Supporting Information

Title: Lipid peroxide-derived short-chain aldehydes are involved in aluminum toxicity of wheat (*Triticum aestivum*) roots

Running title:

Aldehydes affecting Al toxicity

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Aldehydes confirmation by UPLC-TOF-HRMS

Seedlings were treated with 0 or 30 μM $AlCl_3$ for 24 h. Then root tips (0.3 g, 0-10 mm) of seedlings were sliced for aldehyde analysis. Aldehydes were extracted from roots and derivative with 2,4-dinitrophenylhydrazine according to the method of Mano et al (Mano and Biswas, 2018). Prior to injection, the samples were filtered through a BondEluteC18 cartridge (sorbent mass 100 mg; Agilent). For the UPLC-TOF-HRMS analysis, a Waters UPLC system (Waters Corp., Milford, MA, USA) coupled to UV detector and an AB Triple TOF 5600^{plus} System (AB SCIEX, Framingham, USA) mass spectrometry was used, and the separation was achieved on an ACQUITY UPLC HSS T3 column (1.7 μm, 2.1 × 150 mm; Waters Corp.) at a column temperature of 30℃. The mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The linear gradient programs were as follows: 0-2 min,40-45% B; 2-30 min, 45-85% B; 30-35 min, 85% B; 35-36 min, 85-95% B; 36-37 min, 95-40% B. The follow rate was set to 0.3mL min^{-1} and the Sample injection volume was 5μL. The instrument was operated in the negative mode to produce $[M-H]^-$ ions. The source voltage was -4500 V and the source temperature was 550℃. The pressure of gas 1 (air) and gas 2 (air) were set to 50 psi. The pressure of Curtain Gas (N_2) was set to 35 psi. The maximum allowed error was set to ±5 ppm. Declustering potential (DP) was 100 V and the collision energy (CE) was 10 V. For MS/MS acquisition mode, the parameters were almost the same except that the CE was set at 40 ± 20 V, ion release delay (IRD) at 67, ion release width (IRW) at 25. The scan range of m/z of precursor ion and product ion was set as 100-2000 Da and 50-2000 Da, respectively. The exact

mass calibration was performed automatically before each analysis employing the Automated Calibration Delivery System. Dinitrophenylhydrazone derivatives of aldehydes were accurately identified as compared with aldehydes-DNPH standards (CRM47285, Sigma-Aldrich).

Reference:

Mano, J.i. and Biswas, M.S. (2018) Analysis of reactive carbonyl species generated under oxidative stress. Methods in molecular biology (Clifton, N.J.), 1743, 117-124.

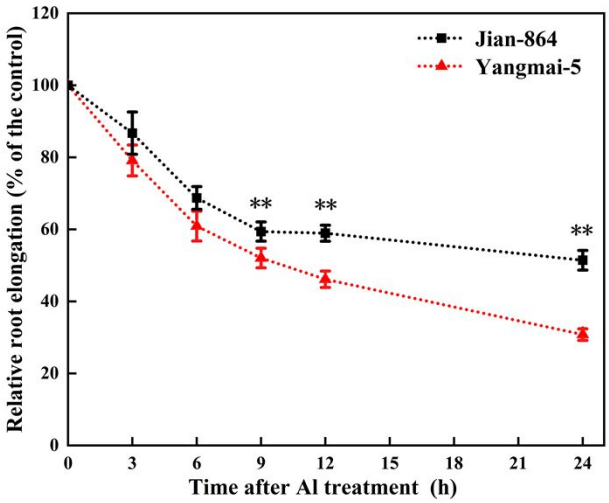

Figure S1. Relative root elongation rates at different times. Root growth of 3-day-old seedlings treated with or without 30 μM $AlCl_3$ for 24 h were measured at 0, 3, 6, 9, 12 and 24 h. The data means ± SD (n=20). Different letters mean significant differences at $p < 0.05$. Asterisks (**) indicated there were significant differences between genotypes.

Table S1. qPCR primers for genes of wheat aldehydes detoxifying enzymes.

Gene	Plant Ensemble ID	Primer sequences		Note
		Forward (5'-3')	Reverse (5'-3')	
TaAKR1	TraesCS3B02G395600.1	GCTGGAACATTCAGATGGGT	ATCGTGGGTCTTGTAAACGC	Encoding aldo-keto reductase (AKR)
TaAKR2	TraesCS3A02G038400.1	TTACATGATGCTGGCAAAGC	TGAACCTAGTGGCGAGTAAGC	Encoding aldo-keto reductase (AKR)
TaAOR	TraesCS6D02G122800.1	GACTGTGCCAGGGTACGAC	AGGACCTTCTCGCTGATGTG	Encoding alkenal/alkenone reductase (AOR)
TaAER	TraesCS5B02G303700.1	TGGCTGCTATGTGGTTGGTA	GCATCGTCAAACCCAAACTT	Encoding alkenal reductase (AER)
Ta30797		GCCGTGTCCATGCCAGTG	TTAGCCTGAACCACCTGTGC	Housekeeping gene; encoding phosphogluconate dehydrogenase

The primers' sequence of *TaAKR1*, *TaAKR2*, *TaAOR*, and *Ta30797* were refered to previous reports (Majlath *et al*., 2020; Paolacci *et al.*, 2009). The *TaAER* primer sequence was designed according to arabidopsis gene At5g16970 (Mano *et al*., 2005; Youn *et al*., 2006).

Reference:

Majlath, I.; Eva, C.; Tajti, J.; Khalil, R.; Elsayed, N.; Darko, E.; Szalai, G.; Janda, T., Exogenous methylglyoxal enhances the reactive aldehyde detoxification capability and frost-hardiness of wheat. Plant Physiol. Biochem. 2020, 149, 75-85.

Mano, J., Belles-Boix, E., Babiychuk, E., Inzé, D., Torii, Y., Hiraoka, E., … Kushnir, S. (2005). Protection against photooxidative injury of tobacco leaves by 2-alkenal reductase. Detoxication of lipid peroxide-derived reactive carbonyls. Plant Physiology, 139, 1773-1783.

Paolacci AR, Tanzarella OA, Porceddu E, Ciaffi M. 2009. Identification and validation of reference genes for quantitative RT-PCR normalization in wheat. BMC Molecular Biology 10, 11.

Youn, B., Kim, S.-J., Moinuddin, S. G. A., Lee, C., Bedgar, D. L., Harper, A. R., … Kang, C. (2006). Mechanistic and structural studies of apoform, binary, and ternary complexes of the Arabidopsis alkenal double bond reductase At5g16970. The Journal of Biological Chemistry, 281, 40076-40088.

Table S2. Molecular weight (WM), retention time (RT), precursor ions (Q1), product ions (Q3) and collision energy (CE) for 14 species of aldehyde DNPH derivatives detected by HPLC-MS/MS

Compounds	MW	RT (min)	Q1 (m/z)	Q3 (m/z)	CE (V)
Formaldehyde	210	9.550	209	163	4
Acetaldehyde	224	13.225	223	122	4
Acrolein	236	16.829	235	158	8
Propionaldehyde	238	17.995	237	122	20
Crotonaldehyde	250	20.674	249	181	24
Butyraldehyde	252	21.872	251	122	24
Isovaleraldehyde	266	24.757	265	122	32
Valeraldehyde	266	25.397	265	152	32
(*E*)-2-hexenal	278	27.517	277	200.1	8
Hexanal	280	28.358	279	122.1	20
Benzaldehyde	286	24.050	285	163	12
Heptaldehyde	294	30.814	293	152	20
2-Ethylhexanal	308	32.094	307	152	16
Nonyl aldehyde	322	35.552	321	122	40

Table S3. Identities of aldehyde-DNPH derivatives in wheat roots using UPLC-TOF-HRMS

Number	t_R (min) TOF	Formula	Precursor ions (m/z)	Product ions (m/z)	Error (ppm)	Confidence	Corresponding aldehydes	References
1	2.98	C7H6N4O4	209.0322	135.0217, 117.0192, 181.0148, 162.0305	2.7	level 1 (confirmed)	Formaldehyde	
2	3.87	C8H8N4O4	223.0481	122.0235, 181.0099, 163.0252, 163.0513, 164.0321	3.7	level 1 (confirmed)	Acetaldehyde	
3	5.05	C9H8N4O4	235.0478	65.0164 , 88.0195 , 109.0177 , 170.0341 , 181.0115 , 235.0458	2.2	level 1 (confirmed)	Acrolein	
4	5.05	C9H10N4O4	237.0626	95.0154 , 109.0162 , 163.0253 , 183.0037 , 182.0197 , 183.0223 , 291.0721	-1.4	level 1 (confirmed)	Propionaldehyde	
5	6.72	C10H10N4O4	249.0627	76.0317 , 123.0263 , 163.0252 , 182.0150 , 183.0236 , 190.8562 , 310.1221	-0.9	level 1 (confirmed)	Crotonaldehyde	
6	7.25	C10H12N4O4	251.0786	122.0254 , 151.0128 , 163.0242 , 181.0119 , 205.0601	0.1	level 1 (confirmed)	Butyraldehyde	
7	8.95	C11H14N4O4	265.0940	152.0217 , 181.0115 , 265.0927	-0.9	level 1 (confirmed)	Valeraldehyde	
8	9.38	C11H14N4O4	265.0943	76.0210 , 122.0245 , 152.0217 , 163.0253 , 265.1489	0.3	level 1 (confirmed)	Isovaleraldehyde	
9	11.76	C12H16N4O4	279.1098	122.0242 , 152.0217 , 163.0250 , 181.0114 , 205.0614	-1	level 1 (confirmed)	Hexanal	
10	8.82	C13H10N4O4	285.0624	120.0134 , 163.0233 , 186.8666 , 238.0608 , 285.0670	-1.9	level 1 (confirmed)	Benzaldehyde	
11	11.11	C12H14N4O4	277.0937	156.9201 , 163.0280 , 181.0140	-1.9	level 1 (confirmed)	(*E*)-2-hexenal	
12	14.17	C13H18N4O4	293.1250	96.9677 , 152.0236 , 221.1519	-1.8	level 1 (confirmed)	Heptaldehyde	
13	19.17	C15H22N4O4	321.1558	122.0249 , 152.0220 , 163.0235 , 274.1527	-3.2	level 1 (confirmed)	Nonylaldehyde	
14	8.81	C15H20N4O5	335.1353	138.0179 , 152.0218 , 167.0078 , 182.0191 , 288.1330	-2.4	level 2 (probable)	4-hydroxy-(E)-2-nonenal	(Tang *et al.*, 2021; Liu *et al.*, 2020)
15	4.02	C12H14N4O5	293.0885	163.0267 , 167.0098 , 182.0207 , 221.1547	-2.2	level 2 (probable)	4-hydroxy -(E)-2-hexenal	(Liu *et al.*, 2020)
16	15.58	C14H20N4O5	307.1405	76.0221 , 122.0249 , 152.0223 , 181.0121 , 219.0767	-2.2	level 2 (probable)	Octanal	(Liu *et al.*, 2020)
17	14.76	C14H20N4O4	307.1407	102.0217 , 122.0232 , 152.0215 , 181.0104 , 219.0751 , 277.1407	-1.6	level 3 (unknown)	unknown	
18	15.43	C14H20N4O5	307.1406	76.0224 , 122.0245 , 152.0219 , 181.0114 , 219.0756 , 277.1421	-1.9	level 3 (unknown)	unknown	
19	4.29	C11H12N2O12	363.0320	167.0070, 240.0389, 287.0399, 317.0395	0.7	level 3 (unknown)	unknown	
20	15.65	C14H20N4O4	307.1406	76.0221 , 122.0246 , 152.0217 , 181.0117 , 219.0761	-1.9	Internal standard	2-ethylhexanal	

Reference:

Liu ZY, Zhou DY, Li A, Zhao MT, Hu YY, Li DY, Xie HK, Zhao Q, Hu XP, Zhang JH, Shahidi F. 2020. Effects of temperature and heating time on the formation of aldehydes during the frying process of clam assessed by an HPLC-MS/MS method. Food Chemistry 308, 125650.

Tang Y, Zhao Y, Wang P, Sang S. 2021. Simultaneous determination of multiple reactive carbonyl species in high fat diet-induced metabolic disordered mice and the inhibitory effects of rosemary on carbonyl stress. Journal of Agricultural and Food Chemistry 69, 1123-1131.