

# Identification of lipids and lipid-binding proteins in phloem exudates from *Arabidopsis thaliana*

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## Supplementary Figure legends

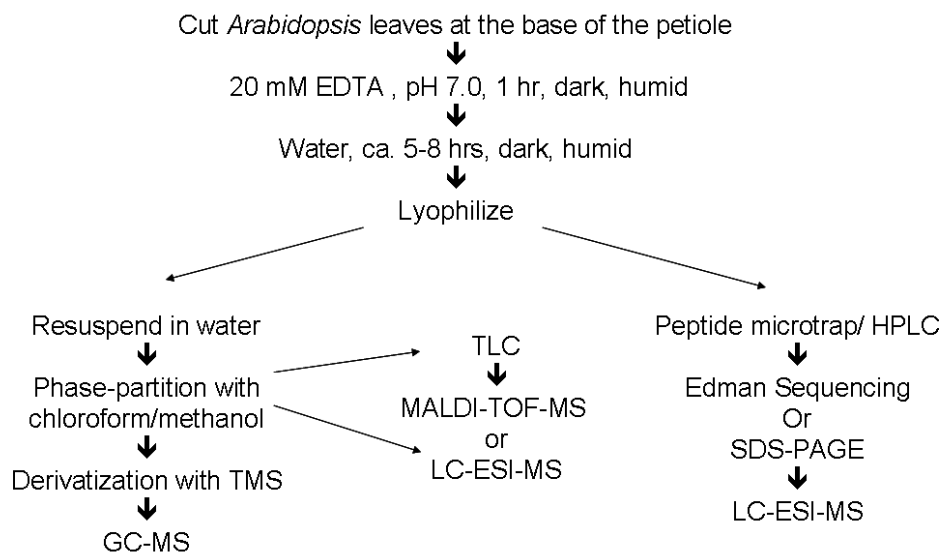
Supplementary Figure S1: Flow chart of phloem extraction and experimental setup. For preparation of phloem RNA, 100U/ml RNase inhibitor (Roche) was added to the deionized water into which the phloem exudate was collected.

Supplementary Figure S2: Time course of the appearance of proteins collected into water. Lane 1 contains the protein standard. Phloem exudate was collected for 1, 5, and 9 hours into deionized water (lanes 4, 3, and 2 respectively).

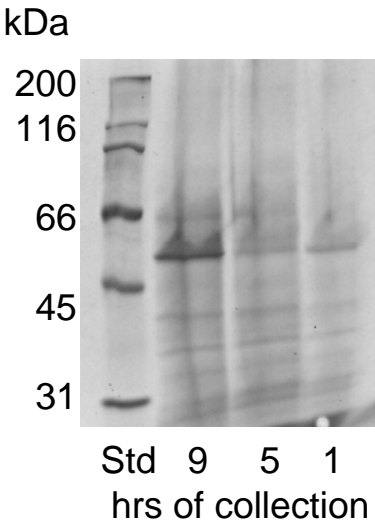
Supplementary Figure S3: GC-MS profile of total (non-phase-partitioned) phloem exudates after one hour of exudation into water (after EDTA treatment). Peaks marked with an asterisk are reagent peaks or column bleed; numbers indicate identified compounds and are cross-referenced in table 1.

Supplementary Table 1: Proteins identified in *Arabidopsis thaliana* phloem exudate; TF indicates the number of distinct tryptic fragments identified

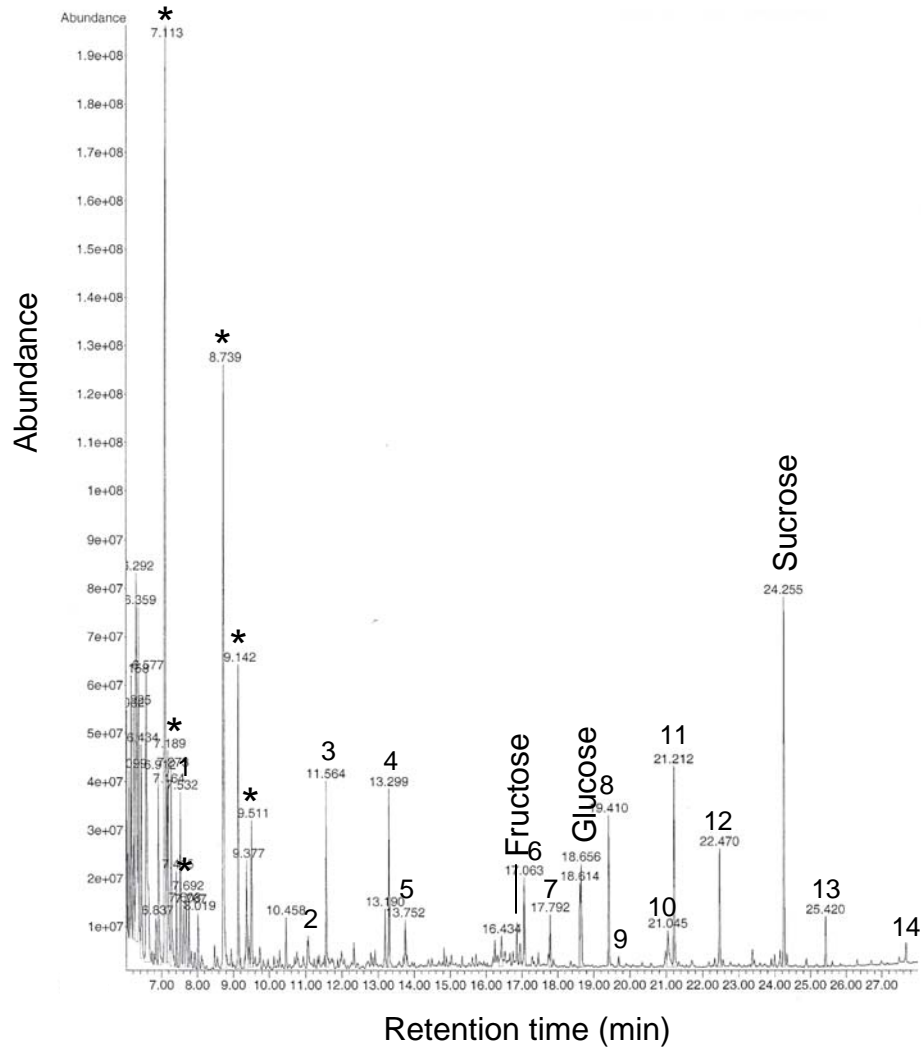
**Collection and Extraction of Arabidopsis Phloem Sap**



Guelette et al., Supplementary Figure 2



Guelette et al., Supplementary Figure S3



**Supplementary table 1:** Proteins identified in *Arabidopsis thaliana* phloem exudate; TF indicates the number of distinct tryptic fragments identified. UPP: unidentified phloem protein; \* homologous to proteins found in Brassica phloem exudates (Giavelisco et al., 2006)

Identified Proteins	# of TF	Accession Number	MW (kDa)	Tryptic fragments	Additional Information
Fd-dependent glutamate synthase	2	At5g04140	180	GNADIIQISGHDGGTGASPISSIK LVAEAGIGTVASGVAK	Redox reactions
Resistance protein homolog RPP1-WsB RPP8	3	At5g43470 AAC72978	141	YITSLKELKIKEMK KQTGEFGKAFTK HQLLVGERDICEVLNDDTIDSR	Nucleotide binding; pathogen response; LRR domain
Ubiquitin-specific protease 26	4	At3g49600	120	DCLMDGAR AMLEASDSSFVDR TIVQDLFSGSVSHVTTCSK ASPEICEECIGER	Protein turnover
Putative acid phosphatase/ Phosphoglycerate mutase-like	3	NP_186780 At3g01310	119	VQLKPLK VQLKPLKWVK MCLDAKAPHLSSSTLPPTLPW	Phosphate homeostasis Histidine phosphatase domain
Cysteine-rich receptor kinase	2	At4g38830	74	LLGYSIEGTER ILMTMSSYSSNMIK	Protein phosphorylation
Putative Receptor Kinase (Rice)	4	Q9AUQ7 At3g17840	72	ASAEVLGKGAFGTAY VSQKADVYSFGVLI LPGAALAGRVEGT	Protein phosphorylation
HSA-like protein UPP1	2	NP_001005208	69	HLVDEPQNLIK KVPQVSTPTLVEVSR	Albumin domain; lipid-binding site
Tetratricopeptide repeat prot.	1	At5g58350		RRLSSLSSSLPR	Protein phosphorylation
Hyp. Protein UPP2	2	At5g48850	63	ELVTGMRSAASK SYMSFISSASISASK	
Thioglucosidase/myrosinase*	2	At5g26000	61	GYALGTDAPGR LFNSGNFEK	Defense; growth regulation

Hypothetical protein UPP3	2	At1g33020	61	MKYNDR TVELYMWVLEDVER	F-box domain; receptor for ubiquitinated targets; prot.interaction
Lipase class 3 family protein	2	At4g16820	58	VPGIFADNDK VDMKMSPYLK	triacylglycerol lipase activity
RING zinc-finger protein	2	At4g01270	56	QKCSLK DPCRLYFQSSGNQTDSIASDK	Protein binding
Aspartic protease	1	At4g04460	56	LN DENADMVPLK	Proteolysis, lipid metabolic processes, endomembrane system
Glycine-rich protein (oleosin) GRP17	3	At5g07530	53	KKKCMSGGMSGSEEGMSGSEGGMS SGGGSK DNPPPAGLPPNSGAGAGGAQSLIKKS K KSMMSGMSGSEEGMSGSEGGMSSGG GSK	Oleosin domain/lipid storage ATP/GTP-binding motif; lipid binding site
Ala-aminotransferase	3	At1g23310	53	LLEATGISTVPGSGFGQK LPTGALQAAK DGYPSDPELIFLTDGASK	Induced under anaerobic stress; gluconeogenesis
Putative kinase	3	At2g16750	53	GFLEDGKGVAVK TSISSDSPRGQESI GVAVKTLKPSVK	Protein phosphorylation
RubisCo LSU*	11	NP_051067	53	DLAVEGNEIIR LSGGDHXHAGTVVGK VALEACVKAR TFQGPPHGIQVER LTYYTPEYETK GGLDFTKDDENVNSQPFMR YGRPLLGCTIKPK DTDILAAFR	Photosynthesis

				DDENVNSQPFMR PLLGCTIKPK DNGLLLHIHR	
UDP-Glc-pyrophosphorylase*	2	At3g03250	52	VLQLETAAGAAIR IQTPTDEIVVPYEK	Glucose-metabolism; phosphate homeostasis
MS5	2	At4g20900	51	CRLFEEETR SGRIIEEA V LLEHK	Pollen development
SNM1	2	At3g26680	50	RLLLLHLHR KRILLHIHR	Sensitive to N <sub>2</sub> ; repair of oxidative DNA damage
Rubisco activase*	2	At2g39730	50	GLAYDTSDDQQDITRGK MEKFYWAPTR	Photosynthesis
Put. PIG-P	Ed	At2g39435	50	YDQQNFKSK	Phosphatidylinositol synthesis
Myrosinase-binding Protein like*	2	At3g16470	48	TSQPFGLTSGEEAELGGGK VYVGQGSVVYVK	Defense; JA-inducible; lectin
Enolase	2	At2g36530	48	VNQIGSVTESIEAVK AGAVVSGIPLYK	Glycolysis; glucose metabolism
Monodehydroascorbate reductase-like protein*	2	At3g52880	46	EFANGQGVQPGELAVISK TSVPDVYAVGDVATFPLK	Oxidative stress; redox functions, salt tolerance
Sqd1	4	At4g33030	44	QASSFYHLSK VEAEEHYNAK VHDSHNIAFTCK FCVQAAVGHPLTVYGK	Sulfolipid biosynthesis
Put. Fructose biphosphate aldolase *	3	At4g38970	43	GILAMDESNATCGK MVDVLVEQNIVPGIK LDSIGLENTEANR	Some forms diurnal and circadian regulated
Fructose-1,6-bisphosphatase	3	At3g55800	42	GFPGTHEFLLLDEGK GIFTNVTSPAK YTGGNMVPDVMQIIVK	Similar to FBpase; gluconeogenesis; pentose- phosphate pathway
Hydroxy methylbilane synthase	2	At5g08280	41	GSPLALAQAYETR YPALHVEENPR	Protoporphyrin synthesis

Zn-bind. dehydrogenase/ oxidoreductase	2	At1g23740	41	VVAAALNPVDAK VVALTGAVTPPGFR	IAA-induced
GDSL motiv Lipase /hydrolase	1	At1g29660	40	ITFSGQVENYK	Pathogen-response; signaling
Glyceraldehyde-3-P- dehydrogenase*	5	At1g13440	37	TLLFGEKPVTVFGIR VVDLIVEHMSK SSIFDAK AATYDEIKK YDSVHGQWK	Gluconeogenesis; cytosol
GAPC-1*	6	At3g04120	37    37	YDSVHGQWK FGIVEGLMTTVHSITATQK DAPMFFVVGVEHEYK VPTVDVSVVDLTVR GILGYTEDDVVSTDFVGDNR NPEDIPWAEAGADYVVESTGVFT	Gluconeogenesis; cytosol
Carbonic anhydrase	1	At3g01500	37	EKYETNPALYGELAK	Stress response; zinc ion binding; membrane bound
Annexin*	4	At1g35720	36	TGTDEGALTR YGDDHGEEILK TLDKELSNDFER TSTQLLHAR	Phospholipid binding
THI1	2	At5g54770	36	ALDMNTAEDAIVR LFNAVAAEDLIVK	Thiamine synthesis; DNA repair
Cysteine synthase	2	At3g04940	35	EGLLVGISSGAAAR VVLTMPSSMSLERK	Cysteine biosynthesis
Oxygen-evolving enhancer protein	5	At5g66570	35	GTGTANQCPTIDGGSETFSFKPGK GGSTGYDNAVALPAGGR SKPETGEVIGVFESLQPSDTDLGAK RLTYDEIQSVK GDEEELVKENVK	Manganese-stabilizing protein in PSII
Putative 3-beta hydroxysteroid	2	At2g37660	35	ELLVGKDDELLETETR	Rossmann- fold NADP+



dehydrogenase/isomerase				ALDLASKPEGTGTPTK	binding proteins; epimerase;
Putative protein UPP4	2	At5g02240	35	ELLVGKDELLELQTDTK ALFSQVTSRF	Rossmann- fold NADP+ binding protein; ABA-ind.
Inorganic phosphatase-like protein	2	At5g09650	33	VQEEGPAESLDYR AHLVNDVEDVEK	PP cleavage; metabolism
Glyoxylase I-homologue	5	At1g11840	30	GNAYAQIAIGTDDVYK FLHVVYR IVNQELGGK DPDGYTFELIQR TIEFYTEVFGMK	Drought and salt tolerance
Ribonucleoprotein UPP5	2	At2g37220	29	VNAGPPPPK VSEAEARPPR	RNA-binding protein
Isomerase ROC 4 (cyclophilin)-like	3	At3g62030	29	FEDENFTLK IVMGLFGEVVPK VYFDVEIGGEVAGR HTGPGILSMANAGPNTNGSQFFICTV	Protein folding
CYP2 (cyclophilin)*	3	At2g21130	18	K FEDENFER IVMELYTDK HVVFQVVEGLDVVK	
Ethylene-responsive RNA helicase	2	CAA09215 At5g63120	29	LLTLLK LLTLLKQLMDGSK	Nucleic-acid binding, ATP- dependent helicase; DEAD- box; nucleoplasmic transport; transcription/translation /splicing; ethylene response
14-3-3-like protein GF14 epsilon psi	2 1	At1g22300 At2g10450	28 9	ILSSIEQK DSTLIMQLLP DSTLIMKILR	Cis-acting regulatory element, protein binding
Chlorophyll a/b binding protein	5	At2g34420	28	ELEVIHSR FGAEVWFK	

				GPSGSPWYGSDR WAMLGALGCVFPELLAR LAMFSMFGFFVQAIVTGK	
Cytosolic triosephosphate isomerase	4	At3g55440	27	NVASDVAATTR EAGSTMDVVAQTK VTNWSNVVIAIYEPVWAIGTGK ELGGQADVDGFLVGGASLKPEFIDIK	Carbon metabolism
Glutathione S-transferase 8	3	At1g78380	27	FANFSIESEVPK VTEFVSELR	Toxin catabolism; auxin regulated
Thaumatococcus-like protein PR5	7	At1g75040	26	LGDGGFELTPGASR YAGCVSDLNAACPDMLK TGCNFDASGKGR NNCPTTVWAGTLAGQGPK QLTAPAGWSGR CVTGDCGGLR VMDQNNVVACK	Osmotin; pathogenesis related
Protein with FHA domain UPP6	2	At2g21530	26	WLLKPVGDGDTR ENEQISNISFLAK	Signal transduction; Forkhead associated domain; DNA/transcript. reg.
Cu/Zn Superoxide Dismutase CSD2	3	At2g28190	22	HAGDLGNINANADGVAETTIVDNQIP LTGPNSVVGR GTSDVEGVVTLTQDDSGPTTVNVR GGHELSTTGNAGGR	
Lipid-associated family protein	1	At4g39730	20	IYDKDGDYIGIK	lipid/protein binding
Rubisco SSU*	6	At1g67090	20	IIGFDNTR LPLFGCTDSAQVLK KKFETLSYLPDLTDSELAK LPLFGCTDSAQVK MQVWPPIGK QVQCISFIAYKPPSFTG	Carbon fixation

L-PSP family protein	2	At3g20390	20	FVSESVEDQTEQVLK ANNLVFLSGVLGLIPETGK	Endoribonuclease;
Thioredoxin m	2	At1g03680	20	LNTDESPATPGQYGVR DTIIGAVSK	
Major latex protein*	5	At1g70890	18	SFAFTLQVTPK VLEGDLMNEYK NKIEAVDPEK IEAVDPEK EIDEHLLAEE	Pathogenesis related
Bet vI allergen-like protein	2	At1g23130	18	ERIEAVDQEK VGSVIFWNYAIDGQPK	Pathogenesis related
GRP7 (CCR2)*	4	At2g21660	17	SITVNEAQRS GFGFVTFKDMK SGGGGGYSGGGGSYGGGGGR ALETAFAYGDYIDSK	RNA-binding; circadian oscillations
Cu/Zn Superoxide dismutase CSD1	2	At1g08830	15	GGHELSTATGNAAGGR AAVVVHADPDDLK	Response to abiotic and biotic stress; cytosolic
Thioredoxin h*	2	At5g42980	13	VDVDELNTVAEEFK EGEIKETVVGAAK	Protection from oxidative damage
Putative CP12 protein	2	At2g47400	13	AGGSDPLEEYCNDNPETDECR FGSMKRMVVVK	Light regulation of calvin cycle; redox equivalents