

Both cold and sub-zero acclimation induce cell wall modification and changes in the extracellular proteome in *Arabidopsis thaliana*

Daisuke Takahashi¹, Michal Gorka¹, Alexander Erban¹, Alexander Graf¹, Joachim Kopka¹, Ellen Zuther¹ and Dirk K. Hinch^{1*}

¹Max-Planck-Institut für Molekulare Pflanzenphysiologie, Am Mühlenberg 1, D-14476 Potsdam, Germany

* hinch@mpimp-golm.mpg.de

Corresponding author:

Dirk K. Hinch

Max-Planck-Institut für Molekulare Pflanzenphysiologie

Am Mühlenberg 1, Potsdam, 14476, Germany

Tel/Fax: +49-(0)331-567-8253

E-mail: hinch@mpimp-golm.mpg.de

Supplemental data legends

Supplementary Fig. S1. Diagram overview of sequential cell wall fractionation adapted from Ruprecht et al. (2011), Neumetzler et al. (2012) and Pettolino et al. (2012).

Supplementary Fig. S2. Amounts of crystalline cellulose and uronic acid in total cell wall material isolated from Col-0 and N14. (a) Proportions of crystalline cellulose in total cell wall material. (b) Proportions of uronic acid in total cell wall material. Weight % were calculated relative to total cell wall weight (A and B). Error bars indicate $\pm$ s.e.m. (n =3-4). Significant differences between treatments within an accession at $p<0.05$ (Tukey-Kramer test) are marked with different letters above the bars.

Supplementary Fig. S3 Amounts of crystalline cellulose in the four sub-fractions isolated from Col-0 and N14. Weight % were calculated relative to weight of the insoluble (a), CDTA-soluble (b), Na_2CO_3 -soluble (c) and KOH-soluble (d) cell wall sub-fractions. Error bars indicate $\pm$ s.e.m. (n =3-4). Significant differences between treatments within an accession at $p<0.05$ (Tukey-Kramer test) are marked with different letters above the bars.

Supplementary Fig. S4. Amounts of uronic acid in the four sub-fractions isolated from Col-0 and N14. Weight % were calculated relative to weight of the insoluble (a), CDTA-soluble (b), Na_2CO_3 -soluble (c) and KOH-soluble (d) cell wall sub-fractions. Error bars indicate $\pm$ s.e.m. (n =3-4). Significant differences between treatments within an accession at $p<0.05$ (Tukey-Kramer test) are marked with different letters above the bars.

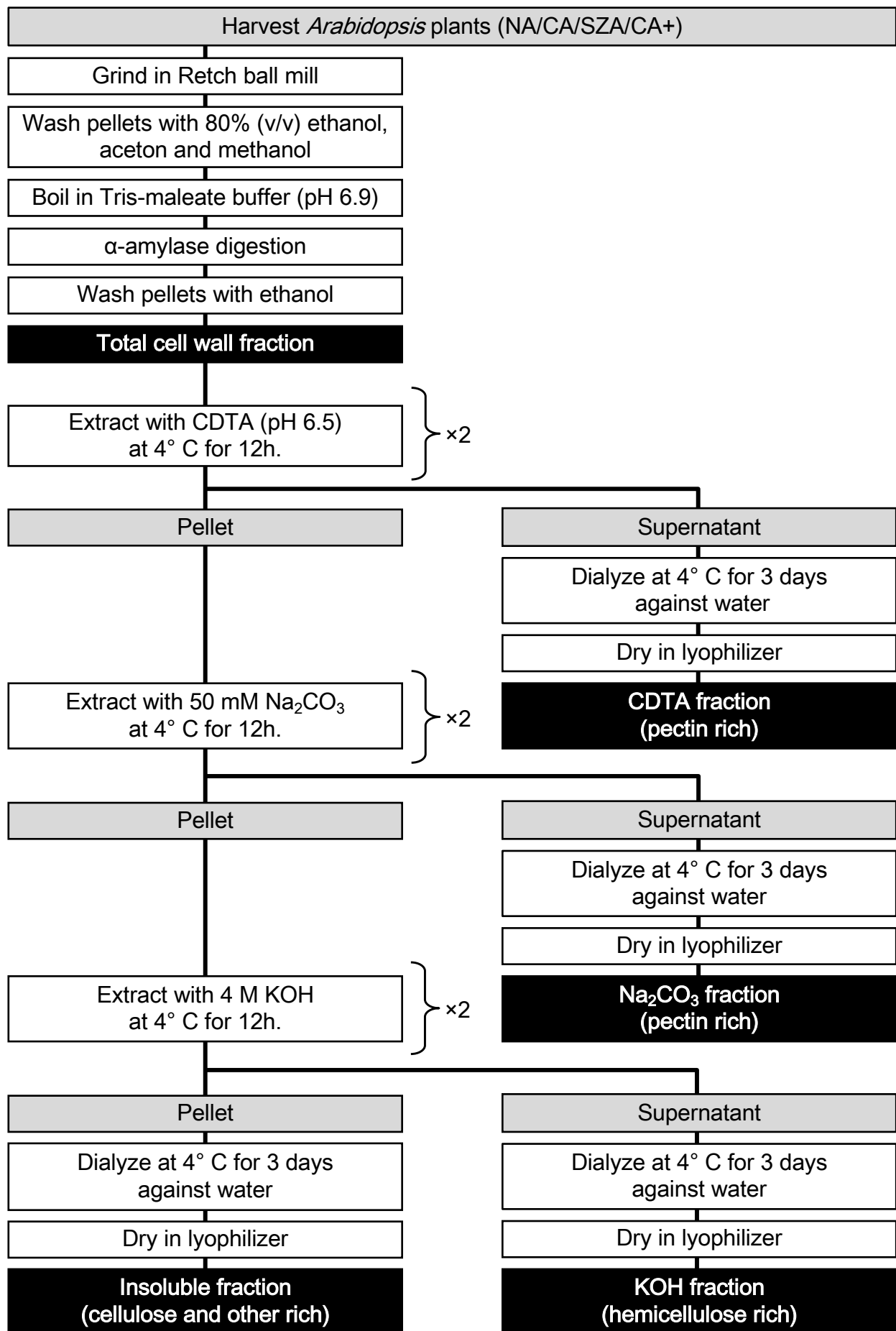
Supplementary Fig. S5. Compositions of cell wall monosaccharides isolated from N14 under NA, CA, SZA and CA+ conditions. Monosaccharide composition of total cell wall material (a) and the insoluble (b), CDTA-soluble (c), Na_2CO_3 -soluble (d) and KOH-soluble (e) cell wall sub-fractions was characterized by GC-MS. The contribution of the single sugars is expressed as mol% of the total amount of all seven monosaccharides in each fraction. Error bars indicate $\pm$ s.e.m. (n =3-4). Significant differences (Student's *t*-test) between NA and CA or SZA and CA+ samples are indicated by asterisks above the bars of CA or SZA samples, respectively (* $p<0.05$, ** $p<0.01$).

Supplementary Table S1. Subcellular localization of identified proteins. Potential protein location were predicted by SUBA3. Each value indicates percent of each predicted targeting site calculated from normalized abundance of each proteins identified.

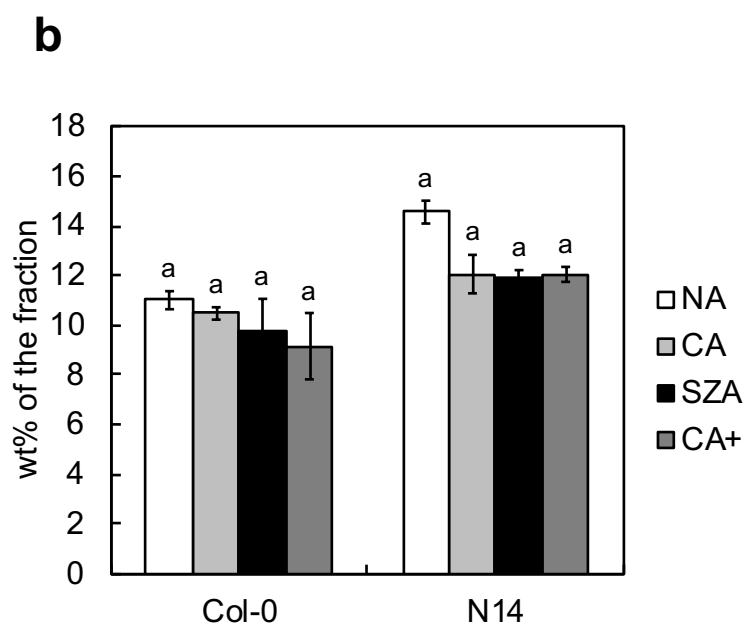
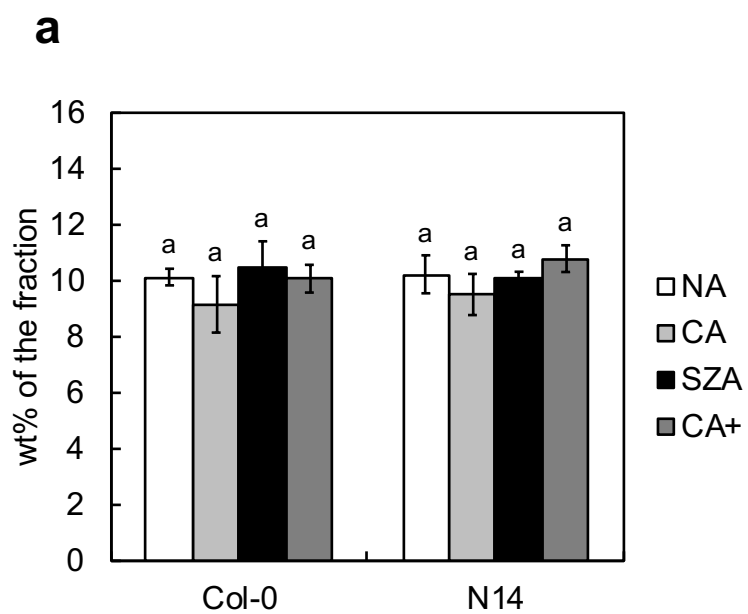
Supplementary Table S2. Top 10 significantly differentially abundant apoplastic proteins under SZA compared to CA+ conditions in N14. Significantly more or less abundant apoplastic proteins were defined as $\text{SZA/CA} > 2.0$ and $\text{SZA/CA} < 0.5$, respectively, with $p<0.05$ in both cases. All listed proteins were identified with at least two unique peptides. Trend lines show quantitative changes of protein abundances expressed as log2-fold change compared to NA samples.

Supplementary Table S3. List of identified and quantified peptides in *Arabidopsis* apoplastic fluids. ^aModification type and its location in peptide sequence identified, ^bAssigned proteins identified by MASCOT search engine, ^cWhether or not to use peptide in quantification with ProgenesisQI.

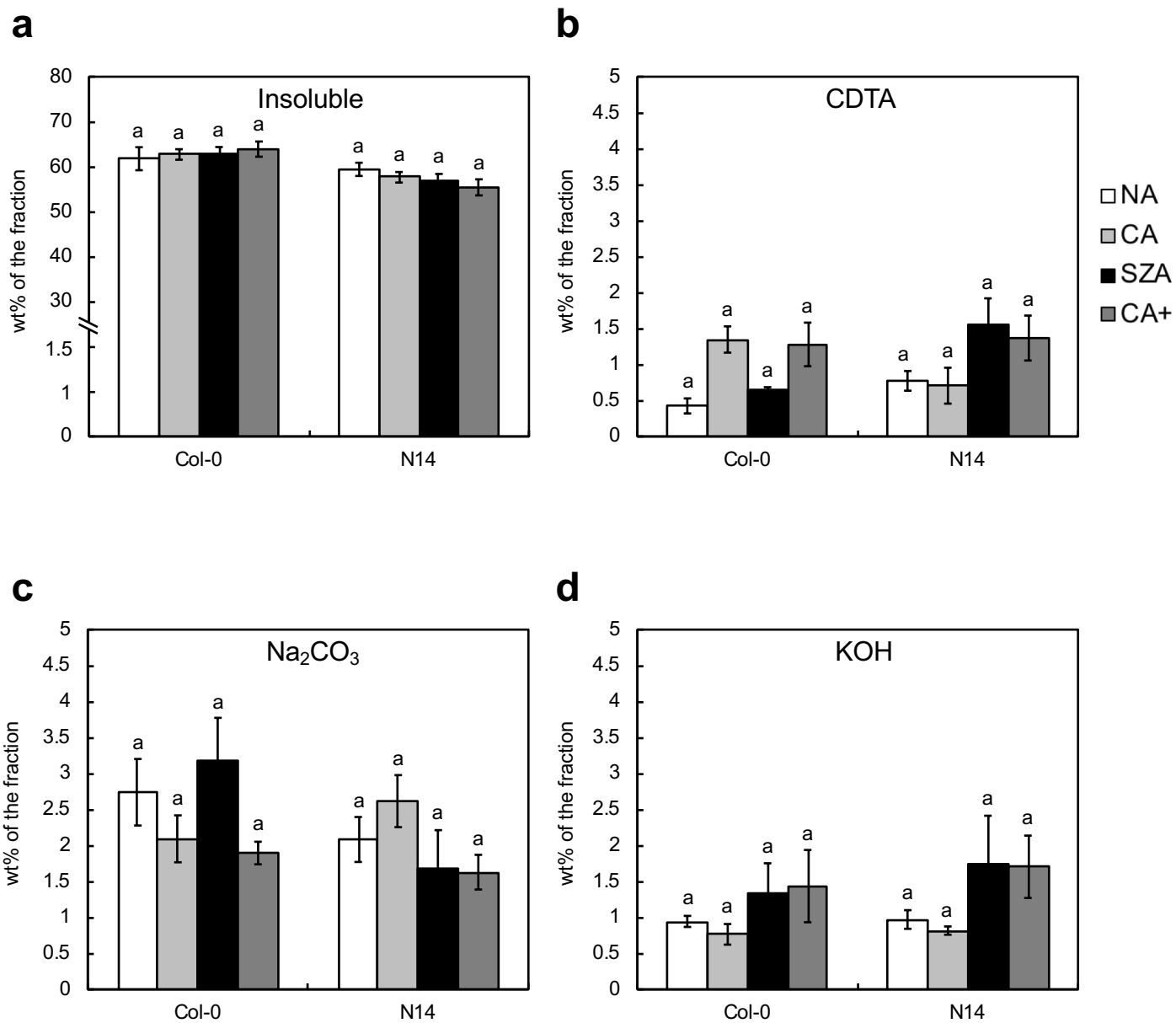
Supplementary Table S4. List of identified and quantified proteins in *Arabidopsis* apoplastic fluids. ^aAll peptide assigned to individual proteins. ^bUnique peptide assigned to individual proteins. ^cConfidence score generated by Progenesis QI, ^dPotential protein location predicted by SUBA3.



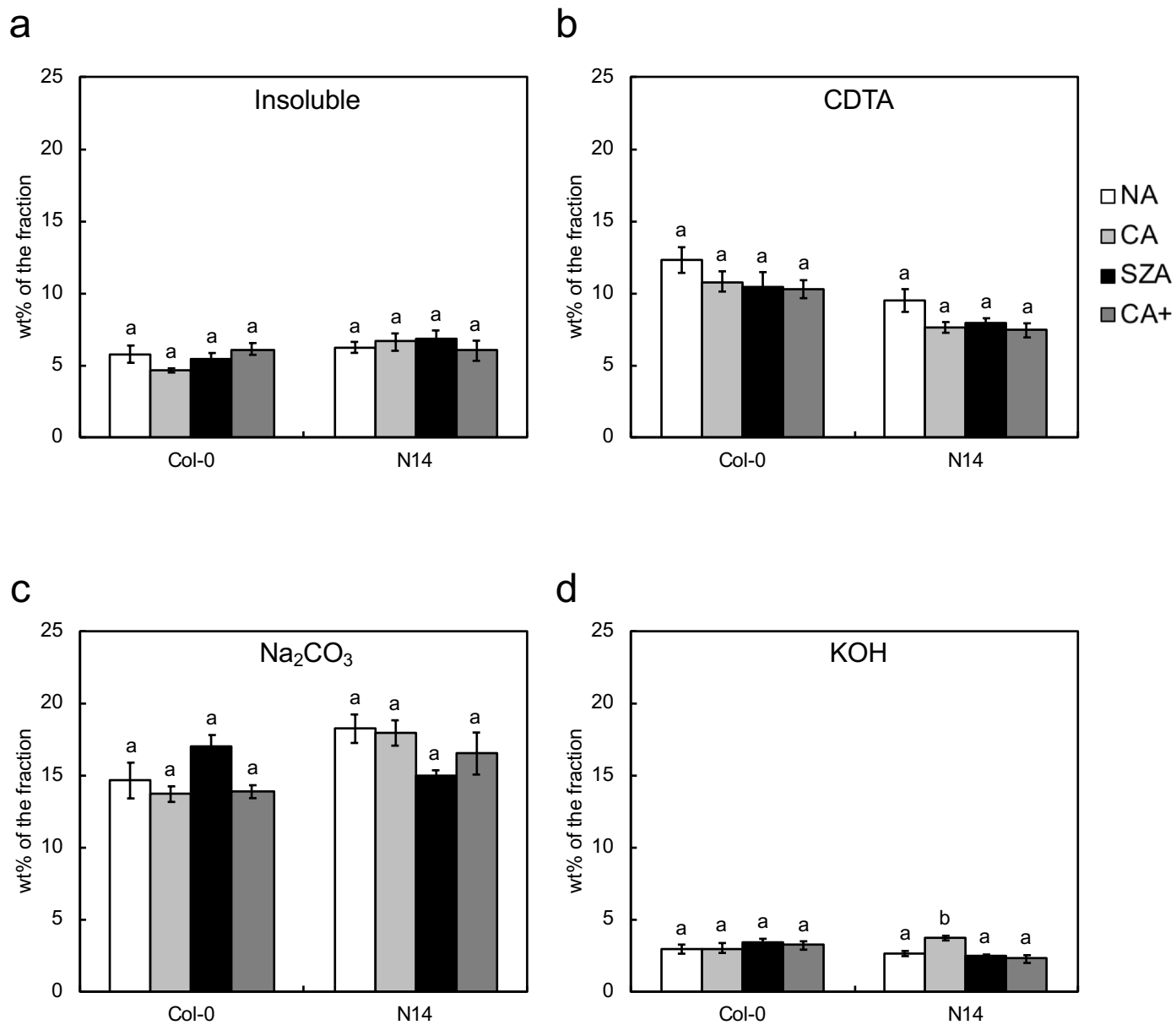
Supplementary Fig. S1.



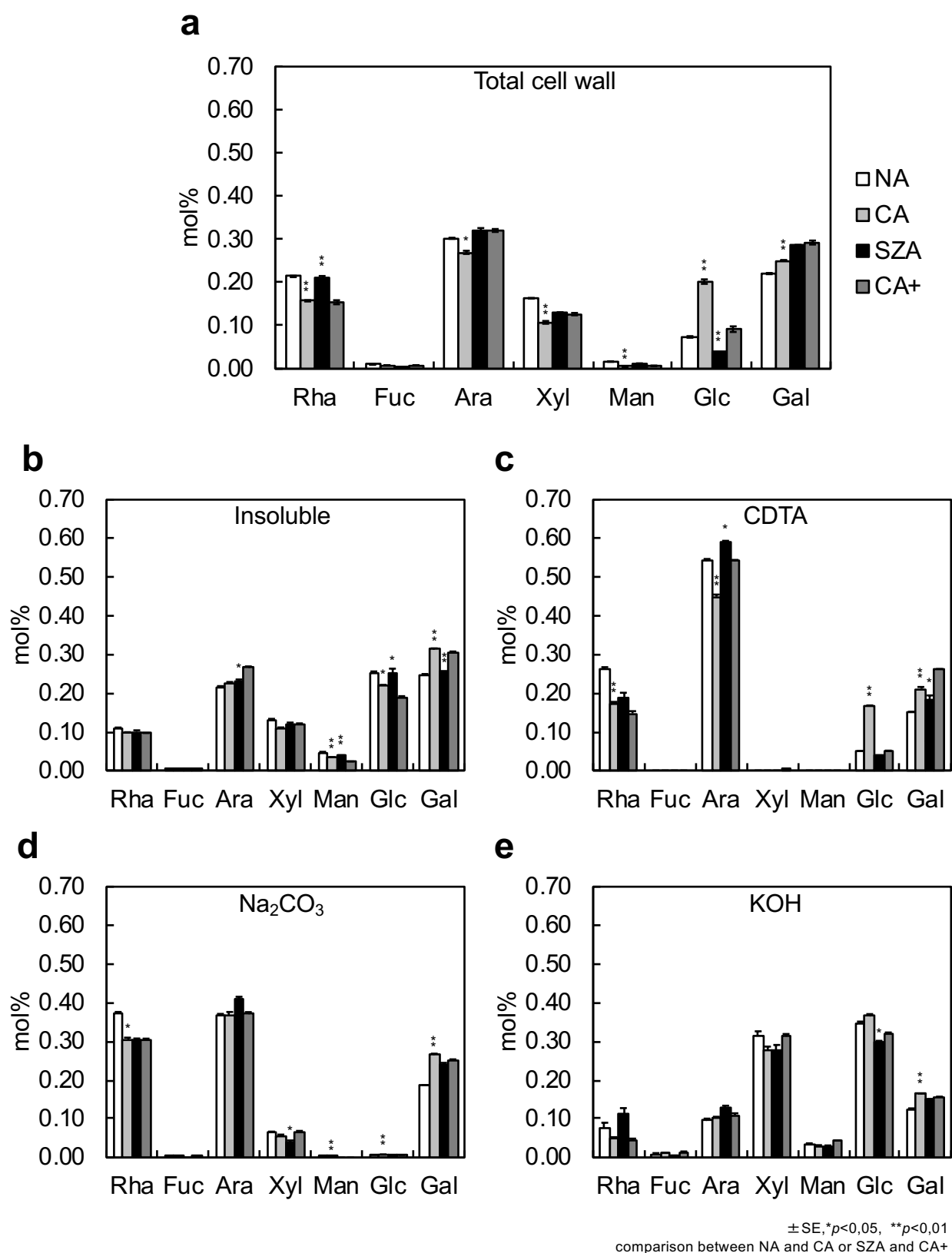
Supplementary Fig. S2.



Supplementary Fig. S3.



Supplementary Fig. S4.



Supplementary Fig. S5.

Supplementary Table S1.

	Col-0										N14									
	Apoplastic fluid (+CaCl ₂)					Apoplastic fluid (-CaCl ₂)					Apoplastic fluid (+CaCl ₂)					Apoplastic fluid (-CaCl ₂)				
	NA	CA	SZA	CA+		NA	CA	SZA	CA+		NA	CA	SZA	CA+		NA	CA	SZA	CA+	
Extracellular	76.23	77.17	71.65	74.83		24.08	14.03	13.21	17.41		77.70	77.08	68.82	79.98		15.27	21.85	19.24	29.78	
Extracellular,ER	0.01	0.01	0.01	0.01		0.05	0.03	0.02	0.02		0.04	0.02	0.01	0.02		0.13	0.07	0.05	0.08	
Extracellular,Plasma Membrane	0.72	0.75	0.59	0.67		0.74	0.73	0.63	0.65		0.56	0.71	0.50	0.65		0.56	0.48	0.39	0.45	
Cytosol,Extracellular	4.95E-03	0.01	0.02	0.01		0.01	0.05	0.04	0.03		0.01	0.01	0.01	0.01		0.04	0.06	0.04	0.04	
Mitochondrion,Extracellular	0	0	0	0		1.09E-03	1.23E-03	4.74E-03	2.06E-03		0	0	0	0		0	0	0	0	
Extracellular Total	76.96	77.94	72.27	75.53		24.88	14.84	13.90	18.11		78.32	77.82	69.35	80.67		15.99	22.46	19.73	30.35	
Cytosol	2.49	2.61	3.11	2.31		6.72	12.27	12.28	11.23		2.61	2.64	3.49	2.13		10.52	12.92	12.10	9.21	
Cytosol,Mitochondrion	0	0	0	0		0.05	0.08	0.07	0.05		0.02	0.01	0.01	0.01		0.07	0.07	0.06	0.05	
Cytosol,Nucleus	0.09	0.11	0.14	0.09		0.24	0.52	0.50	0.34		0.23	0.16	0.21	0.18		0.31	0.67	0.42	0.53	
Cytosol,Plasma Membrane	0.04	0.07	0.09	0.07		0.20	0.43	0.45	0.42		0.05	0.08	0.11	0.06		0.25	0.41	0.44	0.40	
Cytosol,Plastid	1.54E-04	5.33E-04	1.85E-03	5.32E-04		0.01	0.02	0.01	0.01		1.56E-03	1.42E-03	2.80E-03	1.02E-03		0.02	0.02	0.02	0.02	
Cytosol,Plastid,Nucleus	3.24E-03	3.78E-03	0.01	2.50E-03		0	0	0	0		0	0	0	0		0	0	0	0	
Cytosol,Vacuole	0	0	0	0		0.01	0.02	0.02	0.02		1.68E-03	1.35E-03	3.88E-03	1.08E-03		0.02	0.02	0.02	0.02	
ER	0.07	0.09	0.14	0.08		0.14	0.27	0.30	0.28		0.09	0.13	0.17	0.11		0.14	0.25	0.29	0.24	
ER,Golgi	0	0	0	0		0	0	0	0		0	0	0	0		0.01	0.02	0.02	0.01	
ER,Peroxisome	0	0	0	0		0.01	0.01	0.01	0.01		0	0	0	0		1.66E-03	3.98E-03	2.46E-03	2.10E-03	
Golgi	0	0	0	0		0	0	0	0		0.02	0.01	0.01	0.02		0	0	0	0	
Mitochondrion	1.99	1.81	1.96	2.44		2.96	2.40	2.52	2.43		1.03	1.04	1.53	0.92		2.24	2.36	2.71	2.19	
Mitochondrion,Plastid	0.01	0.01	0.02	0.01		0.06	0.08	0.07	0.04		0.01	0.02	0.02	0.01		0.06	0.07	0.07	0.07	
Nucleus	0.37	0.56	0.79	0.53		0.30	0.60	0.82	0.46		0.86	0.98	1.59	1.40		0.29	0.52	0.86	0.45	
Nucleus,Plasma Membrane	0.01	0.01	0.02	0.01		0.01	0.01	0.01	0.01		1.60E-03	3.97E-03	0.01	3.45E-03		2.47E-03	5.00E-03	2.45E-03	1.08E-03	
Peroxisome	0.33	0.35	0.41	0.28		1.08	1.23	1.27	1.58		0.24	0.29	0.36	0.21		1.08	1.32	1.34	0.99	
Plasma Membrane	1.45	1.35	1.28	1.54		1.08	0.92	0.88	0.84		5.15	1.77	1.53	2.04		0.88	0.93	0.91	1.03	
Plasma Membrane,ER	9.18E-04	1.75E-03	0.01	2.10E-03		4.69E-03	0.01	0.01	3.82E-03		1.06E-03	2.99E-03	0.01	2.13E-03		4.07E-03	0.01	0.01	0.01	
Plasma Membrane,Golgi	5.45E-05	4.73E-04	1.26E-03	1.04E-03		0	0	0	0		0	0	0	0		0	0	0	0	
Plastid	14.96	13.54	18.25	15.39		61.69	65.67	66.18	63.22		9.98	13.19	19.96	10.03		67.32	57.04	59.95	53.41	
Plastid,Peroxisome	0	0	0	0		0.02	0.02	0.02	0.02		0	0	0	0		0.01	0.02	0.02	0.01	
Vacuole	1.17	1.47	1.45	1.61		0.49	0.51	0.56	0.78		1.35	1.81	1.55	2.11		0.71	0.70	0.85	0.87	
Vacuole,ER	0.03	0.03	0.03	0.06		0.03	0.02	0.03	0.06		0.03	0.04	0.04	0.07		0.02	0.03	0.03	0.05	
Vacuole,Golgi	0.02	0.02	0.03	0.03		0.02	0.04	0.05	0.04		0.02	0.01	0.02	0.02		0.02	0.04	0.04	0.03	
Vacuole,Plasma Membrane	2.14E-03	1.52E-03	1.57E-03	9.55E-04		0.02	0.02	0.02	0.01		1.50E-03	9.52E-04	1.22E-03	9.43E-04		0.03	0.11	0.09	0.05	
Total	100	100	100	100		100	100	100	100		100	100	100	100		100	100	100	100	

Supplementary Table S2.

		Trend line			
		NA	CA	SZA	CA+
Apoplastic fluid (+CaCl ₂)	AGI code	Description			
	AT4G02290.1	Glycosyl hydrolase 9B13 (GH9B13)			
	AT5G01930.1	Endo-beta-mannase 6 (MAN6)			
	AT5G58390.1	Peroxidase superfamily protein			
	AT5G57560.1	Xyloglucan endotransglucosylase/hydrolase family protein (XTH22)			
	AT4G00230.1	Xylem serine peptidase 1 (XPS1)			
	AT3G14310.1	Pectin methylesterase 3 (PME3)			
	AT3G11780.1	MD-2-related lipid recognition domain-containing protein			
	AT3G14067.1	Subtilase family protein			
	AT3G62020.1	Germin-like protein 10 (GLP10)			
	AT3G04720.1	Pathogenesis-related 4 (PR4)			
	AT2G45220.1	Plant invertase/pectin methylesterase inhibitor superfamily			
	AT2G37130.1	Peroxidase superfamily protein			
	AT2G02990.1	Ribonuclease 1 (RNS1)			
	AT3G47380.1	Plant invertase/pectin methylesterase inhibitor superfamily protein			
	AT1G30720.1	FAD-binding Berberine family protein			
	AT5G39580.1	Peroxidase superfamily protein			
	AT3G61490.1	Pectin lyase-like superfamily protein			
	AT4G37530.1	Peroxidase superfamily protein			
	AT2G41800.1	Protein of unknown function, DUF642			
	AT1G30730.1	FAD-binding Berberine family protein			
Apoplastic fluid (-CaCl ₂)	AT3G14310.1	Pectin methylesterase 3 (PME3)			
	AT3G16370.1	GDSL-like Lipase/Acylhydrolase superfamily protein			
	AT1G31710.1	Copper amine oxidase family protein			
	AT5G63810.1	β-galactosidase 10 (BGAL10)			
	AT3G54400.1	Eukaryotic aspartyl protease family protein			
	AT1G78060.1	Glycosyl hydrolase family protein			
	AT4G25900.1	Galactose mutarotase-like superfamily protein			
	AT1G78850.1	D-mannose binding lectin protein with Apple-like carbohydrate-binding domain			
	AT2G02850.1	Plantacyanin (ARPN)			
	AT3G44100.1	MD-2-related lipid recognition domain-containing protein			
	AT2G02990.1	Ribonuclease 1 (RNS1)			
	AT4G24040.1	Trehalase 1 (TRE1)			
	AT5G64120.1	Peroxidase superfamily protein			
	AT1G26380.1	FAD-binding Berberine family protein			
	AT4G25260.1	Plant invertase/pectin methylesterase inhibitor superfamily protein			