10.1071/FP22181

Functional Plant Biology

Supplementary Material

Red light-induced inhibition of maize (*Zea mays*) mesocotyl elongation: evaluation of apoplastic metabolites

Elena Sharova[A,*], *Tatiana Bilova*[A,B], *Elena Tsvetkova*[C], *Galina Smolikova*[A], *Andrej Frolov*[B], *and Sergei Medvedev*[A]

[A]Department of Plant Physiology and Biochemistry, Saint Petersburg State University, Saint Petersburg, Russian Federation.

[B]K.A. Timiryazev Institute of Plant Physiology RAS, Moscow, Russian Federation.

[C]Department of Biochemistry, Saint Petersburg State University, Saint Petersburg, Russian Federation.

[*]Correspondence to: Elena Sharova Department of Plant Physiology and Biochemistry, Saint Petersburg State University, Saint Petersburg, Russian Federation Email: e.sharova@spbu.ru

Supplementary information

Directory

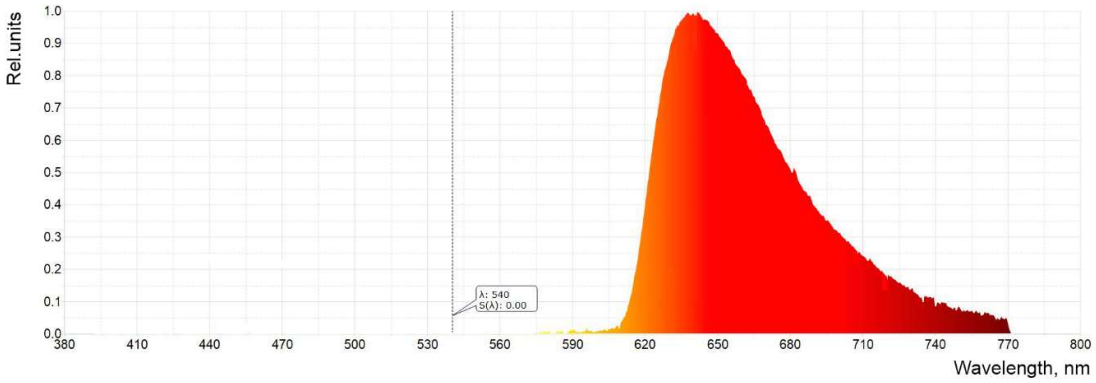

Fig. S1. Light spectrum used for maize seedlings treatment

Table S1. Gas chromatographic (GC) separation conditions and electron ionization-quadrupole-mass spectrometry (EI-Q-MS) settings for analysis of primary polar thermostabile metabolites of apoplastic solution from maize mesocotyl with Shimadzu GC2010 gas chromatograph coupled online to a quadrupole mass selective detector Shimadzu GCMS QP2010 with CTC GC PAL Liquid Injector (Shimadzu Scientific Instruments, Australia)

Parameters	**Setting**
	GC settings
Separation column	HP-5 capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness, Thermo Fisher Scientific, Bremen, Germany)
Carrier gas / carrier gas flow rate	Helium / 1 mL/min
Injector operation mode	Splitless mode (90 s splitless time)
Injector temperature	250°C
Temperature program	1 min at 40°C ramp 15°C/min to 70°C 1 min at 70°C ramp 6°C/min to 320°C 12 min at 320°C
Parameters	**MS settings**
Ionization mode	Electron ionization (EI)
Electron energy	70 eV
Operation mode	scanning at 0.34 sec $scan^{-1}$
m/z range	50 - 700

Table S2. The metabolites identified in the apoplastic fluid obtained by low-speed centrifugation from the mesocotyls of 3-day-old maize seedlings grown in the dark and 4 h after their exposure to red light.

Metabolite[a]	*m/z*[b]	Standard, 50 pmol			AF from the dark grown mesocotyls (D)[f]					AF from mesocotyls after their 4 h red-light illumination (L)					L/D[h]	p^i <0.05
		RT, min	RI	Peak area	RT[c]	RI[d]	Peak area[e], ẍ, n=3	nmol g^{-1} FW[g]	mmol L^{-1} in AF[g]	RT	RI	Peak area, ẍ, n=3	nmol g^{-1} FW	mmol L^{-1} in AF		
Lactic acid (2TMS)	191				9.94	1077	1.53E+06			9.95	1080	1.60E+06			1.05	
Alanine (2TMS)	116	10.81	1115	2.80E+05	10.82	1117	3.09E+06	22.09	0.820±0.102	10.83	1118	3.36E+06	24.02	0.890±0.191	1.09	
Malonic acid (2TMS)	233	13.03	1207	5.16E+04	13.06	1209	2.99E+04	1.10	0.041±0.011	13.06	1209	3.23E+04	1.20	0.044±0.009	1.08	
Valine (2TMS)	144	13.28	1218	6.60E+05	13.30	1219	3.57E+06	10.70	0.400±0.043	13.30	1220	3.97E+06	12.00	0.440±0.108	1.11	
Serine (2TMS)	116	14.20	1258	4.43E+05	14.25	1259	1.12E+07	58.00	2.150±0.123	14.25	1261	1.15E+07	60.00	2.200±0.190	1.03	
Serine (3TMS)	204	16.48	1359		16.50	1359				16.50	1359					
Ethanolamine (3TMS)	174				14.40	1265	2.94E+06			14.40	1266	3.24E+06			1.10	
Leucine (2TMS)	158	14.52	1271	4.57E+05	14.56	1273	7.71E+05	3.38	0.126±0.028	14.56	1274	8.53E+05	3.87	0.142±0.026	1.11	
Phosphoric acid (3TMS)	299				14.62	1276	5.67E+07			14.61	1276	5.98E+07			1.05	
Isoleucine (2TMS)	218	14.99	1292	1.17E+05	15.05	1293	1.37E+05	2.34	0.087±0.019	15.05	1294	1.60E+05	2.80	0.104±0.022	1.17	
Threonine (2TMS)	219	15.01	1292	3.66E+05	15.06	1295	3.63E+06	19.80	0.740±0.137	15.06	1296	3.67E+06	20.80	0.770±0.092	1.01	
Threonine (3TMS)	218	17.01	1382		17.05	1384				17.05	1384					
Glycine (3TMS)	174	15.28	1304	2.06E+06	15.32	1305	6.13E+06	5.95	0.220±0.040	15.32	1305	6.13E+06	5.98	0.220±0.038	1.00	
Succinic acid (2TMS)	247	15.28	1314	1.86E+05	15.53	1315	1.18E+06	12.70	0.470±0.132	15.55	1316	1.39E+06	14.80	0.550±0.242	1.12	
Glyceric acid (3TMS)	189	15.80	1328	5.65E+05	15.85	1330	2.06E+05	0.74	0.027±0.001	15.86	1330	2.19E+05	0.77	0.029±0.004	1.06	
Fumaric acid (2TMS)	245	16.30	1350	1.34E+06	16.33	1351	7.83E+05	1.18	0.044±0.007	16.33	1351	7.52E+05	1.13	0.042±0.010	0.96	
Aspartic acid (2TMS)	160	17.86	1420	8.65E+05	17.90	1422	4.70E+06	10.97	0.390±0.041	17.91	1423	4.46E+06	10.33	0.390±0.046	0.95	
Aspartic acid (3TMS)	232	19.85	1516		19.93	1519				19.95	1520					
β-Alanine (3TMS)	248	17.93	1424	9.80E+05	17.96	1425	1.15E+06	2.34	0.087±0.003	17.97	1426	1.51E+06	3.13	0.116±0.010	**1.32**	***<0.05***
Malic acid (3TMS)	233	19.21	1485	5.02E+05	19.32	1490	1.47E+07	63.50	2.350±0.359	19.33	1490	1.65E+07	70.80	2.620±0.597	1.12	
Pyroglutamic acid (2TMS)	156	19.84	1515	2.15E+06	19.98	1522	2.74E+07	25.84	0.957±0.028	19.96	1521	2.58E+07	24.22	0.897±0.070	0.94	
GABA (3TMS)	174	20.05	1525	2.04E+06	20.11	1529	1.62E+07	16.10	0.596±0.106	20.13	1529	1.74E+07	17.20	0.637±0.111	1.07	
Glutamine [-H_2O] (2TMS)	155				20.16	1531	1.67E+07			20.16	1531	1.51E+07			0.90	
Glutamine (3TMS)	156				24.75	1774				24.75	1774					
Phenylalanine (1TMS)	120	20.39	1542	1.27E+06	20.46	1546	3.90E+06	6.14	0.227±0.027	20.47	1546	3.73E+06	5.88	0.218±0.013	0.96	
Phenylalanine (2TMS)	218	21.92	1620		21.96	1622				21.98	1623					
2-Oxoglutaric acid (2TMS)	198	20.98	1572	2.41E+05	21.01	1573	3.55E+05	2.96	0.110±0.035	21.02	1574	4.03E+05	3.35	0.124±0.025	1.13	

Metabolite[a]	m/z[b]	Standard, 50 pmol			AF from the dark grown mesocotyls (D)[f]					AF from mesocotyls after their 4 h red-light illumination (L)					L/D[h]	p^i <0.05
		RT, min	RI	Peak area	RT[c]	RI[d]	Peak area[e], ẍ, n=3	nmol/g FW[g]	mmol/L in AF[g]	RT	RI	Peak area, ẍ, n=3	nmol/ g FW	mmol/L in AF		
Asparagine (2TMS)	159				21.43	1594	8.66E+06			21.50	1598	9.40E+06			1.08	
Asparagine (3TMS)	231				22.81	1666				22.89	1670					
Putrescine (4TMS)	174	24.00	1730	5.12E+06	24.03	1732	3.24E+06	1.31	0.049±0.005	24.05	1733	4.09E+06	1.65	0.061±0.004	**1.26**	***<0.05***
***trans*-Aconitic acid (3TMS)**	229				24.31	1747	2.16E+05			24.30	1747	6.25E+05			**2.90**	***<0.05***
C5Furanose 1 (4TMS)	217				24.61	1764	8.23E+06			24.63	1765	8.55E+06			1.04	
C5Furanose 2 (4TMS)	217				24.87	1779	2.12E+06			24.89	1779	2.21E+06			1.04	
Citric acid (4TMS)	273	25.48	1812	1.59E+06	25.51	1814	5.22E+06	6.64	0.246±0.031	25.52	1815	5.26E+06	6.62	0.245±0.060	1.01	
Fructose (1MEOX,5TMS)	307	26.53	1873	5.53E+05	26.46	1869	1.32E+08	481.0	17.80±0.95	26.46	1870	1.33E+08	482.0	17.90±1.49	1.00	
Glucose 1 (1MEOX,5TMS)	319	26.80	1889	1.85E+06	26.92	1896	2.50E+08	369.0	13.65±1.00	26.92	1896	2.56E+08	369.0	13.65±0.29	1.02	
Glucose 2 (1MEOX,5TMS)	319	27.11	1907		27.49	1932				27.49	1932					
Lysine (4TMS)	174	27.24	1915	4.41E+05	27.56	1935	1.26E+06	5.97	0.221±0.047	27.56	1934	1.21E+06	5.63	0.209±0.017	0.96	
Tyrosine (3TMS)	218				27.74	1946	6.07E+06			27.74	1946	5.81E+06			0.96	
Gluconic acid (6TMS)	333	28.45	1989	6.04E+05	28.56	1996	2.21E+05	0.74	0.027±0.002	28.56	1996	2.11E+05	0.71	0.026±0.004	0.96	
Galactaric acid (6TMS)	292	29.16		6.75E+05	29.22	2037	1.30E+05	0.39	0.014±0.002	29.22	2037	1.39E+05	0.41	0.015±0.003	1.07	
Myo-inositol (6TMS)	318	29.90	2080	1.43E+06	29.99	2086	4.63E+06	6.52	0.240±0.046	29.99	2086	4.50E+06	6.30	0.230±0.050	0.97	
trans-Ferulic acid (2TMS)	338	29.98	2086	3.39E+05	30.06	2090	6.44E+04	0.33	0.012±0.001	30.06	2091	6.48E+04	0.33	0.012±0.002	1.01	
Tryptophan (3TMS)	202	31.62	2193	6.48E+05	31.82	2207	5.58E+05	1.76	0.065±0.008	31.83	2208	5.72E+05	1.82	0.067±0.003	1.02	
Fructose 6-P (1MEOX, 6TMS)	315	33.10	2296	1.65E+06	33.13	2298	2.07E+05	0.25	0.010±0.002	33.14	2299	1.93E+05	0.24	0.009±0.002	0.93	
Glucose 6-P (1MEOX, 6TMS)	387	33.27	2308	1.20E+06	33.31	2311	5.10E+05	0.86	0.032±0.004	33.31	2311	4.70E+05	0.79	0.030±0.006	0.92	
Glucose 6-P (1MEOX, 6TMS)	387	33.52	2326		33.56	2329				33.56	2329					
Inosine (4TMS)	217				36.72	2565	1.35E+05			36.73	2565	1.53E+05			1.13	
Sucrose (8TMS)	437	37.50	2626	1.03E+06	37.52	2628	8.91E+05	1.73	0.064±0.031	37.52	2628	7.56E+05	1.45	0.054±0.030	0.85	
Cellobiose (1MEOX, 8TMS)	204				38.33	2694	1.15E+06			38.33	2694	1.10E+06			0.96	
Maltose 1 (8TMS)	361	38.71	2725	2.93E+06	38.75	2728	4.77E+06	3.26	0.121±0.037	38.75	2728	3.86E+06	2.64	0.098±0.010	0.81	
Maltose 2 (8TMS)	361	39.03	2752		39.06	2754				39.06	2754					
Trehalose (8TMS)	361	38.79	2731	6.61E+06	38.82	2734	3.96E+06	1.22	0.045±0.012	38.82	2734	4.00E+06	1.23	0.046±0.007	1.01	

Metabolite[a]	*m/z*[b]	Standard, 50 pmol			AF from the dark grown mesocotyls (D)[f]					AF from mesocotyls after their 4 h red-light illumination (L)					L/D[h]	*p*[i] *<0.05*
		RT, min	RI	Peak area	RT[c]	RI[d]	Peak area[e], ẍ, n=3	nmol/g FW[g]	mmol/L in AF[g]	RT	RI	Peak area, ẍ, n=3	nmol/ g FW	mmol/L in AF		

[a] – The metabolites were identified as trimethylsilyl (TMS) or metoxyamine, trimethylsilyl (MEOX,TMS) derivatives by co-elution and spectral similarity with authentic standards or by retention index and spectral similarity using electron ionization mass-spectral libraries (EI-MS) – NIST 8.0 (National Institute of Standards and Technology) or GMD (GolmMetabolom Database, http://gmd.mpimp-golm.mpg.de). The metabolites are arranged in order of their retention time (RT) increase.

[b] – the *m/z* refers to compound-specific fragment ions, selected for quantification by integration of peak areas at characteristic extracted ion chromatograms.

[c] – Retention time (min).

[d] – Retention index calculated by *RT* of C_{10} – C_{40} alkanes using Automated Mass Spectral Deconvolution and Identification System (AMDIS, www.amdis.net) software.

[e] – the peak area integrated at characteristic extracted ion chromatograms and used for quantification.

[f] – AF – apoplastic fluid obtained by low-speed centrifugation from maize mesocotyl growth zone (two consecutive 5-mm-long segments cut 1.5mm below the coleoptile node).

[g]– The nmol g FW^{-1} and mmol L^{-1} in AF presents results of semi-quantitative analysis of the metabolite content and concentration in AF, respectively, which were calculated on the basis of an integrated peak area obtained for a standard used in concentration of 50 pmol μL^{-1}. Metabolite concentrations (mmol L^{-1}) in AF are expressed as mean ± standard deviation.

[h] – The fold change was calculated as abundance (peak areas at characteristic extracted ion chromatograms) ratio L/D.

[i] – *p*-value. The metabolites which have a significant increase (*p*-value<0.05) in L/D ratio are highlighted with bold font.

Table S3. Glucose-6-phosphate dehydrogenase (G6PDH) activity in the homogenate and apoplastic fluid isolated from maize mesocotyl segments. The data represent the mean ± standard error of three biological replicates.

Mesocotyl segment	G6PDH activity, μmol min^{-1} g FW^{-1}		H / AF, %
	Homogenate (H)[a]	Apoplastic fluid (AF)[b]	
1	1.654 ± 0.072	0.0016 ± 0.0002	0.10
2	0.974 ± 0.029	0.0007 ± 0.0001	0.07
3	0.906 ± 0.032	0.0007 ± 0.0001	0.08
4	0.752 ± 0.015	0.0002 ± 0.0001	0.03