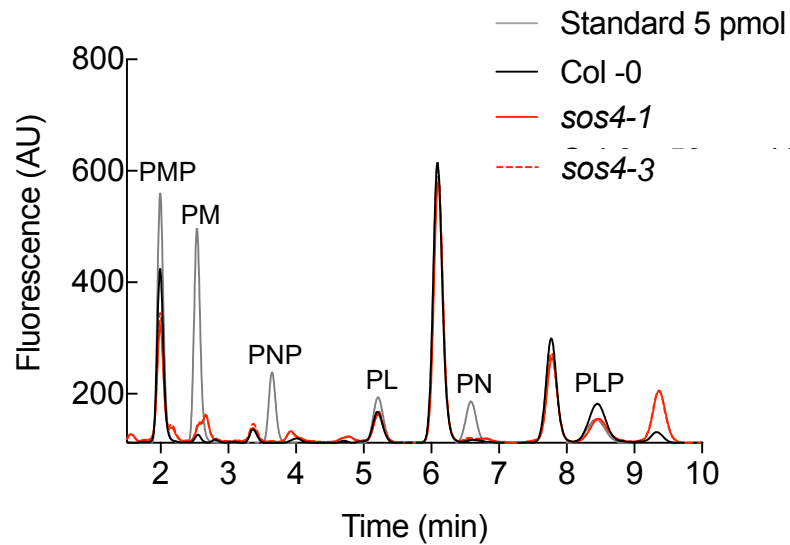
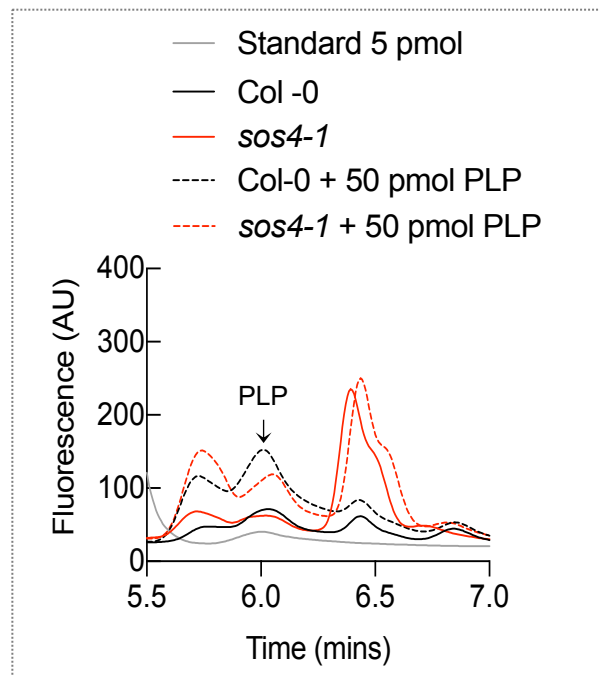
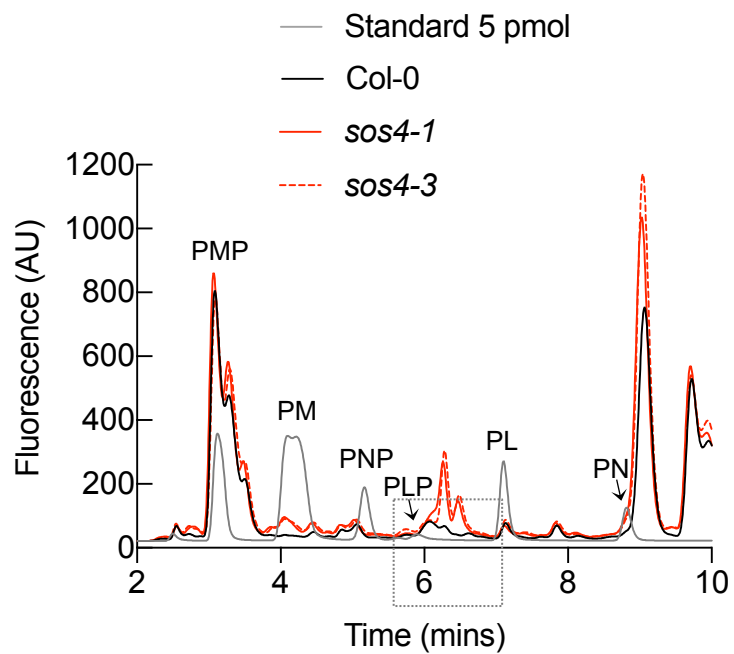
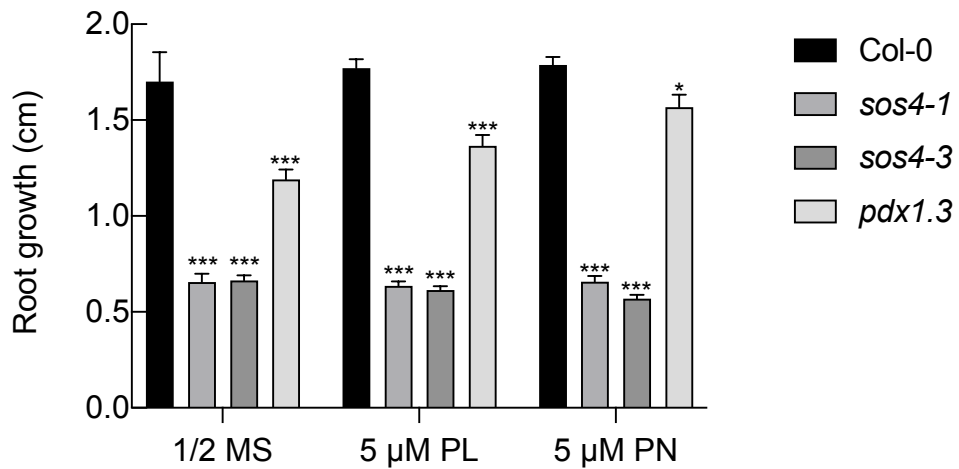


A**B**

Supplemental Figure S1. Comparison of HPLC methods for assessment of B₆ vitamers.

(A) A representative HPLC chromatogram of B₆ vitamers with the method established in our study. The individual vitamer standards are pyridoxal 5'-phosphate (PLP), pyridoxamine 5'-phosphate (PMP), pyridoxine 5'-phosphate (PNP), pyridoxal (PL), pyridoxamine (PM), pyridoxine (PN). In each case 5 pmol was loaded. In the case of the plant extracts, the material used from each line was shoots (25 mg) of 3-week-old plants grown on soil in individual pots in 16 hr light (120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 21°C), 8 hr dark (at 18°C) cycles. The key indicates the color coding used for each line.

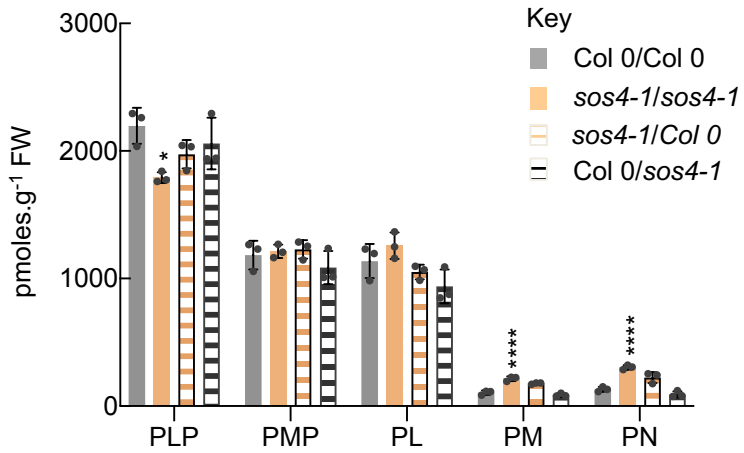
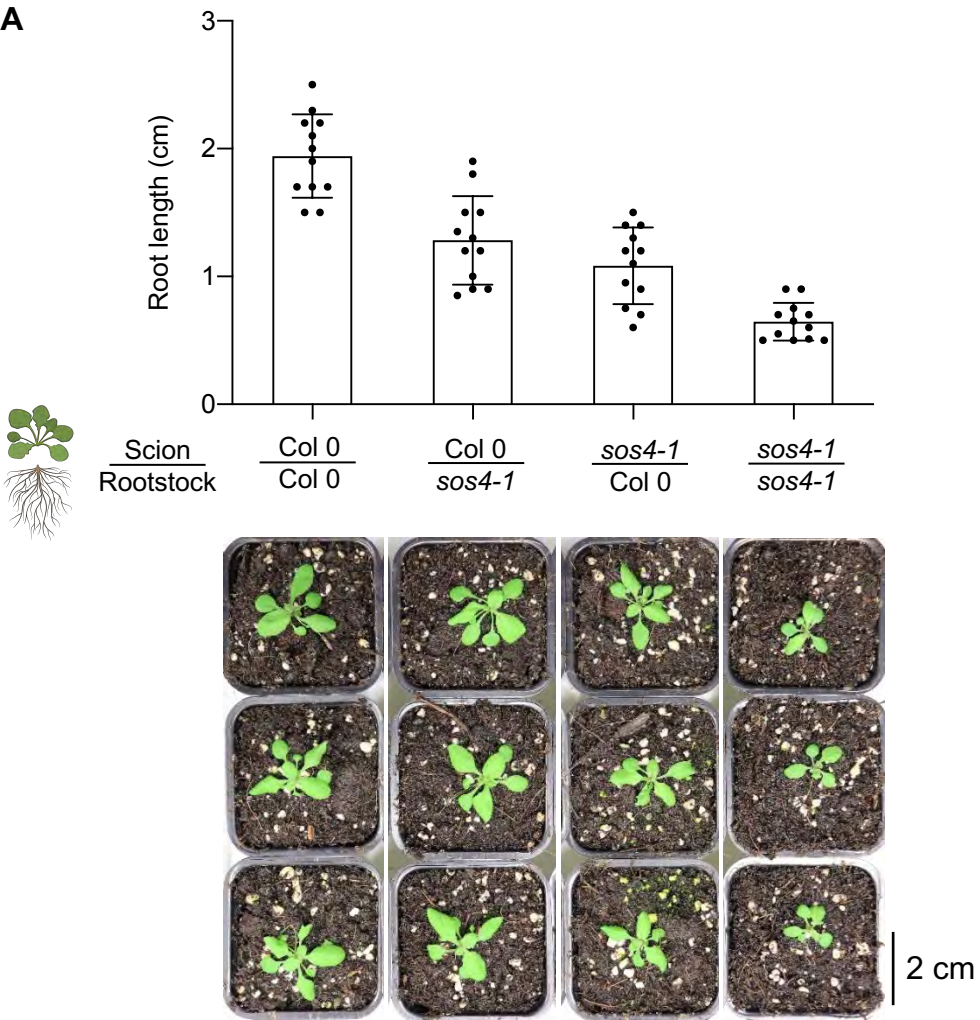
(B) A representative HPLC chromatogram of B₆ vitamers employing the method described in González E et al 2007 Plant Physiol. The individual vitamer standards are as in **(A)**. To aid visualization of the profile around PLP (hashed frame on the left chromatogram), the area from retention time 5.5 to 7 min is shown on the right on a smaller scale. The plant extracts used from each line was lyophilized shoots (25 mg) of 36-day-old plants grown on soil in individual pots 8 hr light (120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 21°C), 16 hr dark (at 18°C) cycles. The key indicates the color coding used for each line. Plant extracts that were spiked with 50 pmol PLP are also shown.

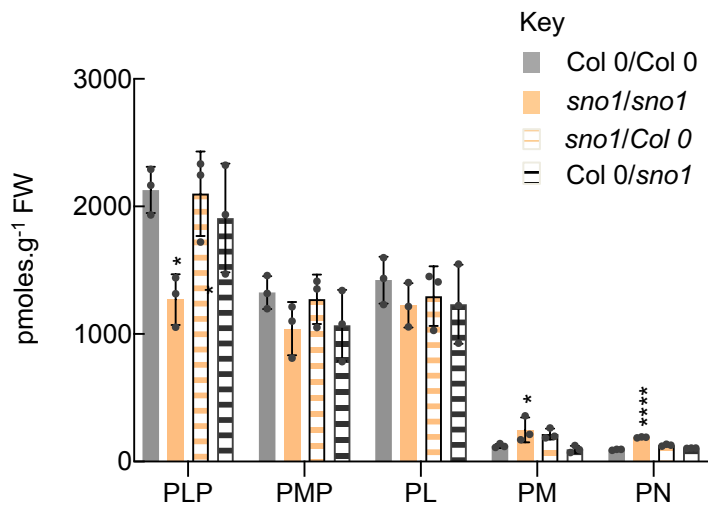
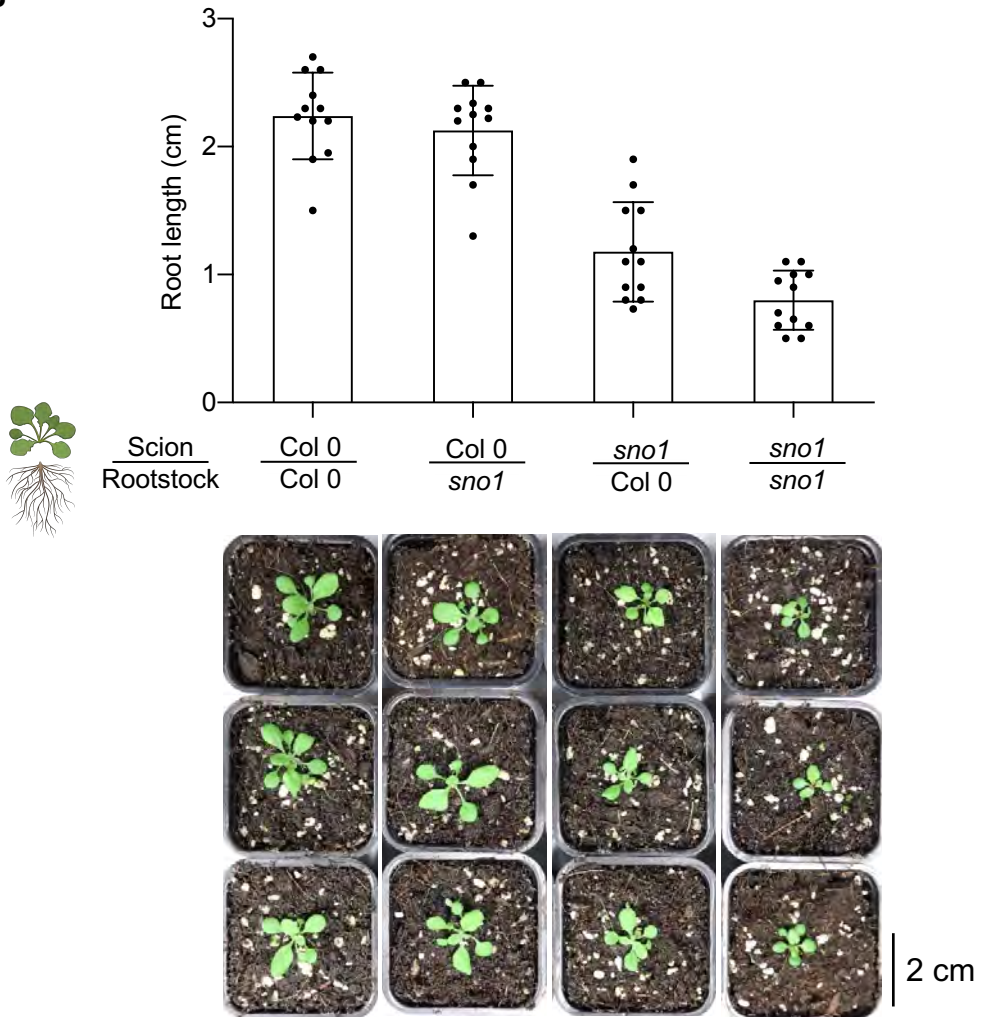


Supplemental Fig. S2. Effect of external application of PN or PL on *sos4* root growth.

Elongation of roots of 5-day-old seedlings of *sos4* alleles or the biosynthesis *de novo* mutant *pdx1.3* compared to wild type (Col-0) grown in sterile culture medium (1/2 MS) in the absence or presence of either PN or PL (5 μ M in each case). The data represent the mean \pm SD of three biological replicates (each comprising \geq 8 plants). The asterisks indicate significance by one-way ANOVA tests (* P value < 0.05, *** P value < 0.001). A separate ANOVA was used for each line in comparison with the wild type.

A

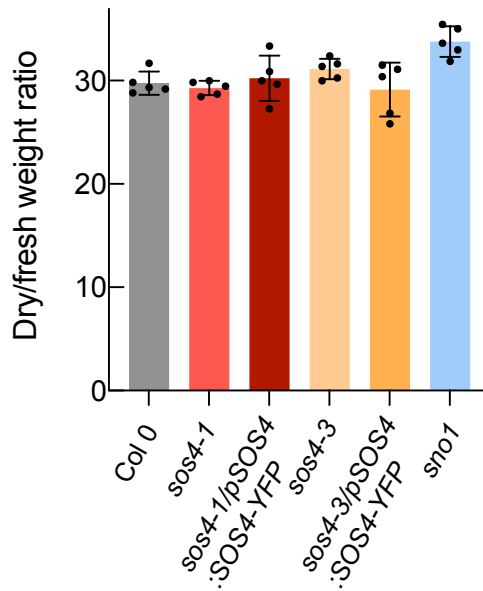


B

Supplemental Figure S3. Reciprocal micrografting of *sos4* lines and wild type.

(A) and **(B)** The histogram at the top shows the elongation of micrografted roots at 7 days after grafting. The data are the mean \pm SD of three biological replicates (each comprising ≥ 12). The scion and rootstock used are as indicated below. Below this are photographs of micrografted seedlings initially grown on sterile culture medium for 7 days and then transferred to soil for 14 days, in each case under 16 hr light ($120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 21°C), 8 hr dark (at 18°C) cycles. The scion and rootstock used are as indicated above. The histogram at the bottom shows the individual B₆ vitamer content in the shoots of plants grown as shown in the photographs. The individual vitamers are pyridoxal 5'-phosphate (PLP), pyridoxamine 5'-phosphate (PMP), pyridoxal (PL), pyridoxamine (PM), pyridoxine (PN). The key indicates the color coding used for each micrografted line.

The data are represented as the mean \pm SD of three biological replicates (each comprising ≥ 15 plants grown in individual pots). Asterisks indicate significance by one-way ANOVA test (* P value < 0.05 , ** P value < 0.01 , *** P value < 0.001). A separate ANOVA was used for each line in comparison with the wild type.



Supplemental Figure S4. Determination of the dry-to-fresh mass ratio of genotypes.

Seedlings were grown on sterile culture under 16 hr light ($120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 21°C), 8 hr dark (at 18°C) cycles for 2 weeks after which dry and fresh weight of shoots was determined. The data are mean dry to fresh weight ratios \pm SD of five biological replicates (each comprising shoots of 5 seedlings).

Supplemental Table S1. Elemental content of lines relative to wild type.

Inome profile of shoots and roots of 2-week-old plants grown on soil in 16 hr light (either 120 or 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 21 °C), 8 hr dark (at 18 °C) cycles. Elements were determined by ICP-MS. The data are the mean log values \pm SD of three biological replicates (each comprising \geq 20 plants). Significance was determined by one-way ANOVA tests for p value $<$ 0.01.

Roots 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

Element	<i>sos4-1</i> vs wild type			<i>sos4-3</i> vs wild type			<i>sno1</i> vs wild type		
	mean	SD	P value	mean	SD	P value	mean	SD	P value
Li 7	-1,15	0,07	<0,0001	-0,56	0,27	0,0042	-0,07	0,09	0,8904
B 11	0,78	0,39	0,0137	-0,01	0,31	0,9996	-0,22	0,055	0,6079
Na 23	-0,33	0,15	0,0062	-0,42	0,09	0,0015	-0,29	0,04	0,0125
Mg 24	-0,38	0,14	0,0019	-0,32	0,08	0,0047	0,03	0,078	0,9272
P 31	-0,00	0,12	>0,9999	-0,19	0,00	0,012	-0,15	0,03	0,0386
S 34	-0,01	0,17	0,9955	-0,25	0,03	0,0193	-0,18	0,04	0,092
K 39	-0,30	0,07	0,0015	-0,14	0,08	0,0717	-0,05	0,07	0,6312
Ca 43	-0,32	0,37	0,1902	-0,55	0,09	0,0241	-0,29	0,10	0,2387
Mn 55	0,56	0,04	0,0002	0,69	0,03	<0,0001	0,61	0,17	<0,0001
Fe 56	0,02	0,07	0,9155	-0,08	0,07	0,2562	-0,14	0,06	0,0501
Co 59	0,60	0,10	<0,0001	0,58	0,09	<0,0001	0,40	0,09	0,0008
Ni 60	0,56	0,06	<0,0001	0,46	0,02	<0,0001	0,49	0,08	<0,0001
Cu 63	-0,13	0,16	0,4373	-0,25	0,06	0,071	-0,28	0,16	0,0454
Zn 66	0,20	0,16	0,1436	0,13	0,15	0,4287	-0,18	0,08	0,2039
Mo 98	0,14	0,19	0,5609	0,35	0,13	0,0547	0,44	0,19	0,0192

Shoots 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

Element	<i>sos4-1</i> vs wild type			<i>sos4-3</i> vs wild type			<i>sno1</i> vs wild type		
	mean	SD	P value	mean	SD	P value	mean	SD	P value
Li 7	-0,36	0,03	<0,0001	-0,22	0,19	0,0027	-0,03	0,08	0,8599
B 11	-0,16	0,13	0,1309	0,00	0,20	>0,9999	-0,30	0,12	0,0031
Na 23	-0,19	0,04	0,0093	-0,10	0,18	0,2358	0,01	0,08	0,9883
Mg 24	-0,17	0,04	0,0128	-0,17	0,15	0,0135	0,02	0,09	0,9628
P 31	0,24	0,03	0,0006	0,10	0,17	0,1481	-0,10	0,06	0,149
S 34	-0,23	0,07	<0,0001	-0,29	0,09	<0,0001	0,02	0,07	0,8716
K 39	0,26	0,07	0,001	0,06	0,19	0,6577	0,07	0,05	0,5614
Ca 43	0,17	0,03	<0,0001	0,15	0,03	<0,0001	0,15	0,03	<0,0001
Mn 55	0,21	0,16	0,345	0,36	0,36	0,053	0,11	0,29	0,7812
Fe 56	0,09	0,17	0,3852	0,17	0,17	0,0696	-0,13	0,06	0,1876
Co 59	0,23	0,19	0,1901	0,14	0,27	0,5732	0,17	0,29	0,3992
Ni 60	-0,16	0,11	0,282	-0,12	0,27	0,4899	0,23	0,21	0,0864
Cu 63	0,30	1,00	0,6145	0,03	0,10	0,9994	0,01	0,07	>0,9999
Zn 66	-0,17	0,07	0,7059	-0,14	0,13	0,8022	0,13	0,64	0,8381
Mo 98	-0,32	0,04	<0,0001	-0,65	0,11	<0,0001	-0,14	0,14	0,0434

Roots 120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

Element	<i>sos4-1</i> vs wild type			<i>sos4-1/pSOS4:SOS4-YFP</i> vs wild type			<i>sos4-3</i> vs wild type			<i>sno1</i> vs wild type		
	mean	SD	P value	mean	SD	P value	mean	SD	P value	mean	SD	P value
Li 7	-0,10	0,19	0,9156	0,11	0,53	0,8666	0,15	0,18	0,7375	0,03	0,17	0,998
B 11	0,28	0,09	0,0828	0,10	0,22	0,7888	0,09	0,14	0,8583	0,36	0,36	0,0166
Na 23	-1,17	0,17	<0,0001	0,24	0,35	0,2108	-1,20	0,15	<0,0001	-0,77	0,27	<0,0001
Mg 24	-0,72	0,19	<0,0001	-0,06	0,28	0,9295	-0,49	0,15	0,0003	-0,69	0,16	<0,0001
P 31	-0,39	0,08	<0,0001	0,02	0,07	0,9883	-0,29	0,09	<0,0001	-0,29	0,11	<0,0001
S 34	0,02	0,04	0,9073	0,03	0,05	0,87	0,10	0,11	0,04	0,09	0,07	0,0856
K 39	-0,07	0,06	0,5098	1,97E-04	0,13	>0,9999	-0,08	0,03	0,3129	-0,04	0,13	0,8709
Ca 43	-0,32	0,05	<0,0001	-0,08	0,14	0,3631	-0,13	0,13	0,065	-0,36	0,06	<0,0001
Mn 55	1,45	0,39	0,0001	0,17	0,41	0,9292	1,05	0,36	0,0038	1,82	0,87	<0,0001
Fe 56	0,16	0,15	0,3703	-0,08	0,15	0,6555	0,03	0,11	0,9771	0,17	0,16	0,0993
Co 59	1,27	0,39	0,0002	0,11	0,34	0,9767	0,94	0,31	0,0042	1,58	0,79	<0,0001
Ni 60	1,53	0,33	<0,0001	0,03	0,31	0,9998	1,23	0,31	0,0001	1,79	0,76	<0,0001
Cu 63	0,51	0,19	<0,0001	0,07	0,09	0,7286	0,36	0,09	0,0002	0,36	0,16	0,0002
Zn 66	0,59	0,31	0,0446	0,06	0,21	0,995	0,27	0,25	0,5647	0,83	0,73	0,0034
Mo 98	0,59	0,35	0,0028	0,29	0,43	0,195	0,15	0,16	0,752	0,62	0,16	0,0018

Shoots 120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$

Element	<i>sos4-1</i> vs wild type			<i>sos4-1/pSOS4:SOS4-YFP</i> vs wild type			<i>sos4-3</i> vs wild type			<i>sno1</i> vs wild type		
	mean	SD	P value	mean	SD	P value	mean	SD	P value	mean	SD	P value
Li 7	-0,37	0,07	<0,0001	-0,03	0,04	0,7819	-0,03	0,09	0,8084	0,13	0,06	0,0046
B 11	0,28	0,09	<0,0001	-0,10	0,05	0,0263	0,3	0,05	<0,0001	0,34	0,08	<0,0001
Na 23	-0,40	0,36	0,0175	-0,13	0,26	0,724	0,41	0,18	0,0139	0,91	0,17	<0,0001
Mg 24	-0,28	0,056	<0,0001	-0,05	0,06	0,5203	-0,01	0,08	0,998	0,15	0,08	0,0013
P 31	-0,83	0,17	<0,0001	0,00	0,07	0,9998	-1,10	0,08	<0,0001	-1,00	0,06	<0,0001
S 34	-0,39	0,10	<0,0001	-0,02	0,05	0,951	-0,21	0,04	0,0001	-0,33	0,09	<0,0001
K 39	-0,13	0,03	0,0004	0,07	0,04	0,0713	-0,08	0,05	0,0498	-0,31	0,08	<0,0001
Ca 43	-0,23	0,05	<0,0001	0,03	0,04	0,6207	-0,30	0,08	<0,0001	-0,20	0,03	<0,0001
Mn 55	0,26	0,08	<0,0001	-0,02	0,04	0,9728	0,12	0,12	0,0373	0,34	0,08	<0,0001
Fe 56	0,49	0,07	<0,0001	-0,05	0,02	0,4271	0,33	0,08	<0,0001	0,26	0,08	<0,0001
Co 59	0,87	0,17	<0,0001	-0,18	0,07	0,0372	0,57	0,14	<0,0001	0,68	0,09	<0,0001
Ni 60	0,37	0,09	<0,0001	0,02	0,12	0,9829	0,29	0,11	0,0003	0,36	0,16	<0,0001
Cu 63	0,10	0,10	0,0691	0,06	0,09	0,3931	0,14	0,09	0,0139	-0,24	0,04	<0,0001
Zn 66	0,05	0,09	0,5801	-0,08	0,03	0,1486	-0,49	0,06	<0,0001	-0,29	0,10	<0,0001
Mo 98	-0,77	0,14	<0,0001	0,08	0,17	0,6001	-0,77	0,16	<0,0001	-0,82	0,08	<0,0001

Supplemental Table S2. Oligonucleotide primers used in this study

Purpose	Gene	AGI number	Sequence	Notes	
Cloning	<i>SOS4</i>	At5g37850	F- CACCATGACGACGCCTCCAG		
			R- GCTGTATCTTTCAGCTTTCAGTTCGACC		
	<i>pSOS4</i>	At5g37850	F- CCGAGCTCTCAAGCTTTTTGATTAGTGTGTGA		-1973 to <i>SOS4</i> start codon
			R- GGACTAGTAGCTTCTGTTTGACCCATGAGAC		
qPCR	<i>SOS4</i>	At5g37850	F- ACGTTGCAGGCGCTTTTGCG		
			R- AGTTCGACCTTCGGGTTGCGA		
	<i>UBC21</i>	At5g25760	F- TAGCATTGATGGCTCATCCTGA		Reference gene
			R- TTGTGCCATTGAATTGAACCC		
Genotyping	<i>SOS4</i>	At5g37850	F- TGGAAACTGTGAACACCGGATAT		
			R- CCAAACCAAACAGTCTAAGGCTG		
			ATATTGACCATCATACTCATTGC		Left border T-DNA