1	*	75	2	20.65	<0.1%	11.06	97.6	(R)D/I V D/A/H Y/K(A)	960.479	1.5
2	*	17	3	20.36	<0.1%	20.36	83.9	(K)D T N W P L G W P I G G F P G P Q G P Y Y C G W G A E K (S)	3034.419	3.8
3	*	100	2	19.5	<0.1%	14.56	89.2	(K)E/G/Y/E/V/I/K(A)	894.457	1.1
4			3	21.65	<0.1%	21.65	98.1	(K)E H V A A Y G E G N E R (R)	1345.613	2
5	*	25	2	24.54	<0.1%	24.54	96.2	(R)H/E T A D I N T F S W G V/A N R(G)	1817.857	4.2
6			2	17.11	<0.1%	17.11	79.8	(R)H/K E H I A A Y G E G N E/R(R)	1610.767	5.4
7	*	100	3	25.71	<0.1%	25.71	97.4	(K)I F S S P E V A A A E I P W I Y G I E Q E Y T I L I Q K (D)	2927.435	5.6
8	*	100	2	23.19	<0.1%	23.19	94.9	(K)I I A E Y I I W I I G G S G M D L R (S)	1793.926	2
9	*3	100	3	18.48	<0.1%	18.48	91.6	(R)R P A S N M D P Y V V T S m I A E T T I I I W K P (-)	2720.379	5.8
10	*4	50	3	14.46	<0.1%	14.46	84.5	(R)R P A S N m D P Y V V T S m I A E T T I I V W K P (-)	2706.363	3.6
11	*4	67	2	23.2	<0.1%	15.29	99.7	(R)T/L P G/P/V T/D P S/K(L)	1111.599	3.2
12	*4	42	2	22.41	<0.1%	15.66	97.1	(R)T/L P G/P V/T/D P S K/L P K(W)	1449.831	3.6
13	*3	92	2	21.17	<0.1%	12.47	100	(R)T/L S G P V T/D P S/K(L)	1101.579	3.9
14	*3	50	2	21.41	<0.1%	16.34	91.9	(R)T L S G P V T/D P S K/L P K(W)	1439.81	3
15			2	24.52	<0.1%	24.52	93.5	(K)W N Y D G S S T G Q A I P G E D S E V I I L Y I P Q I A I I F K (D)	2972.395	3.2

Additional Table A6

SPOT 5 – GS1-5

>gi|1169926|sp|P38563.2|GLNA5_MAIZE RecName: Full=Glutamine synthetase root isozyme 5; AltName: Full=GS117; AltName: Full=Glutamate--ammonia ligase

MASLTDLVNLDSLSDCTDKIAEYIWVGSGIDLRSKARTVKGPITDPSQLPKWNYDGSSTGQAPGEDSEVILYPQAIKDPFRKGNLIL
VMCDCYTPQGEPIPSNKRKYKAATVFSHPDVAAEVPWYGIEQEYTLQKDLWSWPLGWVPGGYPGPQGPYYCAAGADKAFGRDVVDA
HYKACLYAGINISGINGEVMPPGWQVEFQVGPVSGISAGDEIWWARYILERITEMAGIVLSLDPKPIKGDWNGAGAHTNYSTKSMREAGG
YEVKEAIEKLGRRHREHIAAYGEGNERRLTGRHETADINTFKWGVANRGASIRVGRDTEKEGKGYFEDRRPASNMDDPYVVTGMIA
DTILWKG

184/357 AA: 51.5% coverage

Total peptides (unique): 301 (14)

Isoform specific peptides: 6

Specificity reproducibility (%): 100%

n	s	%f	z	Score	Local FDR (%)	Database Fwd-Rev Score	SPI (%)	Sequence Map	MH ⁺ Matched (Da)	MH ⁺ Error (ppm)
1	*	100	3	24.7	<0.1%	17.33	100	(K)AATVFSHPDVAAEVPWYGIQEYTLQK(D)	3162.578	6.3
2			2	21.17	<0.1%	13.49	97.5	(R)DMVVDIA/HY/K(A)	946.463	0.9
3			2	18.47	<0.1%	11.95	88.7	(R)E A/G/G/Y/E/V/I/K(A)	965.494	1.1
4	*	8	3	14.67	<0.1%	9.3	79.2	(R)E A/G/G/Y/E/V I/K E A I E/K(L)	1535.795	3.2
5			3	20.92	<0.1%	20.92	98.5	(K)E HVA A/Y/G/E/G/N E/R(R)	1345.613	1.7
6			3	12.05	<0.1%	12.05	70.6	(K)E HII/A A Y/G E/G N E R/R(L)	1501.714	1.1
7			2	24.05	<0.1%	24.05	89.4	(K)G DMW/N/G/A/G A/H/T N/Y/S/T/K(S)	1578.693	9.2
8	*	100	2	19.08	<0.1%	19.08	98.4	(K)G/P/II/T/DIP S Q/L/P K(W)	1152.626	3.1
9			2	20.08	<0.1%	20.08	97.7	(R)H/E/T/A D/II/N/T/F/K(W)	1175.569	1.7
10	*	100	4	19.75	<0.1%	19.75	96.5	(R)H R/E HVA A Y/G/E/G/N E/R(R)	1638.773	-1.1
11	*	100	2	23.89	<0.1%	23.89	98	(R)I I/A/E/Y/II/W/V/G/G/S/G I/D/L R(S)	1761.953	2.8
12			3	25.32	<0.1%	21.07	97	(R)I/T/E/M/A/G I/V/L/S/L/D/P K/P I/K(G)	1825.05	2.1
13	*	100	3	24.5	<0.1%	24.5	100	(R)R P A S N M D/P Y/V/V/T/G/m/II/A/D T T/II/W/K(G)	2579.3	5.5
14			3	20.38	<0.1%	20.38	98.3	(K)WNYDGSSTGQAPGEDSEVILYPQAIK(D)	2972.395	4.4

Additional Table A7

SPOT 6 – GS1-1

>gi|585201|sp|P38559.1|GLNA1_MAIZE RecName: Full=Glutamine synthetase root isozyme 1; AltName: Full=GS122; AltName: Full=Glutamate--ammonia ligase

MASLTDLVNLDSLSDCTDRIIAEYIWGGTGIDLR*SKARTVKGPITDPIQLPKWNYDGSSTGQAPGEDSEVILYPQAIKDPFRKGNHILV
MCDCTYPQGEPIPTNKRYSAAKVFSSHPDVAAEVPWYGIEQEYTLQKDVSWPLGWVPGGYPGPQGPYYCAAGADKAFGRDVVDA
HYKACLYAGINISGINGEVMPPGWQVEFQVGPVSGISAGDEIWWARYILERITEMAGIVLSLDPKPIKGDWNGAGAHTNYSTKSMREAGG
YEVKAAIDKLGKRHKEHIAAYGEGNERRLTGRHETADINTFKWGVANRGASIRVGRDTEREGKGYFEDRRPASNMDDPYVVTGMIAE
DTILWNGN

*: with the sequence conflict (I → S) reported in SwissProt Reviewed Database. This mutation does not modify the pI of the protein.

186/357 AA: 52.1% coverage

Total peptides (unique): 255 (17)

Isoform specific peptides: 3

Specificity reproducibility (%): 100%

n	s	%f	z	Score	Local FDR (%)	Database Fwd-Rev Score	SPI (%)	Sequence Map	MH ⁺ Matched (Da)	MH ⁺ Mass Shift (Da)	MH ⁺ Error (ppm)
1			2	21.42	<0.1%	13.78	97.5	(R)DMVVDIA/HY/K(A)	946.463	0.0013	1.3
2			2	18.24	<0.1%	9.68	88.8	(R)E A/G/G/Y/E/V/I/K(A)	965.494	0.0019	2
3			3	21	<0.1%	21	100	(K)E HVA A/Y/G/E/G/N E/R(R)	1345.613	0.0041	3.1
4			3	10.39	<0.1%	10.39	77.4	(K)E HII/A A Y/G E/G N E R/R(L)	1501.714	0.0035	2.3
5			2	24.66	<0.1%	24.66	91.3	(K)G DMW/N/G/A/G A/H T/N/Y/S/T/K(S)	1578.693	0.0016	1
6			2	18.05	<0.1%	18.05	98.1	(K)G/P/II/T/DIP I Q/L/P K(W)	1178.678	0.0022	1.8
7			2	18.37	<0.1%	18.37	99	(K)G/P/II/T/DIP S Q/L/P K(W)	1152.626	0.001	0.9
8			2	20.1	<0.1%	20.1	93.8	(R)H/E/T/A D/II/N/T/F/K(W)	1175.569	0.0004	0.3
9			3	17.88	<0.1%	17.88	90.7	(R)H K/E H I/A/A/Y/G/E/G N E/R(R)	1610.767	0.0053	3.3
10			4	15.79	<0.1%	15.79	86.2	(R)H/K/E HII/A/A/Y/G/E/G N E R R(L)	1766.868	0.0009	0.5
11	*	67	2	21.7	<0.1%	21.7	95.2	(R)I I/A/E/Y/II/W/I/G/G T G I/D/L/R(S)	1789.985	0.003	1.7
12			3	26.59	<0.1%	22.59	97.6	(R)I/T/E/M/A/G I/V/L/S L/D/P K/P I K(G)	1825.05	0.0053	2.9
13	*	33	3	16.51	<0.1%	16.51	92	(R)R P A S N m D P Y/V/V/T/G m/II/A/E/T T I/L/W/N G(N)	2750.328	31.9902	0.1
14			2	17.68	<0.1%	17.68	88.1	(R)T V/K/G/P I/T/DIP S Q/L/P K(W)	1480.837	0.0036	2.4
15	*	100	3	27.52	<0.1%	27.52	95.8	(K)VFSSHPDVAAEVPWYGIQEYTLQK(D)	2919.456	0.0137	4.7
16			2	23.53	<0.1%	23.53	93.6	(K)WNYDGSSTGQAPGEDSEVILYPQAIK(D)	2972.395	0.0113	3.8
17			4	14.64	<0.1%	14.64	74.2	(K)WNYDGSSTGQAPGEDSEVILYPQAIKDPFRK(G)	3615.739	0.0171	4.7

Evaluation of amino acid levels by LC-ESI-MS

Maize plants were grown for 10 days without N sources and then exposed for the last 30 h to absence of N (c), to 10 mM NO₃⁻ (n), to 10 mM NH₄⁺ (a) or to 5 mM NO₃⁻+5 mM NH₄⁺ (na). **TOT**: calculated sum of total amino acids. The table reports the amino acid concentrations (μM) as average values ± SE and the proportion of total amino acids (%). The analyses were conducted on four roots and leaves and three xylem sap biological samples analyzed in duplicate (n=8, n=6). The asterisk indicates significant differences with respect to (c) plants (p column, Student's t-test with: ** p≤0.01, * p≤0.05).

Additional Table A8. Levels of amino acids in roots

	c plants			n plants				a plants				na plants			
	μM	SE	%	μM	SE	%	p	μM	SE	%	p	μM	SE	%	p
GLY	137.5	35.6	3.78	74.8	12.9	1.04		111.0	11.6	0.79		82.9	5.6	0.65	
ALA	1072.2	179.4	29.47	2827.4	354.4	39.37	**	3948.1	559.0	28.07	**	5077.7	727.3	39.86	**
SER	418.6	93.1	11.50	351.2	36.6	4.89		239.6	33.5	1.70		301.1	26.9	2.36	
PRO	9.3	1.7	0.25	6.3	0.6	0.09		9.6	1.5	0.07		6.2	0.7	0.05	
VAL	95.0	4.1	2.61	91.6	9.0	1.28		154.2	9.0	1.10	**	146.8	6.0	1.15	**
THR	93.0	10.6	2.56	78.0	5.6	1.09		86.7	9.2	0.62		93.2	3.9	0.73	
ILE	89.3	4.3	2.45	34.5	2.6	0.48	**	54.0	4.1	0.38	**	65.9	10.2	0.52	*
LEU	170.7	8.3	4.69	38.6	2.2	0.54	**	78.1	8.0	0.56	**	94.0	21.8	0.74	**
ASN	230.5	75.7	6.34	298.2	51.8	4.15		4404.7	634.2	31.32	**	2093.3	323.0	16.43	**
ASP	210.2	26.6	5.78	353.5	29.0	4.92	**	292.5	46.7	2.08		332.0	30.5	2.61	**
GLN	301.2	41.6	8.28	945.2	62.2	13.16	**	3771.8	618.1	26.82	**	3024.3	270.4	23.74	**
LYS	43.7	2.0	1.20	22.4	1.3	0.31	**	38.1	2.3	0.27		37.7	3.0	0.30	
GLU	428.0	94.5	11.76	1856.0	215.1	25.85	**	602.7	156.6	4.28		1098.4	129.0	8.62	**
MET	10.4	0.7	0.29	6.4	0.5	0.09	**	8.0	0.7	0.06	*	7.5	0.9	0.06	*
HIS	135.7	26.9	3.73	42.4	6.0	0.59	**	64.2	9.0	0.46	*	78.2	22.1	0.61	
PHE	36.7	1.3	1.01	29.8	1.7	0.42	**	37.9	1.7	0.27		38.8	2.8	0.30	
ARG	44.9	2.6	1.23	27.7	2.0	0.39	**	44.1	2.9	0.31		44.4	4.4	0.35	
TYR	47.4	4.3	1.30	50.2	4.7	0.70		67.7	3.2	0.48	**	68.5	7.3	0.54	*
TRP	59.7	2.0	1.64	39.7	2.8	0.55	**	46.8	1.4	0.33	**	45.5	3.8	0.36	**
CYS	4.8	0.7	0.13	7.5	2.1	0.10		5.4	1.8	0.04		3.6	0.2	0.03	
TOT	3638.8	376.2	100.0	7181.4	570.6	100.0	**	14065.2	2021.8	100.0	**	12740.0	521.5	100.0	**
Δ%c						+97.4				+286.53				+250.1	

Additional Table A9. Levels of amino acids in xylem sap

	c plants			n plants				a plants				na plants			
	μM	SE	%	μM	SE	%	p	μM	SE	%	p	μM	SE	%	p
GLY	13.5	1.8	0.58	13.5	0.6	0.15		9.5	0.5	0.1	*	7.5	0.3	0.05	**
ALA	615.0	98.6	26.60	2987.7	131.3	32.35	**	3606.2	262.4	29.3	**	6831.2	269.9	43.87	**
SER	303.1	101.3	13.11	269.4	19.6	2.92		73.4	3.2	0.6	*	81.8	3.2	0.53	
PRO	19.8	3.8	0.85	18.9	1.4	0.20		31.0	2.6	0.3	*	22.8	1.4	0.15	
VAL	120.3	19.5	5.21	148.1	10.2	1.60		238.8	21.4	1.9	**	237.4	15.1	1.52	**
THR	112.7	26.0	4.88	207.1	10.3	2.24	**	102.3	3.3	0.8		98.8	1.2	0.63	
ILE	92.8	17.7	4.01	65.2	4.9	0.71		131.5	14.8	1.1		102.9	6.6	0.66	
LEU	91.7	27.5	3.97	52.9	7.4	0.57		125.9	16.7	1.0		75.0	5.6	0.48	
ASN	84.0	32.4	3.64	1042.6	45.3	11.29	**	591.4	23.3	4.8	**	779.6	134.3	5.01	**
ASP	28.9	3.3	1.25	32.2	5.5	0.35		28.3	3.3	0.2		17.4	4.4	0.11	*
GLN	407.6	100.6	17.63	3778.3	256.5	40.91	**	6552.6	281.7	53.2	**	6512.3	197.8	41.82	**
LYS	166.9	27.8	7.22	136.7	7.5	1.48		238.9	19.3	1.9	*	206.3	12.9	1.32	
GLU	30.2	4.4	1.31	71.2	6.4	0.77	**	87.1	4.1	0.7	**	70.8	10.9	0.45	**
MET	8.2	2.1	0.35	49.3	2.8	0.53	**	36.9	4.1	0.3	**	32.5	1.0	0.21	**
HIS	86.3	12.4	3.73	134.0	10.8	1.45	**	88.4	11.9	0.7		125.5	7.7	0.81	*
PHE	25.6	5.9	1.11	44.7	5.2	0.48	*	116.0	11.4	0.9	**	72.2	5.5	0.46	**
ARG	50.2	11.7	2.17	133.7	5.6	1.45	**	152.8	13.6	1.2	**	218.8	12.5	1.41	**
TYR	18.1	4.8	0.78	20.5	0.8	0.22		86.2	12.6	0.7	**	61.5	4.2	0.39	**
TRP	17.2	1.5	0.74	12.4	0.5	0.13	**	17.7	0.6	0.1		12.3	0.7	0.08	**
CYS	19.3	2.5	0.84	16.2	0.5	0.18		8.5	0.5	0.1	**	4.9	0.5	0.03	**
TOT	2311.6	493.5	100.0	9234.7	405.7	100.0	**	12323.3	625.8	100.0	**	15571.4	432.7	100.0	**
Δ% _c						+299.5				+433.1				+573.6	

Additional Table A10. Levels of amino acids in leaves

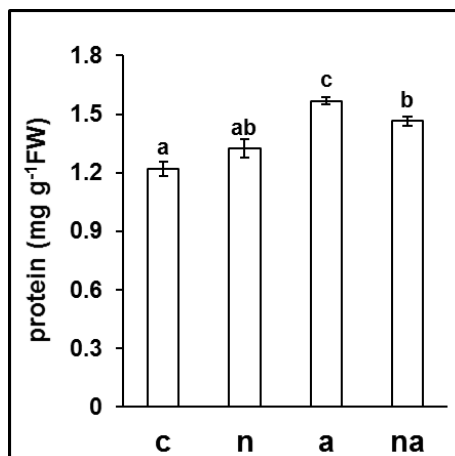
	c plants			n plants				a plants				na plants			
	μM	SE	%	μM	SE	%	p	μM	SE	%	p	μM	SE	%	p
GLY	1896.3	694.1	33.62	870.9	32.4	10.71		2727.3	590.6	14.18		2555.1	311.6	12.35	
ALA	1913.0	161.9	33.92	3461.6	172.0	42.55	**	9786.3	2240.8	50.88	**	11431.6	2011.3	55.24	**
SER	230.7	17.6	4.09	362.5	16.2	4.46	**	606.7	97.8	3.15	**	829.6	41.7	4.01	**
PRO	3.2	1.3	0.06	4.9	1.5	0.06		9.5	2.1	0.05	*	12.1	2.9	0.06	**
VAL	325.6	18.3	5.77	330.1	5.2	4.06		457.7	6.2	2.38	**	433.1	18.4	2.09	**
THR	109.8	4.9	1.95	97.8	2.7	1.20	*	165.6	7.3	0.86	**	157.7	7.0	0.76	**
ILE	9.9	0.3	0.17	9.2	0.3	0.11		37.1	7.3	0.19	**	36.3	1.7	0.18	**
LEU	7.5	1.3	0.13	3.6	0.6	0.04	**	23.0	6.0	0.12	*	21.2	0.9	0.10	**
ASN	42.9	1.7	0.76	41.1	0.9	0.51		399.9	110.8	2.08	**	275.9	78.6	1.33	**
ASP	94.4	2.4	1.67	266.6	27.2	3.28	**	266.3	15.0	1.38	**	262.5	12.3	1.27	**
GLN	60.7	3.2	1.08	194.1	5.9	2.39	**	867.2	212.0	4.51	**	851.4	104.9	4.11	**
LYS	21.8	1.5	0.39	15.8	2.5	0.19	*	36.8	8.8	0.19		32.4	7.3	0.16	
GLU	735.8	170.2	13.04	2265.4	670.0	27.85	*	3519.4	1056.8	18.30	*	3520.0	1012.0	17.01	**
MET	6.0	0.8	0.11	6.1	0.4	0.07		11.4	1.0	0.06	**	12.7	0.5	0.06	**
HIS	114.5	27.2	2.03	138.2	55.6	1.70		161.4	52.5	0.84		107.7	37.5	0.52	
PHE	8.4	0.9	0.15	12.8	0.3	0.16	**	40.3	8.4	0.21	**	38.2	1.9	0.18	**
ARG	1.3	0.1	0.02	1.2	0.1	0.01		1.5	0.2	0.01		3.7	1.8	0.02	
TYR	16.9	0.9	0.30	25.4	0.4	0.31	**	64.0	13.2	0.33	**	67.5	2.1	0.33	**
TRP	37.2	1.9	0.66	24.0	2.6	0.29	**	46.7	4.6	0.24		42.8	3.3	0.21	
CYS	4.8	1.1	0.09	3.7	0.3	0.05		4.5	1.0	0.02		4.1	0.5	0.02	
TOT	5640.6	1008.4	100.0	8135.0	729.8	100.0	*	19232.6	4312.2	100.0	**	20695.8	3458.7	100.0	**
Δ% _c						+44.2				+240.97				+266.9	

Additional Table A11. List of experimental parameters used for amino acid quantitation. The standards were prepared to a final concentration in 0.5 mM TDFHA, 25% (v/v) methanol.

AA	MH ⁺ (m/z)	RT (min)	Equation	R ²	Range
Glycine	76.04	0.92±0.04	$y=125.947x^2+107.416x+0.834$	0.996	1-1000 µM
Alanine	90.06	1.19±0.06	$y=5.449x^3+26.433x^2+460.063x$	0.989	0-6000 µM
Serine	106.05	0.83±0.04	$y=1901.870x^2-349.818x+45.721$	1	25-1000 µM
Proline	116.07	1.25±0.08	$y=3.518x-1.435$	1	1-25 µM
Valine	118.09	6.20±0.80	$y=0.062x^2+6.760x-1.757$	1	1-200 µM
Threonine	120.07	1.00±0.04	$y=1.233x^2+11.335x+4.254$	0.998	10-100 µM
Isoleucine	132.10	12.56±0.50	$y=0.040x^2-4.395x-1.190$	1	1-100 µM
Leucine	132.10	13.62±0.50	$y=0.055x^2+4.043x-1.454$	1	1-100 µM
Asparagine	133.06	0.80±0.04	$y=-0.062x^3+4.649x^2-2.928x+6.740$	1	1-3000 µM
Aspartic acid	134.05	0.68±0.04	$y=-1.068x^2+23.148x+0.478$	0.999	10-100 µM
Glutamine	147.08	0.89±0.04	$y=0.003x^3+0.455x^2+9.584x$	1	0-5000 µM
Lysine	147.11	16.15±0.30	$y=-1.004x^3+10.212x^2+11.380x$	0.999	0-200 µM
Glutamic acid	148.06	0.97±0.04	$y=0.537x^3-5.562x^2+27.782x-9.991$	1	10-1000 µM
Methionine	150.06	7.25±0.90	$y=6.716x$	0.999	0-100 µM
Histidine	156.08	16.05±0.16	$y=12.545x-3.007$	1	1-100 µM
Phenylalanine	166.09	15.70±0.18	$y=0.146x^2+2.900x-0.548$	1	1-100 µM
Arginine	175.12	16.20±0.14	$y=0.644x^2+5.015x$	1	0-200 µM
Tyrosine	182.08	9.53±0.40	$y=-0.526x^2+14.607x-0.512$	1	1-100 µM
Tryptophan	205.10	16.03±0.14	$y=58.193x^3-107.263x^2+67.771x-4.640$	0.999	1-25 µM
Cysteine (2)	241.03	1.13±0.08	$y=537.571x+0.574$	0.999	1-10 µM

Evaluation of soluble protein content

Additional Figure A4. Evaluation of soluble protein content in maize roots. Maize plants were grown for 10 days without N sources and then exposed for the last 30 h to absence of N (c), to 10 mM NO₃⁻ (n), to 10 mM NH₄⁺ (a) or to 5 mM NO₃⁻+5 mM NH₄⁺ (na). Data are expressed as average values ±SE (n=3). The upper letters indicate differences among the four treatments according to Student's t-test (p≤0.01).



List of abbreviations: 1D: mono-dimensional, 2D: two-dimensional, (a): plants exposed to 10 mM NH_4^+ , ALA: alanine, ARG: arginine, ASN: asparagine, ASP: aspartic acid, (c): control plants exposed to N absence, CYS: cysteine, GLN: glutamine, GLU: glutamic acid, GLY: glycine, GS: glutamine synthetase, HIS: histidine, ILE: isoleucine, LC-ESI-MS: Liquid Chromatography-ElectroSpray Ionization-Mass Spectrometry, LC-nESI-MS/MS: Liquid Chromatography-nanoElectroSpray Ionization-Tandem Mass Spectrometry, LEU: leucine, LYS: lysine, (n): plants exposed to 10 mM NO_3^- , N: nitrogen, (na): plants exposed to 5 mM NO_3^- +5 mM NH_4^+ , NH_4^+ : ammonium, NO_3^- : nitrate, PHE: phenylalanine, PRO: proline, MET: methionine, SER: serine, TDFHA: tridecafluoroheptanoic acid, THR: threonine, TRP: tryptophan, TYR: tyrosine, VAL: valine.