

Supplementary Material

Results:

Changes in the cellular contents of other amino acids ([Supplementary Figs. S1 and S2](#) and [Supplementary Table S2, S3 and S4](#)), which are not direct products of the Glu-Orn-Arg-Pro-Put pathway showed that:

- I. Histidine remained mostly unchanged in various treatments; Ser, on the other hand, increased in the presence of Orn (+E or -E)
- II. Cysteine was lower in the presence of Orn but slightly higher at 12 h of induction but no so at 24 h.
- III. There was little change in Phe and Leu+Trp under any treatment conditions.
- IV. Of the three branched chain amino acids, Ala was about 8- to 10-fold higher than Val and 15- to 40-fold higher than Ile in the control plants. The content of Ala increased significantly between 12 to 24 h in most cases, except in the presence of Orn (+ or -E), where it was <25% of the control levels. The addition of Glu resulted in higher Ala content at both times in un-induced plants.
- V. Induced plants had significantly higher amounts of Val at 24 h of induction, and so did the un-induced plants treated with Glu or Arg; however, Orn prevented these changes.
- VI. Isoleucine content was significantly higher upon induction of *mODC* and Orn reversed this trend.
- VII. Cellular Lys was lower in E+Orn treatment and higher in the presence of Glu, but the differences were small.
- VIII. Methionine, which was the lowest in relative amounts (up to 10-fold less than Orn) showed a small but significant increase in response to exogenous Glu at 12 and 24 h in the un-induced plants but was lower in the presence of Orn.
- IX. Tyr was not resolved in any of our samples by the method used here.
- X. Total soluble amino acids (g^{-1} FW) were slightly higher (1.2-fold) in the induced plants vs. the control plants at 24 h, and so were the Glu (-E) treated plants. No significant change was seen in the total amino acids content with other treatments.
- XI. On the basis of their relative abundance, the major (>2% of total at any given time) amino acids in the soluble fraction were Gln, Glu, Arg (+Thr and Gly), Leu (+Trp), Ala, Phe and Ser. All other amino acids constituted <1% each of the total pool; Met always being <0.1% of the total pool.
- XII. Among those whose relative concentrations (as % of total) increased with induction and/or Orn treatment were Glu, GABA and Pro (almost doubled at 24 h), and those that were negatively impacted were Ala, Pro, Val, Cys, and Lys.

Supplementary Table S1. Changes in polyamines and amino acids in 12 d-old seedlings of wild type and 2x35S::mODC transgenic lines 1-7 and 18-2 of *Arabidopsis thaliana*. N= 3 replicates, each consisting of 8-10 seedlings; * denotes $P \leq 0.05$ between WT and transgenic lines as obtained by Student's 't' test.

Metabolite	Concentration ($\mu\text{mol.g}^{-1}$ FW)		
	Wild type	mODC-1-7	mODC-18-2
Put+Cad	0.08 \pm 0.02	3.90 \pm 0.51*	2.38 \pm 0.06*
Spd	0.42 \pm 0.02	0.45 \pm 0.01	0.45 \pm 0.01
Spm	0.11 \pm 0.00	0.14 \pm 0.01*	0.08 \pm 0.00*
Glu	3.48 \pm 0.13	3.44 \pm 0.38	2.91 \pm 0.04
Gln	31.63 \pm 1.56	23.28 \pm 2.86	24.35 \pm 0.96*
Orn	0.70 \pm 0.15	0.03 \pm 0.00*	0.12 \pm 0.02*
Pro	1.41 \pm 0.36	2.01 \pm 0.38	1.47 \pm 0.25
His	0.66 \pm 0.07	0.51 \pm 0.01	0.51 \pm 0.01
Arg	5.25 \pm 0.73	3.68 \pm 0.41	3.96 \pm 0.23
GABA	0.30 \pm 0.04	0.49 \pm 0.05*	0.36 \pm 0.02
Ser	1.94 \pm 0.12	1.88 \pm 0.10	1.55 \pm 0.01*
Gly	1.43 \pm 0.25	1.97 \pm 0.18	1.52 \pm 0.05
Cys+Cystine	0.12 \pm 0.02	0.09 \pm 0.01	0.08 \pm 0.02
Met	0.00 \pm 0.00	0.003 \pm 0.001*	0.001 \pm 0.000*
Lys	0.20 \pm 0.04	0.12 \pm 0.01*	0.11 \pm 0.01
Phe	0.17 \pm 0.00	0.15 \pm 0.01	0.13 \pm 0.00*
Trp	0.01 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00
Ala	0.92 \pm 0.08	1.23 \pm 0.10	1.04 \pm 0.03
Ile	0.06 \pm 0.00	0.07 \pm 0.01	0.06 \pm 0.00
Val	0.20 \pm 0.01	0.26 \pm 0.02*	0.21 \pm 0.00
Leu	0.21 \pm 0.01	0.22 \pm 0.02	0.15 \pm 0.01*
Thr	0.42 \pm 0.04	0.57 \pm 0.03*	0.45 \pm 0.02

Supplementary Table S2. Contents of individual amino acids at 12 h post induction (as % of total PCA-soluble amino acids) in 12 d-old seedlings of *mODC-10-1* line grown in liquid MS medium with various supplements. C= control, E= Induced, Orn (0.1 mM), Glu (1 mM), Arg (0.5 mM). Numbers in red and blue denote amino acids that are up-regulated and down-regulated, respectively with >50% change (N= 4 replicates each consisting of 6-7 seedlings).

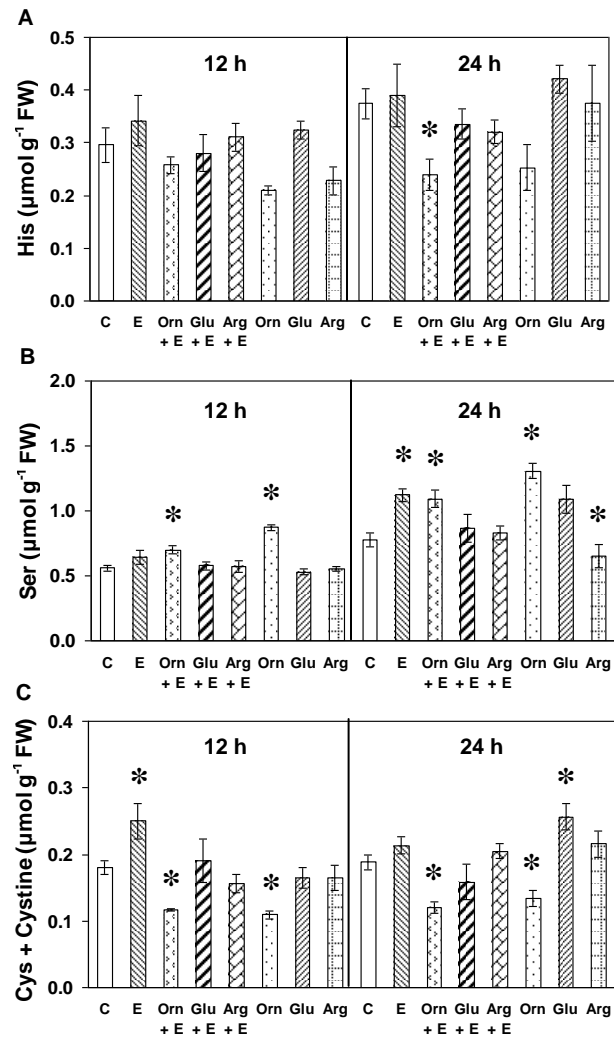
Amino acid	C	E	E + Orn	E + Glu	E + Arg	Orn	Glu	Arg
Glu	5.34 ± 0.12	5.36 ± 0.13	10.50 ± 0.13	5.12 ± 0.02	4.39 ± 0.13	9.98 ± 0.26	5.18 ± 0.14	4.52 ± 0.14
Gln	45.06 ± 1.38	48.57 ± 2.55	50.06 ± 0.66	50.43 ± 2.07	47.21 ± 1.66	49.97 ± 0.48	50.49 ± 1.46	47.77 ± 0.81
Ser	1.66 ± 0.10	1.57 ± 0.08	1.88 ± 0.08	1.53 ± 0.06	1.65 ± 0.05	2.21 ± 0.03	1.42 ± 0.06	1.54 ± 0.05
Arg+Thr+Gly	16.23 ± 0.41	15.95 ± 0.34	12.52 ± 0.39	15.18 ± 0.49	17.6 ± 0.44	12.83 ± 0.64	14.12 ± 0.18	18.23 ± 0.14
Ala	4.04 ± 0.38	3.11 ± 0.66	0.84 ± 0.15	2.69 ± 0.50	3.43 ± 0.37	0.73 ± 0.05	4.57 ± 0.41	3.16 ± 0.42
Pro	0.29 ± 0.01	0.31 ± 0.01	0.22 ± 0.01	0.29 ± 0.01	0.34 ± 0.01	0.21 ± 0.04	0.33 ± 0.01	0.26 ± 0.00
Gaba	0.32 ± 0.01	0.44 ± 0.02	0.43 ± 0.01	0.52 ± 0.03	0.35 ± 0.02	0.34 ± 0.06	0.60 ± 0.03	0.29 ± 0.02
Val	0.49 ± 0.02	0.45 ± 0.03	0.31 ± 0.01	0.40 ± 0.03	0.49 ± 0.02	0.29 ± 0.01	0.52 ± 0.02	0.41 ± 0.03
Met	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
Ile	0.23 ± 0.02	0.26 ± 0.02	0.19 ± 0.00	0.23 ± 0.02	0.29 ± 0.02	0.19 ± 0.01	0.26 ± 0.01	0.25 ± 0.01
Leu+Trp	21.47 ± 0.77	19.12 ± 1.53	19.37 ± 0.12	19.53 ± 1.10	20.19 ± 1.37	18.65 ± 0.46	18.18 ± 0.76	19.69 ± 0.60
Phe	2.35 ± 0.18	2.51 ± 0.07	1.96 ± 0.12	2.05 ± 0.11	1.75 ± 0.10	2.00 ± 0.37	1.77 ± 0.03	2.11 ± 0.27
Cys	0.54 ± 0.03	0.61 ± 0.00	0.32 ± 0.01	0.50 ± 0.05	0.45 ± 0.02	0.28 ± 0.02	0.44 ± 0.04	0.46 ± 0.03
Orn	0.59 ± 0.02	0.44 ± 0.05	0.39 ± 0.05	0.39 ± 0.05	0.52 ± 0.02	1.43 ± 0.21	0.69 ± 0.10	0.35 ± 0.06
Lys	0.43 ± 0.01	0.38 ± 0.05	0.26 ± 0.03	0.33 ± 0.03	0.36 ± 0.02	0.31 ± 0.02	0.49 ± 0.05	0.26 ± 0.03
His	0.88 ± 0.07	0.84 ± 0.15	0.70 ± 0.03	0.74 ± 0.14	0.90 ± 0.06	0.53 ± 0.03	0.87 ± 0.07	0.63 ± 0.08

Supplementary Table S3. Contents of individual amino acids at 24 h post induction (as % of total PCA-soluble amino acids) in 12 d-old seedlings of *mODC-10-1* line grown in liquid MS medium with various supplements. C= control, E= Induced, Orn (0.1 mM), Glu (1 mM), Arg (0.5 mM). Numbers in red and blue denote amino acids that are up-regulated and down-regulated, respectively with >50% change (N= 4 replicates each consisting of 6-7 seedlings).

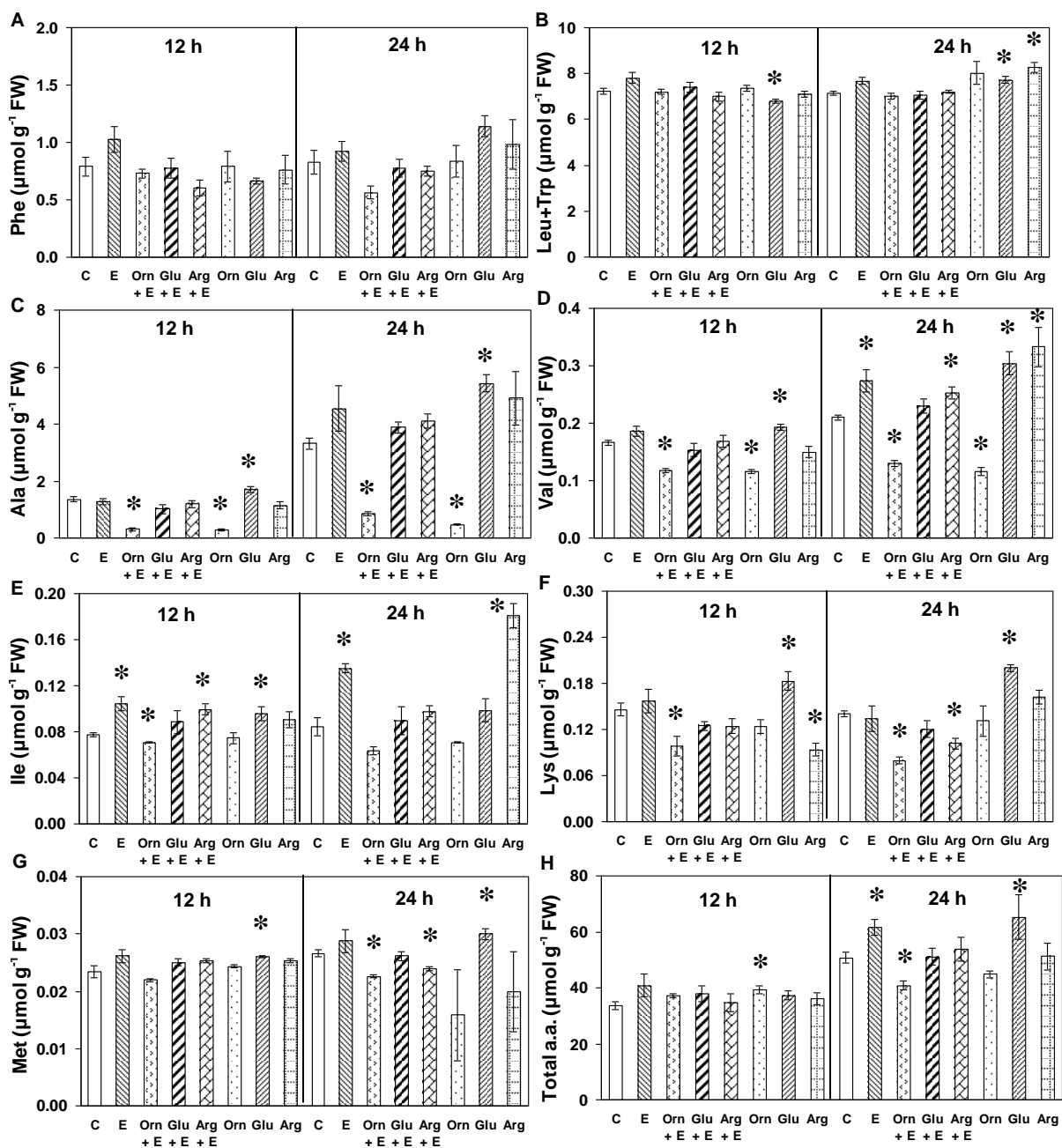
Amino acid	C	E	E + Orn	E + Glu	E + Arg	Orn	Glu	Arg
Glu	4.93 ± 0.06	4.87 ± 0.13	10.97 ± 0.44	5.25 ± 0.18	4.38 ± 0.08	9.50 ± 0.17	5.09 ± 0.05	5.98 ± 0.26
Gln	55.28 ± 1.72	54.91 ± 0.99	49.94 ± 0.71	53.46 ± 1.52	49.77 ± 1.53	48.86 ± 1.42	54.09 ± 1.07	40.63 ± 2.10
Ser	1.53 ± 0.08	1.83 ± 0.09	2.68 ± 0.07	1.69 ± 0.08	1.54 ± 0.09	2.91 ± 0.05	1.67 ± 0.05	1.28 ± 0.06
Arg+Thr+Gly	13.32 ± 0.65	14.29 ± 0.22	13.32 ± 0.18	13.71 ± 0.35	19.18 ± 0.26	14.31 ± 0.12	14.16 ± 0.12	20.28 ± 0.80
Ala	6.54 ± 0.57	7.39 ± 0.99	2.08 ± 0.14	7.60 ± 0.77	7.63 ± 0.68	1.04 ± 0.03	8.31 ± 0.42	9.59 ± 0.91
Pro	0.23 ± 0.01	0.34 ± 0.01	0.24 ± 0.01	0.46 ± 0.12	0.37 ± 0.01	0.19 ± 0.03	0.34 ± 0.01	0.46 ± 0.01
Gaba	0.20 ± 0.01	0.33 ± 0.02	0.31 ± 0.02	0.47 ± 0.01	0.27 ± 0.00	0.37 ± 0.08	0.44 ± 0.03	0.73 ± 0.15
Val	0.41 ± 0.01	0.44 ± 0.01	0.32 ± 0.00	0.45 ± 0.02	0.47 ± 0.02	0.26 ± 0.02	0.47 ± 0.00	0.65 ± 0.04
Met	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.07 ± 0.02	0.05 ± 0.00	0.05 ± 0.01
Ile	0.17 ± 0.01	0.22 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.18 ± 0.00	0.16 ± 0.00	0.15 ± 0.01	0.35 ± 0.04
Leu+Trp	14.08 ± 0.43	12.48 ± 0.35	17.22 ± 0.42	13.76 ± 0.90	13.41 ± 0.75	17.89 ± 1.38	11.81 ± 0.66	16.14 ± 1.84
Phe	1.63 ± 0.24	1.50 ± 0.10	1.38 ± 0.12	1.52 ± 0.03	1.39 ± 0.07	1.86 ± 0.34	1.75 ± 0.05	1.92 ± 0.10
Cys	0.37 ± 0.02	0.35 ± 0.02	0.29 ± 0.02	0.31 ± 0.03	0.38 ± 0.01	0.30 ± 0.03	0.39 ± 0.01	0.42 ± 0.04
Orn	0.23 ± 0.01	0.16 ± 0.03	0.26 ± 0.03	0.20 ± 0.02	0.20 ± 0.02	1.43 ± 0.23	0.34 ± 0.03	0.49 ± 0.11
Lys	0.28 ± 0.01	0.22 ± 0.03	0.20 ± 0.01	0.24 ± 0.01	0.19 ± 0.02	0.29 ± 0.04	0.31 ± 0.02	0.32 ± 0.03
His	0.74 ± 0.06	0.63 ± 0.11	0.59 ± 0.09	0.66 ± 0.03	0.60 ± 0.06	0.56 ± 0.09	0.64 ± 0.07	0.73 ± 0.06

Supplementary Table S4. Changes in polyamines and amino acids in 7 d-old wild type seedlings grown in GM medium (control) and GM medium supplemented with 1 mM Put, 1 mM Spd or 0.5 mM Spm. N= 3-4 replicates, each consisting of 8-10 seedlings; * denotes $P \leq 0.05$ between WT and transgenic lines as obtained by Student's 't' test.

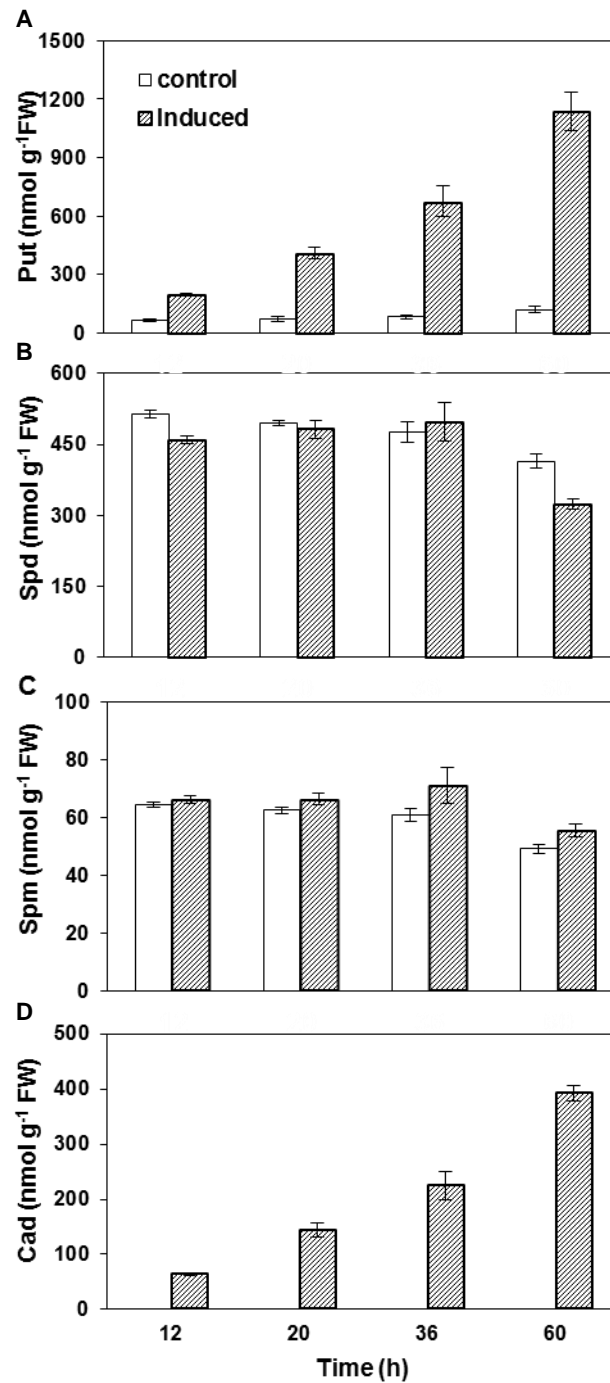
Metabolite	Concentration ($\mu\text{mol.g}^{-1}$ FW)			
	Wild type	1 mM Put	1 mM Spd	0.5 mM Spm
Put	0.04 ± 0.01	$1.18 \pm 0.07^*$	$0.22 \pm 0.01^*$	$0.13 \pm 0.01^*$
Spd	0.43 ± 0.03	$0.33 \pm 0.01^*$	$1.65 \pm 0.07^*$	$0.26 \pm 0.01^*$
Spm	0.08 ± 0.01	$0.04 \pm 0.00^*$	$0.02 \pm 0.00^*$	$2.02 \pm 0.03^*$
Glu	1.25 ± 0.07	1.25 ± 0.13	1.02 ± 0.11	1.01 ± 0.13
Gln	28.05 ± 0.38	38.10 ± 3.62	24.59 ± 1.65	23.21 ± 2.38
Orn	0.06 ± 0.00	$0.08 \pm 0.00^*$	$0.12 \pm 0.02^*$	$0.18 \pm 0.03^*$
Pro	0.19 ± 0.01	$0.06 \pm 0.01^*$	0.21 ± 0.02	$0.26 \pm 0.02^*$
His	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.10 ± 0.01
Arg+Thr+Gly	3.43 ± 0.16	3.51 ± 0.26	$4.45 \pm 0.13^*$	4.76 ± 0.53
GABA	0.22 ± 0.02	0.17 ± 0.01	0.25 ± 0.03	0.27 ± 0.01
Ser	1.02 ± 0.05	$1.31 \pm 0.08^*$	1.13 ± 0.04	1.34 ± 0.15
Cys+Cystine	0.07 ± 0.01	0.12 ± 0.03	0.07 ± 0.01	0.09 ± 0.03
Met	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Lys	0.05 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.07 ± 0.01
Phe	0.17 ± 0.01	$0.09 \pm 0.01^*$	0.14 ± 0.01	0.18 ± 0.01
Trp	0.03 ± 0.00	$0.07 \pm 0.00^*$	0.03 ± 0.00	0.02 ± 0.00
Ala	0.43 ± 0.03	0.31 ± 0.02	0.37 ± 0.07	0.40 ± 0.03
Ile	0.10 ± 0.01	0.13 ± 0.01	$0.12 \pm 0.00^*$	$0.13 \pm 0.01^*$
Val	0.11 ± 0.00	0.10 ± 0.01	0.12 ± 0.00	0.13 ± 0.01
Leu	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00



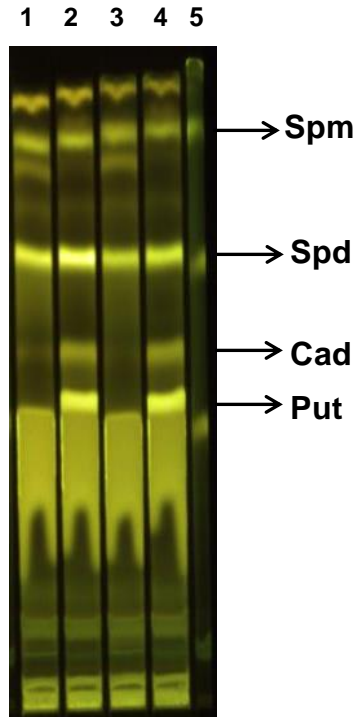
Supplementary Fig. S1 Cellular contents of (A) histidine, (B) serine, and (C) cys+cystine in induced (+E) and uninduced 12 day old *mODC-10-1* transgenic seedlings with or without 0.1 mM Orn, 0.5 mM Arg or 1 mM Glu. Data are mean \pm SE of four replicates; each replicate consists of 6-7 seedlings. * = $P \leq 0.05$ for significant difference between treatment and control at a given time.



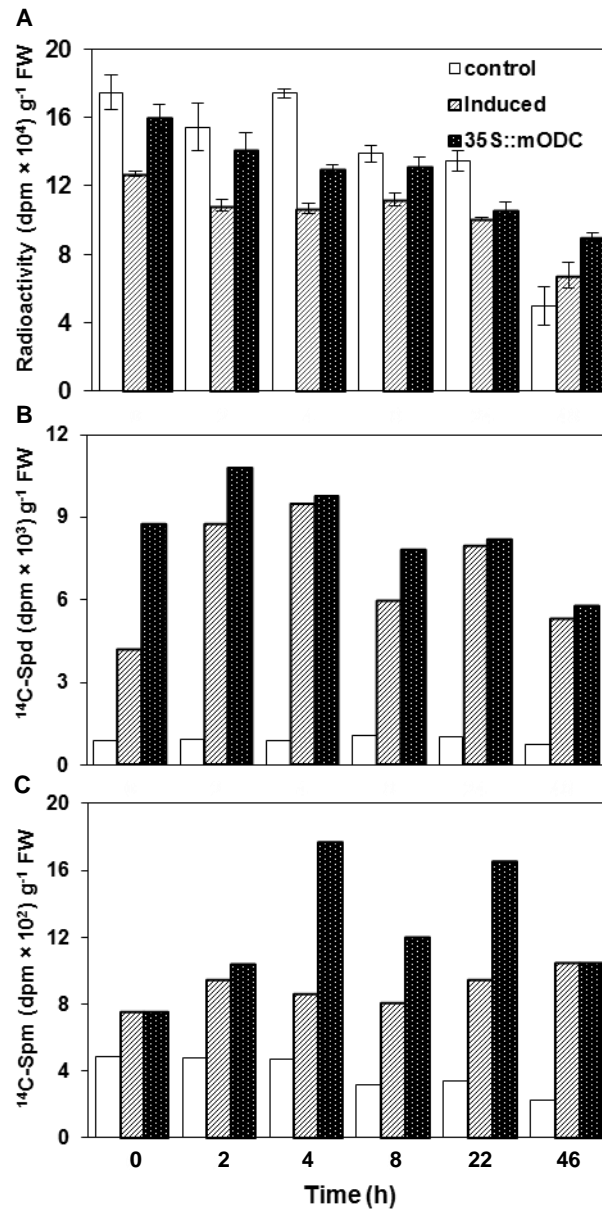
Supplementary Fig. S2 Cellular contents of (A) phenylalanine, (B) leucine+tryptophan, (C) alanine, (D) valine, (E) isoleucine, (F) lysine, (G) methionine, and (H) total PCA soluble amino acids in 12 h and 24 h induced (+E) and uninduced 12 day old *mODC-10-1* transgenic seedlings with or without 0.1 mM Orn, 0.5 mM Arg or 1 mM Glu. Data are mean \pm SE of four replicates; each replicate consists of 6-7 seedlings. * = $P \leq 0.05$ for significant difference between treatment and control at a given time.



Supplementary Fig. S3. Cellular contents of PCA soluble PAs (A) Put, (B) Spd, (C) Spm, and (D) Cad in 2-week old inducible *mODC-10-1* seedlings at different times after induction (+ estradiol) and control (- estradiol). The induction was performed the same way as for ¹⁴C-feeding experiment except no radioisotope was added. PA levels at 12, 20, 36 and 60 h after induction are equivalent to 0, 8, 24 and 48 h after removal of the radioisotope.



Supplementary Fig. S4 Separation of dansyl-polyamines from radioisotope incorporation samples (12-day old seedlings of *mODC-10-1*) on TLC plates as visualized under UV light at 48 and 72 h after transfer to label-free medium. Lanes are: 1 and 3= un-induced, 2 and 4= induced, 5= standards.



Supplementary Fig. S5 Amount of radioactivity in (A) Aqueous fraction representing uptake, (B) [¹⁴C]Spd, and (C) [¹⁴C]Spm at different times after 2-week old seedlings of *mODC-10-1* (induced= +E for 8h, control= -E) and *2×35S::mODC-1-7* (constitutive) were incubated with [U-¹⁴C]Orn for 4 h, washed with label-free medium, and transferred to label-free medium.