

1 **Supplementary file 2.**

2
3 **Multiple strategies for heat adaptation in rice endosperms**
4 **revealed by on-site cell-specific analysis**

5
6 Hiroshi Wada^{1,†,*}, Yuto Hatakeyama^{1,†}, Yayoi Onda², Hiroshi Nonami², Taiken Nakashima³,
7 Rosa Erra-Balsells⁴, Satoshi Morita¹, Kenzo Hiraoka⁵, Fukuyo Tanaka⁶, and Hiroshi Nakano¹

8
9 ¹Kyushu Okinawa Agricultural Research Center, National Agricultural Research
10 Organization, Chikugo, Japan; ²Graduate School of Agriculture, Ehime University,
11 Matsuyama, Japan; ³Faculty of Agriculture, Hokkaido University, Sapporo, Japan;
12 ⁴Department of Organic Chemistry, University of Buenos Aires, Buenos Aires, Argentina;
13 ⁵Clean Energy Research Center, The University of Yamanashi, Kofu, Japan; ⁶Central
14 Region Agricultural Research Center, National Agriculture and Food Research
15 Organization, Tsukuba, Japan

16
17 [†]These authors contributed equally to this manuscript.

18 *For correspondence: hwada@affrc.go.jp

19
20
21

22 Supplementary file 2.

List of amino acids and carbohydrates detected, as independent ions of clusters, in outer endosperm in each treatment using pikoPPESI-MS in negative ion mode.

Metabolites	Ion molecule formula	Ion type detected [M=molecule]	Theoretical ^a m/z	Observed m/z	Δm ppm	Relative abundance (%) ^b			Frequency of detection ^c			
						26°C	34°C	N+34°C	26°C	34°C	N+34°C	
Amino acids	Glycine	C2H5NO2	[M-H]	74.0247	74.0247	0.0	0.15	0.35	0.12	++	++++	+++
	Alanine	C3H7NO2	[M-H]	88.0404	88.0403	1.1	2.41	3.47	0.78	++++	++++	++++
	GABA	C4H9NO2	[M-H]	102.0561	102.0560	1.0	1.30	2.10	0.27	++++	++++	++++
	Serine	C3H7NO3	[M-H]	104.0353	104.0353	0.0	0.89	2.16	0.78	++++	++++	++++
	Serine	C3H7NO3	[M+Cl]	140.0120	140.0119	0.7	0.34	0.12	0.35	+++	+++	++
	Proline	C5H9NO2	[M-H]	114.0561	114.0560	0.9	0.05	0.03	0.07	+	+++	++
	Valine	C5H11NO2	[M-H]	116.0717	116.0716	0.9	0.33	0.55	0.04	+++	++	+
	Threonine	C4H9NO3	[M-H]	118.0510	118.0509	0.8	0.49	0.77	0.21	++++	++++	++++
	Threonine	C4H9NO3	[M+Cl]	154.0276	154.0272	2.6	0.87	0.02	1.73	+++	++	++
	Cysteine	C3H7NO2S	[M-H]	120.0125	120.0125	0.0	-	0.22	0.03	-	+++	+
	Leucine, Isoleucine	C6H13NO2	[M-H]	130.0874	130.0874	0.0	0.48	0.17	0.03	+++	+++	++
	Asparagine	C4H8N2O3	[M-H]	131.0462	131.0463	-0.8	0.30	0.56	0.19	++++	++++	++
	Aspartic acid	C4H7NO4	[M-H]	132.0302	132.0302	0.0	16.36	28.88	15.44	++++	++++	++++
	Aspartic acid	C4H7NO4	[M+Cl]	168.0069	168.0070	-0.6	0.05	-	0.05	+	-	++
	Glutamine	C5H10N2O3	[M-H]	145.0619	145.0618	0.7	25.66	37.25	0.87	++++	++++	++++
	Glutamine	C5H10N2O3	[M+Cl]	181.0385	181.0385	0.0	0.03	-	0.01	+	-	+
	Lysine	C6H14N2O2	[M-H]	145.0983	145.0982	0.7	0.03	0.03	-	+	++	-
	Glutamic acid	C5H9NO4	[M-H]	146.0459	146.0458	0.7	37.90	58.81	29.05	++++	++++	++++
	Glutamic acid	C5H9NO4	[M+Cl]	182.0226	182.0225	0.5	0.07	-	-	+	-	-
	Methionine	C5H11NO2S	[M-H]	148.0438	148.0437	0.7	0.05	0.01	-	+	++	-
	Histidine	C6H9N3O2	[M-H]	154.0622	154.0621	0.6	0.19	0.16	0.30	+++	++++	++++
	Phenylalanine	C9H11NO2	[M-H]	164.0717	164.0715	1.2	0.65	0.51	1.69	++++	++++	++++
	Arginine	C6H14N4O2	[M-H]	173.1044	173.1042	1.2	0.02	0.00	-	+	+	-
Tyrosine	C9H11NO3	[M-H]	180.0666	180.0665	0.6	0.30	0.20	0.16	++++	++++	++	
Tryptophan	C11H12N2O2	[M-H]	203.0826	203.0826	0.0	0.03	0.02	-	+	++	-	
Tryptophan	C11H12N2O2	[M+Cl]	239.0593	239.0595	-0.8	2.56	-	31.66	++++	-	++++	
Organic acid	Malic acid	C4H6O5	[M-H]	133.0142	133.0142	0.0	100.00	84.11	100.00	++++	++++	++++
	Monodehydroascorbate	C6H7O6	[M-H]	174.0170	174.0169	0.6	0.33	2.25	1.48	++++	++++	++++
	Ascorbic acid	C6H8O6	[M-H]	175.0247	175.0247	0.0	20.39	59.64	20.95	++++	++++	++++
Carbohydrate	Hex	C6H12O6	[M-H]	179.0561	179.0560	0.6	2.51	1.31	1.14	++++	++++	++++
	Hex	C6H12O6	[M+Cl]	215.0328	215.0328	0.0	19.16	1.93	2.70	++++	++++	++++
	Hex6P	C6H13O9P	[M-H]	259.0224	259.0222	0.8	1.73	3.34	0.42	++++	++++	++++
	Hex ₂	C12H22O11	[M-H]	341.1089	341.1089	0.0	15.27	12.16	2.21	++++	++++	++++
	Hex ₂	C12H22O11	[M+Cl]	377.0856	377.0858	-0.5	10.01	15.63	5.07	++++	++++	++
	(Hex ₂) ₂	C24H44O22	[M-H]	683.2251	683.2260	-1.3	4.43	4.16	1.29	++++	++++	++++
	(Hex ₂) ₂	C24H44O22	[M+Cl]	719.2018	719.2026	-1.1	1.52	1.64	0.36	+++	++++	++
Peptide	Glutathione	C10H17N3O6S	[M-H]	306.0766	306.0766	0.0	0.12	2.47	0.62	++	++++	++++

23

24

(Continued from previous page.)

cluster ions	Metabolite's cluster	Ion cluster molecular formula	Ion type detected [M=cluster]	Theoretical ^a m/z	Observed m/z	Δm ppm	Relative abundance (%) ^b			Frequency of detection ^c		
							26°C	34°C	N+34°C	26°C	34°C	N+34°C
	Glycine+Hex	C8H17NO8	[M-H]	254.0881	254.0881	0.0	0.08	0.01	0.01	++	++	+
	Alanine+Hex	C9H20NO8	[M-H]	268.1038	268.1037	0.3	0.81	0.19	0.21	+++	++++	++
	GABA+Hex	C10H21NO8	[M-H]	282.1195	282.1194	0.3	0.07	-	-	++	-	-
	Serine+Hex	C9H19NO9	[M-H]	284.0987	284.0988	-0.4	0.65	0.09	0.20	++	+++	++
	Proline+Hex	C11H21NO8	[M-H]	294.1194	294.1191	1.1	0.05	-	-	+	-	-
	Valine+Hex	C11H23NO8	[M-H]	296.1351	296.1349	0.6	0.11	-	-	++	-	-
	Threonine+Hex	C10H21NO9	[M-H]	298.1143	298.1144	-0.2	0.28	0.01	0.07	++	++	++
	Cysteine+Hex	C9H19NO8S	[M-H]	300.0759	300.0759	-0.2	-	0.28	-	-	+	-
	Leucine, Isoleucine+Hex	C12H23NO8	[M-H]	310.1507	310.1510	-0.9	0.13	0.00	-	++	+	-
	Asparagine+Hex	C10H20N2O9	[M-H]	311.1096	311.1095	0.3	0.07	0.00	0.03	++	-	+
	Aspartic acid+Hex	C10H19NO10	[M-H]	312.0936	312.0938	-0.6	0.61	0.10	0.13	++	+++	++
	Glutamine+Hex	C5H11N2O3	[M-H]	325.1252	325.1253	-0.2	0.60	-	0.13	++	-	++
	Glutamic acid+Hex	C11H21NO10	[M-H]	326.1093	326.1092	0.2	2.32	0.48	0.23	+++	++++	++
	Methionine+Hex	C11H23NO8S	[M-H]	328.1072	328.1075	-1.1	-	0.02	0.01	-	++	-
	Phenylalanine+Hex	C15H23NO8	[M-H]	344.1351	344.1349	0.6	0.06	0.00	0.02	++	-	-
	Tyrosine+Hex	C15H23NO9	[M-H]	360.1300	360.1298	0.5	0.06	-	-	+	-	-
	Malic acid+Hex	C10H18O11	[M-H]	313.0776	313.0780	-1.4	1.23	0.05	2.52	++++	+++	+++
	Ascorbic acid+Hex	C12H20O12	[M-H]	355.0881	355.0883	-0.6	0.09	0.02	0.03	+++	+++	+
	Glycine+Hex ₂	C14H27NO13	[M-H]	416.1410	416.1411	-0.2	0.15	0.28	0.07	++	++++	++
	Alanine+Hex ₂	C15H29NO13	[M-H]	430.1567	430.1566	0.2	1.53	3.11	1.25	++	++++	+++
	GABA+Hex ₂	C16H31NO13	[M-H]	444.1724	444.1724	-0.1	0.12	0.14	0.04	++	+++	++
	Serine+Hex ₂	C15H29NO14	[M-H]	446.1516	446.1518	-0.5	1.12	3.30	1.62	++++	++++	++++
	Proline+Hex ₂	C17H31NO13	[M-H]	456.1723	456.1726	-0.6	0.11	0.07	0.03	++	+++	+
	Valine+Hex ₂	C17H33NO13	[M-H]	458.1880	458.1884	-0.9	0.21	0.28	0.08	++	+++	++
	Threonine+Hex ₂	C16H31NO14	[M-H]	460.1672	460.1676	-0.8	0.37	0.95	0.28	+++	++++	++
	Cysteine+Hex ₂	C15H29NO13S	[M-H]	462.1288	462.1292	-1.0	-	0.31	0.09	-	++++	+
	Leucine, Isoleucine+Hex ₂	C18H35NO13	[M-H]	472.2036	472.2039	-0.6	0.18	0.16	0.05	++	+++	+
	Asparagine+Hex ₂	C16H30N2O14	[M-H]	473.1625	473.1625	0.0	0.14	0.42	0.30	++	++++	++
	Aspartic acid+Hex ₂	C16H29NO15	[M-H]	474.1465	474.1466	-0.2	2.48	7.31	2.29	++++	++++	+++
	Glutamine+Hex ₂	C5H11N2O3	[M-H]	487.1781	487.1789	-1.6	1.73	1.94	1.16	++	++++	+++
	Lysine+Hex ₂	C18H36N2O13	[M-H]	487.2145	487.2147	-0.3	0.04	0.06	0.01	+	++	+
	Glutamic acid+Hex ₂	C17H31NO15	[M-H]	488.1622	488.1624	-0.5	8.41	20.25	6.76	++++	++++	++++
	Methionine+Hex ₂	C17H33NO13S	[M-H]	490.1601	490.1591	1.9	-	-	0.01	-	-	+
	Histidine+Hex ₂	C18H31N3O13	[M-H]	496.1785	496.1785	0.0	0.05	0.04	0.03	+	+++	+
	Phenylalanine+Hex ₂	C21H33NO13	[M-H]	506.1880	506.1879	0.2	0.07	0.01	0.02	++	+	+
	Arginine+Hex ₂	C18H36N4O13	[M-H]	515.2207	515.2210	-0.6	0.01	-	-	+	-	-
	Tyrosine+Hex ₂	C21H33NO14	[M-H]	522.1829	522.1827	0.4	0.12	-	0.06	++	-	+
	Glutathione+Hex ₂	C22H39N3O17S	[M-H]	648.1928	648.1935	-1.1	-	0.28	0.08	-	+++	+
	Malic acid+Hex ₂	C16H28O16	[M-H]	475.1305	475.1306	-0.3	4.39	4.57	2.05	++++	++++	+++
	Ascorbic acid+Hex ₂	C18H30O17	[M-H]	517.1410	517.1411	-0.2	0.14	3.55	0.78	++	++++	+++

a All the theoretical values are quoted from Metlin (<http://metlin.scripps.edu/index.php>).

b Values were calculated as a percentage to the base peak.

c Signal detected (+); Signal not detected (-). Frequencies of detection (x) in 4 individual measurements are indicated as: ++++, 75% < x < 100%, +++, 50% < x < 75%, ++, 25% < x < 50%, +, 0% < x < 25%.