

Supplementary Material

Salt-stress induced alterations in the root lipidome of two barley genotypes with contrasting responses to salinity

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Table S1. Lipid standards used for targeted (QQQ) lipidomics analysis and their IUPAC nomenclature

Detected lipid species are annotated as follows: lipid class designation (total number of carbon atoms in the fatty acid chains: total number of double bonds in the fatty acid chains), e.g. PC (32:0). LPC, lysophosphatidylcholine; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; d, deuterated.

Avanti catalog no.	Lipid	IUPAC name
855676	LPC(17:0)	1-heptadecanoyl-sn-glycero-3-phosphocholine
830856P	PA (34:0)	1,2-diheptadecanoyl-sn-glycero-3-phosphate (sodium salt)
860399	PC (34:1)d	1-palmitoyl-d31-2-oleoyl-sn-glycero-3-phosphocholine
830756	PE (34:0)	1,2-diheptadecanoyl-sn-glycero-3-phosphoethanolamine
860384	PG (16:0/18:1)d	1-palmitoyl-d31-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt)
840044P	PI (34:2)	L- α -phosphatidylinositol (Soy) (sodium salt)
840028P	PS (34:0)	1,2-diheptadecanoyl-sn-glycero-3-phospho-L-serine (sodium salt)

Table S2. Fatty acid abbreviations and their IUPAC nomenclature

Fatty acid abbreviation	IUPAC nomenclature
C10:0	C10:0
C11:0	C11:0
C12:0	C12:0
C13:0	C13:0
C14:0	C14:0
C14:1	C14:1 (cis-9)
C15:0	C15:0
C15:1	C15:1 (cis-10)
C16:0	C16:0
C16:1	C16:1 (cis-9)
C17:0	C17:0
C17:1	C17:1 (cis-10)
C18:3n6	C18:3 (all cis-6,9,12)
C18:2n6c	C18:2 (all cis-9,12)
C18:2n6t	C18:2 (all trans-9,12)
C18:3n3	C18:3 (all cis-9,12,15)
C18:0	C18:0
C18:1n9t	C18:1 (trans-9)
C18:1n9c	C18:1 (cis-9)
C20:5n3	C20:5 (all cis-5,8,11,14,17)
C20:3n6	C20:3 (all cis-8,11,14)
(C20:3n3)	C20:3 (all cis-8,11,14)
C20:0	C20:0
C20:4n6	C20:4 (all cis-5,8,11,14)
C20:2	C20:2 (all cis-11,14)
C20:1	C20:1 (cis-11)
C21:0	C21:0
C22	C22:0

C22:1n9	C22:1 (cis-9)
C22:6n3	C22:1 (all cis-4,7,10,13,16,19)
C22:2	C22:2 (all cis-13,16)
C23:0	C23:0
C24:0	C24:0
C24:1	C24:1 (cis-15)

Table S3. Fatty acids identified in barley roots using GC-MS

Control: 0 mM NaCl; Salt: 100 mM NaCl; Average is mean ($n = 6$) in picomoles/mg Fresh weight. Values with black fill indicate significance at $P < 0.05$ based on Student's t -test. SE: standard error; SEM: standard error of the mean.

	Clipper Control				Clipper Salt				
	Average	SE	x-fold	SEM	Average	SE	x-fold	SEM	p-value
C12:0	7.95	0.22	1.0	0.03	8.67	0.13	1.1	0.02	0.02
C14:0	10.32	0.27	1.0	0.03	11.59	0.64	1.1	0.06	0.10
C15:0	22.42	0.96	1.0	0.04	13.15	0.69	-1.7	0.05	<0.00
C16:0	1287.30	74.37	1.0	0.06	1353.65	71.21	1.05	0.05	0.53
C16:1	15.00	1.39	1.0	0.09	7.42	0.83	-2.0	0.11	<0.00
C17:0	6.25	0.10	1.0	0.02	5.81	0.19	-1.	0.03	0.07
C18:0	190.13	13.43	1.0	0.07	238.38	29.58	1.3	0.12	0.17
C18:1n9c	29.47	2.14	1.0	0.07	31.83	4.36	1.1	0.14	0.64
C18:1n9t	21.36	5.35	1.0	0.25	11.31	0.94	-1.9	0.08	0.09
C18:2n6c	1506.67	69.74	1.0	0.05	1483.20	60.76	-1.0	0.04	0.80
C18:3n3	7.47	0.50	1.0	0.07	6.44	0.17	-1.2	0.03	0.08
C18:3n6	837.98	49.00	1.0	0.06	889.52	42.41	1.1	0.05	0.44
C20:0	8.47	0.14	1.0	0.02	9.80	0.33	1.2	0.03	<0.00
C20:1	5.42	0.37	1.0	0.07	5.18	0.27	-1.1	0.05	0.62
C20:2	7.55	0.27	1.0	0.04	7.77	0.43	1.0	0.06	0.68
C20:3n6	4.16	0.07	1.0	0.02	4.20	0.14	1.0	0.03	0.83
C21:0	4.59	0.05	1.0	0.01	4.57	0.08	1.0	0.02	0.89
C22	18.93	0.46	1.0	0.02	20.44	0.80	1.	0.04	0.13
C22:1n9	3.77	0.08	1.0	0.02	3.63	0.09	-1.0	0.02	0.26
C22:2	5.12	0.09	1.0	0.02	5.19	0.14	1.0	0.03	0.67
C22:6n3	6.88	0.27	1.0	0.04	7.97	0.34	1.2	0.04	0.03
C23:0	14.19	0.31	1.0	0.02	13.58	0.55	-1.0	0.04	0.36
C24:0	19.88	0.43	1.0	0.02	19.58	0.63	-1.0	0.03	0.70
C24:1	8.04	0.22	1.0	0.03	7.88	0.24	-1.0	0.03	0.63

	Sahara Control				Sahara Salt				p-value
	Average	SE	x-fold	SEM	Average	SE	x-fold	SEM	
C12:0	8.12	0.21	1.0	0.03	8.49	0.20	1.0	0.02	0.24
C14:0	11.78	0.37	1.0	0.03	11.30	0.82	-1.0	0.07	0.60
C15:0	32.18	1.68	1.0	0.05	27.69	1.92	-1.2	0.07	0.11
C16:0	1346.78	36.63	1.0	0.03	1445.67	35.20	1.1	0.02	0.08
C16:1	13.63	1.09	1.0	0.08	8.40	0.56	-1.6	0.07	<0.00
C17:0	5.50	0.11	1.0	0.02	5.11	0.21	-1.1	0.04	0.13
C18:0	194.97	4.43	1.0	0.02	212.72	12.39	1.	0.06	0.21
C18:1n9c	51.43	5.14	1.0	0.10	37.70	6.07	-1.4	0.16	0.11
C18:1n9t	12.04	0.67	1.0	0.06	12.41	0.85	1.0	0.07	0.73
C18:2n6c	1679.98	61.85	1.0	0.04	1502.17	47.31	-1.1	0.03	0.05
C18:3n3	6.48	0.61	1.0	0.09	5.24	0.19	-1.2	0.04	0.08
C18:3n6	933.28	35.91	1.0	0.04	1234.25	74.34	1.3	0.06	<0.00
C20:0	7.62	0.21	1.0	0.03	7.52	0.40	-1.0	0.05	0.82
C20:1	8.49	0.49	1.0	0.06	8.01	0.66	-1.1	0.08	0.57
C20:2	9.49	0.14	1.0	0.01	9.34	0.29	-1.0	0.03	0.65
C20:3n6	4.23	0.07	1.0	0.02	4.65	0.08	1.1	0.02	<0.00
C21:0	4.02	0.08	1.0	0.02	3.76	0.16	-1.1	0.04	0.18
C22	17.11	0.44	1.0	0.03	16.09	0.76	-1.1	0.05	0.27
C22:1n9	4.24	0.07	1.0	0.02	4.10	0.10	-1.0	0.03	0.31
C22:2	5.35	0.05	1.0	0.01	5.42	0.12	1.0	0.02	0.57
C22:6n3	6.22	0.11	1.0	0.02	5.96	0.08	-0	0.01	0.10
C23:0	9.89	0.32	1.0	0.03	7.77	0.65	-1.3	0.08	0.01
C24:0	18.17	0.37	1.0	0.02	17.89	0.70	-1.0	0.04	0.73
C24:1	10.02	0.26	1.0	0.03	10.41	0.45	1.0	0.04	0.47

Table S4. Lipid profiling features showing significant ($P < 0.01$) fold-changes (FC) ≥ 2 following salt treatment in both Clipper and Sahara roots compared to their respective controls sorted on the basis of P -value (lowest P -value to highest P -value), t -test, $n = 6$ for each treatment group

Clip C: Clipper control; Clip S: Clipper salt treated; Sah C: Sahara control; Sah S: Sahara salt treated. m/z = mass to charge ratio of the observed feature. A positive $\log_2(\text{FC})$ value means the feature was increased in the salt-treated roots relative to the controls and vice versa. P -value according to Student's t -test.

m/z	Clip C vs Clip S		Sah C vs Sah S	
	$\log_2(\text{FC})$	P-value	$\log_2(\text{FC})$	P-value
934.6492	-4.5	2E-09	-2.0	4E-06
801.5507	-4.3	3E-09	-2.2	6E-06
575.5038	-3.8	4E-09	-1.5	6E-06
939.6047	-4.7	4E-09	-2.4	6E-06
775.5349	-4.0	5E-09	-1.1	6E-05
313.2743	-3.9	6E-09	-1.6	4E-06
955.5763	-4.6	1E-08	2.4	3E-05
786.5738	-3.0	1E-08	-1.4	7E-07
925.5860	-2.8	1E-08	-1.2	1E-03
617.5143	-4.3	1E-08	-1.6	1E-05
817.5234	-3.7	2E-08	-1.4	2E-06
920.6311	-2.8	3E-08	-1.2	1E-03
850.5451	2.5	3E-07	1.3	1E-04
573.4894	2.5	6E-07	1.6	2E-05
740.5231	1.2	7E-07	1.2	5E-06
778.5392	1.8	1E-06	1.6	5E-05
413.2132	1.6	2E-06	1.2	4E-04
797.5203	1.4	2E-06	1.1	5E-05
767.4841	2.1	2E-06	1.3	2E-04
728.5231	1.6	2E-06	1.4	1E-06

745.5023	2.2	2E-06	1.6	1E-04
813.4935	1.6	4E-06	1.4	4E-05
762.5296	2.5	4E-06	1.7	7E-05
613.4833	1.4	1E-05	1.2	1E-04
792.5640	1.3	2E-05	1.1	8E-05
778.5412	1.4	6E-05	1.7	1E-05
800.5215	1.8	9E-05	2.5	1E-05
972.5674	1.5	1E-04	1.2	1E-03
788.5448	3.3	1E-04	1.3	3E-04
812.5450	3.1	2E-04	1.2	7E-04
816.4945	1.9	2E-04	2.6	3E-05
599.5040	2.1	2E-04	1.3	9E-04
815.5542	3.8	4E-04	4.0	6E-04
810.5292	2.8	6E-04	2.0	4E-03
736.4929	1.8	2E-03	2.5	1E-03
1560.0990	2.4	2E-03	1.3	2E-03
690.5072	-1.1	3E-03	-1.3	1E-11
1583.0844	2.7	4E-03	1.4	6E-04
1582.0807	2.3	5E-03	1.1	3E-03
662.4761	-1.7	7E-03	-3.0	7E-05
1192.8124	3.4	9E-03	2.0	2E-04

Table S5. Lipid profiling features found to be significantly altered only in Clipper roots following salt treatment ($P < 0.01$, fold-change (FC) ≥ 2) sorted on the basis of P -value (lowest P -value to highest P -value), $n = 6$ for each treatment group

Feature: mass-to-charge ratio of observed feature_associated retention time. Unique to Clipper: 72 down after salt; 39 up after salt. P -value according to Student's t -test.

Feature	$\log_2(\text{FC})$	P -value
1577.0779_12.08	-8.9	2.9E-10
932.634_12.08	-2.3	1.1E-08
791.5078_12.95	-3.8	1.2E-08
796.5935_13.06	-3.5	2.6E-08
591.4986_12.96	-4.3	2.8E-08
591.4989_12.08	-2.3	3.8E-08
963.6026_12.29	-2.3	4.1E-08
958.6475_12.29	-2.1	7.6E-08
335.2586_12.08	-2.0	1.2E-07
937.5886_12.08	-2.2	1.3E-07
337.2743_13.18	-2.6	1.5E-07
763.5336_13.2	-2.4	1.6E-07
815.508_12.07	-2.0	1.8E-07
313.2744_12.08	-1.7	2.1E-07
1576.0745_12.09	-7.5	2.2E-07
867.685_21.84	-1.8	3.0E-07
794.5797_12.07	-1.8	3.5E-07
810.5734_11.14	-2.3	3.8E-07
799.5355_12.07	-1.8	3.9E-07
953.5604_12.08	-2.4	4.6E-07
961.5867_11.43	-1.6	5.6E-07
770.5739_12.97	-2.2	5.8E-07
615.4989_12.07	-1.8	5.9E-07
888.797_21.85	-1.7	7.4E-07

617.5133_12.29	-1.6	9.3E-07
851.7116_21.85	-1.8	1.6E-06
956.6316_11.42	-1.7	1.7E-06
573.4882_12.07	-1.1	2.1E-06
965.6173_13.21	-1.1	2.2E-06
934.6473_13.15	-1.3	2.2E-06
854.724_20.13	-1.8	2.4E-06
702.5074_12.96	-2.5	3.8E-06
793.5236_13.73	-3.7	4.0E-06
772.5944_13.74	-3.7	4.4E-06
960.662_13.22	-1.1	4.9E-06
777.551_13.73	-3.7	4.9E-06
832.7398_21.52	-2.5	4.9E-06
846.5239_12.36	1.0	1.6E-05
1046.7878_16.77	-1.8	1.7E-05
808.5583_10.29	-4.1	2.1E-05
1560.0963_12.42	1.4	2.2E-05
784.5584_11.09	-1.4	2.6E-05
859.6791_20.14	-2.8	2.8E-05
846.7569_21.86	-2.0	2.9E-05
575.5039_13.72	-3.0	4.0E-05
856.74_20.97	-2.1	4.7E-05
848.5397_13.27	1.1	5.0E-05
761.5185_12.29	-1.9	5.2E-05
834.7556_21.97	-2.1	5.7E-05
844.7393_21.4	-1.0	6.7E-05
878.5696_11.09	2.8	7.0E-05
794.5108_12.36	1.1	8.1E-05
791.5467_12.42	1.1	8.2E-05
852.5611_11.79	1.3	1.3E-04
313.1054_2.12	-1.4	1.4E-04

1538.1144_12.43	1.2	1.4E-04
891.6862_21.4	-1.1	1.5E-04
814.5609_9.04	3.4	1.6E-04
814.5607_9.05	3.4	1.6E-04
1561.0997_12.41	1.3	1.6E-04
575.5033_21.42	-1.1	1.8E-04
950.5838_12.37	1.1	1.9E-04
912.7973_21.41	-1.2	2.2E-04
835.534_11.78	1.2	2.3E-04
875.713_21.42	-1.1	2.4E-04
889.6701_20.68	-1.3	2.7E-04
796.5266_13.27	1.2	2.9E-04
810.6016_13.51	1.2	3.3E-04
792.0471_12.42	1.2	3.4E-04
1538.1122_13.27	2.6	3.5E-04
952.6_13.28	1.4	3.5E-04
918.6152_11.5	-4.6	4.0E-04
1182.8284_12.45	1.9	4.3E-04
475.2211_2.63	-1.2	4.8E-04
632.5256_13.37	1.0	5.1E-04
974.5839_11.52	1.2	5.2E-04
1539.1158_13.27	2.4	5.2E-04
974.5809_13.26	1.1	5.5E-04
676.492_12.07	-2.0	5.6E-04
996.5648_11.52	1.1	5.7E-04
870.7579_21.42	-1.2	6.0E-04
1516.1305_13.28	2.6	6.5E-04
818.5107_11.52	1.2	7.3E-04
873.6966_20.69	-1.3	8.5E-04
597.4881_21.42	-1.1	9.2E-04
732.5734_12.06	10.2	9.4E-04

1047.7571_21.41	-1.2	9.8E-04
910.7797_20.71	-1.1	1.1E-03
716.5249_13.41	1.2	1.1E-03
817.6119_19.03	-1.9	1.1E-03
1561.1029_11.52	2.2	1.2E-03
812.6561_19.02	-1.9	1.2E-03
790.561_9.74	2.5	1.4E-03
794.5713_12.53	-2.4	1.4E-03
575.5039_13.41	1.1	2.1E-03
846.526_3.19	-2.3	2.1E-03
868.7416_20.73	-1.3	2.2E-03
910.5516_13.42	1.4	2.4E-03
762.5161_9.73	3.0	2.7E-03
738.5057_13.41	1.3	2.7E-03
869.7009_22.21	-1.2	2.9E-03
806.568_13.43	1.2	3.0E-03
608.5258_14.16	1.1	3.7E-03
978.6138_13.43	1.2	5.6E-03
812.5602_12.13	-1.6	6.1E-03
760.49_11.71	1.3	6.5E-03
732.561_12.05	-2.4	7.3E-03
853.7278_22.21	-1.1	7.5E-03
389.0639_12.06	-5.0	7.6E-03
924.8028_21.98	-1.0	8.5E-03
597.4884_11.71	1.2	8.6E-03

Table S6. Lipid profiling features found to be significantly altered only in Sahara roots following salt treatment ($p < 0.01$, fold-change (FC) ≥ 2) sorted on the basis of p-value (lowest p-value to highest p-value), n = 6 for each treatment group

Feature: mass-to-charge ratio of observed feature_ associated retention time. Unique to Sahara: 9 down after salt; 11 after salt. p-value according to Student's *t*-test.

Feature	$\log_2(\text{FC})$	p-value
766.5369_13.4	-7.8	<0.000
955.5763_13	-2.3	<0.000
518.3232_3.62	1.0	<0.000
711.4078_2.07	-1.4	<0.000
371.0552_10.53	1.0	<0.000
911.6545_18.89	1.1	0.0001
740.5229_11.09	1.3	0.0001
1512.0989_12.36	2.0	0.0001
895.6809_18.89	1.1	0.0001
890.7256_18.89	1.3	0.0001
932.7628_18.89	1.5	0.0001
496.3413_5.29	1.2	0.0002
710.5933_13.39	-4.5	0.0003
602.3579_2.13	-1.2	0.0003
746.5686_14.28	-4.0	0.0006
751.4141_4.62	-1.0	0.0013
1572.0433_11.13	3.1	0.0031
1156.8129_12.34	3.1	0.0044
441.7867_3.36	-1.2	0.0055
798.5653_9.76	-2.2	0.0055

Table S7. Putative lipid assignments for common mass features significantly altered in Clipper and Sahara barley roots after salt-treatment compared to their respective controls (based on LipidMaps search of precursor m/z \pm 0.01Da using LipidMaps online tools) listed in Table S4 as determined by comparison of lipid profiles obtained using HPLC-ESI-QTOF-MS as described in Materials and Methods and listed in order of increasing input mass

A positive $\log_2(\text{FC})$ value, means increased in Clipper_Salt-treated compared to Clipper_Control. Note not all masses listed in Table S4 match to an entry in the LipidMaps database.

Input Mass	Matched Mass	Delta	Name	Class	Main Class	Clipper	Sahara
						(Control vs Salt-treated) $\log_2(\text{FC})$	(Control vs Salt-treated) $\log_2(\text{FC})$
313.2743	313.2737	0.0006	FA(19:0(Ep))	FA	FA	-3.9	-1.6
413.2132	413.217	0.0038	FA(21:0(OH2,Ke2,Ep2, ,cyclo))	FA	FA	1.6	1.2
573.4894	573.4878	0.0016	DG(P-34:4)	DG	GL	2.5	1.6
575.5038	575.5034	0.0004	DG(O-34:4)	DG	GL	-3.8	-1.5
599.504	599.5034	0.0006	DG(P-36:5)	DG	GL	2.1	1.3
613.4833	613.4827	0.0006	DG(36:6)	DG	GL	1.4	1.2
617.5143	617.514	0.0003	DG(36:4)	DG	GL	-4.3	-1.6
662.4761	662.4756	0.0005	PC(27:1)	PC	PL	-1.7	-3.0
690.5072	690.5069	0.0003	PC(29:1)	PC	PL	-1.1	-1.3
728.5231	728.5225	0.0006	PC(32:3)	PC	PL	1.6	1.4

736.4929	736.4912	0.0017	PE(36:6)	PE	PL	1.8	2.5
740.5231	740.5225	0.0006	PC(33:4)	PC	PL	1.2	1.2
745.5023	745.5014	0.0009	PG(34:3)	PG	PL	2.2	1.6
762.5296	762.528	0.0016	PS(34:1)	PS	PL	2.5	1.7
767.4841	767.4858	0.0017	PG(36:6)	PG	PL	2.1	1.3
775.5349	775.5273	0.0076	PA(42:7)	PA	PL	-4.0	-1.1
778.5392	778.5382	0.001	PC(36:6)	PC	PL	1.8	1.6
778.5412	778.5382	0.003	PC(36:6)	PC	PL	1.4	1.7
786.5738	786.5644	0.0094	PS(P-37:2)	PS	PL	-3.0	-1.4
788.5448	788.5436	0.0012	PS(36:2)	PS	PL	3.3	1.3
797.5203	797.5175	0.0028	PI(31:0)	PI	PL	1.4	1.1
800.5215	800.5225	0.001	PC(38:9)	PC	PL	1.8	2.5
801.5507	801.5429	0.0078	PA(44:8)	PA	PL	-4.3	-2.2
810.5292	810.528	0.0012	PS(38:5)	PS	PL	2.8	2.0
812.545	812.5436	0.0014	PS(38:4)	PS	PL	3.1	1.2
813.4935	813.4913	0.0022	LPI(34:6)	LPI	LPL	1.6	1.4
815.5542	815.5586	0.0044	PA(45:8)	PA	PL	3.8	4.0
817.5234	817.5226	0.0008	PI(O-34:4)	PI	PL	-3.7	-1.4
920.6311	920.6375	0.0064	PS(46:6)	PS	PL	-2.8	-1.2
925.586	925.5801	0.0059	PI(41:6)	PI	PL	-2.8	-1.2
934.6492	934.6532	0.004	PS(47:6)	PS	PL	-4.5	-2.0
939.6047	939.5957	0.009	PI(42:6)	PI	PL	-4.7	-2.4

955.5763	955.5672	0.0091	PIP(O-38:3)	PIP	PIP	-4.6	2.4
1560.099	1560.0891	0.0099	CL(80:9)	CL	CL	2.4	1.3
1582.0807	1582.0734	0.0073	CL(82:12)	CL	CL	2.3	1.1

Table S8. Quantitative lipid analysis in Clipper barley roots using HPLC-QQQ-MS

Control: 0 mM NaCl; Salt: 100 mM NaCl; Average is mean ($n = 6$) in picomoles/mg Fresh weight. Values with black fill indicate significance at $P < 0.05$. SE: standard error; SEM: standard error of the mean.

	Clipper Control				Clipper Salt				
	Average	SE	x-fold	SEM	Average	SE	x-fold	SEM	P-value
LPC (17:0)	0.01	0.00	1.0	0.18	0.01	0.00	-1.3	0.17	0.32
LPC (15:0)	0.07	0.01	1.0	0.09	0.04	0.00	-2.0	0.07	<0.00
LPC (16:0)	0.23	0.09	1.0	0.39	0.11	0.03	-2.0	0.23	0.25
LPC (18:1)	0.16	0.04	1.0	0.22	0.30	0.15	1.9	0.48	0.37
LPC (18:2)	1.68	0.18	1.0	0.11	1.73	0.04	1.0	0.02	0.77
LPC (18:3)	1.17	0.17	1.0	0.15	1.40	0.10	1.2	0.07	0.27
LPC (24:4)	0.05	0.01	1.0	0.10	0.04	0.01	-1.2	0.22	0.40
PC (32:1)	0.37	0.03	1.0	0.08	0.49	0.04	1.3	0.07	0.02
PC (32:2)	0.89	0.07	1.0	0.08	1.10	0.07	1.2	0.06	0.06
PC (32:3)	0.44	0.03	1.0	0.07	0.60	0.04	1.4	0.07	0.01
PC (33:1)	0.36	0.02	1.0	0.05	0.34	0.02	-1.1	0.07	0.60
PC (33:2)	4.99	0.25	1.0	0.05	4.63	0.24	-1.1	0.05	0.34
PC (33:3)	3.46	0.11	1.0	0.03	3.09	0.21	-1.1	0.07	0.15
PC (33:4)	0.40	0.02	1.0	0.04	0.42	0.04	1.0	0.08	0.68
PC (34:0)	0.55	0.07	1.0	0.12	0.78	0.06	1.4	0.08	0.02
PC (34:1)	7.01	0.40	1.0	0.06	11.03	0.73	1.6	0.07	<0.00
PC (34:2)	88.56	5.70	1.0	0.06	135.95	9.42	1.5	0.07	<0.00
PC (34:3)	58.10	4.31	1.0	0.07	95.22	7.07	1.6	0.07	<0.00
PC (34:4)	0.91	0.06	1.0	0.07	1.24	0.08	1.4	0.06	0.01
PC (35:1)	0.15	0.02	1.0	0.11	0.16	0.01	1.	0.08	0.62
PC (35:2)	0.83	0.27	1.0	0.33	0.79	0.08	-1.	0.10	0.88
PC (35:3)	0.83	0.07	1.0	0.08	1.11	0.07	1.3	0.06	0.01
PC (35:4)	0.69	0.04	1.0	0.05	0.71	0.05	1.0	0.07	0.83
PC (36:0)	1.03	0.16	1.0	0.16	4.06	0.64	3.9	0.16	<0.00
PC (36:1)	1.05	0.11	1.0	0.10	0.98	0.06	-1.1	0.06	0.58

PC (36:2)	3.72	0.30	1.0	0.08	3.97	0.30	1.1	0.08	0.57
PC (36:3)	18.41	1.17	1.0	0.06	20.10	1.98	1.1	0.10	0.48
PC (36:4)	109.18	3.84	1.0	0.04	130.98	9.00	1.2	0.07	0.05
PC (36:5)	108.30	4.92	1.0	0.05	154.24	11.55	1.4	0.07	<0.00
PC (36:6)	28.50	1.85	1.0	0.06	46.88	3.64	1.6	0.08	<0.00
PC (38:2)	2.37	0.34	1.0	0.14	8.07	1.27	3.4	0.16	<0.00
PC (38:3)	1.02	0.14	1.0	0.14	1.39	0.10	1.4	0.07	0.05
PC (38:4)	1.13	0.10	1.0	0.09	1.63	0.13	1.4	0.08	0.02
PC (38:5)	0.70	0.06	1.0	0.09	0.93	0.06	1.3	0.06	0.02
PC (39:2)	0.28	0.04	1.0	0.14	0.30	0.02	1.1	0.08	0.57
PC (41:6)	0.10	0.02	1.0	0.15	0.13	0.02	1.2	0.19	0.43
PE (32:1)	7.20	1.38	1.0	0.19	1.64	0.18	-4.4	0.11	<0.00
PE (32:2)	5.72	0.83	1.0	0.14	4.89	0.59	0.9	0.12	0.43
PE (33:1)	0.21	0.05	1.0	0.24	0.31	0.08	1.5	0.26	0.33
PE (33:2)	1.43	0.22	1.0	0.15	2.04	0.19	1.4	0.09	0.07
PE (33:3)	2.44	0.23	1.0	0.10	3.10	0.30	1.3	0.10	0.12
PE (34:1)	7.60	0.96	1.0	0.13	14.02	1.65	1.9	0.12	0.01
PE (34:2)	73.18	10.22	1.0	0.14	144.21	15.92	2.0	0.11	<0.00
PE (34:3)	38.64	5.31	1.0	0.14	77.58	8.37	2.0	0.11	<0.00
PE (35:3)	0.32	0.05	1.0	0.14	0.56	0.07	1.8	0.13	0.02
PE (35:4)	0.53	0.05	1.0	0.09	0.65	0.06	1.2	0.09	0.17
PE (36:3)	5.43	0.96	1.0	0.18	8.40	1.12	1.6	0.13	0.07
PE (36:4)	82.67	8.90	1.0	0.11	134.34	13.26	1.6	0.10	0.01
PE (36:5)	23.98	4.22	1.0	0.18	50.06	5.40	2.1	0.11	<0.00
PE (36:6)	16.91	2.32	1.0	0.14	37.76	4.35	2.2	0.12	<0.00
PE (38:4)	1.59	0.23	1.0	0.14	2.63	0.32	1.7	0.12	0.03
PE (38:5)	0.48	0.09	1.0	0.20	0.98	0.13	2.1	0.14	0.01
PG (32:0)	0.57	0.04	1.0	0.07	0.73	0.06	1.3	0.08	0.04
PG (34:1)	2.35	0.12	1.0	0.05	3.37	0.29	1.4	0.09	0.01
PG (34:2)	20.04	1.12	1.0	0.06	32.77	2.51	1.6	0.08	<0.00
PG (34:3)	17.68	1.67	1.0	0.09	37.41	2.81	2.1	0.08	<0.00

PG (36:3)	0.00	0.00	1.0	0.26	0.01	0.00	2.1	0.31	0.14
PG (36:4)	0.20	0.03	1.0	0.15	0.38	0.03	1.9	0.09	<0.00
PG (36:5)	0.62	0.05	1.0	0.08	1.12	0.07	1.8	0.07	<0.00
PI (34:1)	4.82	0.10	1.0	0.02	7.60	0.45	1.6	0.06	0.00
PI (34:2)	52.79	1.11	1.0	0.02	78.13	6.92	1.5	0.09	<0.00
PI (34:3)	44.05	1.46	1.0	0.03	72.72	6.38	1.7	0.09	<0.00
PS (34:2)	0.04	0.00	1.0	0.08	0.09	0.01	2.1	0.14	0.01
PS (40:8)	0.02	0.00	1.0	0.13	0.02	0.00	1.0	0.09	0.79

Table S9. Quantitative lipid analysis in LR Sahara barley roots using HPLC-QQQ-MS

Control: 0 mM NaCl; Salt: 100 mM NaCl; Average is mean ($n = 6$) in picomoles/mg Fresh weight. Values with black fill indicate significance at $P < 0.05$. SE: standard error; SEM: standard error of the mean.

	Sahara Control				Sahara Salt				P-value
	Average	SE	x-fold	SEM	Average	SE	x-fold	SEM	
LPC (17:0)	0.01	0.00	1.0	0.24	0.01	0.00	1.9	0.34	0.23
LPC (15:0)	0.10	0.01	1.0	0.06	0.17	0.02	1.7	0.12	0.01
LPC (16:0)	0.15	0.01	1.0	0.06	0.22	0.01	1.5	0.04	<0.00
LPC (18:1)	0.18	0.01	1.0	0.08	0.26	0.02	1.4	0.06	<0.00
LPC (18:2)	1.74	0.09	1.0	0.05	2.00	0.18	1.2	0.09	0.21
LPC (18:3)	1.08	0.05	1.0	0.05	1.88	0.18	1.7	0.10	<0.00
LPC (24:4)	0.06	0.01	1.0	0.15	0.05	0.01	-1.2	0.12	0.35
PC (32:1)	0.46	0.02	1.0	0.04	0.50	0.02	1.1	0.04	0.12
PC (32:2)	1.21	0.06	1.0	0.05	1.25	0.04	1.0	0.03	0.51
PC (32:3)	0.62	0.03	1.0	0.05	0.88	0.03	1.4	0.04	<0.00
PC (33:1)	0.66	0.02	1.0	0.03	0.61	0.10	-1.1	0.16	0.63
PC (33:2)	8.96	0.38	1.0	0.04	8.60	0.51	-1.0	0.06	0.58
PC (33:3)	6.05	0.21	1.0	0.03	7.06	0.32	1.2	0.05	0.03
PC (33:4)	0.63	0.03	1.0	0.06	0.79	0.04	1.3	0.05	0.01
PC (34:0)	0.86	0.09	1.0	0.10	0.96	0.08	1.1	0.09	0.45
PC (34:1)	10.37	0.44	1.0	0.04	11.01	0.55	1.1	0.05	0.39
PC (34:2)	124.30	4.79	1.0	0.04	135.26	5.38	1.1	0.04	0.16
PC (34:3)	75.95	3.89	1.0	0.05	121.16	6.89	1.6	0.06	<0.00
PC (34:4)	1.29	0.03	1.0	0.03	1.72	0.08	1.3	0.05	<0.00
PC (35:1)	0.11	0.01	1.0	0.06	0.11	0.01	1.0	0.13	1.00
PC (35:2)	1.52	0.27	1.0	0.18	0.98	0.10	-1.6	0.10	0.09
PC (35:3)	0.74	0.03	1.0	0.04	0.75	0.05	1.0	0.06	0.90
PC (35:4)	0.54	0.03	1.0	0.05	0.50	0.03	-1.1	0.06	0.24
PC (36:0)	0.79	0.06	1.0	0.08	1.21	0.08	1.5	0.07	<0.00
PC (36:1)	1.03	0.10	1.0	0.10	0.95	0.04	-1.1	0.04	0.52

PC (36:2)	4.11	0.42	1.0	0.10	4.00	0.23	-1.0	0.06	0.82
PC (36:3)	24.18	1.47	1.0	0.06	23.71	0.94	-1.0	0.04	0.79
PC (36:4)	128.98	4.39	1.0	0.03	119.40	3.73	-1.1	0.03	0.13
PC (36:5)	131.34	5.52	1.0	0.04	169.85	6.74	1.3	0.04	<0.00
PC (36:6)	36.14	2.36	1.0	0.07	71.36	4.62	2.0	0.06	<0.00
PC (38:2)	1.65	0.15	1.0	0.09	2.14	0.17	1.3	0.08	0.05
PC (38:3)	2.24	0.23	1.0	0.10	2.25	0.24	1.0	0.11	0.97
PC (38:4)	1.90	0.15	1.0	0.08	2.45	0.23	1.3	0.09	0.07
PC (38:5)	0.80	0.04	1.0	0.05	0.98	0.04	1.2	0.04	0.01
PC (39:2)	0.26	0.02	1.0	0.07	0.19	0.02	-1.4	0.10	0.03
PC (41:6)	0.11	0.01	1.0	0.11	0.12	0.01	1.1	0.07	0.44
PE (32:1)	6.12	0.48	1.0	0.08	1.14	0.18	-5.4	0.16	<0.00
PE (32:2)	6.05	0.36	1.0	0.06	1.64	0.15	-3.7	0.09	<0.00
PE (33:1)	0.48	0.05	1.0	0.11	0.34	0.05	-1.4	0.13	0.08
PE (33:2)	3.28	0.17	1.0	0.05	2.59	0.35	-1.3	0.13	0.10
PE (33:3)	5.16	0.19	1.0	0.04	5.35	0.45	1.0	0.08	0.71
PE (34:1)	13.93	1.05	1.0	0.08	12.29	1.27	-1.1	0.10	0.34
PE (34:2)	130.06	7.81	1.0	0.06	122.43	12.71	-1.1	0.10	0.62
PE (34:3)	63.88	4.84	1.0	0.08	89.85	9.58	1.4	0.11	0.04
PE (35:3)	0.39	0.05	1.0	0.12	0.24	0.03	-1.6	0.11	0.02
PE (35:4)	0.51	0.02	1.0	0.03	0.36	0.03	-1.4	0.09	<0.00
PE (36:3)	6.91	0.27	1.0	0.04	5.85	0.74	-1.2	0.13	0.21
PE (36:4)	130.00	5.87	1.0	0.05	103.52	8.47	-1.3	0.08	0.03
PE (36:5)	44.76	2.81	1.0	0.06	51.30	6.49	1.2	0.13	0.38
PE (36:6)	30.85	2.60	1.0	0.08	59.52	6.90	1.9	0.12	<0.00
PE (38:4)	3.66	0.27	1.0	0.07	3.71	0.39	1.0	0.11	0.92
PE (38:5)	0.98	0.08	1.0	0.09	1.29	0.16	1.3	0.12	0.11
PG (32:0)	0.61	0.02	1.0	0.04	0.65	0.04	1.1	0.06	0.44
PG (34:1)	2.67	0.11	1.0	0.04	2.30	0.12	-1.2	0.05	0.05
PG (34:2)	26.21	0.88	1.0	0.03	26.84	1.06	1.0	0.04	0.66
PG (34:3)	28.63	1.74	1.0	0.06	42.71	2.90	1.5	0.07	<0.00

PG (36:3)	0.01	0.00	1.0	0.28	0.01	0.00	-1.7	0.16	0.19
PG (36:4)	0.42	0.03	1.0	0.07	0.38	0.03	-1.1	0.07	0.26
PG (36:5)	1.18	0.04	1.0	0.03	1.30	0.06	1.1	0.04	0.13
PI (34:1)	6.96	0.38	1.0	0.05	6.99	0.52	1.0	0.07	0.96
PI (34:2)	67.38	2.99	1.0	0.04	65.71	3.61	-1.0	0.05	0.73
PI (34:3)	53.83	3.11	1.0	0.06	79.43	6.12	1.5	0.08	0.00
PS (34:2)	0.06	0.01	1.0	0.10	0.07	0.01	1.1	0.18	0.63
PS (40:8)	0.02	0.00	1.0	0.12	0.01	0.00	-1.2	0.07	0.21

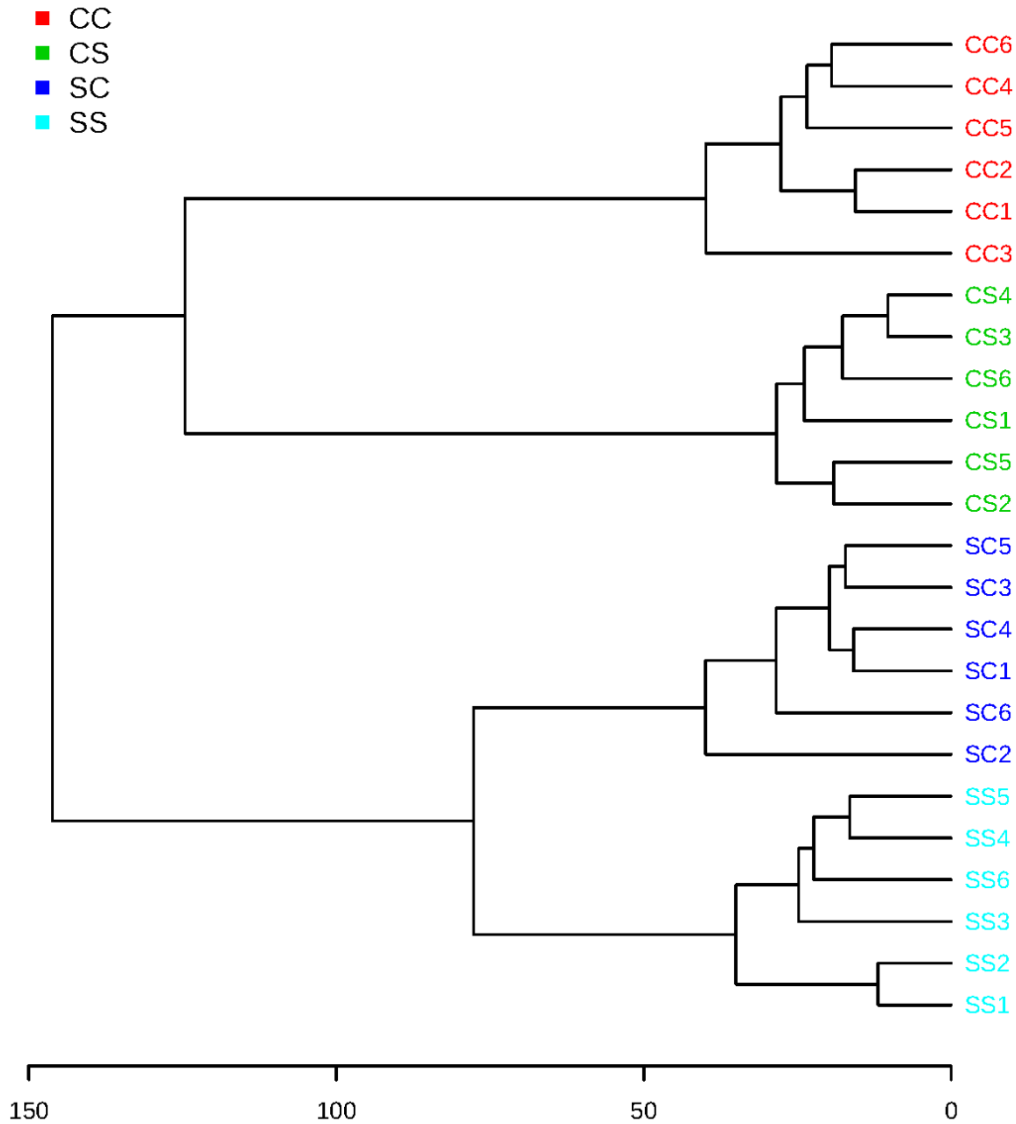


Fig. S1. Hierarchical Clustering Analysis (HCA) untargeted lipid profiling analysis of total barley root lipids from the barley genotypes Clipper and Sahara shown as dendrogram. CC: Clipper control; CS: Clipper salt treated; SC: Sahara control; SS: Sahara salt treated; numbers designate biological replicates. Control (0 mM NaCl) and salt treated (100 mM NaCl) for 5 weeks. Plot created using MetaboAnalyst 3.0 (Xia, J. *et al.*, 2015).