

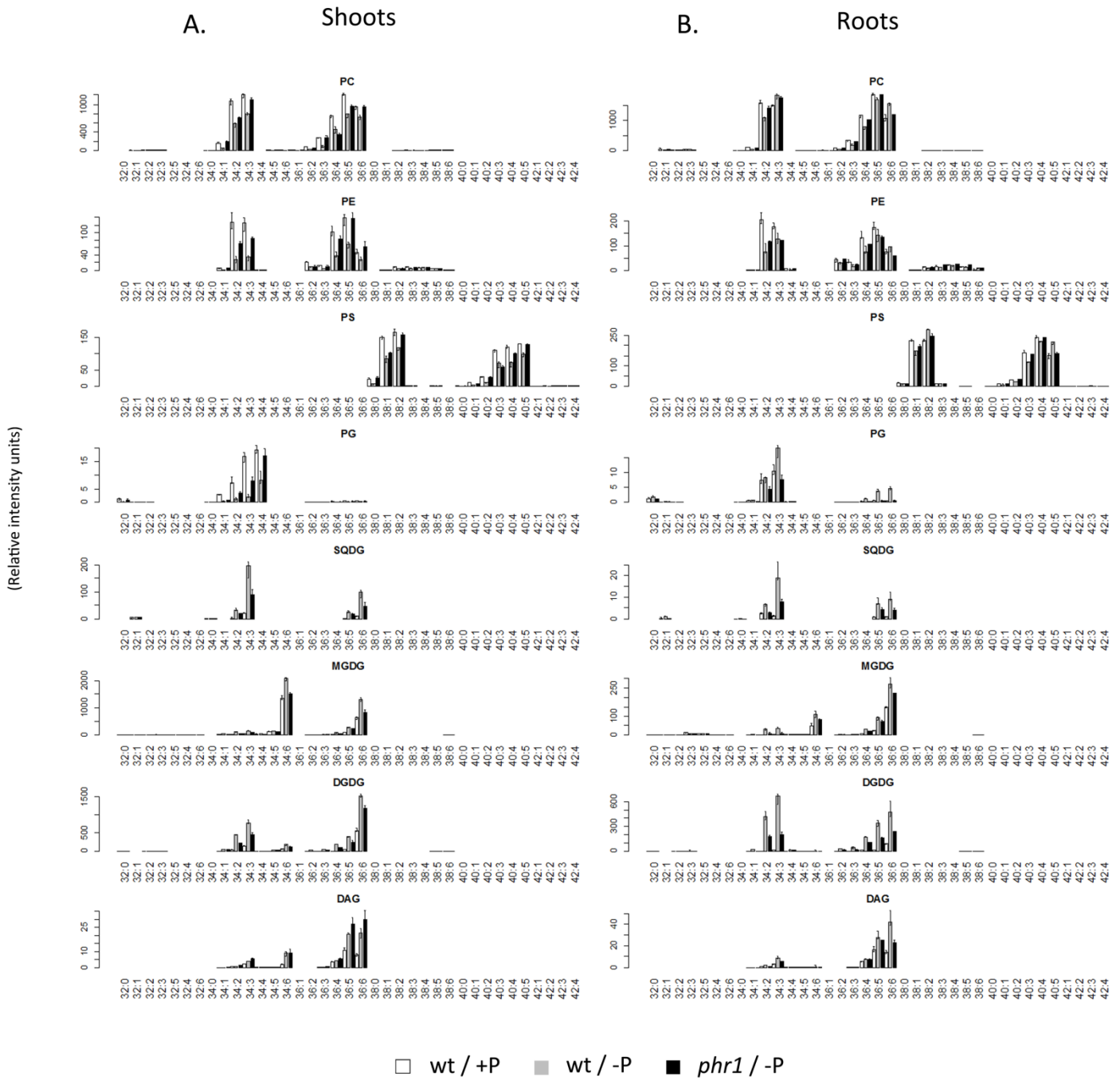
**The transcription factor PHR1 regulates lipid remodeling and triacylglycerol accumulation in *Arabidopsis thaliana* during phosphorus starvation.**

Bikram Datt Pant, Asdrubal Burgos, Pooja Pant, Alvaro Cuadros-Inostroza, Lothar Willmitzer, Wolf-Rüdiger Scheible

SUPPLEMENTARY DATA

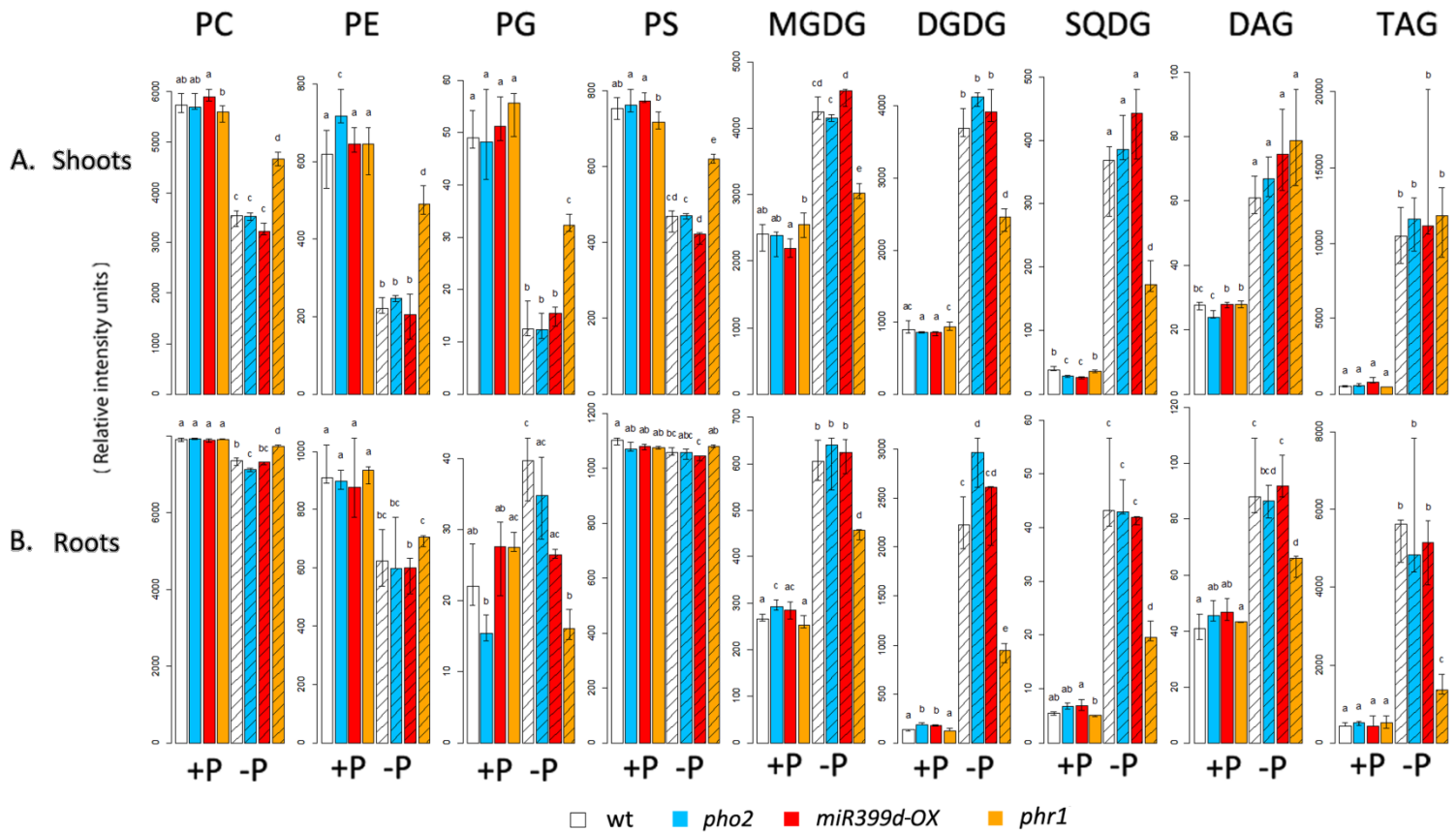
**Supplementary Figure S1.** Effect of P starvation on the relative abundance of glycerolipid species in shoots and roots of *Arabidopsis*.

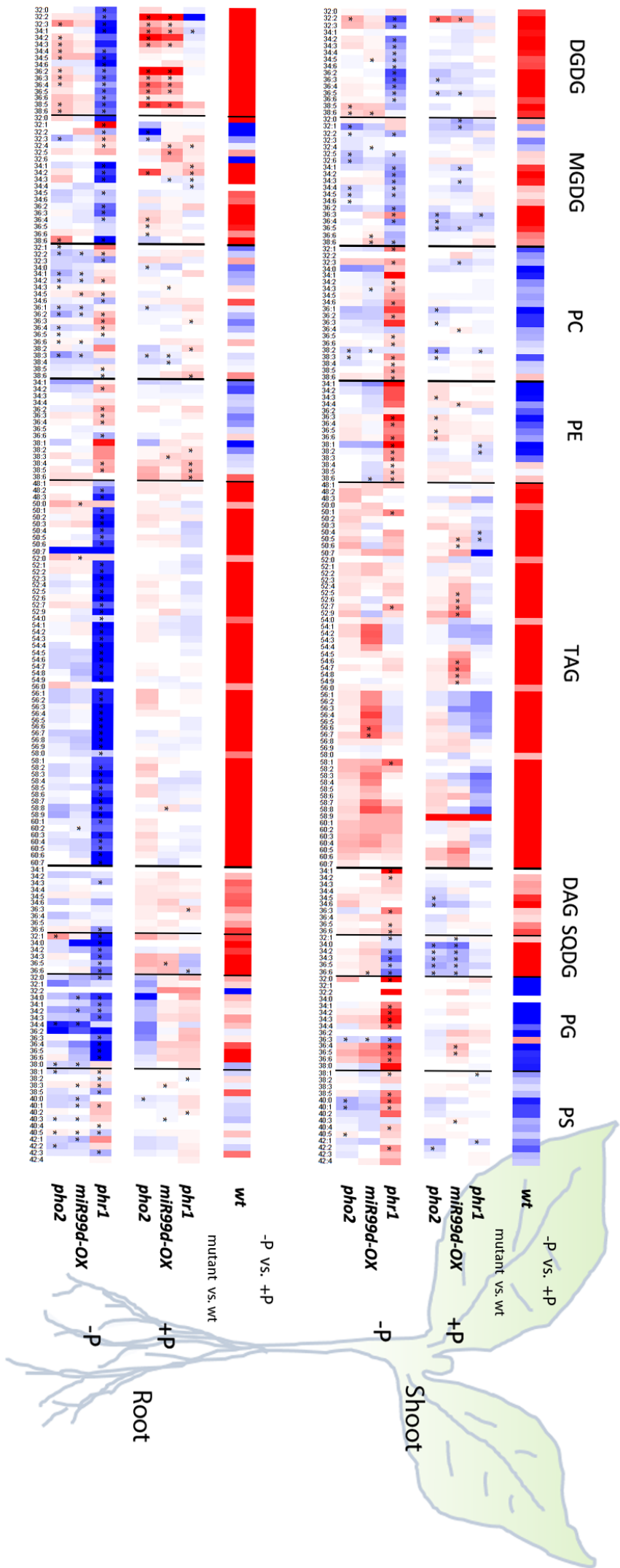
Values shown represent median relative intensities corresponding to shoots (A) and roots (B) of wild-type plants growing at 3mM Pi (white bars) and 0mM Pi (gray bars), and of *phr1* plants at 0mM Pi (black bars). Error bars represent the interval between the 1<sup>st</sup> and the 3<sup>rd</sup> quartiles.



**Supplementary Figure S2.** Effect of *pho2* and *miR399d-OX* genotypes in lipid composition under P starvation.

The mutant *phr1* is included for comparison. Plotted values represent the sum of normalized intensities of all the species belonging to each class. Error bars represent the interval between the 1<sup>st</sup> and the 3<sup>rd</sup> quartiles. Statistical groups calculated with pairwise T-tests are indicated with letters (a-d). Color code: wild type (white), *pho2* (blue), *miR399d-OX* (red), and *phr1* (orange); 3mM Pi (+P, solid bars) and 0mM Pi (-P, dashed bars).





**Supplementary Figure S3.** Changes caused by *phr1*, *mirR99d-OX* and *pho2* genotypes at the species level.

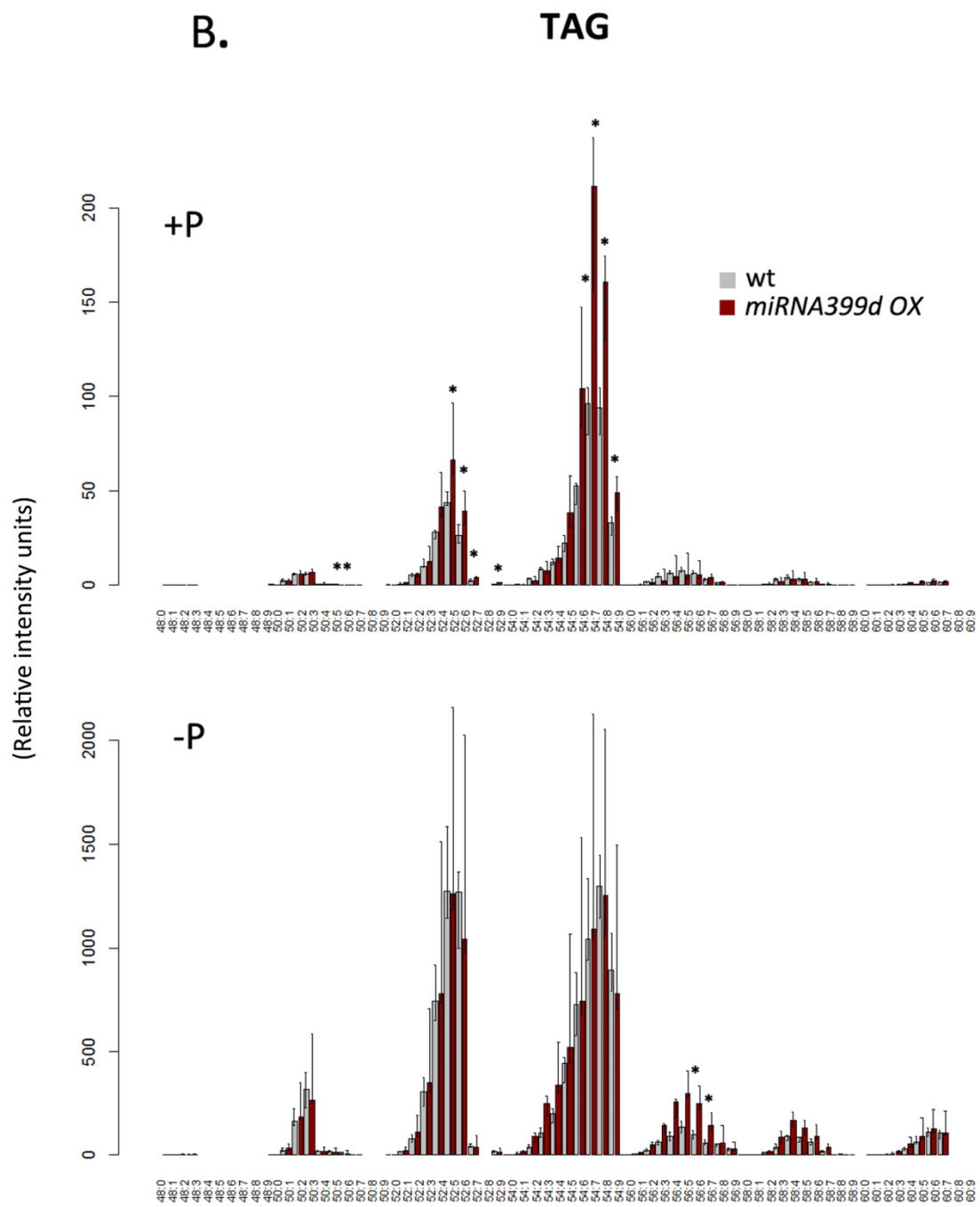
Heatmap colors indicate fold changes. Significant changes relative to the wild type are denoted with asterisks (T-test p-value < 0.05).

**Supplementary Figure S4.** The *miR399d-OX* genetic background has a significant effect on the abundance of a number of TAG species, although total TAG content is not significantly affected.

T-test p-values between wild type and *miR399d-OX* plants at 0mM Pi (-P) and 3mM Pi (+P) (A). Relative abundance of TAG species: wild type (gray), *miR399d-OX* (dark red) (B). Significant changes (p-value < 0.05) are highlighted with stars.

**A.** wt vs. *miRNA399d OX*

	-P	+P
TAG 48:1	0.262	0.653
TAG 48:2	0.334	0.857
TAG 48:3	0.507	0.918
TAG 50:0	0.188	0.265
TAG 50:1	0.319	0.445
TAG 50:2	0.379	0.331
TAG 50:3	0.575	0.179
TAG 50:4	0.445	0.072
TAG 50:5	0.513	* 0.039
TAG 50:6	0.580	* 0.046
TAG 50:7	0.253	0.055
TAG 52:0	0.456	0.103
TAG 52:1	0.200	0.427
TAG 52:2	0.195	0.424
TAG 52:3	0.299	0.296
TAG 52:4	0.399	0.095
TAG 52:5	0.517	* 0.044
TAG 52:6	0.538	0.025
TAG 52:7	0.398	* 0.019
TAG 52:9	0.642	* 0.001
TAG 54:0	0.192	0.077
TAG 54:1	0.077	0.447
TAG 54:2	0.075	0.442
TAG 54:3	0.065	0.429
TAG 54:4	0.110	0.386
TAG 54:5	0.226	0.142
TAG 54:6	0.348	* 0.021
TAG 54:7	0.352	* 0.005
TAG 54:8	0.510	* 0.006
TAG 54:9	0.569	* 0.018
TAG 56:0	0.520	0.821
TAG 56:1	0.130	0.415
TAG 56:2	0.126	0.413
TAG 56:3	0.071	0.431
TAG 56:4	0.057	0.430
TAG 56:5	0.061	0.420
TAG 56:6	* 0.049	0.447
TAG 56:7	* 0.047	0.461
TAG 56:8	0.179	0.331
TAG 56:9	0.290	0.309
TAG 58:0	0.859	0.057
TAG 58:1	0.271	0.323
TAG 58:2	0.169	0.417
TAG 58:3	0.098	0.434
TAG 58:4	0.088	0.454
TAG 58:5	0.209	0.473
TAG 58:6	0.221	0.476
TAG 58:7	0.052	0.572
TAG 58:8	0.062	0.612
TAG 58:9	0.151	0.801
TAG 60:1	0.250	0.380
TAG 60:2	0.224	0.372
TAG 60:3	0.170	0.434
TAG 60:4	0.133	0.438
TAG 60:5	0.216	0.451
TAG 60:6	0.344	0.214
TAG 60:7	0.345	0.095



### Supplementary Table S1. Fragmentation of 52-C and 54-C TAG species.

With the aim of exploring the composition of the most abundant TAG acyl groups, the masses of TAG fragments of 30 to 46 acyl carbons, with 0 to 6 saturations, were queried to TAG species MSMS spectra. The hits shown in the table had a maximum retention time (RT) difference of  $\pm 0.01$  min to the TAG species fragmented. For 52-C species there were only 34-C and 36-C fragments, implying 18-C and 16-C losses. It is well known that the bulk of PC and DAG, TAG precursor classes, contains either 34 or 36 acyl carbons, with a composition of 18-C + 16-C and 18-C + 18-C fatty acids, respectively (Browse and Somerville 1991). As only 16-C or 18-C fatty acid losses could be detected, the composition of 52-C TAG can be deduced as 18-C + 18-C + 16-C.

TAG species	formula	m/z (M + NH <sub>4</sub> )	RT	TAG fragment	formula	m/z (M + H)	RT	Predicted loss
52:0	C55H106O6	880.8347	15.8050	34:0	C37H70O4	579.5324	15.7979	18:0
52:1	C55H104O6	878.8105	15.3403	34:0	C37H70O4	579.5327	15.3352	18:1
52:1	C55H104O6	878.8105	15.3403	34:1	C37H68O4	577.5188	15.3371	18:0
52:2	C55H102O6	876.8018	15.3429	34:0	C37H70O4	579.5327	15.3352	18:2
52:2	C55H102O6	876.8018	15.3429	34:1	C37H68O4	577.5188	15.3371	18:1
52:2	C55H102O6	876.8018	15.3429	34:2	C37H66O4	575.5029	15.3440	18:0
52:3	C55H100O6	874.7854	15.0890	34:0	C37H70O4	579.5327	15.0858	18:3
52:3	C55H100O6	874.7854	15.0890	34:1	C37H68O4	577.5179	15.0816	18:2
52:3	C55H100O6	874.7854	15.0890	34:3	C37H64O4	573.4876	15.0909	18:0
52:3	C55H100O6	874.7854	15.0890	36:3	C39H68O4	601.5188	15.0986	16:0
52:4	C55H98O6	872.7701	14.8094	34:1	C37H68O4	577.5174	14.8149	18:3
52:4	C55H98O6	872.7701	14.8094	34:2	C37H66O4	575.5028	14.8036	18:2
52:5	C55H96O6	870.7544	14.5049	34:2	C37H66O4	575.5029	14.5042	18:3
52:5	C55H96O6	870.7544	14.5049	34:3	C37H64O4	573.4875	14.5038	18:2
52:6	C55H94O6	868.7390	14.1696	34:3	C37H64O4	573.4875	14.1696	18:3
52:6	C55H94O6	868.7390	14.1696	34:4	C37H62O4	571.4718	14.1700	18:2
52:7	C55H92O6	866.7237	13.7994	34:4	C37H62O4	571.4721	13.7918	18:3
52:7	C55H92O6	866.7237	13.7994	34:5	C37H60O4	569.4573	13.8007	18:2
52:9	C55H88O6	862.6936	12.8072	34:6	C37H58O4	567.4408	12.8145	18:3
52:9	C55H88O6	862.6936	12.8072	36:6	C39H62O4	595.4718	12.8154	16:3
54:1	C57H108O6	906.8403	15.5859	36:0	C39H74O4	607.5652	15.5873	18:1
54:1	C57H108O6	906.8403	15.5859	36:1	C39H72O4	605.5495	15.5824	18:0
54:2	C57H106O6	904.8335	15.5894	34:2	C37H66O4	575.5022	15.5983	20:0
54:2	C57H106O6	904.8335	15.5894	36:0	C39H74O4	607.5652	15.5873	18:2
54:2	C57H106O6	904.8335	15.5894	36:1	C39H72O4	605.5495	15.5824	18:1
54:2	C57H106O6	904.8335	15.5894	36:2	C39H70O4	603.5342	15.5890	18:0
54:3	C57H104O6	902.8173	15.3651	34:3	C37H64O4	573.4876	15.3732	20:0
54:3	C57H104O6	902.8173	15.3651	36:0	C39H74O4	607.5650	15.3635	18:3
54:3	C57H104O6	902.8173	15.3651	36:1	C39H72O4	605.5485	15.3561	18:2
54:3	C57H104O6	902.8173	15.3651	36:2	C39H70O4	603.5345	15.3589	18:1
54:3	C57H104O6	902.8173	15.3651	36:3	C39H68O4	601.5185	15.3624	18:0
54:4	C57H102O6	900.8012	15.1174	36:1	C39H72O4	605.5488	15.1106	18:3
54:4	C57H102O6	900.8012	15.1174	36:2	C39H70O4	603.5334	15.1104	18:2
54:4	C57H102O6	900.8012	15.1174	36:4	C39H66O4	599.5030	15.1192	18:0
54:5	C57H100O6	898.7859	14.8508	36:2	C39H70O4	603.5334	14.8536	18:3
54:5	C57H100O6	898.7859	14.8508	36:3	C39H68O4	601.5176	14.8441	18:2
54:5	C57H100O6	898.7859	14.8508	36:5	C39H64O4	597.4879	14.8553	18:0
54:6	C57H98O6	896.7697	14.5520	36:3	C39H68O4	601.5182	14.5559	18:3
54:6	C57H98O6	896.7697	14.5520	36:6	C39H62O4	595.4717	14.5567	18:0
54:7	C57H96O6	894.7540	14.2153	36:4	C39H66O4	599.5028	14.2177	18:3
54:7	C57H96O6	894.7540	14.2153	36:5	C39H64O4	597.4872	14.2097	18:2
54:8	C57H94O6	892.7389	13.8402	36:5	C39H64O4	597.4873	13.8433	18:3
54:8	C57H94O6	892.7389	13.8402	36:6	C39H62O4	595.4719	13.8439	18:2
54:9	C57H92O6	890.7234	13.4045	36:6	C39H62O4	595.4721	13.4048	18:3

**Supplementary Table S2.** Effect of P starvation and different genetic backgrounds (*phr1*, *pho2* and *miR399d-OX*) on the expression of lipid remodeling genes, transcription factors and genes involved in TAG accumulation.

Values are log<sub>2</sub>-transformed fold changes between two conditions/genotypes. These ratios were calculated from average expression levels (two technical and two biological replicates) of 40-ΔCTs. Significant changes (T-test p-value < 0.05) are denoted with stars.

		Shoots				Roots				
		wt -P vs. wt +P	<i>phr1</i> -P vs. wt -P	<i>pho2</i> +P vs. wt +P	<i>miR399d-OX</i> vs. wt +P	wt -P vs. wt +P	<i>phr1</i> -P vs. wt -P	<i>pho2</i> +P vs. wt +P	<i>miR399d-OX</i> vs. wt +P	
Lipid remodeling genes	<i>PLDZ1</i>	AT3G16785	0.41	-0.02	0.38	0.14	0.01	* -0.63	0.07	-0.11
	<i>PLDZ2</i>	AT3G05630	* 4.69	* -1.45	-0.05	0.18	* 3.15	* -2.22	0.40	0.17
	<i>NPC4</i>	AT3G03530	* 8.47	* -3.56	* 0.51	-0.03	* 2.37	* -3.55	0.14	-0.05
	<i>NPC5</i>	AT3G03540	* 9.73	* -1.18	* 1.18	* 1.89	* 2.34	* -3.32	0.12	* 0.65
	<i>PAH1</i>	AT3G09560	* 3.01	* -1.04	-0.03	0.01	* 1.86	* -2.05	0.15	-0.23
	<i>PAH2</i>	AT5G42870	* 0.89	* -0.54	0.17	0.18	0.10	* -0.55	-0.10	-0.20
	<i>PLA2A</i>	AT2G26560	* 1.87	-0.35	* 0.89	* 1.68	* 1.21	* -0.76	-0.05	* 0.90
	<i>GDPD5</i>	AT1G74210	* 2.98	* -1.43	-0.10	0.29	* 0.92	* -1.41	0.28	-0.34
	<i>GDPD6</i>	AT5G08030	* 10.53	* -5.57	0.19	-0.11	* 2.76	* -3.88	-0.08	* -0.53
	<i>MGD2</i>	AT5G20410	* 5.20	* -1.59	0.06	-0.49	* 2.06	* -2.89	0.44	* -1.03
	<i>MGD3</i>	AT2G11810	* 5.35	* -1.04	-0.20	* -1.30	* 4.50	* -3.50	* 1.07	* -0.60
	<i>DGD1</i>	AT3G11670	* 1.32	-0.37	-0.04	-0.21	* 0.79	* -0.81	0.11	-0.28
	<i>DGD2</i>	AT4G00550	* 2.23	* -1.02	0.10	0.01	* 1.50	* -1.86	0.16	-0.39
	<i>SQD1</i>	AT4G33030	* 5.16	* -1.17	-0.12	-0.20	* 3.00	* -1.06	* 0.75	0.35
	<i>SQD2</i>	AT5G01220	* 5.65	* -2.10	-0.10	-0.05	* 3.11	* -2.95	0.35	-0.26
Genes involved in TAG accumulation	<i>DGAT1</i>	AT2G19450	0.36	0.33	-0.15	0.00	-0.13	* -0.69	-0.10	-0.35
	<i>PDAT1</i>	AT5G13640	0.50	0.17	-0.02	-0.16	-0.20	* -0.54	0.05	-0.49
	<i>ROD1</i>	AT3G15820	0.22	-0.04	-0.16	0.08	-0.35	* -0.69	0.02	* -0.70
	<i>LPCAT2</i>	AT1G63050	* -0.54	* 0.97	-0.27	0.06	-0.41	* -0.58	0.09	-0.44
	<i>BCCP2</i>	AT5G15530	* -0.90	* 0.54	-0.20	* -0.63	-0.37	* -1.59	0.26	* -0.62
	<i>PDH-E1α</i>	AT1G01090	-0.40	0.21	0.01	-0.19	* -1.02	-0.46	-0.16	* -0.75
Transcription factors	<i>ABI4</i>	AT2G40220	* -1.92	* 5.70	* 0.73	* -0.56	* -4.49	-0.48	* -3.97	* -4.52
	<i>MYB62</i>	AT1G68320	* 2.70	-0.13	0.40	0.34	0.16	* 2.68	* -1.20	* -0.86
	<i>WRKY75</i>	AT5G13080	-0.31	* 1.46	-0.40	* 1.06	* 1.61	* 0.88	-0.11	0.41
	<i>ZAT6</i>	AT5G04340	* -0.80	* -1.69	* -1.12	* -1.70	-0.25	0.08	* -1.37	* -0.79
	<i>UBQ10</i>	AT4G05320	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

