

Accessory Publication



Fig. S1. Growth habit of *Arabidopsis thaliana* plants transformed with *HvALMT1*. Plants were transformed with *HvALMT1* and selected on media containing kanamycin to produce the T₀ generation shown here. The three plants on the left are representative of the range of growth phenotypes in the T₀ generation as compared to normal wild-type growth (shown on the right). All plants are of approximately the same age.

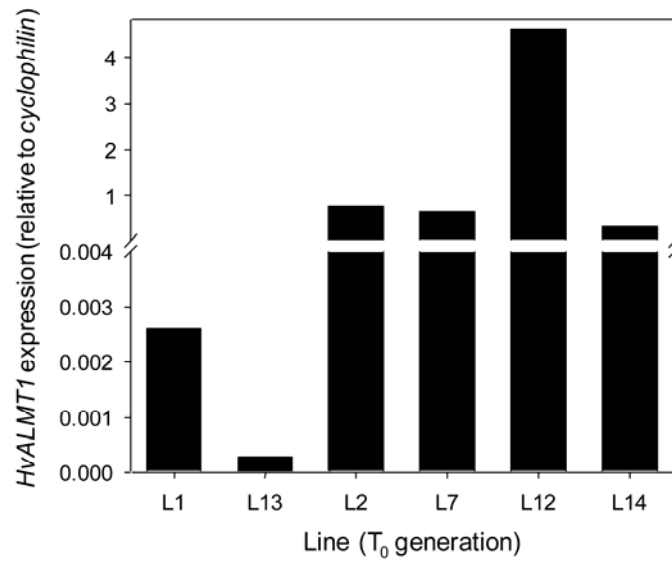


Fig. S2. Expression of *HvALMT1* in transgenic *Arabidopsis thaliana* plants. Expression of *HvALMT1* was determined by qRT-PCR and is shown relative to the internal reference gene cyclophilin. Each bar is the relative expression value of one plant only. A non-specific PCR product with a relative expression of 0.0006 was detected in wild-type plants.

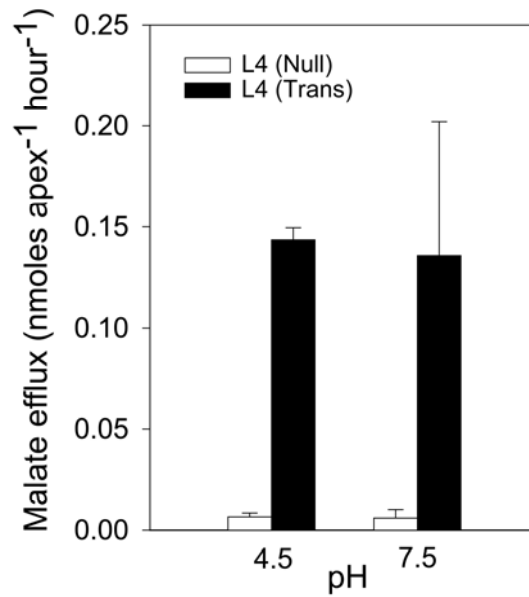


Fig. 3. The pH-independent efflux of malate from transgenic barley (*Hordeum vulgare*) that over-express *HvALMT1*. Plants of the T₁ generation segregating for the presence of the *HvALMT1* transgene (L4 (Trans)) were separated from null segregants of the T₁ generation and plants of the homozygous null line of the T₂ generation (L4 (Null)). Exudates were collected for 3.5 h from root apices of 9-day-old seedlings at pH 4.5 or pH 7.5. Shown is the mean and s.e.m. (for pH 4.5 $n = 2$ for transgenic, $n = 6$ for null; for pH 7.5 $n = 4$ for transgenic, $n = 5$ for null).

Table S1. The concentration of elements in leaves of barley (*Hordeum vulgare*) transformed with *HvALMT1*

Shown is the elemental concentration in the second youngest fully emerged leaf of the T₀ generation of barley transformed with *HvALMT1* (L1 to L6) or non-transformed controls (C1 to C3) expressed on a dry weight basis. For each element, values followed by a different letter are significantly different ($P < 0.05$). There were no significant differences in the concentration of B or Na ($n = 3$ for L5 and $n = 6$ for all other lines)

Line	B ($\mu\text{g g}^{-1}$)	Ca (mg g^{-1})	Cu ($\mu\text{g g}^{-1}$)	Fe ($\mu\text{g g}^{-1}$)	K (mg g^{-1})	Mg (mg g^{-1})	Mn ($\mu\text{g g}^{-1}$)	Na (mg g^{-1})	P (mg g^{-1})	S (mg g^{-1})	Zn ($\mu\text{g g}^{-1}$)
C1	12.4 ± 8.1	4.8 ± 0.5 ^b	17.2 ± 0.7 ^{bc}	83.0 ± 2.6 ^b	48.7 ± 1.3 ^b	2.11 ± 0.07 ^{cd}	28.0 ± 2.7 ^{ab}	0.72 ± 0.08	8.2 ± 0.3 ^{ab}	5.2 ± 0.3 ^b	61 ± 3 ^b
C2	3.7 ± 2.7	4.8 ± 0.2 ^b	18.8 ± 0.6 ^{ab}	92.3 ± 4.0 ^a	50.6 ± 1.0 ^{ab}	2.05 ± 0.03 ^{cd}	27.8 ± 0.9 ^{ab}	0.74 ± 0.03	9.2 ± 0.4 ^b	4.9 ± 0.2 ^b	77 ± 3 ^a
C3	8.3 ± 5.2	4.1 ± 0.5 ^b	16.9 ± 0.5 ^{bc}	80.7 ± 2.9 ^b	49.5 ± 0.8 ^b	1.92 ± 0.09 ^d	26.8 ± 1.1 ^{ab}	0.55 ± 0.06	8.9 ± 0.2 ^{ab}	6.1 ± 0.4 ^a	61 ± 2 ^b
L1	3.9 ± 1.8	6.1 ± 0.8 ^{ab}	15.9 ± 0.4 ^c	76.6 ± 0.8 ^b	48.8 ± 0.7 ^b	2.26 ± 0.09 ^{bcd}	27.1 ± 3.5 ^{ab}	0.85 ± 0.12	8.1 ± 0.5 ^b	4.6 ± 0.2 ^{bc}	49 ± 4 ^c
L2	14.9 ± 12.6	4.2 ± 0.6 ^b	19.3 ± 0.6 ^a	77.6 ± 3.2 ^b	51.8 ± 1.0 ^{ab}	2.08 ± 0.06 ^{cd}	29.3 ± 1.6 ^a	0.70 ± 0.08	9.5 ± 0.5 ^a	5.0 ± 0.2 ^b	67 ± 2 ^{ab}
L3	15.1 ± 7.3	5.2 ± 0.6 ^b	16.9 ± 0.6 ^{bc}	82.2 ± 3.6 ^b	53.6 ± 0.8 ^a	2.12 ± 0.08 ^{cd}	27.7 ± 2.7 ^{ab}	0.75 ± 0.11	9.2 ± 0.3 ^{ab}	5.1 ± 0.3 ^b	62 ± 3 ^b
L4	5.3 ± 4.5	8.3 ± 0.7 ^a	16.0 ± 0.3 ^c	75.4 ± 2.0 ^b	48.0 ± 1.3 ^b	2.50 ± 0.15 ^{abc}	32.6 ± 2.4 ^a	0.91 ± 0.12	8.4 ± 0.4 ^{ab}	4.5 ± 0.1 ^{bc}	62 ± 2 ^b
L5	17.1 ± 6.2	9.2 ± 2.4 ^a	10.7 ± 1.4 ^d	50.1 ± 2.7 ^c	31.1 ± 0.3 ^d	3.11 ± 0.75 ^{ab}	16.5 ± 1.3 ^c	0.78 ± 0.19	4.6 ± 0.6 ^d	3.1 ± 0.2 ^d	37 ± 6 ^d
L6	13.2 ± 7.3	9.6 ± 1.7 ^a	12.0 ± 0.9 ^d	54.3 ± 1.9 ^c	35.7 ± 1.7 ^c	3.17 ± 0.39 ^a	20.4 ± 3.4 ^{bc}	0.94 ± 0.17	6.3 ± 0.3 ^c	3.9 ± 0.2 ^c	42 ± 3 ^{cd}

Table S2. The concentration of metabolites in leaves of barley transformed with *HvALMT1*

Shown is the mean concentration of each metabolite (in arbitrary units) expressed on a fresh weight basis. Data derived from TBS analysis is italicised whereas all other data was derived from TMS analysis. Metabolites highlighted in bold are those that were significantly different ($P < 0.05$) in the transgenic lines expressing *HvALMT1* at a high level (L4, L5 and L6) compared to transgenic lines expressing *HvALMT1* at wild-type levels (L1, L2 and L3) and non-transformed control lines (C1, C2 and C3). The SEM is shown (for TBS data $n = 3$ for L5, $n = 5$ for L3 and $n = 6$ for all other lines; for TMS data $n = 3$ for L5, and $n = 6$ for all other lines). The significance column indicates the statistical significance between lines as determined by ANOVA; ns denotes not significant ($P > 0.05$), * denotes $P < 0.05$, **denotes $P < 0.01$, ***denotes $P < 0.001$. The statistical significance was determined from raw data, or where skewed, from square root or \log_{10} transformed data. Where the identification of compounds was not fully validated the metabolites are marked (put). Compounds with MST in the name (e.g., 126003-101 MST 1428.3) were identified based on matching mass spectra from the Max Plank Institute for Plant Physiology mass spectrum library (<http://csbdb.mpimp-golm.mpg.de/csbdb/dbma/msri.html>) for which the metabolite had not been identified (i.e. known unknowns). Note: values presented for each metabolite are relative only and cannot be compared between metabolites.

Metabolite	C1	C2	C3	L1	L2	L3	L4	L5	L6	Significance
	Avg ± SE	Avg ± SE	Avg ± SE	Avg ± SE	Avg ± SE	Avg ± SE	Avg ± SE	Avg ± SE	Avg ± SE	
1-Monohexadecanoglycerol	0.66 0.03	0.67 0.01	0.64 0.04	1.39 0.71	1.29 0.63	1.38 0.67	0.77 0.04	0.68 0.11	1.24 0.60	ns
1-Monooctodecanoglycerol	1.07 0.03	1.13 0.06	1.08 0.08	2.77 1.46	2.21 1.09	2.49 1.27	1.33 0.08	1.15 0.18	2.03 1.03	ns
2-Butanoic acid	5.3 0.9	7.3 0.8	2.6 0.4	10.8 1.2	6.8 0.7	12.8 1.3	11.0 1.4	31.2 1.5	13.3 2.7	***
2-Keto-gluconic acid	7.5 0.7	8.0 0.7	8.4 0.7	9.2 0.7	8.9 1.2	11.4 0.8	9.9 1.6	23.1 1.6	13.9 3.6	***
2-Oxocaproic acid	0.56 0.12	0.45 0.09	0.86 0.13	0.27 0.03	0.55 0.10	0.27 0.04	0.39 0.10	0.59 0.05	0.31 0.11	***
2-Oxovaleric acid	0.069 0.020	0.058 0.017	0.063 0.015	0.045 0.006	0.060 0.014	0.060 0.009	0.067 0.020	0.040 0.020	0.061 0.019	ns
3-Phosphoglycerate	0.114 0.012	0.077 0.010	0.111 0.007	0.037 0.004	0.058 0.006	0.041 0.004	0.081 0.007	0.082 0.022	0.062 0.007	***
4-Hydroxycinnamic acid	3.6 0.7	3.3 0.6	3.6 0.7	2.6 0.2	2.9 0.5	3.0 0.2	3.5 0.7	2.3 1.0	3.1 0.7	ns
4-Hydroxybenzoic acid	0.57 0.01	0.61 0.03	0.58 0.06	0.49 0.02	0.77 0.03	0.54 0.02	0.74 0.03	0.99 0.20	0.83 0.12	***
5-Oxoproline	11.9 1.6	14.0 1.9	10.2 1.1	7.6 0.9	9.8 2.7	10.9 3.7	20.5 2.6	50.7 10.9	28.8 5.2	***
6-Hydroxynicotinic acid	0.62 0.05	0.55 0.03	0.66 0.11	0.26 0.03	0.49 0.03	0.25 0.05	0.47 0.06	0.44 0.09	0.44 0.04	***
9,12-Octadecanoic acid	0.106 0.026	0.124 0.036	0.162 0.044	0.074 0.016	0.095 0.035	0.048 0.014	0.077 0.027	0.000 0.000	0.000 0.000	***
Acetohydroxamic acid	6.8 3.9	6.1 3.0	5.8 2.9	2.5 0.6	6.1 3.3	2.8 0.4	6.4 3.2	12.1 9.7	6.0 2.9	**
Aconitic acid	1.24 0.10	0.91 0.10	1.07 0.10	1.33 0.08	0.93 0.11	1.45 0.15	0.75 0.12	0.74 0.06	0.46 0.09	***
Adenine	0.34 0.03	0.41 0.04	0.25 0.03	0.15 0.01	0.34 0.02	0.19 0.02	0.43 0.03	0.57 0.08	0.51 0.08	***
α-Ketoglutarate	1.9 0.2	2.3 0.4	1.3 0.3	2.0 0.3	1.5 0.2	1.6 0.1	2.0 0.1	2.0 0.1	1.4 0.3	ns
Alanine	13.0 1.5	16.3 2.6	11.0 0.8	10.6 1.0	10.7 1.1	13.6 3.7	20.1 2.7	30.8 1.5	26.8 8.0	***
β-Alanine	0.77 0.07	0.76 0.16	0.72 0.14	0.24 0.03	0.65 0.13	0.43 0.06	0.53 0.10	0.96 0.15	0.90 0.14	**
Allantoin	1.39 0.30	2.17 0.57	1.04 0.36	0.73 0.45	1.50 0.46	0.24 0.07	1.94 1.00	31.02 4.38	21.83 7.95	***
Aminoisobutyric acid	0.08 0.01	0.09 0.00	0.11 0.01	0.05 0.01	0.08 0.00	0.07 0.01	0.12 0.02	0.23 0.01	0.13 0.02	***
Arabinose	0.33 0.05	0.29 0.04	0.52 0.07	0.36 0.07	0.74 0.16	0.26 0.03	0.33 0.05	2.68 0.30	2.04 0.33	***
Ascorbic acid	0.68 0.18	0.58 0.16	0.45 0.13	0.19 0.09	0.72 0.30	0.86 0.65	2.78 0.93	7.95 2.84	3.14 0.91	***
Asparagine	2.0 0.4	5.0 1.5	1.9 0.4	1.4 1.1	3.7 2.3	0.6 0.2	3.8 0.9	116.9 56.6	36.5 9.0	***
Aspartic acid	14.6 0.9	15.7 1.0	13.0 1.2	9.5 1.0	9.5 0.7	11.5 1.1	17.4 1.3	19.3 2.6	11.3 1.8	***
Citrate	164 33	110 16	214 42	202 38	175 35	267 33	160 52	321 57	77 45	**
Cysteine	0.053 0.004	0.068 0.009	0.065 0.004	0.027 0.002	0.058 0.005	0.045 0.010	0.083 0.005	0.204 0.032	0.222 0.065	***
Digalactosylglycerol	0.75 0.26	0.45 0.11	0.69 0.13	0.87 0.19	0.31 0.02	0.59 0.12	0.41 0.11	1.91 0.78	0.94 0.32	***
Docosanoic acid	0.60 0.03	0.49 0.02	0.69 0.05	0.20 0.01	0.40 0.02	0.25 0.03	0.48 0.04	0.33 0.05	0.33 0.04	***
Dodecanoic acid	0.36 0.03	0.34 0.01	0.47 0.05	0.24 0.02	0.37 0.04	0.26 0.03	0.40 0.06	0.41 0.03	0.33 0.05	***
Erythronic acid (put)	4.4 0.4	4.7 0.6	5.7 0.8	4.8 0.7	4.8 0.7	6.7 0.3	7.1 1.1	19.4 7.7	17.1 7.1	***
Ethylene glycol (put)	96 10	66 16	72 8	50 7	66 8	58 11	80 9	58 8	66 14	*
Ethylphosphoric acid	1.83 0.22	1.62 0.19	2.05 0.37	0.84 0.24	1.37 0.35	0.70 0.21	1.89 0.68	1.17 0.30	0.92 0.27	***
FA 16:0	39 1	40 1	37 2	42 2	40 2	41 2	44 3	37 5	36 2	ns
FA 18:0	80 3	85 1	72 3	89 3	88 4	89 3	97 5	80 10	78 3	***
FA 20:0	1.39 0.09	1.35 0.08	1.26 0.13	1.32 0.04	1.40 0.08	1.27 0.06	1.56 0.12	1.24 0.17	1.20 0.07	ns
Ferulic acid	1.22 0.13	1.09 0.14	1.38 0.11	0.65 0.06	0.53 0.13	1.12 0.08	0.90 0.12	2.01 0.65	0.83 0.33	***
Fructose 6-phosphate	2.1 0.2	2.8 0.6	2.1 0.3	3.3 0.3	3.2 0.7	3.1 0.5	2.7 0.6	3.5 2.7	1.7 0.5	ns
Fructose	9 3	5 1	36 15	12 6	46 15	4 0	10 6	116 20	42 14	***
Fumaric acid	11.4 1.3	12.1 0.5	10.3 0.8	13.1 1.0	9.3 1.3	14.3 1.9	4.7 1.0	2.8 0.3	1.7 0.4	***
GABA (γ-Aminobutyric acid)	0.49 0.04	0.64 0.04	0.27 0.02	1.70 0.12	0.90 0.10	1.54 0.09	0.89 0.12	0.43 0.02	0.59 0.09	***
Galactinol	1.26 0.14	1.38 0.23	1.13 0.25	1.19 0.32	1.45 0.12	0.83 0.10	1.50 0.21	2.93 0.47	2.13 0.33	***
Galactonic acid	2.8 0.2	2.8 0.2	2.7 0.1	2.6 0.2	3.1 0.3	3.0 0.3	2.7 0.4	3.8 0.1	2.2 0.4	*

Gentibiose	0.015 0.002	0.016 0.003	0.020 0.004	0.019 0.003	0.023 0.005	0.020 0.004	0.033 0.008	0.080 0.004	0.054 0.017	***
Glucose 6-phosphate	5.0 0.3	6.6 1.3	4.8 0.3	7.5 0.4	6.6 1.2	7.2 0.9	6.5 0.9	9.2 5.9	4.8 1.5	ns
Glucaric acid (put)	1.28 0.13	1.37 0.16	1.40 0.15	1.35 0.11	1.53 0.20	1.77 0.19	2.11 0.35	4.49 0.08	3.12 0.65	***
Glucose	13.3 5.8	20.4 14.9	48.0 16.4	49.9 31.1	63.9 10.0	4.8 0.3	12.2 6.6	355.2 118.9	146.0 47.6	***
Glutamate	89 3	86 5	88 5	54 5	68 4	71 2	102 6	100 2	79 8	***
Glutamine	0.092 0.008	0.128 0.036	0.021 0.004	0.070 0.024	0.107 0.052	0.138 0.085	0.234 0.060	2.657 0.608	0.600 0.193	***
Glyceric acid	4.6 1.2	4.8 0.6	5.6 0.6	5.4 0.7	4.7 1.1	6.2 0.9	7.0 1.5	10.1 2.3	3.7 1.2	**
Glycerol	0.86 0.05	0.92 0.10	2.35 1.48	0.77 0.14	0.50 0.15	0.75 0.15	0.92 0.06	1.01 0.29	1.22 0.16	*
Glycerol 3-phosphate	8.9 0.8	9.5 1.5	7.7 1.3	9.0 0.6	13.1 1.2	8.6 0.2	15.1 1.4	23.6 10.7	14.6 2.5	***
Glycerophosphorylglycerol	2.3 0.5	2.7 0.6	2.3 0.5	2.9 0.7	2.6 0.3	1.8 0.3	2.4 0.6	4.0 2.7	3.8 0.8	ns
α-Glycerophosphoglycerol	5.9 1.3	6.7 1.8	5.8 1.2	7.1 1.7	6.4 0.8	4.3 0.7	6.0 1.4	10.4 7.0	9.7 2.1	ns
Glycine	2.6 0.3	3.3 0.3	2.6 0.5	2.1 0.2	2.2 0.3	2.8 0.8	4.4 0.4	13.9 1.4	8.6 1.9	***
Glycolic acid	3.4 0.3	3.4 0.2	3.8 0.3	4.0 0.2	3.5 0.2	5.6 0.5	3.7 0.3	11.0 1.4	5.4 0.9	***
Heptadecanoic acid	2.8 0.1	2.4 0.1	3.2 0.2	1.4 0.1	2.2 0.1	1.7 0.2	2.7 0.2	2.8 0.6	2.2 0.3	***
Hexadecanoic acid	68 3	63 3	66 3	46 2	59 3	51 5	68 4	33 4	45 10	***
Histidine	0.30 0.08	0.39 0.08	0.16 0.07	0.24 0.08	0.63 0.13	0.44 0.12	0.85 0.16	24.14 11.60	8.95 3.29	***
Homoserine	0.142 0.075	0.139 0.050	0.145 0.089	0.017 0.017	0.032 0.022	0.094 0.078	0.387 0.202	1.863 1.378	0.572 0.181	***
Iminodicetic acid	0.026 0.004	0.025 0.005	0.033 0.004	0.015 0.004	0.022 0.005	0.017 0.003	0.022 0.004	0.035 0.009	0.028 0.004	**
Isocitrate	31 6	19 3	41 6	16 3	22 4	18 4	16 4	30 9	11 3	***
Isoleucine	0.62 0.12	0.62 0.06	0.85 0.15	0.29 0.04	0.82 0.09	0.35 0.03	0.92 0.10	3.69 1.88	1.85 0.46	***
Lactic acid	11.5 2.2	10.7 2.0	12.0 2.3	7.3 0.4	9.4 1.7	8.4 0.4	10.8 2.1	6.1 2.6	9.3 2.1	**
Leucine	2.2 1.0	1.8 0.2	4.2 1.3	1.8 0.7	3.6 1.1	2.0 0.6	4.7 1.0	12.2 3.6	10.2 3.4	***
Lysine	0.58 0.10	0.49 0.07	0.45 0.12	0.22 0.04	0.63 0.13	0.30 0.03	0.68 0.07	3.19 0.74	2.31 0.44	***
Malate	117 11	125 11	142 20	181 34	133 6	211 18	101 25	119 23	66 20	***
Maleic acid	0.25 0.02	0.22 0.02	0.26 0.03	0.54 0.02	0.19 0.01	0.70 0.09	0.17 0.02	1.58 0.96	0.60 0.45	***
Malonic acid	0.82 0.10	0.55 0.04	1.14 0.13	0.32 0.04	0.82 0.11	0.35 0.01	0.47 0.05	0.69 0.09	0.72 0.23	***
Mannitol	0.22 0.03	0.21 0.04	0.52 0.20	0.41 0.15	0.57 0.22	0.66 0.48	0.77 0.58	1.00 0.11	0.90 0.17	***
Melibiose	1.26 0.15	1.17 0.17	0.64 0.10	0.99 0.13	0.39 0.03	1.25 0.16	0.79 0.18	1.05 0.05	0.80 0.16	**
Methionine	0.21 0.03	0.22 0.03	0.22 0.02	0.08 0.01	0.21 0.03	0.13 0.02	0.35 0.03	1.73 0.92	0.68 0.21	***
Monomethylphosphate	1.8 0.3	2.0 0.4	1.6 0.2	0.5 0.1	0.9 0.1	0.6 0.0	1.8 0.3	13.0 5.7	3.3 0.8	***
Muconic acid	0.63 0.08	0.49 0.10	0.85 0.12	0.26 0.04	0.25 0.03	0.59 0.12	0.51 0.09	2.94 0.83	0.74 0.51	***
myo-Inositol	23 2	21 2	30 3	25 4	28 4	31 2	23 2	45 6	26 5	***
Nonanoic acid	0.35 0.03	0.29 0.01	0.48 0.11	0.24 0.02	0.30 0.03	0.29 0.04	0.37 0.05	0.41 0.03	0.32 0.06	**
Octadecanoic acid	108 4	102 3	103 5	74 4	95 4	83 7	110 7	92 11	90 13	***
Oxalic acid	0.53 0.07	0.74 0.15	0.60 0.05	0.22 0.02	0.47 0.07	0.28 0.03	0.72 0.06	0.84 0.01	0.73 0.20	***
Oxaloacetic acid	1.33 0.23	1.59 0.38	1.90 0.17	0.65 0.06	0.76 0.14	0.97 0.11	1.37 0.14	2.59 0.57	1.01 0.36	***
p-Coumaric acid	0.13 0.03	0.15 0.05	0.15 0.03	0.12 0.03	0.11 0.04	0.11 0.01	0.11 0.04	0.13 0.08	0.04 0.02	ns
Pentadecanoic acid	0.77 0.02	0.71 0.02	0.94 0.04	0.37 0.03	0.63 0.04	0.46 0.05	0.76 0.05	0.63 0.09	0.62 0.07	***
Pentanoic acid	0.117 0.029	0.106 0.026	0.084 0.013	0.041 0.005	0.113 0.034	0.084 0.027	0.211 0.030	0.462 0.148	0.371 0.084	***
Phenylalanine	2.6 0.3	2.8 0.1	3.1 0.2	1.5 0.1	2.6 0.3	1.8 0.2	4.2 0.5	11.8 5.2	5.0 0.9	***
Phosphate	74 8	80 10	94 14	123 11	90 9	140 14	75 11	75 47	49 17	***
Phytanic acid	4.8 0.2	4.3 0.1	5.2 0.3	2.3 0.1	3.5 0.2	2.5 0.3	4.2 0.4	3.3 0.5	3.3 0.5	***
Proline	2.6 0.8	2.2 0.1	5.5 2.2	4.0 1.7	3.7 0.9	3.7 1.1	4.6 0.6	115.2 6.8	37.9 18.0	***
Pyroglutamate	265 18	340 48	237 32	470 31	294 11	528 42	458 49	803 85	512 21	***
Pyruvate	0.072 0.036	0.388 0.283	0.039 0.011	0.273 0.057	0.255 0.102	0.135 0.024	0.147 0.053	0.209 0.152	0.148 0.085	ns

Quinic acid	36 8	43 13	30 15	32 10	17 6	16 4	22 6	17 14	11 6	ns
Raffinose	87 10	82 13	51 8	97 20	20 2	124 8	95 12	315 18	86 17	***
Ribonic acid	2.6 0.2	2.7 0.3	3.0 0.2	2.5 0.2	2.9 0.5	3.3 0.4	3.5 0.4	6.5 0.4	3.6 0.3	***
Ribose	1.42 0.10	1.34 0.12	1.48 0.14	1.19 0.17	2.13 0.31	1.17 0.10	1.23 0.07	0.71 0.86	2.01 0.70	*
Serine	9.9 1.4	9.8 0.7	8.2 0.5	5.6 0.6	7.7 1.0	6.2 1.7	18.3 2.1	27.0 3.5	22.6 2.0	***
Shikimic acid	0.66 0.12	0.36 0.12	0.85 0.24	0.26 0.04	0.27 0.04	0.18 0.03	0.39 0.07	0.77 0.37	0.33 0.10	**
Sinapic acid	2.2 0.3	1.7 0.3	2.9 0.6	1.0 0.2	1.2 0.1	2.1 0.4	3.0 0.3	7.2 1.0	4.7 0.8	***
S-methyl-cysteine (put)	0.40 0.06	0.73 0.13	0.26 0.05	0.63 0.22	0.66 0.29	0.72 0.25	0.86 0.18	71.86 54.84	11.57 4.08	***
Succinic acid	4.7 1.0	4.1 0.5	3.6 0.5	5.3 0.8	3.8 0.4	4.7 0.2	5.4 1.4	6.3 0.6	3.2 0.4	ns
Sucrose	41 3	50 7	39 6	93 8	42 3	94 8	66 6	103 15	75 11	***
Tetracosanoic acid	0.047 0.003	0.041 0.003	0.082 0.015	0.018 0.002	0.040 0.006	0.021 0.002	0.037 0.004	0.037 0.005	0.026 0.004	***
Tetradecanoic acid	0.57 0.08	0.53 0.05	0.73 0.09	0.30 0.04	0.30 0.03	0.37 0.05	0.63 0.09	1.21 0.09	0.64 0.23	***
Threonic acid	7.7 0.9	7.1 0.8	10.1 1.0	8.5 0.5	10.6 0.9	8.6 1.2	6.8 1.1	7.9 1.4	4.7 1.7	*
Threonic acid-1,4-lactone	0.26 0.02	0.34 0.05	0.26 0.04	0.40 0.04	0.35 0.04	0.49 0.05	0.44 0.02	0.75 0.04	0.50 0.09	***
Threonine	1.9 0.3	2.8 0.4	1.7 0.1	1.3 0.2	1.9 0.1	2.0 0.4	4.2 0.4	7.6 0.7	5.7 0.6	***
Tricosanoic acid	0.031 0.002	0.031 0.002	0.047 0.006	0.014 0.001	0.026 0.003	0.019 0.002	0.032 0.004	0.043 0.012	0.042 0.008	***
Tryptophan	0.19 0.06	0.19 0.02	0.21 0.08	0.03 0.02	0.22 0.16	0.10 0.02	0.28 0.04	6.96 5.39	1.14 0.44	***
Tyrosine	1.76 0.40	1.54 0.12	2.13 0.32	0.80 0.16	1.92 0.38	0.94 0.12	2.23 0.28	6.71 2.48	3.89 1.06	***
Uracil	0.14 0.02	0.21 0.04	0.09 0.03	0.32 0.07	0.21 0.03	0.24 0.04	0.25 0.04	0.54 0.25	0.38 0.04	***
Valine	1.35 0.15	1.46 0.10	1.53 0.18	0.64 0.06	1.21 0.06	0.77 0.04	1.92 0.15	8.69 4.17	3.29 0.43	***
Xylose	0.19 0.01	0.22 0.03	0.21 0.02	0.20 0.03	0.21 0.03	0.23 0.04	0.18 0.02	0.40 0.01	0.24 0.07	*
126003-101_MST_1428.3	1.31 0.08	1.25 0.13	1.29 0.13	1.62 0.14	1.66 0.13	1.70 0.11	1.53 0.16	1.54 0.08	0.97 0.28	*
126004-101_MST_1257.2	62 6	62 5	68 13	83 7	62 6	78 5	74 3	67 5	67 5	ns
136002-101_MST_1337.3	1.10 0.39	0.97 0.33	1.41 0.46	3.59 1.00	3.65 1.05	1.36 0.31	1.69 0.51	5.58 6.16	0.57 0.13	*
140003-101_MST_1376.5	0.18 0.01	0.21 0.03	0.24 0.02	0.23 0.03	0.15 0.02	0.30 0.02	0.25 0.05	0.13 0.02	0.13 0.02	***
143004-101_MST_1419.6	0.30 0.05	0.45 0.13	0.42 0.08	0.56 0.09	0.34 0.08	0.66 0.16	0.26 0.07	0.49 0.49	0.25 0.10	ns
148010-101_MST_1483.8	2.5 0.4	2.3 0.3	3.0 0.3	3.3 0.3	2.8 0.3	3.1 0.5	2.1 0.4	3.2 0.0	1.7 0.4	*
174001-101_MST_1751	1.8 0.4	1.9 0.5	3.0 0.8	2.1 0.6	1.4 0.3	4.3 1.2	4.1 0.8	18.5 9.7	13.3 7.5	***
176011-101_MST_1768.8	4.3 2.9	5.9 4.1	1.5 0.5	8.6 6.1	4.1 2.6	2.3 1.2	1.6 0.2	8.2 6.8	0.9 0.3	ns
179010-101_MST_1792.2	2.0 0.3	3.0 0.8	3.1 0.8	3.0 0.7	3.3 1.1	6.2 1.1	6.9 1.9	11.9 5.3	6.6 2.3	**
192018-101_MST_1926.7	14 1	18 2	13 1	13 1	20 4	24 2	22 1	26 1	23 2	***
197007-101_MST_1979.3	3.8 0.6	5.5 1.7	5.8 1.7	6.2 1.6	6.5 1.8	12.8 2.1	13.9 3.9	26.7 6.6	16.8 8.4	***
212003-101_MST_2120.8	0.57 0.08	0.59 0.06	0.33 0.05	0.68 0.13	0.55 0.06	0.46 0.04	0.39 0.03	0.96 0.75	0.26 0.06	**
214003-101_MST_2140.2	6 1	7 2	9 1	8 1	17 4	14 2	16 3	45 15	36 12	***
239008-101_MST_2395.4	5.9 1.0	4.9 0.9	6.1 1.3	6.6 1.3	7.6 0.7	4.5 1.0	4.8 1.3	23.5 3.9	14.9 5.4	***
256004-101_MST_2565.3	4.7 0.4	5.2 0.7	4.6 0.3	6.3 0.6	7.1 0.9	7.5 0.7	7.8 0.8	17.7 2.9	11.1 1.2	***

Table S3. The concentration of metabolites in roots of barley (transformed with *HvALMT1*)

Shown is the mean concentration of each metabolite (in arbitrary units) from the roots of the T₀ generation of barley transformed with *HvALMT1* segregating as either null segregants for the transgene (Null), heterozygous for the transgene (Het) or homozygous for the transgene (stunted individuals; Hom). The data is expressed on a fresh weight basis and where derived from the TBS analysis is italicised whereas all other data was derived from the TMS analysis. Metabolites highlighted in bold are those that were significantly different ($P < 0.05$) in the segregants of the T₀ generation of line L4 that contained the *HvALMT1* transgene compared to null segregants. The standard error of the mean (SE) is shown and the significance column indicates the statistical significance between lines as determined by ANOVA; ns denotes not-significant ($P > 0.05$), *denotes $P < 0.05$, **denotes $P < 0.01$, ***denotes $P < 0.001$ ($n = 6$ for L4 (Null), $n = 10$ for L4 (Het) and $n = 2$ for L4 (Hom)). The statistical significance was determined from raw data or log₁₀ transformed data. When transformation was unable to yield normally distributed data a Kruskal-Wallis ANOVA on ranks was undertaken. ** The identification of galactinol was not fully validated. Note: values presented for each metabolite are relative only and cannot be compared between metabolites.

Metabolite	L4 (Null)	L4 (Het)	L4 (Hom)	Significance
	Avg ± SE	Avg ± SE	Avg ± SE	
1-Monooctodecanoglycerol	0.0020 0.0005	0.0028 0.0005	0.0064 0.0024	***
3-O-beta-D-galactose-D-arabinose	0.0089 0.0033	0.0089 0.0022	0.0202 0.0049	***
Adenosine	0.012 0.004	0.013 0.001	0.032 0.000	***
<i>Alanine</i>	0.034 0.009	0.033 0.005	0.064 0.023	***
β -alanine	0.013 0.002	0.010 0.002	0.017 0.002	***
Allantoin	0.051 0.019	0.012 0.004	0.101 0.032	***
Arabinose	0.033 0.007	0.019 0.003	0.038 0.016	ns
Arabitol	0.17 0.06	0.21 0.03	0.45 0.19	***
Arginine/Ornithine	0.0068 0.0027	0.0055 0.0009	0.0183 0.0126	***
<i>Asparagine</i>	0.16 0.03	0.10 0.02	0.29 0.03	***
<i>Aspartate</i>	0.070 0.014	0.034 0.005	0.064 0.001	***
Benzoate	0.099 0.038	0.124 0.022	0.273 0.098	***
<i>Butyrate</i>	0.061 0.011	0.037 0.005	0.093 0.007	***
Citrate	0.079 0.029	0.043 0.009	0.064 0.016	ns
Dehydroascobate	0.0154 0.0052	0.0063 0.0012	0.0120 0.0065	*
Diethylene glycol	0.34 0.12	0.42 0.06	0.88 0.43	***
Digalactosylglycerol	0.0080 0.0029	0.0045 0.0006	0.0079 0.0025	ns
Eicosanoate	0.011 0.004	0.011 0.002	0.027 0.011	***
Erythronate	0.024 0.005	0.029 0.003	0.041 0.009	***
Ethanolamine	0.23 0.02	0.19 0.02	0.30 0.07	***
Fructose	7.5 2.7	5.0 1.0	13.1 7.2	ns
Fumarate	0.025 0.007	0.031 0.004	0.041 0.019	***
GABA	0.20 0.05	0.16 0.03	0.21 0.05	**
Galactinol**	0.0063 0.0017	0.0041 0.0011	0.0129 0.0100	ns
Galactitol	0.0036 0.0016	0.0034 0.0008	0.0069 0.0004	***
Galactonate	0.016 0.004	0.017 0.002	0.028 0.005	**
Galactosylglycerol	0.037 0.008	0.021 0.004	0.040 0.013	*
Galacturonate	0.0067 0.0017	0.0058 0.0008	0.0175 0.0117	**
Gentibiose	0.095 0.041	0.035 0.006	0.143 0.079	ns
Glucarate	0.067 0.012	0.132 0.019	0.235 0.005	**
Gluconate	0.016 0.003	0.019 0.002	0.028 0.005	***
Glucose	2.7 1.2	1.4 0.3	4.2 2.5	ns
Glucose-6-phosphate	0.0077 0.0031	0.0045 0.0008	0.0174 0.0081	***
Glucuronate	0.0048 0.0013	0.0056 0.0007	0.0176 0.0092	***
<i>Glutamate</i>	0.14 0.03	0.11 0.02	0.17 0.02	***
<i>Glutamine</i>	0.017 0.002	0.010 0.002	0.013 0.004	**
Glycerate	0.016 0.004	0.014 0.002	0.027 0.011	**
<i>Glycerol</i>	0.0079 0.0013	0.0074 0.0008	0.0175 0.0058	***
Glycerol-3-phosphate	0.0060 0.0020	0.0049 0.0009	0.0199 0.0128	***
Glycine	0.0089 0.0022	0.0090 0.0010	0.0159 0.0026	*
Heptadecanoate	0.0063 0.0025	0.0070 0.0011	0.0173 0.0078	***
<i>Hexadecanoate</i>	0.56 0.16	0.52 0.07	1.91 0.45	***
Homoserine	0.0028 0.0008	0.0015 0.0002	0.0047 0.0021	***
Hydroquinone-beta-glucopyranose	0.014 0.005	0.020 0.003	0.052 0.016	***
Inositol	0.187 0.069	0.093 0.012	0.321 0.187	***
Isocitrate	0.0030 0.0010	0.0021 0.0005	0.0061 0.0041	***
<i>Isoleucine</i>	0.090 0.012	0.054 0.006	0.121 0.008	***
Itaconate	0.0021 0.0006	0.0035 0.0006	0.0016 0.0008	ns
Lactose	0.0024 0.0009	0.0021 0.0006	0.0122 0.0088	ns
Laminaribiose	0.0070 0.0022	0.0049 0.0007	0.0115 0.0046	**
<i>Leucine</i>	0.068 0.013	0.041 0.005	0.105 0.009	***
Lysine	0.073 0.020	0.046 0.006	0.190 0.075	***
Malate	0.76 0.25	0.62 0.16	0.76 0.33	ns
Maleate	0.011 0.002	0.013 0.003	0.011 0.006	ns
Maltose	0.0049 0.0015	0.0124 0.0047	0.0528 0.0485	**
Mannitol	0.0075 0.0021	0.0043 0.0006	0.0121 0.0023	***
Melezitose	0.017 0.006	0.014 0.003	0.100 0.081	***
Melibiose	0.022 0.006	0.026 0.004	0.061 0.027	***

<i>Methionine</i>	0.0095	0.0010	0.0109	0.0017	0.0253	0.0092	***
Monomethylphosphate	0.042	0.012	0.041	0.007	0.104	0.009	**
N-acetyl glucosamine	0.010	0.003	0.010	0.002	0.022	0.012	***
<i>Octadecanoate</i>	0.68	0.17	0.71	0.10	2.36	0.26	***
Octadecanol	0.0015	0.0004	0.0018	0.0003	0.0039	0.0015	***
Oxalate	0.040	0.009	0.051	0.010	0.107	0.070	***
<i>Phenylalanine</i>	0.0075	0.0017	0.0058	0.0007	0.0133	0.0028	***
Phosphate	0.49	0.13	0.54	0.09	1.88	0.85	***
Pipecolate	0.029	0.008	0.041	0.017	0.073	0.025	*
Proline	0.019	0.006	0.017	0.009	0.099	0.064	*
Putrescine	2.6	0.6	1.3	0.2	3.1	1.0	***
Pyroglutamate	1.14	0.34	0.69	0.14	1.22	0.31	***
Pyrrole-2-carboxylate	0.0021	0.0007	0.0036	0.0007	0.0055	0.0018	***
Quebrachitol	0.0038	0.0021	0.0028	0.0007	0.0096	0.0092	ns
Quinate	0.047	0.009	0.051	0.007	0.088	0.042	ns
Raffinose	0.79	0.21	0.44	0.08	1.31	0.83	*
Rhamnose	0.0048	0.0009	0.0031	0.0004	0.0088	0.0046	***
Ribonate	0.16	0.03	0.16	0.02	0.31	0.05	***
<i>Serine</i>	0.027	0.005	0.023	0.003	0.060	0.003	***
Shikimate	0.020	0.005	0.020	0.003	0.052	0.027	**
Sorbitol	0.0106	0.0032	0.0089	0.0015	0.0170	0.0036	***
Succinate	0.033	0.010	0.035	0.005	0.074	0.022	***
Sucrose	7.5	1.9	5.7	0.8	11.8	6.9	**
Tetradecanoate	0.019	0.007	0.023	0.003	0.050	0.022	***
Threonate	0.0070	0.0015	0.0066	0.0008	0.0118	0.0040	***
<i>Threonine</i>	0.0099	0.0016	0.0084	0.0010	0.0203	0.0040	***
<i>Tryptophan</i>	0.012	0.001	0.011	0.003	0.045	0.022	*
Turanose	0.014	0.007	0.016	0.003	0.037	0.020	**
<i>Tyramine</i>	0.025	0.007	0.024	0.003	0.043	0.002	***
Tyrosine	0.077	0.026	0.046	0.008	0.146	0.041	***
Uracil	0.0018	0.0008	0.0021	0.0003	0.0038	0.0019	***
Urea	0.025	0.007	0.030	0.007	0.072	0.009	***
<i>Valine</i>	0.039	0.007	0.026	0.003	0.057	0.011	***
Xylitol	0.0027	0.0005	0.0020	0.0003	0.0056	0.0026	***
Xylose	0.038	0.010	0.034	0.006	0.091	0.057	***